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1. Introduction

One of the important regulators for growth and development of the human body is the endocrine system. The endocrine system is composed of glands that secrete hormones into the circulatory system, which are then distributed throughout the body, regulating the function of tissues and maintaining homeostasis. Among these hormones are the insulin-like growth factors (IGF), similar in molecular structure to insulin and playing an important role in cell growth, proliferation, differentiation (LeRoith & Roberts, 2003).

A complex network of molecules, including its binding proteins, proteases and receptors, which together comprise the 'IGF system', modulates the biological function of the IGFs. This system comprises the following components (Figure 1): (i) Two peptide hormones, IGF-1 and -2, (ii) type 1 and type 2 IGF receptors, (iii) six IGF-binding proteins (IGFBP; numbered 1-6) and (iv) IGFBP proteases. IGF-1 and -2 are small signalling peptides (~7.5 kDa) that stimulate action by binding to specific cell surface receptors (IGF-1R) evoking subsequent response inside the cell. Six soluble IGF binding proteins, the IGFBPs, which range in size from 22-31 kDa and share overall sequence and structural homology with each other, regulate the activity of the IGFs. IGFBPs bind strongly to IGFs (K_D ~ 300-700 pM) to ensure that the majority of circulating IGF in the blood stream is sequestered and at the tissue level inhibit the action of IGFs by blocking their access to the receptors. Proteolysis of the IGFBPs dissociates IGFs from the complex, enabling them to bind and activate the cell surface receptors (Figure 1). In tissues, IGFs form a binary complex with IGFBPs, whereas circulating IGFs are associated in ternary complexes containing IGFBP-3 (and IGFBP-5) and a third protein known as the acid-labile subunit (ALS). The ternary complex has a molecular mass of 150 kDa. The most abundant IGF-binding protein in the circulation is IGFBP-3 followed by IGFBP-2.

In recent years, the IGF system in general and IGFBPs in particular have become the focus as clinically important targets of cancer therapeutics (Chan et al., 1998; Harrison et al., 1996; LeRoith & Roberts, 2003; Ma et al., 1999; Rosenzweig & Atreya, 2010; Wu et al., 2004; Yu et al., 1999). Different strategies have been proposed to inhibit cancer growth by blocking IGF-I-R binding and function (recently reviewed by (Rosenzweig & Atreya, 2010)). In this regard, the therapeutic potential of the IGFBPs in inhibiting IGF-1/IGF-2 activity and thereby inhibiting cancer cell growth has been demonstrated. Notably, the IGFBPs do not bind insulin and thus do not interfere with insulin-insulin receptor interactions.
1.1 IGF-binding proteins (IGFBPs)

While extensive studies have been carried out on the role of IGFs in different biological systems and under diseased conditions, a molecular-level understanding of IGF-IGFBP interactions is lacking. The three-dimensional (3D) structures have not yet been determined for any of the full-length IGFBPs. Based on sequence analysis, it is now understood that all IGFBPs contain three structural domains of nearly equal size (Firth & Baxter, 2002; Krywicki & Yee, 1992; LeRoith & Roberts, 2003; Rosenzweig, 2004). The N-and C-terminal domains are highly conserved in sequence across the IGFBPs. They contain 16-18 cysteine residues, forming 8-9 disulfide bonds. Their disulphide bonding indicates that the IGFBPs are thyroglobulin type-1 domain homologues. In recent years, structural studies have been carried out on individual domains in IGFBP-1, -2, -4, -5 and -6 (Kalus et al., 1998; Kibbey et al., 2006; Kuang et al., 2006; Sala et al., 2005; Sitar et al., 2006; Siwanowicz et al., 2005). Studies involving site directed mutagenesis have identified key residues in IGFBPs that are required for binding the IGFs (Clemmons, 2001). These studies have also revealed that both
the N- and C-terminal domains in IGFBPs are essential for IGF-1/2 binding (Clemmons, 2001; Kibbey et al., 2006; Siwanowicz et al., 2005). The central ‘linker’ domain which is structurally disordered has been proposed to be site where most of the post-translational modifications take place. This is also the region where proteases act to cleave the IGFBPs. IGFBP levels are regulated by proteolysis following their secretion from the cell, which dissociates the IGFBP-IGF complex resulting in an increase in IGF-1/2 available for interacting with the IGF-1R (Bunn & Fowlkes, 2003) (Figure 1). This is evidenced from the differential effects of IGFBP-3 in tumor vs. normal prostate cells, wherein IGF-1 bioavailability is increased via IGFBP proteolysis (Miyamoto et al., 2004).

1.2 Role of IGF system in HIV & AIDS

1.2.1 Involvement of growth hormone/IGF axis in AIDS

In addition to its involvement in various cancers, the IGF-system has also been implicated in diabetes, uremic cachexia, muscle wasting in congestive heart failure (CHF), aging, human immunodeficiency virus (HIV), acquired immunodeficiency syndrome (AIDS), and AIDS cachexia (Hambrecht et al., 2002). It is now well understood that the growth hormone (GH)/IGF axis is significantly affected in HIV and AIDS patients (Meininger & Grinspoon, 2001). Patients with HIV infection or AIDS are known to have multiple growth hormone (GH)/IGF axis related defects which include: abnormal GH secretion, profound decrease in serum levels of IGF-1 and IGF-II, abnormal post-translational modifications of IGF binding proteins, increased concentration of IGFBP-2 and reduced IGFBP-1/-3 concentration (Meininger & Grinspoon, 2001). In patients with AIDS wasting syndrome acquired GH resistance has been found to result in increased GH concentrations which occurs as a function of weight loss and loss of lean body mass. Conversely in patients with HIV lipodystrophy syndrome GH concentrations are reduced due to increased abdominal visceral adiposity. The GH/IGF axis has also been implicated in HIV associated osteopenia in patients with HIV-1-infection but without any symptoms of AIDS-associated wasting (Stagi et al., 2004). Thus, due to its significant role IGF levels are closely monitored in HIV/AIDS patients and help to track disease progression (see review by Congote 2005).

1.2.2 Role of IGF-binding proteins in HIV & AIDS

In addition to changes in IGF levels, the concentration of IGFBPs has also been observed to vary in patients with AIDS or HIV infection compared to healthy individuals. It has been observed that the levels of IGFBP-3 decrease whereas those of IGFBP-1 and -2 increase in HIV/AIDS patients (Congote, 2005). The levels of IGF-1/2 and those of IGFBP-1, -2 and -3 in HIV-infected children and adults vary throughout the course of the disease. Administration of GH significantly increases the levels of IGFBP-3 in all HIV infected patients except for patients with AIDS wasting (Mynarcik et al., 1999). Disease progression is associated with decrease IGF-2 levels and increase in IGFBP-2 & IGFBP-3 protease activity, infact IGFBP-2 levels are one of the first parameters to increase after HIV infection, before the development of AIDS (Helle et al., 2001). The elevated IGFBP-3 protease activity is somewhat restored in patients undergoing antiretroviral therapy treatment (Helle et al., 2001). The proteolysis of IGFBP-3 causes IGF to be released, which is captured by IGFBP-2. It has been hypothesized that the low IGF and high IGFBP-2 levels found in HIV infection may contribute to enhanced lymphocyte apoptosis. This may in turn lead to immune dysfunction in patients. In another study, IGFBP-1 was observed to be highly phosphorylated and IGFBP-3 ternary
complexes were formed with reduced ability in AIDS patients with wasting (Frost et al., 1996; Gelato & Frost, 1997).

1.3 IGF-system in treatment of HIV & AIDS

Given the significant role played by GH, IGFs and IGFBPs in HIV and AIDS, different approaches for treatment based on the GH/IGF-system have been proposed. We discuss here the two approaches, which underscore the importance of this system.

1.3.1 Treatment with growth hormone

In our body carbohydrates are the main source of energy needed for survival. Insufficient carbohydrate intake causes the body to burn the reserved fats followed by burning the muscle for energy which leads to loss of lean body mass (LBM). Muscle wasting or Wasting Syndrome (WS) is the most frequent problem in the patients with HIV infection and results in significant loss of body mass (Dudgeon et al., 2006). There are a variety of reasons why patients continue to lose weight, including: loss of appetite, increased metabolism, altered hormone levels, increased cytokine production which produces more fats than proteins. This is further aggravated by different drugs which cause nausea resulting in decreased food intake by patients and poor nutrient absorption which are necessary to maintain body mass. HIV-associated adipose redistribution syndrome (HARS) is an HIV-associated disorder characterized by excess truncal fat, including visceral adipose tissue (VAT). Muscle wasting, and particularly loss of metabolically active lean tissue, contributes to increased mortality, accelerated disease progression, and impairment of strength and functional status. The effect of treatment with protein anabolic agents, including GH, IGF-I, testosterone, nandrolonedecanoate, oxandrolone, and oxymetholone, have been studied in patients with HIV associated wasting. These studies have demonstrated that this treatment can increase lean body mass (LBM) and in some cases provide functional benefits and improvements in quality of life (Mulligan & Schambelan, 2002; Spinola-Castro et al., 2008). The immunologic effects of recombinant human growth hormone (rhGH), recombinant human insulin-like growth factor-1 (IGF-1), or their combination, in patients with moderately advanced HIV infection has been studied. The treatment with a combination of rhGH/rhIGF-1 and low dose of rhGH is reasonably well tolerated, resulting in increased body weight and modest improvements in HIV-specific immune function (Lee et al., 1996; Nguyen et al., 1998). Patients treated with rhGH sustain losses in VAT and truncal fat with no effect on subcutaneous fat in the abdomen or limbs. It has also been observed that non-high-density lipoprotein cholesterol (non-HDL-C) decreases significantly with rhGH treatment (Grunfeld et al., 2007).

1.3.2 Treatment with IGF-1 and IGF: IGFBP complexes

The observation that improved muscle mass, but not linear growth is associated with normalized IGF-1 concentration suggests that IGF-1 may be a potential therapeutic strategy to improve lean body mass in HIV-infected children (Chantry et al., 2008). Treatment with low dose recombinant IGF-1 produces significant, but transient, nitrogen retention. Alternate routes of IGF-1 administration or co-administration with GH prevents attenuation of IGF-1 action (Lieberman et al., 1994). However, administration of IGF-1 has its own set of problems. It causes lowering of glucose levels, which restricts its dosage, and thereby it’s anabolic potential. Further, high levels of IGF-1 are warning signs for the increased risk of
malignancy. In the case of cancer cachexia, which includes muscle wasting and anorexia, the growth of the tumor is associated with increased IGF-1 levels. Interestingly, it was found that administration of IGF-I in complex with IGFBP-3, but not free IGF-1, is a potent stimulator of muscle protein synthesis in rats with chronic undernutrition (Zdanowicz & Teichberg, 2003). In contrast to free IGF-1, significantly higher dosage levels can be used. Administration of IGF-1 in this form increases the bioavailability of IGF-1. Moreover, a high dosage level of this complex does not result in hypoglycemic condition owing to the fact that IGF-1/IGFBP-3 complex does not interact with the insulin receptor. In the case of cancer cachexia, the IGF-1/IGFBP-3 complex fails to alter tumor growth, but improves the tumor-host nutritional state by improving food intake, attenuating weight loss and improving glucose metabolism (Wang et al., 2000). Thus treatment with IGF-1/IGFBP-3 complex seems to be a promising approach to improve whole-body glucose uptake and glucose tolerance, while increasing hepatic glucose production (Congote, 2005; Rao et al., 2010).

1.4 Structural studies of IGFBP-IGF interactions for developing potent therapeutics

In order to improve the efficacy of treatment involving the administration of IGF-1/IGFBP-3 complex discussed above, it is important to understand the structural aspects of IGF-IGFBP interactions. Such studies help in enhancing the binding affinity of IGF-1 to IGFBP-3 and also aid in engineering IGFBPs to be protease resistant. We discuss below a thermodynamic approach that we have adopted to study IGF-IGFBP interactions. This brings out the utility of such studies in designing new forms of IGFBPs with enhanced IGF binding affinity thereby serving as potent therapeutics.

1.4.1 Structural characterization of IGFBP-2 and its fragments

While structures of individual domains are known, 3D structures of full-length IGFBPs and/or their complex with IGF-1 have not yet been determined. This has been due to the difficulty in expressing full-length IGFBPs at milligram quantity levels required for X-ray-crystallography or NMR spectroscopy. All IGFBPs contain 16-18 cysteines bridged by disulphide bonds, which makes them difficult to be expressed in bacterial systems. These proteins thus tend to precipitate inside bacterial cells resulting in inclusion bodies. We recently reported the first high-yield expression and structural characterization of functional full-length recombinant human IGFBP-2 (rIGFBP-2) in E. Coli (Swain et al., 2010a). Figure 2 shows the 2D $[^{15}N-^1H]$ NMR spectrum of bacterially expressed IGFBP-2. A good dispersion of peaks seen in the spectrum indicates a well-folded conformation of the protein. The secondary structural content estimated based on the NMR spectrum was found to be consistent with those observed in the individual domains. In cysteine rich proteins, a key requirement is to conserve the pattern of intra-molecular disulphide bonds required for the protein function. Often scrambling (mis-pairing) of disulphide bonds result during purification resulting in heterogeneity of conformations. In our study, we employed an efficient denaturing-refolding protocol. This involved first denaturing the protein in presence of the a reducing agent such as β-mercaptoethanol or DTT followed by slow removal of the denaturing agent and the reducing agent through dialysis resulting in a unique pattern of intra-molecular disulphide bonds. This is evident from the single set of peaks seen in the 2D NMR spectrum shown in Figure 2 (Swain et al., 2010a).
Fig. 2. Two dimensional [\textsuperscript{15}N-\textsuperscript{1}H] HSQC NMR spectrum of full length hIGFBP-2 (33 kDa) which correlates the polypeptide backbone [\textsuperscript{15}N chemical shift with its directly attached \textsuperscript{1}H. The spectrum was acquired at 288 K at \textsuperscript{1}H resonance frequency of 700 MHz with a ~1 mM sample concentration. A good dispersion of peaks indicates a well-folded conformation of the protein.

In order to understand the mechanistic aspects of IGF-IGFBP interactions, we have undertaken the study of different domains and fragments of IGFBP-2. Biochemical studies reveal that removal of 41 residues (249-289) from the C-terminal tail of full-length hIGFBP-2 (hereafter denoted as IGFBP-2\textsubscript{249-289}) significantly increases the rate of IGF dissociation, in turn abolishing the ability of the truncated protein to effectively bind IGF (Kibbey et al., 2006). Wild type IGFBP-2\textsubscript{249-289} contains two cysteines. However, due to an artifact of cloning full length IGFBP-2 and subsequently the C-terminal polypeptide IGFBP-2\textsubscript{249-289}, our recombinant species all have an additional cysteine at position 281. This resulted in three cysteines in IGFBP-2\textsubscript{249-289} raising the possibility of forming dimers or higher order aggregates. In the presence of reducing agents such as \textbeta-mercaptoethanol (which are known to reduce disulphide bonds) the protein remained as a monomer. However, upon removal of \textbeta-mercaptoethanol by dialysis and/or ultrafiltration, it was found that the polypeptide self-assembled spontaneously into soluble nanotubes several micrometers long (Swain et al., 2010b) (see Figure 3). These tubular structures were studied using different biophysical techniques such as transmission electron microscopy (TEM), NMR spectroscopy, fluorescence and circular dichroism (CD). The observation that formation/dissociation of such nanotubes is reversible (they exchange between monomeric and polymeric forms in presence/absence of reducing agents) and their high mechanical stability due to covalent interaction between the individual components offers new avenues for designing novel IGFBP-based self-assembling nanotubes for biomedical applications.
1.4.2 IGFBP-2 as a biomarker for monitoring disease progression

Bacterial expression of functional full length human IGFBP-2 opens up new avenues to carry out structure-based functional studies in this protein family. One promising application is to generate/engineer antibodies against human IGFBP-2 and use it for detection of IGFBP-2 in HIV/AIDS patients. As mentioned above, it is now established that IGFBP-2 levels are significantly elevated in HIV/AIDS patients (Congote, 2005) and hence IGFBP-2 can be used as a bio-marker for diagnosing or tracking the progression of this disease. In recent years, several bio-markers have been proposed or developed for monitoring HIV infection. These include: CD4 count (Smurzynski et al., 2010), TNF-alpha receptor type 2 as a useful serum marker for metabolic dysfunction (Gelato et al., 2002), fibroblast growth factor-21 (FGF21) (Domingo et al., 2010), levels of iron bound and iron-related proteins in urine to identify HIV-infected children at risk of developing HIVAN and HIV-HUS (Soler-Garcia et al., 2009), plasma levels of high sensitivity C reactive protein (hsCRP), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1) (Padilla et al., 2011), vascular cell adhesion molecule-1 (sVCAM-1) and plasminogen activator inhibitor-1 (PAI-1) (Padilla et al., 2011). Elements of the IGF system have also been found to be promising bio-markers. However, the detection of proteins by antibodies is the most efficient and sensitive method. This will serve to detect/monitor variations in IGFBP-2 levels in patients with HIV/AIDS. Further, once the structural details of IGFBP2-IGFBP-2 antibody interactions are defined, the antibodies can be engineered to have tight binding to IGFBP-2 which in turn will enhance the sensitivity of IGFBP-2 detection.

1.4.3 Structural features of IGF-IGFBP interactions

Structural studies of individual domains of the IGFBPs in free and complexed form with IGF-1 has provided considerable insights into their interactions (Kalus et al., 1998; Kibbey et al., 2006; Kuang et al., 2006; Sala et al., 2005; Sitar et al., 2006; Siwanowicz et al., 2005). As mentioned above, IGFBP's contain three structural domains of nearly equal size (these are denoted as N-terminal, middle or L-domain and C-terminal domains, respectively). It is
now established that both N- and C-domains in IGFBPs are involved in binding IGF-1 with the central domain structurally disordered. High-resolution 3D structures are available for the following IGFBP domains in uncomplexed form: (i) N-terminal domain of IGFBP-1, (ii) C-terminal domain of IGFBP-2, (iii) N-terminal domain of IGFBP-5 and (iv) C-terminal domain of IGFBP-6. In IGF-bound form, structures are available for N-terminal domain of IGFBP-4 and -5 and C-terminal domain of IGFBP-1.

The relative affinities of IGFs vary for the different IGFBPs with IGFBP-1,3,4 having higher affinities for IGF-1 compared to IGF-2 and vice-versa for IGFBP-2,5,6 (Kiefer et al., 1992; Roghani et al., 1991). The salient features of these structural interactions are: (i) the individual domains of different IGFBPs are similar in structure with root mean square deviation (RMSD) < 2-3 Å; (ii) the structures of N- and C-domains of IGFBPs in free and in complex with IGFs are similar indicating that the domains do not undergo a significant conformational change upon binding; (iii) there is a cooperativity between the N- and C-domains of IGFBPs in binding IGF (Kuang et al., 2007). That is, binding of IGF-1 to one of the domains enhances its binding to the other domain. This is presumably due to conformational change or stabilization of IGF-1 upon binding to one domain, which renders its conformation suitable for binding the other domain; (iv) the individual domains bind IGFs with much lower affinity than the full-length protein; (v) the IGF-receptor binding sites of IGFs are masked upon binding IGFBPs. This explains why IGFs do not bind the receptor in IGFBP bound form; (vi) upon binding IGFBP the structurally flexible or disordered regions of IGFs are stabilized. Figure 4 illustrates the mode of IGF binding to IGFBP along with structures of N- and C-domains of IGFBP-5 and IGFBP-1, respectively, in complexed and uncomplexed forms.

![Fig. 4. Three-dimensional structures of IGFBPs in complexed and uncomplexed forms. (a) A ternary complex consisting of the N-domain of IGFBP-4 (orange), C-domain of IGFBP-4 (grey) bound to IGF-1 (light blue). The two domains clasp IGF-1 binding it tightly and blocking its interaction with IGF-1 receptor. (b) Superimposition of 3D the structures of the N-terminal domain of IGFBP-5 in complex and uncomplexed form (RMSD = 1.9 Å) and (c) Superimposition of the 3D structures of the C-terminal domain of IGFBP-1 in complex and uncomplexed form (RMSD = 1.3 Å). The low RMSD values indicate that the conformation of IGFBPs do not change significantly upon binding IGF-1.](www.intechopen.com)

### 1.4.4 Improving IGF-IGFBP interaction

One of the goals of our work is to engineer IGFBPs in order to improve their IGF binding affinity. This will be useful in therapeutics discussed above, which involves the administration of IGF-IGFBP complex rather than free IGF-1 alone. Towards this end, we have carried out structure-based thermodynamic studies of IGFBP in complex with IGF-1 to
evaluate the extent of change in stability of the protein complex upon mutation of key residues in IGFBP. The residues chosen were those that have been verified experimentally to be involved in binding IGFs. A large body of work has been carried out in the past wherein different site-specific mutants, deletion mutants and/or truncated forms of the IGFBPs have been tested for their IGF-1 binding activities (Clemmons, 2001). Many of these residues are known to be conserved across all six IGFBPs. With this information in hand, our objective is to map on the 3D structures of IGFBP mutations that are known to destabilize IGF-IGFBP interactions.

In recent years, computational methods have been proposed to design specific mutants with enhanced ligand binding affinity (Sammond et al., 2007). These are structure-based methods that systematically predict single mutations at protein-protein interfaces which enhance binding affinities. This is based on the hypothesis that increasing the buried hydrophobic surface area or reducing buried hydrophilic surface area leads to enhanced affinity if steric clashes are avoided and all polar groups buried in the core of the protein have a hydrogen bond partner. In the 3D structures of IGFBPs in complex with IGF-1, we mutated residues, which are known to be involved in binding IGF-1, and evaluated the resulting change in thermodynamic stability (via free-energy change). We observed that mutation of residues important for binding IGF-1 increases the free energy of the complex resulting in the destabilization and weakening of IGF-IGFBP interaction. This implies that thermodynamic stability of the IGF-IGFBP complex can be used as an indicator of IGF-1 binding affinity. The study was carried out in 3 stages: first, the change in free energy of the IGF-IGFBP complex upon mutation of residues in IGFBP was predicted. This was carried out using the program I-MUTANT (Caprioti et al., 2005). Next, based on these results, the structure of the complex containing residues in IGFBP that resulted in lowering or increasing the stability of the complex was structurally modeled using the software ROSETTA-DOCK (Lyskov & Gray, 2008). Finally, the modeled structures were subjected to energy minimization followed by 10 ns MD simulations using the program GROMACS (Van der Spoel et al., 2005). In order to understand the structural basis of increased or decreased thermodynamic stability of the IGF-IGFBP complex, the hydrophobic and polar surface areas accessible were evaluated using the program NACCSESS (Hubbard et al., 1993). Two protein complexes of IGFBP with IGF-1 and two uncomplexed forms of the same protein were used for the analysis namely, IGFBP5 N-terminal and IGFBP1 C-terminal having PDB ID 1H59, 1BOE (representing the complexed form) and 2DSQ, 1ZT3 (representing the uncomplexed form), respectively. These two proteins were chosen due to the fact that high-resolution structures of IGF-bound and unbound forms are currently only available for these proteins.

### 1.4.4.1 Energy calculations

Using I-MUTANT 2.0, each residue within the predicted binding sites and conserved regions of N- and C-domain of IGFBP-4 and IGFBP-1, respectively, was mutated to the 19 other possible amino acids at that position and the change in free energy for each mutation was identified based on the ΔΔG value (defined below) predicted by the software. Based on this, 5 residues from the N-domain of IGFBP5 and 2 residues from the IGFBP1 that gave the highest number of stable predictions among all the 19 possible substitutions were identified. The free energy calculations were done as follows:

\[
\text{Overall} \Delta \Delta G \text{ (mutation) } = [\Delta \Delta G \text{ (mutation)}]_{\text{complex}} - [\Delta \Delta G \text{ (mutation)}]_{\text{uncomplexed}}
\]
$\Delta \Delta G$ (mutation) is in general the free energy change of a given structure (complexed or uncomplexed) upon mutation of a given residue. The value of $\Delta \Delta G$ (mutation) either in complex or uncomplexed form (indicated in the subscript above) was obtained from I-MUTANT 2.0 by specifying the desired residue to be mutated. Thus, the overall $\Delta \Delta G$ (mutation) for a desired mutation depends on the free energies of both complexed and uncomplexed forms. A positive value of the overall $\Delta \Delta G$ (mutation) indicates that the particular mutation stabilizes the complex, whereas a negative value for the overall $\Delta \Delta G$ (mutation) indicates de-stabilization.

Figure 5 shows an example of two mutations (one in the N-domain of IGFBP-5 and the second in the C-domain of IGFBP-1), which show de-stabilization upon mutation to any of the other 19 amino acids. This indicates that these residues are very important for binding and mutation of these residues lowers IGF-binding affinity. The lowering of binding strengths upon mutation of these residues has been verified experimentally in the past. For instance L-70 was mutated to Glu in one study (Imai et al., 2000) and C226 was mutated to Tyr in another study (Brinkman et al., 1991). Since cysteines are highly conserved across IGFBPs and are involved in extensive intra-molecular disulphide bonds, their mutation to any other amino acid causes a reduction of IGF-1 binding. This is corroborated by the thermodynamics analysis presented here.

Fig. 5. Thermodynamic analysis of mutations in IGFBP which cause de-stabilization or weakening of the IGF-IGFBP complex. The $\Delta \Delta G$ (mutation) values (defined above) are shown for two residues: L-70 of IGFBP-5 and C-226 of IGFBP-5 which are highly conserved across all IGFBPs and involved in binding IGF-1. Their mutation to any of the other 19 amino acid types results in de-stabilization of the interaction of IGFBP with IGF-1. Thus, these residues are important for binding IGF-1.
Figure 6 illustrates mutation of residue G-57 of the N-domain of IGFBP-5, resulting in enhancement of binding with IGF. Thus, if carried out this mutation will strengthen the IGF-IGFBP complex.

1.4.4.2 Structural basis for increase/decrease IGF-binding affinity in mutant IGFBP

In order to understand the structural basis of our findings above, the structure of IGF-IGFBP complex containing the mutations (L70Q and G57K) were constructed using ROSETTA-DOCK software and subjected to energy minimization and MD simulations. The solvent accessible areas of hydrophobic residues at the interface were then evaluated as follows:

\[
\text{Exposed hydrophobic surface area at interface} = \left[ \text{Exposed hydrophobic surface area of IGF} \right]_+ - \left[ \text{Exposed hydrophobic surface area of IGFBP} \right] - \left[ \text{Exposed hydrophobic surface area of IGF – IGFBP Complex} \right] \]

In general, a large exposure of hydrophobic surface area in the binding interface of two proteins (that is, an increase in solvent accessibility of non-polar residues) is known to result in destabilization of protein interactions (Jones et al., 2008; Sammond et al., 2007; Vallone et al., 1998). On the other hand, decreases in hydrophobic surface area at the binding interface upon mutation indicates that the complex formed is more stable than the wild type. Figure 7
shows the result of analyzing the solvent accessible areas of the two mutations described above.

![Graph showing accessible surface area of IGF-IGFBP-5 interfaces for G57K and L70Q mutations.]

**Fig. 7.** The change in hydrophobic surface area of the IGF-IGFBP-5 interfaces upon mutation. G57K is a stabilizing mutation for the IGF-IGFBP-5 complex due to a large decrease in non-polar surface area at the interface for IGF-1. On the other hand, the L70Q mutation renders the IGF-IGFBP complex unstable due to a large exposure of hydrophobic surface area in IGFBP-5.

In the case of L70Q mutation, the exposed hydrophobic surface areas of both IGFBP-5 and IGF-1 are increased at the interface. This together results in weakening of the complex and hence the IGF-binding affinity of IGFBP-5 is reduced. In the case of G57K, the exposed surface area of IGF-1 at the interface is reduced considerably while that of IGFBP-5 increases slightly. Significantly, the overall change in surface areas is favorable for enhanced binding of IGF-1 with IGFBP-5. This underscores the importance of thermodynamic studies in evaluating ligand binding affinities in this class of proteins.

### 2. Conclusion

AIDS is a debilitating disease with more than 25 million people having succumbed since the start of the epidemic. In order to combat this disease, multiple approaches have been proposed and needs to be taken. New findings related to diagnosis and disease progression continue to emerge. A key finding in the past decade, which is now well established, is the involvement of the hormonal peptides IGF-1 and IGF-2 and the IGF-binding proteins in...
various stages of the disease with different manifestations. The IGF system in general has been extensively studied in this context. While the system is complex in nature with many different proteins interacting to form a regulatory network, the key players have been the IGFs themselves and their binding proteins, the IGFBPs. The recent finding that administration of IGF-1: IGFBP-3 complexes improves whole body glucose uptake is a promising step towards treatment of HIV-associated wasting. In this context, it is important to understand the structural basis of IGF-IGFBP interactions in general, which has been elusive due to the difficulty in producing functional human IGFBPs in large quantities. In our laboratory, we have been successful for the first time in producing bacterially expressed human IGFBP-2 in soluble, functional and monomeric form. This opens up new avenues to study IGF-IGFBP interactions at the atomic level. Further, human IGFBP-2 antibodies can now be generated and used for detection of IGFBP-2 in HIV patients. High-resolution structural studies of IGF in complex with IGFBP will help us to design improved IGFBP species with improve interactions and enhanced binding affinity. All six IGFBPs in the human body are similar in nature as far as their interaction with IGF is concerned. Thus, findings from one IGFBP may be extended to other IGFBPs as well. The available structures of individual domains of IGFBPs are thus very helpful and will aid in future unraveling in detail, of the modes of the interaction of the full length proteins with the IGFs. Efforts are underway in this direction in our laboratory. While much work still needs to be done, the light at the end of tunnel is getting brighter.

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The continuing AIDS pandemic reminds us that despite the unrelenting quest for knowledge since the early 1980s, we have much to learn about HIV and AIDS. This terrible syndrome represents one of the greatest challenges for science and medicine. The purpose of this book is to aid clinicians, provide a source of inspiration for researchers, and serve as a guide for graduate students in their continued search for a cure of HIV. The first part of this book, from the laboratory to the clinic, and the second part, from the clinic to the patients, represent the unique but intertwined mission of this work: to provide basic and clinical knowledge on HIV/AIDS.

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