BIOLOGICAL PHYSICS

Third International Symposium

Santa Fe, NM 1998

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AIP CONFERENCE PROCEEDINGS 487

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American Institute of Physics **Melville, New York**

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L.C. Catalog Card No. 99-066559
ISBN 1-56396-874-6
ISSN 0094-243X
DOE CONF- 9809152

Printed in the United States of America

BIOLOGICAL PHYSICS

PREFACE

Major goals of biological physics are the understanding of biological systems in physical terms and the study of concepts and laws of complex systems. Working towards these goals requires a close collaboration among biologists, chemists, computer scientists, mathematicians, and physicists. Biological physics is a two-way street: physicists can learn a great deal from biology concerning unexpected properties that emerge from complex, interacting systems, and biologists can learn in-depth treatments of such systems from the physical sciences. Close contact between the two sides is, however, still too rare. The symposia on biological physics are designed to help close the gap.

The Commission C6, Biological Physics, of the International Union of Pure and Applied Physics has sponsored a series of International Symposia on Biological Physics to foster interactions between the biological and physical sciences. The first was held in 1993 in Szeged, Hungary, the second in 1995 in Munich, Germany, and the third in Santa Fe, New Mexico, USA in 1998. The fourth will take place in Kyoto, Japan, from 30 July to 3 August 2001.

The present volume contains most of the papers that were presented at the Third International Symposium on Biological Physics, held from 20 to 24 September at the Hotel La Fonda in Santa Fe, New Mexico, USA. The conference was planned by a local organizing committee and by an international advisory committee. The members of these committees are listed below. Also listed are the sponsors without which the Symposium could not have taken place.

Perusal of the papers will show that part of the goal is within reach: Scientists from the different fields indeed talk to each other, not just concerning the simplest biological systems, such as proteins and nucleic acids, but also about much more complex systems, such as cells. Much remains to be done, however, and we hope that the Fourth Symposium in the next century will provide further evidence of growing collaborations between the biological and the physical sciences.

Hans Frauenfelder Gerhard Hummer Roderick Garcia

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Protein Structures in Solution: (1) Effects of Solvation; (2) Studies of Large Molecules

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Abstract. The general theme of this presentation is nuclear magnetic resonance (NMR) spectroscopy in structural biology. Two aspects of special interest to biological physicists are discussed. These are, firstly, the results of NMR studies on the solvation of proteins and nucleic acids in solution and, secondly, the physical foundations of a novel NMR approach for studies with very large particles in solution, transverse relaxation-optimized spectroscopy (TROSY).

HYDRATION OF PROTEINS, NUCLEIC ACIDS AND PROTEIN–DNA COMPLEXES IN SOLUTION

The hydration of proteins and nucleic acids can be studied by observation of ^1H–^1H nuclear Overhauser effects (NOE) between the NMR lines of the individually assigned protons of the biomacromolecules and the water protons (1). NOE measurements in the laboratory frame (NOESY) and the rotating frame of reference (ROESY) recorded without presaturation of the water signal reveal two different time scales for solvent–solute interactions (2): (i) The NOE interaction between the macromolecule and the water is characterized by NOESY cross peaks that have the same sign as the diagonal peaks. This situation has been observed for water molecules located in the interior of proteins (1), for water that is stably bound in the minor groove of B-DNA duplexes (3,4), and in rare cases for water molecules located in grooves on the protein surface (*e.g.*, 5). (ii) Surface hydration waters typically show NOESY cross peaks with opposite sign relative to the intramolecular cross peaks between different protons of the biomacromolecule (2, 6). The different sign shows that compared to the intramolecular proton–proton NOEs, a different effective time modulation prevails for the dipole–dipole coupling between surface hydration water protons and solute protons. Model calculations showed that this experimental observation cannot be explained by modulation of the dipole–dipole coupling governed by the combination of overall rotational motions and intramolecular motions

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in hydrated biomacromolecules, but is readily rationalized by the spectral density functions calculated for free translational diffusion of spheres of different sizes representing the macromolecule and the water, respectively (2). The diffusion coefficients of the surface hydration waters estimated from the NMR data with the use of this model are about 4 to 8 times smaller than the self-diffusion coefficient of pure water.

The model calculations in ref. (2) were based on the assumption that the hydration water molecules spend a defined life time in discrete hydration sites. Alternative models considering delocalized reduced diffusion on the macromolecular surface have more recently been described (7).

The high resolution NMR approach discussed in ref. (1) to (6) is unique in combining atomic resolution spatial information on hydration with data on the kinetics of exchange processes. For "long–lived" interior waters the latter consists of an upper limit on the lifetimes of individual hydration waters in the ms range (8), and a rather precise lower limit in the ns range (2). For surface hydration waters quite precise life times in the range from about 20 ps to 300 ps can be derived (2). In applications with the *Antennapedia* homeodomain bound to its operator DNA the experimental limits on the hydration water life times (9) have been complemented with data obtained from a 2 ns molecular dynamics simulation (10), which indicate that the actual life times of the water molecules bound in the water–DNA interface are close to the lower limit, *i.e.*, in the ns range. This then represents the time scale of part of the protein–DNA contacts that mediate specificity of DNA recognition by proteins in transcriptional regulation (10).

Recent advances in high resolution NMR studies of biomacromolecular solvation include studies of interactions with multiple components in mixed solvents, in particular with chemical denaturants (11, 12), the introduction of diffusion filters for unambiguous identification of NOE cross peaks with the solvent (13), the use of diffusion-based, more precise determination of the upper limit on the life times of "long-lived" hydration water molecules (14, 15), and a study of potential artefacts in the technically demanding experiments needed for these studies (16).

Only a brief reference can be made to complementary methods for studies of macromolecular hydration. X-ray or neutron diffraction studies can provide detailed accounts on the hydration of protein and nucleic acid single crystals, usually with higher spatial resolution and more precise identification of the number of hydration sites than is possible with solution NMR, but they cannot provide the temporal resolution afforded by NMR. A paper comparing the NMR and X-ray data on the *Antennapedia* homeodomain–DNA complex has just appeared and may serve as an illustration (17). Nuclear magnetic relaxation dispersion measurements can yield more precise upper limits on the life times of "long-lived" hydration water than the high resolution NMR approach, but they do not provide, independently of NMR or X-ray structural data, spatial information on hydration (*e.g.*, 18).

TROSY FOR SOLUTION NMR STUDIES OF VERY LARGE MOLECULAR SIZES

Rapid transverse relaxation by dipole–dipole (DD) coupling and chemical shift anisotropy (CSA) modulated by rotational molecular motions limits the size of biomacromolecular structures that can be studied by NMR spectroscopy in solution. Transverse relaxation-optimized spectroscopy (TROSY) (19) uses spectroscopic means to reduce transverse relaxation based on the fact that cross-correlated relaxation caused by interference of DD coupling and CSA gives rise to different relaxation rates of the individual multiplet components in a system of two coupled spins 1/2, *I* and *S*, such as, for example, the ^{15}N–^{1}H fragment of a peptide bond. Theory shows that highly efficient cancellation of transverse relaxation effects in ^{15}N–^{1}H or aromatic ^{13}C–^{1}H moieties can be achieved for one of the four multiplet components (19, 20). TROSY observes exclusively this narrow component, for which the residual linewidth is then mainly due to DD interactions with remote hydrogen atoms in the protein, and possibly to conformational or chemical exchange. The use of TROSY enables high resolution solution NMR studies of particles in the range well above 100 000 Dalton (19, 21), results in improved quality of NMR structure determinations when isotope labeling with ^{15}N and ^{13}C is used (20), and largely extends the molecular size range for the use of triple resonance experiments (22).

ACKNOWLEDGMENTS

Financial support was obtained from the ETH Zürich and the Schweizerischer Nationalfonds. I would like to acknowledge the graduate students, postdoctoral fellows and senior associates who are listed as authors in the references to work from my research group.

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Protein-Solvent Interactions and Biological Functions. Models From Statistical Physics.

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Abstract. We consider two simple biosystems, both exhibiting biological function at room temperature: (1) hydrated protein powders, and (2) nearly anhydrous enzymes in alcohol, and we propose a common structure of the solvent-protein interface. We extend our previous treatment of percolation to the "blobs, nodes and links" model, to describe the surface as patches of H-bonded OH groups, singly connected in the percolating backbone. A dynamical pathway between surface and core is identified in few strong H-bonds between charged side chains and protein secondary structure. Since recent work in collaboration with F. Bruni and G. Consolini shows dielectric behaviour typical of proton glasses, we model proton frustration in blobs by a 2D Ising net, and in collaboration with D. Stauffer we find by Q2R cellular automata that this system fails to reach internal equilibrium. A functional relevance for non-ergodicity in enzymatic activity is proposed.

INTRODUCTION

It is a widespread opinion that solvent molecules bound to the surface of globular proteins are essential for biological function. In this paper we shall introduce a simplified structure of the solvent-protein surface to be modelled by statistical physics of condensed matter, to see if this modelling can offer consistent suggestions about protein dynamics in the low frequency scale where biological function is often displayed.

A Simplified Structure Of Protein Surface

The purpose of this section is to propose a simplified version of the solvent-protein surface to be considered as a disordered two-dimensional (2D) system. To this end we consider two simple biosystems active at room temperature and where proton exchange with solvent is prevented, to identify a simplified but general picture of an active protein surface.

One simple system to be considered is an hydrated lysozyme powder, as studied by our group in the last years and widely reviewed (1) (2). Here both catalytic activity and physical properties can be followed by controlling one parameter only, the water content. The main result is that the 2D percolation threshold for bound water coverage coincides with the onset of biological function, likely because the charge distribution on the protein surface can reach equilibrium thanks to proton migration. The other simple biosystem to be considered is chymotrypsin dissolved in octanol, as first studied by Klibanov'group (3), where activity was detected in nearly dry state. The apparent contradiction between these two results can be overcome by proposing a structure of the solvent-protein surface common to both of them.

A look at fig.1 shows that a layer of hydroxyls is the common feature of both these two biosystems. Moreover from current knowledge of Hydrogen-bonded structures (e.g.

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