Meeting report

PROTEIN–LIGAND INTERACTIONS

A report of a symposium held at the University of Konstanz, West Germany, September 2–6, 1974*

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1. Introduction

Protein–ligand interactions are of great biological significance and cover a wide spectrum of systems. They range from respiration to the action of receptors and repressors and to the subtle influence of electrolytes on proteins. The last decade has witnessed great progress in a molecular approach to biopolymer–small molecule interactions, which includes the application of thermodynamics, quantum mechanics and modern kinetics to protein–ligand interactions, particularly to enzymatic reactions. A discussion of protein–ligand interactions on a more general basis has not been the subject of a previous symposium, although many of those working in this field have realized the need for a detailed discussion of the problems concerning the interaction between proteins and small molecules. Therefore, a symposium on protein–ligand interactions was organized at the University of Konstanz.

The purpose of the symposium was to assemble both biologically and physicochemically oriented scientists, in order to discuss current topics in the wide field of protein–ligand interactions. It was the organizers' hope to promote an interdisciplinary interaction and stimulation of ideas between scientists looking at specific problems from different viewpoints. All participants had received the printed lectures beforehand and, therefore, the emphasis during the symposium was on discussion, which had no time limit. Financial support for the symposium was provided mainly by the Stiftung Volkswagenwerk, by the Gesellschaft der Freunde und Förderer der Universität Konstanz and by the Byk Gulden Lomberg Chemische Fabrik GmbH, Konstanz. The programme was divided into six sections.

1. General

This general section included two purely thermodynamic contributions. A thermodynamic analysis of allosteric macromolecular systems (Colosimo, Brunori and Wyman, Rome) dealt with the relationship among the oxygen binding sites and the Hill parameter, particularly in the extremely large respiratory proteins. Some general remarks (Wyman) on the thermodynamics of biological polyfunctional macromolecules preceded the first section. In addition, there was a general discussion (G. Weber, Urbana, Illinois) of free-energy coupling between different ligands bound to a protein, with particular reference to oligomeric proteins. Problems of energy transfer and of structural fluctuations in proteins and experimental methods for their detection were reviewed. Ligand binding to multienzyme complexes was demonstrated (Kirschner, Weischet and Wiskocil, Basel) by tryptophan synthetase from *E. coli*. Kinetic studies help to elucidate the mechanisms of these interesting systems. Circular dichroism has been used as a very sensitive structural probe in protein–ligand systems. Previous calculations of rotational strengths as the sum of individual contributions of interactions between prosthetic groups and proximate aromatic amino acid residues were extended (Woody, Tempe,

* The complete proceedings will be published together with the discussions in a book, entitled Protein–Ligand Interactions, edited by H. Sund and G. Blauer, Walter de Gruyter, Berlin–New York, 1975.

Arizona) to hemoglobin and to flavodoxin. 'Subunit Exchange Chromatography' involving protein subunits bound covalently to a Sepharose matrix and polymeric proteins, such as oxyhemoglobin, was described (Antonini, Rossi-Fanelli and Chiancone, Rome). The preparative and analytical advantages of this method were discussed.

2. Enzymes

In the first four lectures dealing with enzymes the binding of nucleotides to proteins was discussed. The X-ray structure analyses of various dehydrogenases (alcohol, glyceraldehyde-3-phosphate, lactate and malate dehydrogenase) have shown (Buehner, Würzburg) that the coenzyme is always bound in the 'open' conformation and that the coenzyme binding domains of the investigated dehydrogenases have very similar secondary and tertiary structures. A dominant feature is a parallel six-stranded pleated sheet which is mostly connected by α-helices. The two coenzyme binding sites of beef-liver glutamate dehydrogenase, the active and the non-active site, exhibit different specificities with respect to the adenine moiety of the coenzyme (Koberstein, Dieter and Sund, Konstanz). The active site is rather non-specific and, therefore, ε-NAD(P) can act as the coenzyme, whereas the non-active site appears to be highly specific and requires an intact adenine moiety for binding. A detailed study of the interaction of ATP and its analogs with myosin and the modification by actin (Goody and Mannherz, Heidelberg) leads to a model of the myosin cross-bridge cycle as the molecular force generator, with four states which include the various steps of the enzymatic ATP hydrolysis. The first state is characterized by the cross-bridge conformation detached from the actin filament. In the second state, the cross-bridge, still in the T-conformation, is now attached to the actin. The conformational change from T to R (state 3) produces the power stroke. Detachment of the cross-bridge leads to state four. From experiments with GTP analogs and membrane-bound adenylate cyclase from pigeon erythrocytes, it was assumed (Pfeuffer and Helmreich, Würzburg) that adenylate cyclase is inhibited in the membrane and that inhibition is released by interaction of a nucleotide (GTP) with a regulatory subunit, the guanylnucleotide-binding protein; this mechanism is analogous to that of the activation of protein kinases by c-AMP.

The study of the binding of amino acids to isoleucyl-tRNA synthetase shows (Flossdorf, Prätorius and Kula, Braunschweig-Stöckheim) that the binding capacity for amino acids is an intrinsic property of the synthetase and does not depend on ATP or tRNA. The gain in binding energy is determined exclusively by the size and shape of the aliphatic side chain. From the influence of small molecules (2,3-diphosphoglycerate, phosphate) on the affinity of individual polypeptide chains in hemoglobin and on their cooperativity, it was suggested (Winterhalter, Mansouri and Di Iorio, Basel) that only HPO_4^{2-}, and not $H_2PO_4^-$, is capable of affecting the cooperativity and that its binding is regulated by the $T \rightleftharpoons R$ equilibrium analogous to the binding of organic phosphorus compounds. The problem of cooperative interactions without symmetry or subunits was discussed briefly (Steinhardt, Washington, D.C.) The use of NMR spectroscopy in structural determinations of protein–ligand interactions was illustrated by the system manganese carboxypeptidase and carbobenzoxyglycine, one of its activators (Kushnir and Navon, Tel-Aviv). It appears that the ligand is bound with the carboxylate group close to the metal ion, but is not bound directly in the first coordination sphere of the metal ion. Furthermore, it is reasonable to assume that the activator, in contrast to inhibitors, does not replace the water of hydration on the metal ion.

3. Repressors

In this section only the *lac* operon system was discussed. The sequences of operator constitutive mutants do not support the hypothesis that the repressor simply recognizes a symmetric region on DNA (Gilbert and coworkers, Cambridge, Massachusetts). Rather, they show that the protein 'senses' the detail of an irregular sequence of bases. The interaction may have a symmetric component, but this is not dominant. On recognizing and binding to the operator, the *lac* repressor covers a sequence transcribed into the initial portion of the *lac* messenger. Thus, the repressor functions by blocking the access of the RNA polymerase to its initiation site. The active site of the *lac* repressor is located between amino acids 1 and 58

(Müller-Hill et al., Köhn); tyr-7, tyr-17, gln-18, ser-21, asn-25, gln-54 and gln-55 may be involved in the binding, and the N-terminal part of one polypeptide chain winds itself 'ivy-like' around one half of the operator. Similar studies on the *lac* repressor (K. Weber et al., Cambridge, Massachusetts have shown that the sixty N-terminal amino acid residues form a loop or globule which is available to trypsin or chymotrypsin and which is not necessary for folding, stabilization of the quaternary structure or IPTG binding, but is required for DNA binding. Translational reinitiation past a nonsense codon appears to be a common event in *E. coli*, since there are at least three such sites in the first 70 in-phase codons of the i-gene messenger RNA and all amber mutants bound in this region induce translational restarts. Suppressed nonsense mutations were used to generate altered *lac* repressor molecules (Miller et al., Genève). This allows, for instance, one to determine which substitutions do not produce detectable changes in the activity of the protein and, therefore, to elucidate the structure-function relationship of the *lac* repressor.

The effect of alterations in the *lac* operator DNA on repressor binding (Bourgeois et al. San Diego, California) provides, among others, information on the possible role of the symmetry of the operator in its recognition by the repressor. This symmetry may allow the binding of two or four repressor polypeptide chains, in order to amplify the affinity and the specificity of the interaction, but does not serve the purpose of forming a Gierer-type structure upon the repressor. The binding probably has little effect on the base pairing of the DNA strands.

A study of the interaction of the *lac* repressor with non-specific DNA binding sites yields molecular information related to the *specific* binding of repressor to operator (Von Hippel et al., Eugene, Oregon); e.g., the size of the non-specific binding site (25 to 30 base pairs) is comparable to that of the operator region and the melting data reveal that the repressor binds preferentially to double-helical, non-operator DNA.

4. Receptors

This section dealt mainly with the cholinergic receptor from *Torpedo*. From the investigation of the binding and functional states, it was concluded (Changeux et al. Paris) that the receptor protein possesses several properties which are typical of globular proteins, that it behaves like an 'integral' membrane protein, that its binding properties are under the control of its membrane environment and that it is a *'membrane-bound regulatory protein'*. It is remarkable that there are several states of affinity of the receptor for cholinergic agonists and that, following the efflux of $^{22}Na^{+}$ the half-maximal response is obtained with ~10 μM carbamylcholine, while the dissociation constant for the complex of carbamylcholine with the same membrane fragments is 0.5 μM. This may be explained on the basis of high- and low-affinity sites.

Studies on the subunit structure and binding sites of the acetyl–choline receptor (Hucho, Gordon and Sund, Konstanz) showed that the receptor protein contains two different types of polypeptide chains, but only one of these appears to bind the toxin from *Naja naja*. With the acetylcholine receptor from *Electrophorus* it was possible to incorporate the purified receptor protein into phospholipid vesicles (McNamee, Weill and Karlin, New York, New York). Biochemical and biophysical characterization of the acetylcholine receptor from *Torpedo californica* was carried out by a variety of methods (Raftery et al., Pasadena, California) including ligand binding, optical methods, re-association with phospholipids and X-ray diffraction. In a tentative model, a structure was suggested which is built up of three layers, each containing two 'monomers' having a mol. wt of 40 000. The important features of such a model are that the structure located in the membrane is polar and that it is presumably limited in size by the presence of the bilayer.

5. Antibodies, drugs and metabolites

This section contained an investigation (Pecht, Rehovot) of the binding of various haptens to myeloma proteins by kinetic (temperature jump) and optical (circular polarization of fluorescence) methods. A study of the antibiotic action on enzymes involved in peptidoglycan synthesis (Perkins et al., London) included investigations by optical methods of the interaction of transpeptidases with β-lactam antibiotics. Binding data, obtained by equilibrium dialysis, on the specific interaction between a globulin and steroids

were presented (Lutz, Wiegand and Weder, Zürich). The physiological and pharmacological significance of these studies, which include the binding of sex hormones to human plasma proteins, was discussed. The effects of various lower aliphatic alcohols on the specific complex of human serum albumin with the bile pigment bilirubin was investigated mainly by circular dichroism (Blauer and Lavie, Jerusalem). It appears that the optical activity of a bound protein ligand serves as a sensitive measure of the protein interactions with other ligands.

6. Detergents and electrolytes

This section included a concise survey (Steinhardt, Washington, D.C.) of the specific and non-specific interactions of detergents with proteins. Various thermodynamic and structural features associated with these interactions and analytical methods, including dye solubilization in micelles, were discussed. In the interaction of laurylpyridinium halides with ferrimyoglobin, large effects of the halide counterions were recorded spectrophotometrically; (Yonath and Blauer, Jerusalem) these effects follow the Hofmeister series. Since the interaction increases above the critical micelle concentration of the detergent, micellar structures of the detergent may be formed on the protein.

The influence of ions on the kinetics of unfolding and refolding reactions in globular proteins such as chymotrypsinogen has been followed (Pohl, Konstanz) by temperature-jump experiments at various pH values. The influence of salts on electrostatic interactions in a protein was evaluated with reference to the kinetics of denaturation. A review of neutral salt effects on the conformational stability of biopolymers (Von Hippel, Eugene, Oregon) summarized the present concepts and approaches in this field. The latter include model transfer experiments of protein residues from one medium to another and evaluation of binding constants of ions by recycling chromatography, leading to information about the sites and mechanism of ion binding.