Biomolecular Structure and Dynamics

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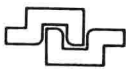

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PREFACE

This book contains the formal lectures and contributed papers presented at the NATO Advanced Study Institute on Biomolecular Structure and Dynamics : Recent Experimental and Theoretical Advances. The meeting convened at the city of Loutraki, Greece on 27 May 1996 and continued to 6 June 1996.

The material presented describes the fundamental and recent advances in experimental and theoretical aspects of molecular dynamics and stochastic dynamics simulations, X-ray crystallography and NMR of biomolecules, structure prediction of proteins, time resolved Fourier transform infrared spectroscopy of biomolecules, computation of free energy, applications of vibrational circular dichroism of nucleic acids and solid state NMR spectroscopy.

In addition, recent advances in UV resonance Raman spectroscopy of biomolecules semiempirical molecular orbital methods, empirical force fields, quantitative studies of the structure of proteins in water by Fourier transform infrared spectroscopy, density function theory (DPT) were presented.

Metal-ligand interactions, DFT treatment of organometallic and biological systems, simulation versus X-ray and far-infrared experiments are also discussed in some detail. In addition, a large proportion of program was devoted to current experimental and theoretical studies of the structure of biomolecules and intramolecular dynamic processes.

The purpose of the proceedings is to provide the reader with a rather broad perspective on the current theoretical aspects and recent experimental findings in the field of biomolecular dynamics. Moreover, the material presented in the proceedings should make apparent the future trends for research in this field, as well as could provide grants for collaborative research between theoreticians and experimentalists in areas of importance to the understanding of biomolecular structure and dynamics.

The proceedings should be of interest to graduate and postgraduate students who are involved or starting research in these areas, and to scientists who are actively pursuing research in biomolecular structure and dynamics.

Appreciable part of the information contained in the proceeding has not yet been published in books on biomolecular structure and dynamics.

G. Vergoten

T. Theophanides

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We express our appreciation to the local organizing committee. Special thanks are due to all lecturers and authors for their cooperation in preparing the manuscripts, excellent delivery of lectures and dedication to the meeting. Mrs Françoise Bailly provided invaluable secretarial assistance in preparation for the meeting and during the meeting.

Last, but not least, we should like to express our gratitude to the NATO Scientific Affairs Division for granting financial support for the meeting.

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MODELING AND COMPUTER SIMULATIONS

THE PHYSICAL CHEMISTRY OF SPECIFIC RECOGNITION

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1. Introduction

> It is our opinion that the processes of synthesis and folding of highly complexes molecules in living cells involve, in addition to covalent bonds, only the intermolecular interactions of van der Waals attraction and repulsion, electrostatic interactions, hydrogen-bond formation, etc., which are now well understood. These interactions are such as to give stability to a system of two molecules with *complementary* structures in juxtaposition...
> In order to achieve maximum stability, the two molecules must have complementary surfaces, like die and coin, and also a complementary distribution of active groups.
> L. Pauling & M. Delbrück (1940) [1]

The reader of these remarkable sentences should remember that they were composed four years before Avery, McLeod & McCarty showed DNA to be the molecule genes are made of, fifteen years before Fred Sanger sequenced insulin, and twenty years before Max Perutz & John Kendrew obtained the X-ray structure of myoglobin. In 1940, Linus Pauling and Max Delbrück had no experimental evidence whatsoever to support their statements. They were addressing colleagues in physics and chemistry rather than biologists who, in these times, seldomly spoke in terms of atomic interactions. Still, in the US at least, biologists were ready to consider physical chemistry as a partner science in the study of the mechanisms that rule the cell and the organism. Figures like Pauling and Delbrück were in the lead, and they were so fully right in this particular case that we find not a word must be changed in their definition of complementarity, which makes it possible for two (macro)molecules to assemble into a specific stable complex. The only question we may ask at the end of this century, is

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whether we can make Pauling and Delbrück's definition quantitative and find numbers that express specificity and stability.

2. Affinity and the law of mass action: equilibrium and rate constants

For stability, the answer seems an easy yes. A non-covalent complex being ruled by the Gulberg-Waage law of mass action, the reaction formula:

$$A + B \underset{k_d}{\overset{k_a}{<\!\!-\!\!-\!\!-\!\!-\!\!-\!\!->}} AB \qquad (1)$$

implies a relationship between the equilibrium concentrations of components A and B and complex AB:

$$K_d = \frac{1}{K_a} = \frac{k_d}{k_a} = \frac{[A][B]}{[AB]} \qquad (2)$$

K_a and K_d are the two equilibrium constants, k_a and k_d the two rate constants for association and dissociation. K_d (or its reciprocal K_a) measure the stability of complex AB and the affinity of A and B for each other. K_d values in the micro- or nanomolar range can be derived by measuring concentrations at equilibrium. This is no longer possible when the affinity is much higher. Then, it is more practical to measure the two rate constants, the ratio of which yields K_d values in the picomolar range or below.

The second order rate constant k_a has an upper value that comes from the stochastic diffusion of molecules in solution: $k_a \approx 10^9\ M^{-1}.s^{-1}$ in water at 25°C. Table 1 quotes rate constants for typical specific protein-protein complexes: two enzyme-inhibitor and one antigen-antibody complexes. The enzyme is bovine trypsinin one case, a bacterial ribonuclease, barnase, in the other. The antigen is hen lysozyme, the antibody, a covalent pair of *E. coli* expressed variable domains (single chain Fv). Affinities cover six orders of magnitude with $K_d = 10^{-8}$ -10^{-14} M, mostly due to k_d, k_a being 10^6-$10^8\ M^{-1}.s^{-1}$. Barnase, barstar and the Fv fragment have been subjected to site-directed mutagenesis. In mutant R59A of barnase, part of a long series analyzed by Schreiber & Fersht [2-3], the point substitution makes the affinity drop by a factor of 10^4. Variant M3 of the Fv fragment has been selected by phage display to raise the affinity for lysozyme by a factor of 5 [4]. Similar changes are observed upon point substitution in other systems.

Table 1: Experimental rate and equilibrium constants in some protein-protein complexes

Complex	k_a	k_d	K_d	ΔG_d	$\Delta\Delta G_d$
	($M^{-1}.s^{-1}$)	(s^{-1})	(M)	(kcal.mol^{-1})	
Trypsin-PTI [a]	$1.1.10^6$	$6.6.10^{-8}$	6.10^{-14}	18.1	-
Barnase-barstar [b]	$3.7.10^8$	$3.7.10^{-6}$	1.10^{-14}	19.0	-
R59A variant	$3.4.10^7$	$2.4.10^{-3}$	7.10^{-11}	13.8	5.4
Lysozyme-Fv D1.3 [c]	$1.8.10^6$	6.10^{-3}	3.10^{-9}	11.7	-
M3 variant	$1.6.10^6$	1.10^{-3}	6.10^{-10}	12.6	-0.9

Values near 25°taken from:
(a) Vincent & Lazdunski [21]; (b) Schreiber & Fersht [2]; (c) Hawkins and al. [4]

The higher affinity of variant M3 is entirely due to the lower rate of dissociation. In contrast, barnase mutation R59A both increases k_d by a factor of 10^3 and lowers k_a by a factor of 10. It should be stressed that barnase-barstar association is extremely fast, with k_a near the diffusion limit for molecules having $M_r \approx 10$ kDa. Nearly every collision between barnase and barstar must yield a specific stable complex. This may seem absurd if we consider that the contact region (covering the enzyme active site) is no more than 10-15% of each component surface. A mutation such as R59A that modifies the net electric charge of barnase as well as the k_a value, shows that the association between barnase and barstar is electrostatically assisted [2-3]. At very high ionic strength, long-range electrostatic interactions are shielded and k_a drops by over four orders of magnitude to $\approx 10^5$ $M^{-1}.s^{-1}$, a value compatible with the precise geometry observed in the complexe.

3. Enthalpies, free enthalpies and entropies

Affinity may also be defined in terms of the usual thermodynamic parameters, the enthalpy H (internal energy at constant pressure), the entropy S and the free enthalpy G (Gibbs energy). Changes in these parameters are quoted in reference to a 'standard'

state, per mole of product of reaction (1) and in either direction. We choose to quote values for dissociation, and signs must be changed for association:

$$\Delta G_d = - RT \ln \frac{K_d}{c_\phi} \qquad (3)$$

Here, R is the gas constant (≈2 cal.mol^{-1}.K^{-1}), T the temperature and c_ϕ the concentration taken to be the standard state. For solution studies, the usual convention is c_ϕ=1 M, yet this is an arbitrary choice and c_ϕ=55,5 M, the molar concentration of pure water, is sometimes used. Moreover, tabulated values almost never use this convention: they relate to the pure liquid or solid chemical species, not to aqueous solution. The c_ϕ convention is unimportant when comparing the affinity of two different ligands for the same site or, as in Table 1, the affinity of a mutant and and the wild type of the same protein. The dissociation changes from K_d to K'_d, the free enthalpy change from ΔG_d to $\Delta G_d+\Delta\Delta G_d$:

$$\Delta\Delta G_d = RT \ln \frac{K'_d}{K_d} \qquad (4)$$

The free enthalpy of dissociation ΔH_d does not depend on c_ϕ. It can be derived from K_d measurements made at several temperatures by applying Van t'Hoff law:

$$\Delta H_d = \frac{d(\Delta G_d/T)}{d(1/T)} = - R \frac{d(\ln K_d)}{d(1/T)} \qquad (5)$$

Then, the entropy of dissociation ΔS_d (which does depend on c_ϕ) is derived from:

$$\Delta G_d = \Delta H_d - T\Delta S_d \qquad (6)$$

In recent years, a direct determination of ΔH_d can be made by isothermal mixing calorimetry as the heat evolved when two solutions are mixed [5]. By performing measurements at several temperatures, the heat capacity of dissociation ΔC_d comes out as:

$$\Delta C_d = \frac{d(\Delta H_d)}{dT} = T \frac{d(\Delta S_d)}{dT} \qquad (7)$$

Assuming ΔC_d to be a constant in the temperature range under study, one may integrate Eq. 7 and predict ΔH_d, ΔS_d and ΔG_d at all temperatures knowing K_d and ΔH_d at 25°C (T_0=298K) only. Fig. 1 shows the result for a lysozyme-antibody HyHEL5 complex [6]. In this particular case, ΔC_p, ΔH_d and ΔS_d all have positive values: association releases heat, a favourable enthalpy stabilizes the complex and a

unfavourable entropy fights it. In other systems, negative values of ΔH_d or ΔS_d can be observed at 25°C. Moreover, ΔC_d is high. Therefore, both the enthalpy and the entropy vary quickly and change sign with temperature. In the lysozyme-HyHEL5 complex, ΔS_d is negative below 0°C. Then, entropy favours complex formation - but that statement is valid only at concentrations above $c_\emptyset$=1M !

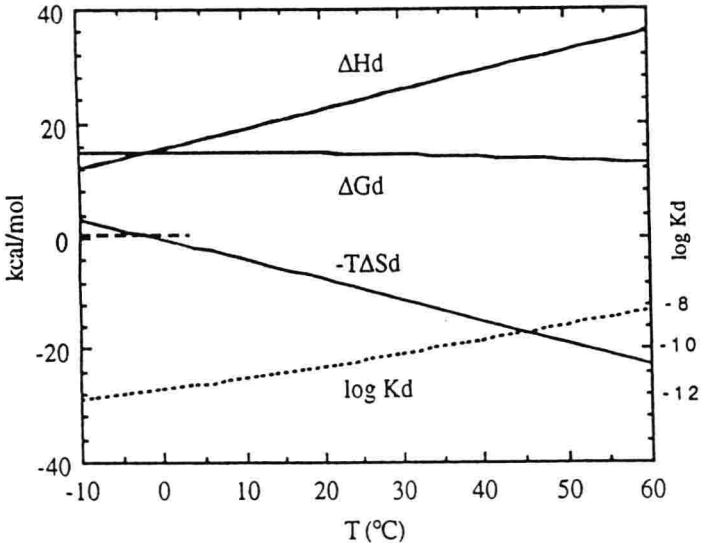

Figure 1: Temperature dependence of thermodynamic parameters for the lysozyme-antibody HyHEL5 complex. ΔH_d was measured by isothermal mixing calorimetry at several temperatures between 10° and 37°C yielding ΔC_d=0.34 kcal.mol^{-1}.K^{-1} [6] and the dissociation constant at T_0=278K (25°C). Assuming ΔC_d to be temperature-independent, we have at all temperatures:

$$\Delta H_d(T) = \Delta H_d(T_0) + (T-T_0)\,\Delta C_d \quad \text{and} \quad \Delta S_d(T) = \Delta S_d(T_0) + \Delta C_d \ln \frac{T}{T_0}$$

The temperature dependence of enthalpy and entropy almost exactly compensate each other; thus, ΔG_d varies by <1 kcal.mol^{-1} between 0 and 37°C, whereas K_d (dashes) changes by a factor of 100.