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Effects of Bioactive Natural and Synthetic Compounds with Different Alkyl Chain Length on Photosynthetic Apparatus

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1. Introduction

Many compounds containing alkyl substituent(s) in their molecule exhibit a wide spectrum of biological activities. Such compounds, mainly water soluble amphiphilic compounds are frequently used in the industry and households as detergents or as disinfectants due to their solubilizing and antimicrobial properties. Consequently they can enter in the environment via waste waters and so affect photosynthetic processes in algae and plants. In general, the biological activity of compounds with alkyl substituent(s) was found to depend on the alkyl chain length. Good correlations between photosynthesis-inhibiting and antimicrobial activity were estimated. For amphiphilic compounds such as surfactants quasi-parabolic course of the dependence of biological activity on the length of alkyl substituent is characteristic. The decrease of biological activity observed from certain chain elongation is called “cut-off” effect. The main mechanism of biological action of the discussed compounds is closely connected with their membrane-damaging effects (Devínsky et al., 1990; Balgavý & Devinsky, 1996).

In photosynthetic electron transfer from water to NADP⁺ three integral membrane protein complexes operating in series: the photosystem 2 (PS 2) reaction centre, the cytochrome bf complex and the photosystem 1 (PS 1) reaction centre are involved. In two reaction centres primary charge separation occurs, in which light energy or excitation energy is transformed into redox free energy (Whitmarsh, 1998). Using an artificial electron acceptor, e.g. 2,6-dichlorophenol-indophenol (DCPIP) with the known site of action in plastoquinone on the acceptor side of PS 2 (Izawa, 1980), inhibition of photosynthetic electron transport (PET) in PS 2 by PET inhibitors can be monitored. On the other hand, application of artificial electron donors with known site of action to chloroplasts activity of which was inhibited by PET inhibitors enables more nearly to specify the site of action of tested inhibitors.

EPR is very useful method for investigation of the effects of organic and metal inhibitors in the photosynthetic apparatus because intact chloroplasts of algae and vascular plants exhibit EPR signals in the region of free radicals (g = 2.00), which are stable during several hours and could be registered at laboratory temperature by conventional continual wave EPR apparatus. EPR spectrum of intact chloroplasts is composed of two components, so-called signal I and signal II, belonging to PS 1 and 2, respectively (Hoff, 1979). Signal II consists of two parts, namely from (i) EPR signal II slow (g = 2.0046, ΔBpp = 1.9 mT) which is clearly
visible in Fig. 1a (full line) and belongs to the intermediate $D'$, i.e. to the tyrosine radical in the 161th position of $D_2$ protein on the donor side of PS 2 (Debus et al., 1988a) and (ii) EPR signal $II_{very fast}$ ($g = 2.0046$, $\Delta B_{pp} = 1.9$ mT) which is observable as an increase of signal II in light (Fig. 1a, difference between the dashed and full lines). This signal belongs to the intermediate $Z'$, i.e. to the tyrosine radical in the 161th position of $D_1$ protein (Debus et al., 1988b) which is also situated on the donor side of PS 2. Further EPR signal is associated with cation--radical of chlorophyll (Chl) a dimer situated in the core of PS 1 (Hoff, 1979).

Due to treatment of plant chloroplasts and algae with PET inhibitors the intensity and the shape of the above mentioned EPR signals can be changed (Fig. 1b). From these changes the site of action of studied inhibitors can be determined. Due to interaction of inhibitors with the oxygen evolving complex (OEC) also release of manganese $\text{Mn}^{2+}$ ions from OEC into interior of thylakoid membranes can occur, which can be registered by EPR spectroscopy. This contribution will be focused on comprehensive review related to inhibition of photosynthetic electron transport by inhibitors of natural origin as well as synthetic inhibitors containing alkyl chain(s) of different length in their molecules. The mechanism and the site of their action in the photosynthetic apparatus will be discussed as well.

2. Inhibitors of photosynthetic electron transport of natural origin

Natural products represent a vast repository of materials and compounds with evolved biological activity, including phytotoxicity. The two fundamental approaches to the use of natural products for weed management are their application as herbicides or leads for synthetic herbicides and their use in allelopathic crops or cover crops (Duke et al., 2002a, 2002b). Structures of some further discussed PET inhibitors of natural origin with alkyl substituent are presented in Fig. 2.

2.1 Fatty acids

Natural fatty acids are important components of biological membranes. They commonly have a chain of 4 to 28 carbons (usually unbranched and even numbered), which may be
saturated or unsaturated. In natural unsaturated fatty acids the double bonds are all cis and are usually not conjugated (Bhalla et al., 2009).

The saturated fatty acids (palmitic acid [16:0] as well as stearic acid [18:0]) applied at concentration 20 and 50 μmol dm⁻³ did not inhibit electron transport activity, whereas the unsaturated fatty acids (oleic acid [18:1], linoleic acid [18:2] and α-linolenic acid [18:3]) inhibited electron transport activity by ~50%. The monounsaturated fatty acid completely inhibited electron transport at 50 μmol dm⁻³. It could be stressed that the extent of PET-inhibiting activity was not dependent on the degree of unsaturation since all the unsaturated fatty acids inhibited PET to the same magnitude (Peters & Chin, 2003).

Krogmann & Jagendorf (1959) observed PET inhibition by unsaturated C₁₈ fatty acids. A few years later McCarty & Jagendorf (1965) and Molotkovsky & Zheskova (1965) showed that linolenic acid (LA) can induce damage in freshly isolated chloroplasts resembling that in chloroplasts after inactivation by gentle heating. Katoh & San Pietro (1968) suggested that high concentrations of LA inhibit PS 1 as well as PS 2. Brody (1970) observed a decline in the population of PS 2 reaction centres due to treatment with LA. Golbeck et al. (1980) localized the site of LA inhibitory action on the donor side of PS 1 and at two functionally distinct sites in PS 2. A reversible site and an irreversible site of inhibition have been located in PS 2. At the irreversible site a time-dependent loss of the loosely bound pool of Mn in the oxygen evolving complex occurred whereas at the reversible site, the photochemical charge separation was rapidly inhibited (<20 s) but after washing of LA-treated chloroplasts a resumption of artificial donor activity from diphenylcarbazide (DPC) to DCPIP was observed. The fact that inhibition of the Hill reaction by linolenic acid may be partially reversible was described also by Okamoto & Katoh (1977) and Okamoto et al. (1977).

According to Golbeck et al. (1980) the mechanism of inhibition of the photoactivity may ultimately lie in the ability of LA to penetrate its hydrophobic tail into the lipid membrane and change the orientation of electron donor and acceptor complexes relative to one another. Alternatively, the hydrophobic tail might interact with the antenna chlorophylls and inhibit transfer of energy to the photoactive trap. Due to direct interaction of free fatty acids with membrane proteins conformational changes in these proteins occur. Since PS 2 and its accessory pigments are most likely bound in a membrane protein complex, it could be supposed that alteration of the membrane structure would induce organizational changes in the associated peptides resulting in inhibition of charge separation between electron donor and acceptor complexes.

Linolenic acid was found to exhibit several effects on thylakoid membrane resulting in: i) modification of the membrane surface-charge density; ii) uncoupling of photophosphorylation (McCarty & Jagendorf, 1965); iii) release of manganese ions from water-oxidizing complex of PS 2 (Golbeck et al., 1980); iv) inhibition of artificial donor-assisted electron transport in PS 1 and PS 2 (Golbeck et al., 1980; Siegenthaler, 1974). Whereas Golbeck & Warden (1984) situated the site of LA action in Qₐ on the donor side of PS 2, Vernotte et al. (1983) stated that its site of action is similar to that of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), namely Q₈. Warden and Csatornai (1987) indicated that interactions of the unsaturated fatty acid, linoleic acid, with PS 2 have two principal regions of inhibition: one associated with the donor complex (signal Hₘₐ or Dₗ) to the reaction centre, and the other located on the reducing side between plastocyanin and Qₐ, whereby linoleic acid inhibits secondary electron transport in PS 2 via displacement of endogenous quinone Qₐ from quinone binding peptides.
Peters & Chin (2003) found that palmitoleic acid (PA), a monounsaturated fatty acid [16:1], caused rapid PET inhibition (within 30 s). The oxidizing side of PS 2 was up to 90% inactivated, whereas no inhibition occurred on the reducing side of the PS 2 complex and PS 1 activity was ~65% inhibited. These researchers did not observe correlation between PET inhibition and lipid peroxidation. On the other hand, PA caused the loss of proteins from the thylakoid membrane which was exacerbated by the light. The proteins were found to be lost in the following order: plastocyanin (PC) < 1 min, manganese stabilizing protein (MSP) ~5 min, cytochrome f (Cyt f) ~10 min, D1 protein ~60 min and D2 protein ~60 min. The timing of the loss of a PS 1 associated protein, PC, overlapped with that of the inhibition of PS 1. According to Peters & Chin (2003) the loss of PC is the cause of PS 1 inhibition. Because MSP loss from the OEC occurs later than the inhibition, it could not be considered as the cause of PET inhibition by PA on the oxidizing side of PS 2 and inhibition occurs due to the loss of Mn²⁺ ions.

Unsaturated fatty acids have been found to remove the Mn²⁺ ions from OEC (Kaniuga et al., 1986) and depletion of Mn²⁺ is correlated to inactivation of oxygen evolution (Garstka & Kaniuga, 1988) and to an inhibition of PS 2 activity (Krieger et al., 1998). Because addition of Mn²⁺ ions can effectively reverse PET inhibition at the donor side of PS 2 caused by PA, it can be assumed that loss of Mn²⁺ was the cause of this inhibition. It can be also supposed that at removing of Mn²⁺ ions form OEC the negatively charged unsaturated fatty acids may act as chaotropic agents or as chelating agents (Peters & Chin, 2003). Nakai et al. (2005) found that Myriophyllum spicatum released fatty acids, specifically nonanoic, tetradecanoic, hexadecanoic and octadecanoic acids. Anti-cyanobacterial effects of these fatty acids considerably depended upon their chain length: nonanoic, cis-6-octadecenoic, and cis-9-octadecenoic acids significantly inhibited growth of Microcystis aeruginosa, whereas tetradecanoic, hexadecanoic and octadecanoic acids did not show any effect.

2.2 Fischerellin, sorgoleone and resorcinolic compounds isolated from sorghum

Fischerellin A, a secondary metabolite of the benthic cyanobacterium Fischerella muscicola (Thur.), was found to inhibit the photosynthetic but not the respiratory electron transport of cyanobacteria and chlorophytes and its site of action is located in PS 2. It is the most active allelochemical component of F. muscicola, which is toxic to other cyanobacteria and photoautotrophic organisms (Bagchi & Marwah, 1994; Hagmann & Jüttner, 1996). Structural elements of fischerellin A ((3E)-1,5-dimethyl-3-{(3R,5S)-3-methyl-5-[(4E)-2-methylpentadec-4-ene-6,8-diyn-1-yl]pyrrolidin-2-ylidene}pyrrolidin-2,4-dione) are an enediyne moiety and two heterocyclic ring systems. This compound exhibits a unique structure composed of two cyclic amines and a C₁₅ substituent that contains a double bond in the (Z)-configuration and two triple bonds. Srivastava et al. (1998) found that fischerellin A affects the fluorescence transients, as well as oxygen evolution by the cyanobacterium Anabaena P9. The green alga, Chlamydomonas reinhardtii, and higher plants were also affected by fischerellin A in a concentration- and time-dependent fashion. It acts at several sites which appear with increasing half-time of interaction in the following sequence: (i) effect on the rate constant of Q₅ reoxidation; (ii) primary photochemistry trapping; (iii) inactivation of PS 2 reaction centre; (iv) segregation of individual units from grouped units. However, fischerellin A does not affect the photosynthetic activity of purple bacteria, Rhodospirillum rubrum. Fischerellin B was determined to be (3R,5S)-3-methyl-5-[(5E)-pentadec-5-ene-7,9-diynyl]-pyrrolidin-2-one.
Sorgoleone (2-hydroxy-5-methoxy-3-[(8´Z,11´Z)-8´,11´,14´-pentadecatriene]benzo-1,4-quinone) is one of the major components of the oily substance exuding from the roots of sorghum (*Sorghum bicolor* (L.) Moench) and it is one of the most studied allelochemicals (Dayan et al., 2010). This natural herbicide (bio herbicide) repressing the growth of other plants present in its surroundings (Dayan, 2006) was found to be a potent inhibitor of PS 2 in isolated chloroplasts, being as effective as diuron at inhibiting photosynthetic electron transport (*IC*₅₀ ~ 100 nmol dm⁻³) (Gonzalez et al., 1997). Its efficacy is not reduced in triazin-resistant pigweed (Dayan et al., 2009) because the common mutation of Ser264 to Gly or Ala in PS 2 causes resistance to triazines, but not to the quinone inhibitors (Oettmeier et al., 1982). Czarnota et al. (2001) performed three-dimensional structure analysis to characterize sorgoleone's mode of action and the results of their studies indicated that sorgoleone required about half the amount of free energy (493.8 kcal mol⁻¹) compared to plastoquinone (895.3 kcal mol⁻¹) to dock into the Q_b-binding site of the PS 2 complex of higher plants. Rimando et al. (2003) observed PS 2 inhibition by resorcinolic compounds having the characteristic three double bonds in terminal methylene lipid side chain as sorgoleone, i.e. 4,6-dimethoxy-2-[(8´Z,11´Z)-8´,11´,14´-pentadecatriene]resorcinol (*IC*₅₀ = 0.09 μmol dm⁻³) and 4-methoxy-6-ethoxy-2-[(8´Z,11´Z)-8´,11´,14´-pentadecatriene]resorcinol (*IC*₅₀ = 0.20 μmol dm⁻³). A new benzoquinone derivative, 2-hydroxy-5-ethoxy-3-[(8´Z,11´Z)-8´,11´,14´-pentadecatriene]-rho-benzoquinone, which was isolated from the root exudates of sorghum was found to be less effective PS 2 inhibitor than sorgoleone (Rimando et al., 1998).

![Fig. 2. Structures of some PET inhibiting compounds of natural origin: palmitoleic acid (I), linoleic acid (II), tenuazonic acid (III), sorgoleone (IV), fischerellin A (V), fischerellin B (VI) and platelet activating factor (1-O-alkyl-2-acetyl-sn-glycerol-3-phosphocholine) (VII).](image-url)

2.3 Hydroxydietrichequinone, tenuazonic acid and platelet activating factor

The natural quinone, hydroxydietrichequinone ((8Z)-3-heptadec-8-enyl-2-hydroxy-5-methoxybenzo-1,4-quinone) is a secondary metabolite of *Cyperus javanicus*. This natural
quinone has a long aliphatic chain \( (C_{17}) \) including an unsaturated bond at its midpoint. Morimoto et al. (2001) found that this quinone inhibited both mitochondrial respiration and photosynthesis in their electron transport systems. In chloroplasts prepared from spinach leaves this natural quinone inhibited PET in PS 2 in a similar way to that of the triazin herbicide, atrazine, which belongs to PS 2 herbicides.

Tenuazonic acid (TeA), a non-host-specific phytotoxin produced by Alternaria alternata, is the first toxin from a phytopathogen which was reported as a natural PS 2 inhibitor with several action sites (Chen et al., 2007, 2008). This bioherbicide with relatively short alkyl chain (sec-butyl) mainly interrupts PS 2 electron transport beyond \( Q_A \) (primary quinone acceptor) by competing with \( Q_B \) (secondary quinone acceptor) for \( Q_B \)-niche of the \( D_1 \) protein. Competition experiments between non-labeled TeA and \( ^{14} \text{C} \) atrazine showed that TeA has a similar site of action as atrazine, which binds to the \( Q_6 \)-site since atrazine binding to \( Q_6 \)-site could be prevented by TeA (Chen et al., 2007). After TeA treatment an increase of non-\( Q_A \) reducing centres was observed. Non-\( Q_A \) reducing centres, also so-called heat sink centres or silent centres, are radiators and often are used to protect the system from over excitation and over reduction which would create dangerous reactive oxygen species (ROS) (Chen et al., 2010). TeA also had a visible effect on electron flow at PS 1 acceptor. Because TeA interrupts PS 2 electron flow and ATP synthesis, it is regarded as an inhibitor of redox energy conservation and therefore also is expected to increase the energization levels in thylakoid, which can result in a large generation of ROS (Chen et al., 2010).

Barr et al. (1988) observed very efficient PET inhibition in PS 2 of spinach chloroplasts by the platelet activating factor 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine, a phospholipid, which is an ether analogue of phosphatidylcholine. Application of 2.8 to 3.5 \( \mu g \) cm\(^{-3} \) of this compound resulted in > 90% PET inhibition. The inhibition site for platelet activating factor was localized close to the reaction centre of PS 2, based on the inhibition of the donor reaction, DPC \( \rightarrow \) DCPIP, in Tris-treated chloroplasts. On the other hand, treatment by phorbol myristate acetate, 1,2-dipalmitin or fatty acid esters gave up to 17–32% PET inhibition, which was observed only at higher concentrations of these compounds.

2.4 Rhamnolipid biosurfactants produced by Pseudomonas aeruginosa

Wang et al (2005) investigated the algicidal activity of the rhamnolipid biosurfactants (the mixture of mono and dirhamnolipids Rha-Rha-C\(_{10}\)-C\(_{10}\) and Rha-C\(_{10}\)-C\(_{10}\)) produced by Pseudomonas aeruginosa. These biosurfactants were found to have potential algicidal effects on Heterosigma akashiwo. The growth of H. akashiwo was strongly inhibited in medium containing rhamnolipids (0.4–3.0 mg dm\(^{-3}\)) and at higher concentrations (≥ 4.0 mg dm\(^{-3}\)) the rhamnolipids showed strong lytic activity toward H. akashiwo. The extent of ultrastructural damage of the alga was severe at high concentrations of rhamnolipids and during extended periods of contact, whereby the plasma membrane was partly disintegrated. The lack of membrane facilitated entry of the rhamnolipid biosurfactants into the cells and allowed damage to other organelles, which resulted in the injury of chloroplasts.

3. Synthetic inhibitors of photosynthetic electron transport

Many synthetic compounds disrupt photosynthesis by blocking electron transfer in photosynthetic apparatus. Herbicides which inhibit Hill reaction belong to the group of PS 2 herbicides. Experimental studies have established that many PS 2 herbicides bind non-
covalently to a 32 kDa protein in the PS 2 complex and inhibit electron transfer between primary electron acceptor – quinone QA and the secondary electron acceptor – quinone QB on the reducing side of PS 2 (Shipman, 1981). Many PS 2 herbicides contain hydrophobic components as well as a flat polar component. The function of the hydrophobic components is to increase the lipid solubility of the entire herbicide molecule and to fit the hydrophobic surface of the herbicide binding site. It is assumed that the flat polar component binds electrostatically at a highly polar protein site (Shipman, 1981).

3.1 Alkyl substituted 2,4,6-trihydroxybenzamides and thiobenzamides

Highly potent PET inhibitors, alkyl substituted derivatives of 3-nitro-2,4,6-trihydroxybenzamide (NTHBA) and thiobenzamide (NTHTBA) (alkyl = ethyl – pentadecyl, benzyl, phenyl) were synthesized and tested by Honda et al. (1990a, 1990b). Comparison of PET-inhibiting activity expressed by pI50 value (negative logarithm of IC50, i.e. compound concentration causing 50 % inhibition with respect to the control) showed that dodecyl (pI50 = 8.4) and tridecyl (pI50 = 8.1) NTHBA derivatives as well as octyl (pI50 = 8.7) and decyl (pI50 = 8.3) NTHTBA derivatives were ten times more active than DCMU (pI50 = 7.3) (Honda et al., 1990a). In general, thiobenzamide derivatives were found to be more active than benzamide derivatives, presumably due to greater variance in the electron withdrawing potency of the two groups on the nucleus. For both series the PET-inhibiting activity largely depended on the overall lipophilicity of the molecules, however optimal chain length in NTHBA and NTHTBA was different. High activity of NTHBA and their thioamide analogues (NTHTBA) for PET inhibition indicated that they should interact with D1 protein of the PET system in a specific manner (Honda et al., 1990b). Free amino hydrogen atom in NTHBA and NTHTBA may be needed for binding to the receptor site, possibly by forming a hydrogen bond. N-octyl-3-nitro-2,4,6-trihydroxybenzamide (PNO8), classified as a phenol-type PS 2 inhibitor, caused degradation of the D1 protein of PS 2 reaction centre into two fragments of 23 and 9 kDa in complete darkness while the D2 protein was not affected at all by incubation with this inhibitor. Occupation by another PS 2 inhibitor, DCMU, of the binding site of the secondary quinone acceptor, QB, prevented the D1 protein from PNO8-induced degradation. These results indicate a selective and specific cleavage of the D1 protein triggered by binding of PNO8 to the QB site (Nakajima et al., 1995).

Yoneyama et al. (1989a, 1989b) synthesized and tested a set of alkyl substituted 3-acyl-2,4,6-trihydroxybenzamides and thiobenzamides for their PET-inhibiting activity. The thioamide derivatives were found to be much more active than the corresponding amide derivatives and some of the compounds were as active as DCMU. The activity of the 3-propionyl-2,4,6-trihydroxybenzamide derivatives with varying N-alkyl group was enhanced by increasing the length of the N-alkyl group reaching maximum with the N-heptyl group (pI50 = 7.4) and then remaining at constant level (pI50 = 7.3) until the chain reached C10. The activity of the N-phenylalkyl derivatives was also enhanced by increasing number of methylene groups indicating that the PET-inhibiting activity of these compounds depended largely on the overall lipophilicity of the amide derivatives (Yoneyama et al., 1989a). Slightly lower activity of thiobenzamide compound which had long side chains in both functional groups (R1 = R2 = hexyl) compared with that which had the same total number of carbon atoms in the side chains (R1 = ethyl, R2 = decyl), suggested that an asymmetric distribution of lipophilic groups about the phloroglucinol nuclei might be preferable for high activity (Yoneyama et al., 1989b).
3.2 2-Hydroxy-3-alkyl-1,4-naphthoquinones and n-alkyl-substituted ubiquinones

Jewess et al. (2002) found that the main mode of herbicidal activity of 2-hydroxy-3-alkyl-1,4-naphthoquinones is the inhibition of PS 2. The length of the 3- n-alkyl substituent for optimal activity differed between herbicidal and in vitro activity. The maximum in vitro activity was given by the nonyl to dodecyl homologues (log \(K_{ow}\) between 6.54 and 8.12), whereas herbicidal activity peaked with the n-hexyl compound (log \(K_{ow} = 4.95\)). The compounds did not show any activity on PS 1 and did not generate toxic oxygen radicals by redox cycling reactions.

Warncke et al. (1994) studied the influence of hydrocarbon tail structure on quinone binding and electron transfer performance at the QA and QB sites of the photosynthetic reaction-center protein isolated from Rhodobacter sphaeroides and solubilized in aqueous and in hexane solutions. It was found that contributions of the same tail structures to the binding free energies of quinones at the QA and QB sites are comparable, suggesting that the binding domains share common features. Comparison of the affinities of a homologous series of 10 \(n\)-alkyl substituted ubiquinones resolves the binding forces along the length of the tail binding domain and shows that strong steric constraints oppose accommodation of the tail in its extended conformation. One- and two isoprene-substituted quinones bind more tightly than analogues substituted with saturated alkyl tail substituents. Thus, the sites exhibit binding specificity for the native isoprene tail structure. Calculations indicated that the binding specificity aroused primarily from a lower integrated torsion potential energy in the bound isoprene tails.

3.3 Triazine and phenylurea derivatives with tail-like substituents

Reifler et al. (2001) investigated effects of tail-like substituents on the binding of competitive inhibitors to the QB site of PS 2. They synthesized triazine and phenylurea derivatives with tail-like substituents and tested the effects of charge, hydrophobicity and size of the tail on binding properties. If the tail was attached to one of the alkylamino groups of triazine-type herbicides or to the para position of phenylurea-type herbicides, loss of binding was not observed. Consequently, the herbicides must be oriented in the QB site such that these positions point toward the natural isoprenyl tail-binding pocket that extends out of the QB site. The requirement that the tail must extend out of the QB site, constrains the size of the other herbicide substituents in the pocket. When longer hydrophobic tails are used, the binding penalty that occurs upon adding a charged substituent at the distal end is reduced. This allows the use of a series of tail substituents possessing a distal charge as an approximate molecular ruler to measure the distance from the QB site to the aqueous phase.

3.4 Alkyl-N-phenylcarbamates and thiocarbamates and amphiphilic alkoxyphenyl carbamates

Derivatives of phenylcarbamic acids, which contain biologically active–NH-CO- group are biologically active compounds applied mainly as herbicides (Moreland, 1993). Phenylcarbamates were found to be mitotic poisons that killed roots by inhibiting cell division (e.g. Nurit et al., 1989).

The action of alkyl-N-phenylcarbamates on the photolytic activity of isolated chloroplasts was already studied in the late 50s of last century by Moreland & Hill (1959). Later it was found that alkyl-N-phenylcarbamates (alkyl = methyl - octyl) and alkyl-N-
phenylthiocarbamates (alkyl = methyl – butyl) interact with the intermediate $D^*$ situated in $D_1$ protein on the donor side of $PS_2$, however OEC, the intermediate $Z^*$ and $PS_1$ were not injured (Šeršen et al., 2000).

The most active PET inhibitor was methylthio derivative ($IC_{50} = 8.5 \mu mol dm^{-3}$), whereby for compounds with linear alkyl substituent PET inhibiting activity showed a linear decrease with increasing lipophilicity of the studied compounds. The inhibitory activity of compounds with branched alkyl substituents ($R = \text{isopropyl, tert-butyl, isobutyl}$) was lower than that of their linear isomers. The lower effectiveness can be connected with the fact that for achievement of the site of action in the photosynthetic apparatus, the branched substituents represent a higher hindrance than their linear isomers (Šeršen et al., 2000). On the other hand, Hansch & Deutsch (1966) found that the inhibition of the Hill reaction in chloroplasts produced by ethyl and isopropyl derivatives of $N$-phenylcarbamates with different substituents in positions 3 and 4 on the benzene ring showed an increase with increasing lipophilicity of the compounds. This indicates that for PET-inhibiting activity of these compounds not only the lipophilicity of the compounds but also electronic properties of the substituents were determinant.

Esters of 2-, 3- and 4-alkoxy substituted phenylcarbamic acids (alkyl = methyl – decyl) were found to inhibit photosynthetic electron transport in spinach chloroplasts and to reduce chlorophyll content in alga *Chlorella vulgaris*. The inhibitory effectiveness strongly depended on the alkyl chain length of the alkoxy substituent showing a typical quasi-parabolic dependence with maximum effect at 6-8 carbon atoms in the alkyl chain of piperidinothylesters (Kráľová et al., 1992a), 7-9 carbon atoms in the alkyl chain of dimethylaminoethylesters (Kráľová et al., 1992b) and 8-9 carbon atoms in the alkyl chain of piperidinopropylesters of alkoxyphenylcarbamic acids (Kráľová et al., 1995a; Šeršen & Kráľová, 1996). Similar results were obtained with morpholinoothylesters of 2-, 3- and 4-alkoxy substituted phenylcarbamic acids (Kráľová et al., 1994a) for which bilinear dependence of photosynthesis-inhibiting activity upon lipophilicity of compounds was confirmed. The alkoxy substitution in the position 2 decreased the inhibitory activity of the compounds when compared with their 3- and 4-substituted analogues. Similar dependences of photosynthesis-inhibiting activity on the length of alkoxy substituent were obtained also for 22 alkyl substituted aryloxyaminopropanols (Mitterhauszerová et al., 1991a). The dependence of photosynthesis-inhibiting activity of 1,3-diamino-2-propylesters of 2- and 3-substituted alkoxyphenylcarbamic acids on the dibasic part of the molecule was weak and the activity was more strongly affected by the position of the alkoxy substituent on the benzene ring of molecule as well as by the length of the alkoxy chain (Mitterhauszerová et al., 1991b).

The PET-inhibiting and algicidal activity of $N$-alkyl-$4$-piperidinothylesters (alkyl = ethyl – butyl) and $N$-ethylypyrrolidinylmethylesters of 2- and 3-substituted alkoxyphenylcarbamic acids (alkoxy = butyloxy – heptyloxy) strongly depended on the lipophilicity of the whole molecule, whereby lower inhibitory activity was determined for 2-alkoxy substituted derivatives (Kráľová et al., 1992c). Strong effect of the chain length of alkyl substituent on photosynthesis-inhibiting activity was found also for quaternary ammonium salts of heptacaine, i.e. for $N$-[2-(2-heptyloxyphenylcarbamoyloxy)-ethyl]-$N$-alkylpiperidinium bromides (Kráľová et al., 1994b). Moreover, it was confirmed that piperidinopropylesters of 2-, 3- and 4-alkoxy substituted phenylcarbamic acids stimulated oxygen evolution rate
(OER) in spinach chloroplasts at relatively low effector concentration, causing photophosphorylation uncoupling due to protonophore properties of these amphiphilic compounds (Šeršen & Kráľová, 1996).

Using EPR spectroscopy it was confirmed that the above mentioned alkoxyphenylcarbamates interacted with Z*/D* intermediates situated on the donor side of PS 2 and with OEC, causing release of Mn2+ ions into interior of thylakoid membranes (Mitterhauszerová et al., 1991b; Kráľová et al., 1992a, 1992c; Šeršen & Kráľová, 1996). The quasi-parabolic course of the dependence of P parameter evaluated from EPR spectra of effector-treated chloroplasts (which could be considered as a measure of photosynthesis inhibition in plant chloroplasts) on the length of alkoxy substituents of tested alkoxyphenyl carbamates correlated with dependences obtained for inhibitory activity of 1,3-diamino-2-propylesters of 2- and 3-alkoxyphenylcarbamic acids (Mitterhauszerová et al., 1991b).

P parameter was evaluated from the ratio of EPR signal I intensity determined for effector-treated chloroplasts in the light and in the dark related to such ratio obtained for untreated chloroplasts. From the above results it can be concluded that lower inhibitory activity of more lipophilic compounds with long alkyl substituents can be connected with the fact that these compounds predominantly remain incorporated in the lipid part of the membrane and only limited number of effector molecules will reach the membrane proteins. On the other hand, low lipophilicity of the compounds with short alkyl chains will result in their limited transition through membrane and in their more difficult access to interaction with PS 2 proteins situated on the inner side of thylakoid membranes.

3.5 2-Alkylsulfanyl-4-pyridinecarbothioamides and anilides of 2-alkylsulfanylpyridin-4-carboxylic acids

The IC50 values related to PET inhibition in spinach chloroplasts by 2-alkylsulfanyl-4-pyridinecarbothioamides (APCT) varied for the investigated set (alkyl = methyl – dodecyl) in the range from 72 μmol dm-3 (for octyl) to 6.24 mmol dm-3 (for methyl derivative) and it was found that the Hansch’s parabolic model is suitable for description of the correlation between photosynthesis-inhibiting activity and lipophilicity of APCT. For more precise determination of the site of APCT action in the photosynthetic apparatus of spinach chloroplasts EPR spectroscopy was used (Kráľová et al., 1997).

Fig. 1 presents EPR spectra of untreated spinach chloroplasts (Fig. 1a) and of chloroplasts treated with 0.05 mol dm-3 of 2-n-butylsulfanyl derivative (Fig. 1b). From Fig. 1b it is evident that the intensity of EPR signal II, mainly of its constituent signal II_slow, has been decreased by the studied compound indicating that APCT interact with D* intermediate. Due to the interaction of APCT with this part of PS 2, PET between PS 2 and PS 1 is impaired and consequently a pronounced increase of signal I intensity in the light (Fig. 1b, dashed line; g = 2.0026, ΔB = 0.7 mT) belonging to chlorophyll a dimer in the core of PS 1 can be observed. Upon addition of DPC, an artificial electron donor with the known site of action in the intermediate Z*/D* on the donor side of PS 2 (Izawa, 1980) to chloroplasts activity of which was inhibited by APCT, PET was practically completely restored. Consequently, it can be assumed that in the presence of APCT the own core of PS 2 (P 680) and a part of the electron transport chain – at least up to plastoquinone – remain intact.

Anilides of 2-alkylsulfanylpyridin-4-carboxylic acids were found to inhibit oxygen evolution rate in C. vulgaris, whereby the lipophilicity of the compound was determining for OER-inhibiting activity (Kráľová et al., 2001). They inhibited also PET in spinach chloroplasts and
the corresponding IC\textsubscript{50} values varied in the range from 4.8 \(\mu\)mol dm\(^{-3}\) to 69.1 \(\mu\)mol dm\(^{-3}\) and the lipophilicity of the most active compounds was about \(\log P = 5.0-5.5\) (Miletin et al., 2001). EPR spectroscopy confirmed that these anilides interacted with the intermediate D\(^*\) (Tyr\(_D\)) and in a pronouncedly lesser extent also with the intermediate Z\(^*\) (Tyr\(_Z\)). The intensive interaction of these compounds with Tyr\(_D\) which is situated on the donor side of PS 2 in less polar environment of the thylakoid membranes can be connected with the presence of hydrophobic alkylsulfanyl substituent in their molecules (Miletin et al., 2001).

### 3.6 Alkyl substituted benzothiazole derivatives

Several series of alkyl substituted benzothiazole derivatives were investigated also for their photosynthesis-inhibiting activity (Krá\'gová et al., 1992d, 1993; Sid\'ová et al., 1998, 1999; Šeršeň et al., 1993). The dependence of the negative logarithm of IC\textsubscript{50} values on the alkyl chain length of 2-alkylthio-6-R-benzothiazoles determined in the system of plant chloroplasts with partially damaged membranes showed a significant role of the substituent in position 6 with respect to the studied inhibitory activity. The increasing of its lipophilicity in comparable series leads to higher activity of compounds having shorter alkyl chains, with subsequent strong decrease of the activity with the further prolongation of the alkyl chain. The drop of the activity at derivatives with longer alkyl chains was the most pronounced in series having the most lipophilic substituent in position 6 (see Fig. 3A) (Krá\'gová et al., 1992d).

On the other hand, the inhibition of chlorophyll production in algae C. vulgaris was in the case of 6-formamido-, 6-acetamido- and 6-benzoylamino derivatives more strongly affected by the presence of compounds with lower lipophilicity of the substituent in position 6 (the inhibitory efficiency decreased in the order 6-formamido-, 6-acetamido- and 6-benzoylamino derivatives) (Fig. 3B). Thus, it can be assumed that higher lipophilicity of the substituent in the position 6 at equal alkyl chain length of the alkyl substituent diminishes the possibility of the compounds to penetrate through the intact outer algal cell membrane resulting in decreased inhibitory activity of the compounds. All bicyclo[2.2.1]hept-5-ene-2,3-dicarboximidomethylamino derivatives showed higher photosynthesis inhibition in plant chloroplasts as well as in C. vulgaris than the corresponding bicyclo[2.2.1]hept-5-ene-2,3-dicarboximido derivatives (Fig. 3) (Krá\'gová et al., 1992d).

Inhibition of PET in spinach chloroplasts and chlorophyll synthesis in C. vulgaris was observed also with 2-alkylthio-6-aminobenzothiazoles and their 6-N-substituted derivatives 3-(2-alkylthio-6-benzothiazolaminomethyl)-2-benzothiazolinethiones and 3-(2-alkylthio-6-benzothiazolinone)-6-bromo-2-benzothiazolinones (Krá\'gová et al., 1993). The dependence of inhibitory activity of these compounds on the alkyl chain length of the thioalkyl substituent showed quasi-parabolic course indicating decrease of biological activity for compounds with higher lipophilicity. It was found that in the presence of these compounds no reduction of PS 1 occurred, however interaction with the intermediates Z\(^*\)/D\(^*\) was not confirmed. Since DPC practically completely restored PET through PS 2 in spinach chloroplasts activity of which was inhibited by these inhibitors it can be assumed that these benzothiazole derivatives did not damage PET between photosynthetic centres PS 2 and PS 1 and their site of action is situated at the donor side of PS 2 (Krá\'gová et al., 1993).

PET inhibition in spinach chloroplasts was observed also by 2-(6-acetamido-benzothiazolethio)acetic acid esters (Sid\'ová et al., 1998). The dependence of PET inhibiting
activity on the lipophilicity of the derivatives with \( R = n \)-alkyl and allyl showed a quasi-parabolic course, the most active compound was the hexyl derivative (IC\(_{50} = 47 \mu\text{mol dm}^{-3}\)). The effect of 2-(alkoxycarbonylmethylthio)-6-aminobenzothiazoles on photosynthetic apparatus was similar to that of above discussed 2-alkylthio-6-R-benzothiazoles and as probable site of their action the oxygen evolving complex was suggested (Šeršen et al., 1993).

Fig. 3. Dependence of negative logarithm of IC\(_{50}\) values related to PET inhibition in spinach chloroplasts on the number of carbons of the alkyl chain of 2-alkylthio-6-R-benzothiazoles (A) and dependence of inhibition of chlorophyll production in *C. vulgaris* in the presence of 0.1 mmol dm\(^{-3}\) of 2-alkylthio-6-R-benzothiazoles (B). (R = formamido (○), acetamido (□), benzoylamino (◊), bicyclo[2.2.1]hept-5-ene-2,3-dicarboximino (●) and bicyclo[2.2.1]hept-5-ene-2,3-dicarboximidomethylamino (▲). (Source: Krafrová et al., 1992 d).

### 3.7 Alkyl substituted N-oxides

Amine oxides, also known as amine-N-oxides or N-oxides are chemical compounds which contain the functional group \( R\text{N}^{+}\text{-O}^- \), an N-O bond with three additional hydrogens and/or hydrocarbon side chains attached to nitrogen atom. The structure of alkyl substituted N-oxides predestine them to have membrane damaging as well as PET-inhibiting properties. Avron (1961) found that the Hill reaction and associated phosphorylation in Swiss chard chloroplasts were sensitive to heptyl- and nonylhydroxyquinoline-N-oxides. Among the photosynthetic reactions studied, light-induced cyclic phosphorylation with phenazine methosulfate as cofactor was least sensitive to the inhibitors, whereas the most sensitive was the Hill reaction and coupled phosphorylation in the presence of ferricyanide. Avron suggested that these inhibitors blocked electron transport at a site similar to that of DCMU. Bamberger et al. (1963) also showed that NADP\(^{+}\) photoreduction with reduced DCPIP as electron donor was highly resistant to these inhibitors, whereas photoreduction through the normal Hill reaction system was not. Izawa et al. (1966) suggested that the inhibition site of 2-heptyl-4-hydroxyquinoline-N-oxide (HOQNO) in non-cyclic electron flow in chloroplasts is somewhat different from that of DCMU, as inferred from the difference in dependency of the actions of these two inhibitors on light intensity. Later, Gromet-Elhanan (1969) described HOQNO as acting at two different sites in the electron transport chain. At a low
concentration it inhibited near PS 2, and at higher concentrations, somewhere near PS 1, what was effectively by-passed by phenazine methosulfate. The latter inhibition site of HOQNO has also been suggested by Hind & Olson (1966) who showed that HOQNO increased the magnitude of reversible changes of cytochrome b6 and proposed that the inhibitor blocked electron flow between cytochrome b6 and PS 1.

![EPR spectra of spinach chloroplasts](image1)

**Fig. 4.** EPR spectra of spinach chloroplasts (I) registered in the dark (full line) and under irradiation (dotted line) for control sample (A) and for chloroplasts treated with 0.01 mol dm$^{-3}$ 1-octylpiperidine-N-oxide (B) or 1-dodecylpiperidine-N-oxide (C); EPR spectra of Mn$^{2+}$ ions (II) in untreated spinach chloroplasts (A) and in chloroplasts treated with 0.05 mol dm$^{-3}$ 1-hexyl-1-ethylpiperidium bromide (B) or with 1-tetradecyl-1-ethylpiperidinium bromide (C). (Source: Kráľová et al. 1992e).

Surfactants of homologous series of 1-alkylpiperidine-N-oxides (APNO) with alkyl = hexyl – octadecyl were found to inhibit PET in spinach chloroplasts and chlorophyll synthesis in algal suspensions of C.vulgaris (Kráľová et al., 1992e). The dependence of algicide effects of APNO (expressed by minimum inhibitory concentration, MIC) on the surfactant alkyl chain length showed quasi-parabolic course and the highest algicide activity (MIC about 10 µmol dm$^{-3}$) exhibited derivatives in which alkyl chain varied from tridecyl to hexadecyl. The algicide effect of surfactants was not connected with their associative properties since all MIC values were far below the critical micelle concentration of these compounds. Similar results were obtained also for PET inhibiting activity of APNO in spinach chloroplasts whereby the more pronounced decrease of activity was associated with the prolongation of the alkyl chain (C$_{15}$-C$_{18}$).

Decreased intensity of both components of signal II as well as the rise of signal I in the light in EPR spectra of APNO-treated chloroplasts indicated that these compounds caused damage of PS 2 and electron flow to PS 1 was interrupted (Fig. 4(I), C). From this Figure it is evident that the changes in EPR spectra caused by dodecyl derivative were considerably higher than those caused by octyl derivative, which is in accordance with the results related to algicide and PET-inhibiting activity of APNO. Moreover, in EPR spectra of APNO-treated chloroplasts occurrence of six lines of fine structure belonging to free Mn$^{2+}$ ions was observed (similarly as it is documented in Fig. 4(II), C), indicating injury of OEC, which is
situated on the donor side of PS 2 (Kráfová et al., 1992e). The amount of released Mn$^{2+}$ ions from the above mentioned OEC – at constant chlorophyll and surfactant concentration - is proportional to the inhibitory activity of surfactant.

$N$-alkyl-$N,N$-dimethyldiamine oxides (ADAO) (alkyl = hexyl – octadecyl) were also found to inhibit PET in plant chloroplasts and chlorophyll synthesis in green alga C. vulgaris (Šeršen et al., 1992). The dependence of the biological activity (expressed by log $IC_{50}$) on the length of alkyl substituent showed quasi-parabolic course with maximum activity for tetradecyl derivative. From EPR spectra it was evident that the site of ADAO action is PS 2, mainly $Z^*/D^*$ intermediates or its neighbouring surroundings. The release of Mn$^{2+}$ ions into thylakoid membranes due to ADAO treatment was also confirmed. In order to find what effects are exhibited by ADAO on chloroplast membranes, an EPR study using spin labels CAT 16 (N-hexadecyl-N-tempoyl-$N,N$-dimethylammonium bromide) and 16 DSA (16-doxylstearic acid) was performed. The motion of spin labels after their incorporation into membranes will be limited and consequently changes in their EPR spectra occur.

Incorporation of ADAO into thylakoid membranes causes also perturbation in membrane structure depending on ADAO concentration and it is evident that the arrangement of thylakoid membrane expressed by order parameter $S$ with increasing ADAO concentration decreased (Fig. 5A). The order parameter $S$ evaluated from the above mentioned changes in EPR spectrum (in detail see in Šeršen et al. (1989)) reflects relative membrane perturbation. From the dependence of order parameter $S$ of hexyl-, dodecyl- and hexadecyl derivatives (obtained with the spin label CAT 16) on the ADAO concentration it is evident that the order parameter $S$ decreased as follows: hexyl, hexadecyl and dodecyl.

Fig. 5. Dependences of the order parameter of thylakoid membranes $S$ (determined from EPR spectra of CAT 16) on the concentration of ADAO for hexadecyl- (C), dodecyl- (o) and hexyl- (△) derivatives (A) and on the alkyl chain length of ADAO at the constant concentration of ADAO 50 μmol dm$^{-3}$ (B); $S$ was evaluated from EPR spectra of CAT 16 (o) and 16 DSA (C) spin labels. (Source: Šeršen et al., 1992).

Fig. 5B presents dependence of order parameter $S$ on the alkyl chain length of ADAO at constant compound concentration (50 mmol dm$^{-3}$). The experiments with both spin labels confirmed that the most effective derivative related to perturbation of membrane arrangement was dodecyl, which is in accordance with the above mentioned results obtained for PET inhibition in spinach chloroplasts and chlorophyll content reduction in
Effects of Bioactive Natural and Synthetic Compounds with Different Alkyl Chain Length on Photosynthetic Apparatus

alga. After adding of ADAO to chloroplasts containing spin labels, an increase in the rate of molecular reorientation of spin label was observed. This was manifested by a decrease in the rotational correlation time values ($\tau_c$) with increasing ADAO concentration (Fig. 6A). The rotational correlation time is linearly proportional to the microviscosity of the environment in which the spin label is located, i.e. ADAO decrease the microviscosity of thylakoid membranes. The course of $\tau_c$ of 16 DSA spin label located in the thylakoid membrane on the alkyl chain length of ADAO at constant compound concentration 50 mmol dm$^{-3}$ (Fig. 6B) was similar to that obtained for order parameter $S$ (Fig. 5B), i.e. the lowest $\tau_c$ exhibited dodecyl derivate. The dependence of $IC_{50}$, $S$ and $\tau_c$ (characterizing the effect of ADAO on photosynthetic apparatus) on the alkyl chain length showed a typical "cut-off" dependence with maximal effects for dodecyl or tetradecyl derivates. This can be explained by the incorporation of ADAO in the lipid phase of membranes whereby the greatest perturbation in the membrane is caused by ADAO with middle chain length (approximately dodecyl), which create sufficiently great free volume in the membrane and their partition coefficients between chloroplast organelles and aqueous phase have also sufficiently high values.

Fig. 6. Dependences of rotational time $\tau_c$ of 16 DSA spin label located in the thylakoid membranes on the concentration of N-dodecyl-N,N-dimethylamine oxide (A) and on the alkyl chain length of ADAO at the constant concentration of ADAO 50 $\mu$mol dm$^{-3}$ (B).
(Source: Šeršeň et al., 1992).

3.8 Cationic surfactants of the type of alkyl substituted quaternary monoammonium and diammonium salts

Lower concentrations of 1-alkyl-1-ethylpiperidinium bromides (AEPBr; alkyl = hexyl – octadecyl) stimulated OER in spinach chloroplasts (Šeršeň & Devínsky, 1994; Šeršeň & Lacko, 1995) indicating their activity with respect to thylakoid membranes. Previously it was found that anionic surfactant sodium dodecyl sulphate (SDS), if applied at low concentrations stimulated photoreduction of potassium ferricyanide, but application of higher surfactant concentrations exhibited inhibitory effects. Stimulation was caused by increased permeability of chloroplast envelope membrane and inhibitory effects were connected with changes in the chloroplast membrane organization, induced by treatment with surfactants. Application of SDS led to an inhibition of the light-induced proton uptake ($\Delta$pH) due to deterioration of ATP-ase (Apostolova, 1988). The possibility that AEPBr enhance OER by a damage of ATP-ase and so prevent photophosphorylation is unlikely, because all AEPBr derivatives stimulated Hill reaction irrespective of their alkyl chain

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length (Šeršeň & Devínsky, 1994). It is also unlikely that AEPBrs can act as protonophores because their structure excludes such effect. Thus, it is probable that AEPBr after their incorporation into thylakoid membranes cause changes in their organization leading to an increase of OER. Similar effect was observed with other surfactants (Apostolova, 1988) and linolenic acid (Golbeck et al., 1980) but changes in the arrangement of the membrane caused by structurally different surfactants need not be the same. Siegenthaler & Packer (1965) found that higher concentrations of decenyl and dodecenyl succinic acids inhibited ferredoxin-NADP photoreduction and photophosphorylation but the application of low concentrations increased photophosphorylation and NADP photoreduction as well.

The arrangement of the thylakoid membranes in spinach chloroplasts was investigated by the spin label method, using CAT 16 as spin label. For characterization of the arrangement of thylakoid membranes the order parameter \( S \) was calculated from EPR spectrum of spin label incorporated into thylakoid membranes according to Šeršeň et al. (1989). The dependences of order parameter \( S \) and OER on the concentration of 1-dodecyl-1-ethylpiperidinium bromide (DEPBr) is shown in Fig. 7. At certain DEPBr concentrations enhancement of both parameters (\( S \) and OER) was observed related to control samples. This stimulating effect was observed at different DEPBr concentrations in OER and in EPR experiment, which was connected with different chlorophyll content in chloroplast suspensions used for individual experiments. Using DEPBr partition coefficient between chloroplast organelles and aqueous environment, DEPBr concentration within chloroplast organelles was calculated. In such terms, OER stimulation and increase of order parameter \( S \) occurred in the same concentration range (Fig. 7B). Based on these findings it could be assumed that OER stimulation is caused by changes in the arrangement of thylakoid membranes.

As mentioned above, the interaction of amphiphilic molecules, including alkyl substituted quaternary ammonium salts, with the hydrophobic parts of cell membranes leads to damage

Fig. 7. The dependence of the DCPIP reduction (\( \bullet \) and the order parameter \( S \) (\( \circ \)) expressed as % of control sample upon concentration of 1-dodecyl-1-ethylpiperidinium bromide (DEPBr) in chloroplast suspension (A) and in chloroplast organelles (B). (Source: Šeršeň & Lacko, 1995).

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of membrane structure (Apostolova, 1988; Devínsky et al., 1990; Balgavý & Devínsky, 1996). Detergent properties of surfactants enable to solubilize functional components bonded with the organized structures (Loach, 1980) and to form mixed micelles with them (Hermann et al., 1988). This can affect the key biochemical and energy yielding processes and subsequently cause biostatic and biocidal effects in lower organisms.

AEPBr (alkyl = hexyl – octadecyl) applied at higher concentrations inhibited PET in spinach chloroplasts and reduced chlorophyll content in algal suspensions of *C. vulgaris*. The photosynthesis-inhibiting activity of these surfactants showed quasi-parabolic dependence upon the length of alkyl substituent (Kráľová et al., 1992e). From changes in EPR spectra of spinach chloroplasts treated with AEPBr *P* parameter was evaluated which is available as a measure of photosynthesis inhibition in plant chloroplasts for homologous series of inhibitors having the same site and mechanism of action. The value of *P* parameter showed very strong dependence on the alkyl chain length of AEPBr. While the values of *P* parameter for AEPBr with shorter alkyl chain (pentyl, hexyl, heptyl) were < 1 and for octadecyl derivative *P* = 6 was determined, the highest value of this parameter was obtained for tetradecyl derivative (*P* = 27.7). These results are in accordance with the finding related to PET inhibition in spinach chloroplasts determined by the use of artificial electron acceptor DCPIP.

The decrease of a biological activity observed for amphiphilic compounds upon elongation of their hydrophobic (hydrocarbon) part is called “cut-off” effect (Devínsky et al., 1990; Balgavý & Devínsky, 1996). These hydrophobic parts of surfactants interact with lipid parts of biological (including thylakoid) membranes. However, penetration of surfactants with longer alkyl through hydrophilic (aqueous) regions of biological membranes is restricted due to their low aqueous solubility causing lower concentration of such surfactants in the membrane in comparison with the concentration of surfactants with shorter alkyl chain. Consequently, the biological activity of long-chain surfactants decreases. It is suggested that the lateral expansion of the phospholipid bilayer of biological membranes caused by the intercalation of long-chain amphiphilic molecules between the phospholipid molecules and the mismatch between their hydrocarbon chain lengths results in the creation of free volume in the bilayer hydrophobic region. According to the free volume theory the extent of membrane disturbance due to surfactant incorporation depends on the size of free volume created under its alkyl chain which can be then filled up with chains of neighbouring lipids as well as on the partition coefficient of the surfactants, i.e. on the number of surfactant molecules in the membrane (Devínsky et al., 1990; Balgavý & Devínsky, 1996).

From EPR spectra of AEPBr-treated chloroplasts it was evident that these compounds interacted with Z*/D* intermediate on the donor side of PS 2 and with OEC as well (Kráľová et al., 1992e). Due to interaction of AEPBr with OEC, Mn²⁺ ions were released into interior of thylakoid membranes, which was manifested in EPR spectra of AEPBr-treated chloroplasts by the presence of six lines of fine structure (Fig. 4(II), C). In OEC, which is situated on the donor side of PS 2, four ions of manganese are bound in the 33kDa protein (Blankenship & Sauer, 1974; Cheniae, 1980), however due to intense spin-spin interaction of protein-bound manganese in untreated chloroplasts is the EPR signal of Mn²⁺ ions very low (Fig. 4(II), A). The amount of released manganese ions (at constant chlorophyll and surfactant concentration) was proportional to the inhibitory activity of surfactant (compare corresponding signals for hexyl (Fig. 4(II), B) and tetradecyl (Fig. 4(II), C) derivatives).
Several homologous series of cationic gemini surfactants, namely \( \text{N,N}^-\text{bis(alkyldimethyl)}-1,6\text{-hexanedianmonium dibromides (HDDBr) (Kráľová & Šeršen, 1994)}, \) isosteric \( \text{N,N}^-\text{(alkyldimethyl)}-3\times1,5\text{-pentanediammonium dibromides (PDDBr) (Kráľová et al., 1995b)} \) and \( 3,8\text{-diaza-4,7-dioxodekane-1,10-diylbis(alkyldimethylammonium) bromides (DDDBr) (Kráľová et al., 2010)} \) were tested for their PET-inhibiting activity and also for inhibition of chlorophyll synthesis in \( \text{C. vulgaris.} \)

For isosteric \( \text{N,N}^-\text{(alkyldimethyl)}-3\times1,5\text{-pentanediammonium dibromides (PDDBr)} \) with \( X = \text{CH}_2, \text{NCH}_3, \text{O or S} \) it was found that their critical micelle concentration did not reflect small differences in lipophilicity of the compounds with a very similar structure sensitively enough. The differences in biological activities (IC\(_{50}\) values related to PET inhibition in spinach chloroplasts and MIC values related to reduction of chlorophyll content in \( \text{C. vulgaris} \) of PDDBr isosters with the same alkyl chain were very small as well, indicating that modification of spacer did not affect the mode of action of these gemini surfactants (Kráľová et al., 1995b).

Similarly to AEPAbr, the above mentioned gemini surfactants (PDDBr, HDDBr and DDDBr) interacted with \( \text{Z}/\text{D}^- \) intermediates situated on the donor side of PS 2 and with OEC as well. For HDDBr surfactants the highest \( P \) values (about 16.7) reached undecyl and tridecyl derivatives which were found to be the most effective PET inhibitors (Kráľová & Šeršen, 1994; Kráľová et al., 2010). The \( P \) parameter determined for dodecyl derivative of DDDBr was 12.4 indicating that insertion of two NHCO groups into spacer resulted in partial decrease of this parameter as well as in the decrease of PET inhibition (Kráľová et al., 2010). For ascertaining whether two CONH groups in the spacer of DDDBr could affect the resulting inhibitory activity of these surfactants, DCPIP photoreduction by the base \( \text{N,N}^-\text{bis(2-dimethylaminoethyl)ethanediame} \) was estimated (Kráľová et al., 2010). This compound did not contain long alkyl chain and so its PET-inhibiting activity is connected only with two CONH groups in its molecule. While the determined IC\(_{50}\) value for this diamide was found to be 4.0 mmol dm\(^{-3}\) and the corresponding IC\(_{50}\) value estimated for nonyl derivative was only 1.74 mmol dm\(^{-3}\), it can be supposed that CONH groups in the spacer participate on the resulting inhibitory effects. Moreover, the extent of the contribution of amide groups to the total inhibitory effect of DDDBr surfactants with longer alkyl chains was much lower, which was reflected in the corresponding IC\(_{50}\) values of these surfactants (e.g. 69.7 \( \mu \text{mol dm}^{-3} \) for dodecyl derivate). Using EPR spectroscopy it was found that the interaction of tested base with the \( \text{Z}^- \) and \( \text{D}^- \) intermediates was much weaker than this of DDDBr and not even release of Mn\(^{2+}\) ions into thylakoid membrane was observed after diamide treatment. Weaker interaction was reflected also by very low value of \( P \) parameter (\( P = 2.9 \)) obtained with tested base. These results indicate that for amphiphilic compounds from the group of cationic gemini surfactants it is more easy to reach the sites of their action in the photosynthetic apparatus (which can be situated in the regions of thylakoid membranes with different polarity) than for their non-polar bases.

4. Conclusion

Herbicidal effects of compounds having alkyl chain(s) in their molecule are caused either by interaction of the compound with membrane (destruction, re-arrangement, change of the viscosity, etc.) or by its interaction with proteins occurring in plant cells. Interaction with membranes is characteristic mainly for amphiphilic compounds (amine oxides, quaternary
ammonium salts and fatty acids) in which alkyl chains could be incorporated into membrane, what results in structure modification of photosynthetic proteins, which bind individual components of the PET chain. Due to such changes the photosynthetic electron transport through photosynthetic centres will be interrupted what results in the decrease of OER. The most effective disturbance of the membrane and thus the highest inhibitory effect will be exhibited by compounds with middle alkyl chain length ensuring not only sufficiently high free volume under alkyl chain but also high concentration of the surfactant in the membrane due to suitable value of surfactant partition coefficient. Moreover, it was found that due to interaction of amphiphilic membrane-active compounds with manganese cluster release of manganese ions from the oxygen evolving complex occurs and dysfunction of intermediates $Z^*/D^*$ occurring at 161th position of $D_1$ and $D_2$ proteins situated on the donor side of PS 2 is manifested. However, herbicides can act also by direct interaction with cell proteins. Such herbicides have in their structure certain functional groups which can interact with some amino acid residues of proteins. Due to such interaction interruption of PET through photosynthetic centres occurs and photosynthesis is inhibited.

Many bioactive natural and synthetic compounds with alkyl chain(s) in their molecules act as PS 2 herbicides. The site of their action is usually situated in Q$_b$ on the reducing side of PS 2 (e.g. sorgoleone, tenuazonic acid, N-octyl-3-nitro-2,4,6-trihydroxybenzamide) and/or in $Z^*/D^*$ intermediates on the donor side of PS 2 (e.g. fatty acids, alkyl-N-phenylcarbamates, amphiphilic alkoxypyrenylcarbamates, 2-alkylsulfanyl-4-pyridinecarbothioamides, alkyl substituted N-oxides and quaternary monoammonium and diammonium salts).

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Photosynthesis is one of the most important reactions on Earth, and it is a scientific field that is intrinsically interdisciplinary, with many research groups examining it. This book is aimed at providing applied aspects of photosynthesis. Different research groups have collected their valuable results from the study of this interesting process. In this book, there are two sections: Fundamental and Applied aspects. All sections have been written by experts in their fields. The book chapters present different and new subjects, from photosynthetic inhibitors, to interaction between flowering initiation and photosynthesis.

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