-formaldehyde, adding two methyl groups to the presence of varying GAG concentrations, the interacting amino acid could be identified.

We introduce a new ITC competition assay that overcomes this limitation, thereby allowing for a precise thermodynamic description of high- and low-affinity protein-ligand interactions involving poorly water-soluble compounds. We discuss the theoretical background of the approach and demonstrate some practical applications using examples of both high- and low-affinity protein-ligand interactions.

A Chemical Proteomics Approach toward Identification of Human Abscisic Acid-Binding Proteins

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Abscisic acid (ABA) is a plant hormone present in all plant-based food and has no known toxic effects. Recently, ABA was shown to modulate various aspects of human disease states in model systems. Presumably, ABA mediates its responses through binding to receptors of as yet unknown identity. Using an ABA microarray, high-throughput proteomics approach we identified the 78 kilodalton glucose regulated protein 78 (GRP78; HSPA5; Bip) and 70 kilodalton heat shock protein (HSP70-2) as putative human ABA-binding proteins. Both of these proteins have intracellular roles in protein folding and are also known to be involved in extracellular receptor activities at the cell membrane. Characterization of the binding of ABA to the proteins of the heat shock protein 70 (HSP70) family, HSP70-1 and GRP78 was carried out by surface plasmon resonance spectroscopy (SPR) in the presence and absence of co-chaperone, DnaJ. DnaJ is known to control substrate binding of heat shock proteins. The binding affinities of both GRP78 and Hsp70-1 for ABA were found to be in the μM range and increased dramatically in the presence of the co-chaperone. The lack of SPR signal upon injection of structurally but not functionally related molecule trans-cinnamic acid (CA), highlights the specificity of the ABA interaction with GRP78. Interestingly, ABA had differential effects on modulation of the ATPase activity of these two HSP70 family proteins. Overall this work highlights a novel and potentially important interaction between a dietary plant hormone and human heat shock proteins. Determination of the biological relevance of this ABA/HSP interaction is currently underway.

Investigation of the Influence Glycosaminoglycan Sulfation on the Interaction with Interleukin-8 by Fluorescence and Solution NMR Spectroscopy

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The interactions between glycosaminoglycans (GAGs), important components of the extracellular matrix (ECM), and proteins such as growth factors and chemokines play a critical role in cellular regulation processes. Previous work on the chemokine Interleukin-8 (IL-8) has focused on its interaction with heparin and heparan sulfate, which regulate chemokine function. Nevertheless, low or non sulfated GAGs such as hyaluronan (HA), dermatan sulfate (DS) and chondroitin sulfate (CS), also contained in the extracellular matrix, have so far not been studied with regard to their distinct binding properties towards IL-8.

In this work, we combine fluorescence as well as solution NMR experiments to study the recognition properties of GAG hexasaccharides, including HA, CS, DS and heparin, to IL-8. Using 1H-15N HSQC spectroscopy of IL-8 in the presence of varying GAG concentrations, the interacting amino acid could be identified. Furthermore to observe lysine side chains, a reductive methylation with [13C]-formaldehyde, adding two methyl groups to the ε-NH3+ of lysines without altering the positive charge, was performed. In order to resolve the signals from the lysine side chains, 1H-13C HSQC spectra were recorded and the influence of the GAG concentration was studied. The results show that an increase in GAG sulfation enhances the strength of the binding to the protein. In addition, in cases of equal degree of sulfation the position of the sulfate group plays a crucial role suggesting that interactions between proteins and GAGs are not purely electrostatically driven and steric forces as well as contributions from hydrogen bonds have to be considered. These observations were also confirmed by molecular docking and dynamic simulations providing a structural picture of the interaction of IL-8 with various GAGs.

Fluorescent, Reagentless Biosensors for Phosphate: Altering Properties, Expanding Usefulness

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Two forms of biosensor for inorganic phosphate (Pi) have been developed, based on the phosphate binding protein of Escherichia coli, and are now widely used for time-resolved measurements of enzymes that produce Pi. One form is an adduct with a single coumarin fluorophore. For the second version, two cysteine mutations were introduced and labeled with a rhodamine. When physically close to each other and appropriately oriented, two rhodamine dyes can interact to form a non-covalent dimer. In this state they have little or no fluorescence, unlike the high fluorescence intensity of monomeric rhodamine. The labeling sites were so placed that the distance and orientation between the rhodamines changes with the conformation change associated with Pi binding. This movement alters the extent of interaction between rhodamines and gives a large fluorescence increase as Pi binds. In both these forms, Pi binds rapidly and tightly, making them good probes for rapid reaction assays, where all Pi can be bound to the biosensor: that is, the probe must be at least stoichiometric with the Pi. The rhodamine version has a large advantage over coumarin in terms of sensitivity and photobleaching. However, to make the probe more useful for direct measurements such as high-throughput, the biosensor would best be used sub-stoichiometrically. This is more efficient in terms of usage amounts and has the potential of measuring over a wider range of Pi concentrations. To achieve this, several strategies were examined to locate mutations on this two domain protein to weaken the Pi binding while maintaining the fluorescence response.

Heme Proteins

Porphyrin-Mediated Photoinduced Conformational Changes to Human Serum Albumin at Physiological and Acidic pH

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Many of the biological and non-biological roles and uses of protoporphyrin-IX (PPIX) depend on its ability to bind large macromolecules such as human serum albumin (HSA). HSA is relevant to both biomedical and technological applications of PPIX (both free-base and metal), and its binding site for PPIX derivatives is well established. Once bound, PPIX is known to be photochemically activated in the protein. The reaction of the porphyrin in HSA can be shown capable of transiently bestowing altered ligand-binding and pseudo-enzymatic properties to HSA that could be linked to biological functions. The irradiation of PPIX non-covalently bound to Beta-Lactoglobulin (BLG) is known to produce protein conformational changes that appear to be pH-dependent due to BLG’s intrinsic conformational transitions. These processes have not been extensively studied in non-physiological pH conditions for FePPIX or PPIX bound to HSA. This study implemented a combination of optical methods and computational simulations to compare the binding characteristics of hemin and PPIX to HSA as well as examine the structural effects of light-dose irradiation of the ligand on the protein at different pH. Spectroscopic data suggests that irradiation of the porphyrins’ Soret band, when bound to HSA, is capable of modifying the globular protein structure by direct charge transfer mechanisms at both physiological and acidic pH conformations. Computational docking simulations predict lower free energy of binding for PPIX than for heme.

How Does Hemoglobin Create Such Diverse Functionality of Physiological Relevance?

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Hemoglobin (Hb) without heterotrophic effectors or stripped Hb, is an O2-carrier with a high-affinity (P50 < 1 μM), and a modest cooperativity (K, = 10 μM), and a minimal Boltzmann sigmoidal form (ΔH = −0.84 kJ/mol). Thus, though the cooperative mechanism of Hb without consideration of hetero-otic effects, may be of academic interest, stripped Hb is not a viable O2-carrier under physiological conditions of PO2 from 7 to 23 μM at 15°C (or from 30 to 100 μM at 37°C). Such a benign functionality of stripped Hb is greatly