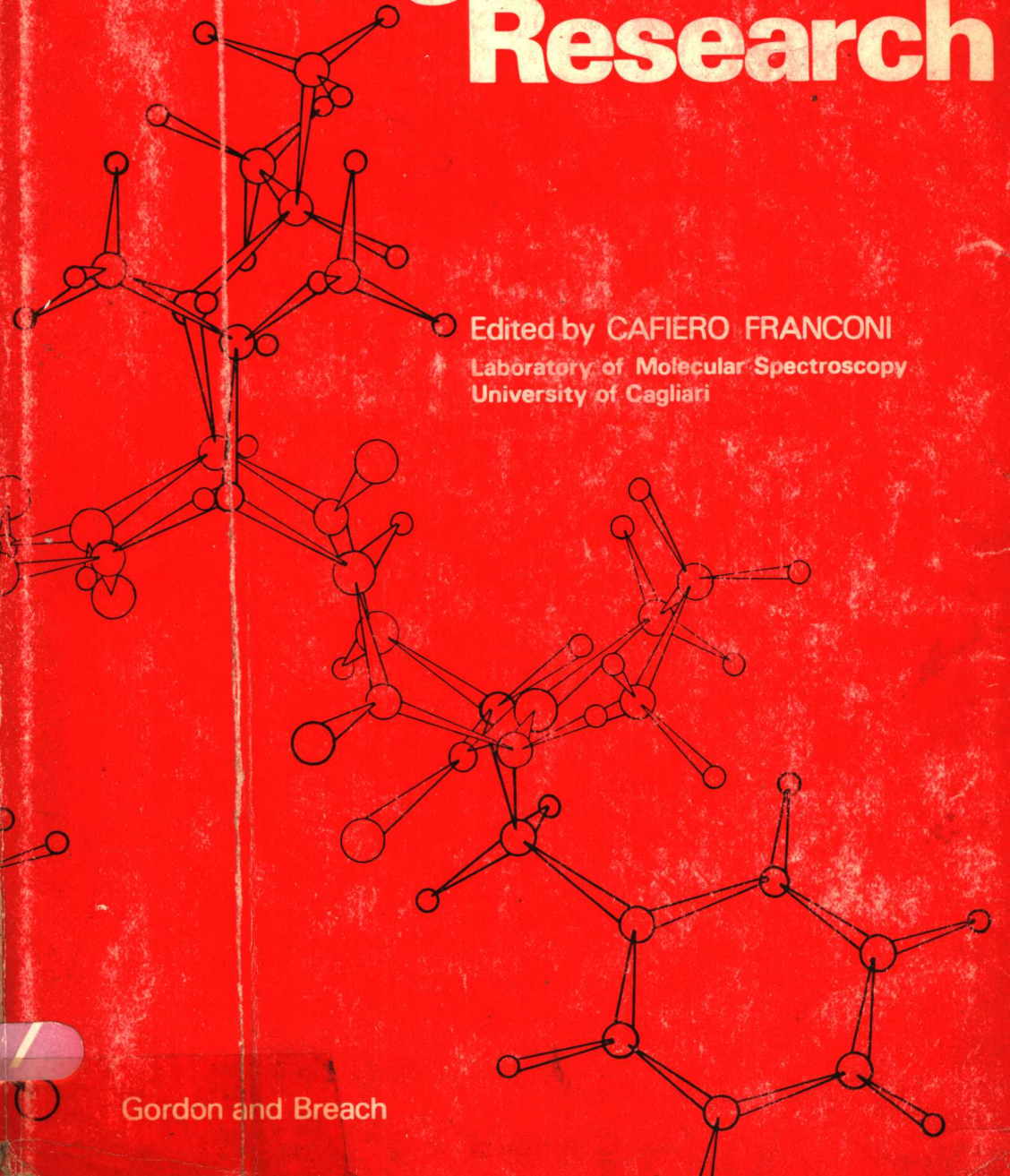
Magnetic Resonances in Biological Research



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Preface

This book contains the reports of physicists, chemists and biologists working in the field of magnetic resonance spectroscopy applied to systems of biological interest. These reports were read at the Third International Conference on Magnetic Resonances in Biological Research, held at S. Margherita of Cagliari, Italy.

Among the radiofrequency spectroscopies, magnetic resonance spectroscopies play a very important role chiefly because they involve electron and nuclear spins and their associated magnetic moments. In fact many spin magnetic interactions are present at molecular level and the results of their study have been quite rewarding so that new fields of research are now fully developed regarding studies of structure, conformation and electronic distribution of molecules, molecular and latticesymmetry and dynamics.

Also molecules of biological interest together with biological systems were subjected to analysis by magnetic resonance spectroscopies and many significant data at molecular level of interest to biochemists have already been collected. In fact in recent years parallel developments have occurred in the understanding of both molecular biology and spin magnetic interactions, so that today – given also the tremendous improvement of the basic instrumentation – it is possible to describe many biologically important problems in molecular terms which can be studied by magnetic resonance spectroscopies. Thus the latter methods of investigation have become a unique tool for taking further steps toward a better definition of major problems such as the relationships between molecular properties and biological specificity.

It appeared that 1969 was the right year in which to collect and review the main results of the research in the field, since the previous meeting had occurred in 1966 and a three year gap seemed long enough for such a rapidly growing field. However, besides a review of recent developments, it seemed also opportune to have a first hand account of the attempts made toward new directions in order to have an assessment of its future, given also the large accumulation of data relative to new problems of molecular biology occurred in this period. These feelings have been shared by many scientists working in the field so that with their enthusiasm and cooperation it was a simple task to organize a conference on this subject.

The reviews and reports read at the conference and included in this volume concern chiefly studies on the interactions of small molecules with proteins,

ESR studies of metalloproteins and in particular of hemoglobin, NMR structural and conformational studies of polypeptides and proteins. It must at once be said that the papers read at the conference confirmed the first expectations that new horizons in molecular biology are being opened by the particular view points of the magnetic resonances methods.

The success of the conference was assured first by the close cooperation of the members of the scientific committee: M. S. Blois, W. E. Blumberg, O. Jardetzky, B. G. Malmström, B. Mondovì, W. D. Phillips and L. H. Piette, and by the active participation at the conference of the most distinguished experts in the field. To all of them we give our thanks.

The conference was supported by the Società Italiana di Scienze Farmaceutiche (S. I. S. F.); the Assessorato alla Pubblica Istruzione and the Assessorato alla Industria of the Ente Regione Autonoma della Sardegna (E. R. A. S.), the Varian S.p.A. of Turin and the Cagliari Chamber of Commerce. Special thanks are due to Prof. P. Pratesi, President of the S. I. S. F., to Dott. A. Giagu De Martini and Dott. P. Soddu of the E. R. A. S. and to Dott. G. Ferretti of Varian S.p.A., for their earnestness in supporting the conference.

Thanks are also due to Prof. B. G. Marini-Bettolo Director of the National Institute of Health and to Prof. A. Boscolo, President of the Board of Cagliari University for their cooperation.

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P. PRATESI

There are valid reasons for believing that study of the structure of proteins and of their interactions may lead to the formulation of valid hypotheses about the dynamics of the so-called "receptors"; that is, of those particular receptive chemical structures of the biological substrates that interact with drugs and are believed to be responsible for the specificity of the drugs themselves. Indeed, research on the molecular properties which govern the interaction between molecules of biologically active compounds and receptors, carried out on a large number of substances, has led to the conclusion that shape, molecular size and distribution of the electronic charge play a fundamental part in the said interaction, determining the possibility that the molecule of a biologically active substance reaches the critical distance for interaction with the receptor.

Magnetic resonance spectroscopies are powerful means for examining these processes at the protein level. This is the main reason why the Società Italiana di Scienze Farmaceutiche has enthusiastically supported Professor Franconi's proposal to organize this international conference in Italy.

In the name of the Società Italiana di Scienze Farmaceutiche I wish, therefore, to express our thanks to all those taking part in the conference, and our hope that this meeting will stimulate the interest of chemists and physicists in the study of the physico-chemical aspects of the action of drugs. We are particularly grateful to the rector of the University of Cagliari for his support. This meeting well expresses the scientific awareness of the chemistry world at Cagliari. My best compliments to Professor Franconi and to the molecular spectroscopy laboratory people of Cagliari University for having invited the most qualified scientific workers in the field; their presence here guarantees the success of this conference.

Contents

PAPER 1

Intermolecular forces and conformational changes in protein-ligand interactions

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Abstract

Some current problems in the calculation of stable conformations of proteins are outlined, with a list of the factors entering into such calculations. A new model for the thermodynamics of transfer of alcohols from a hydrocarbon solvent into water is presented. It is derived as an extension of the Némethy–Scheraga theory for water structure. A partition function is written for the alcohol molecule in water, based on a distribution over four energy levels, corresponding to three, two, one or zero hydrogen bonds formed by the hydroxyl group. The statistical weighting for each level are derived from those for water in a self-consistent manner. For the alcohol in hydrocarbon, one energy state has to be considered. The theory matches the experimental free energies of transfer within 0.2 kcal/mole, the enthalpies within 0.4 kcal/mole at 25 °C. The empirical binding and interaction constants used in the description of ligand binding, conformational changes, and allosteric models are discussed in terms of free energy changes. The free energy condition on subunit interactions for positive and negative cooperativity is derived. It is shown that an apparently hyperbolic (Michaelis–Menten) ligand saturation curve can be obtained even with strong interactions between subunits for a "simplest sequential model" with equivalent subunits.

I Introduction

THE CONFORMATION of macromolecules in solution is determined essentially by the balance of noncovalent interactions which act both between segments of the macromolecule, and between these segments and solvent molecules surrounding them. Proteins in their native state and polypeptides under some physical conditions take up a well-defined, compact, and more or less rigid shape, i.e. the chain has a definite conformation. The conformation can be described by listing the values of the torsional angles of internal rotation around single bonds of the molecule[1]. In proteins, there are two such angles per residue, ϕ and ψ. The third angle, ω, corresponding to the C–N peptide bond, generally is assumed to be fixed, due to the partial double bond character of the peptide bond (Fig. 1). The conformation of the amino acid side chains is described by a series of torsional angles denoted by χ.

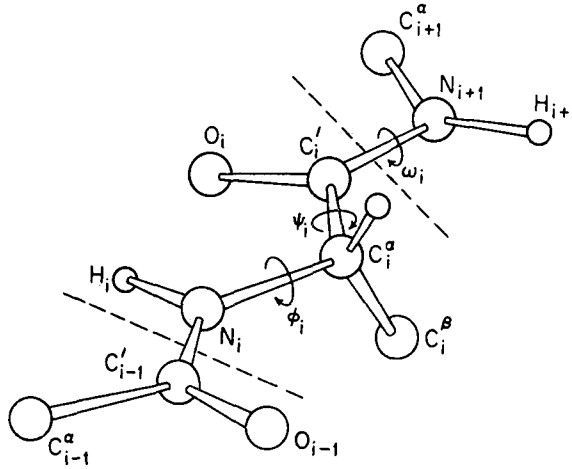

Figure 1 Two peptide units linked together in the fully extended conformation. The torsional angles of internal rotation, ϕ, ψ, and ω, defining the conformation, are shown. The dashed lines delimit an amino acid residue[1]

Conformational changes occur frequently when protein molecules interact with each other (such as in subunit interactions) or with ligand molecules binding to them (substrates, inhibitors, activators). These conformational changes can be of great importance in biochemical functions, such as enzyme specificity[2] or allosteric regulation.[3] The analysis of ligand bind-

ing and regulatory phenomena usually is carried out in terms of phenomenological equilibrium constants.[4,5] However, the binding of ligands and concomitant conformational changes are just as much the result of the balance of noncovalent interactions as is the establishment of stable conformations of the isolated macromolecule. A detailed understanding of binding phenomena, not yet available, would require a description of binding and interaction equilibria in terms of local noncovalent interactions.[6]

II The analysis of stable protein conformations

In recent years, great effort has been expended upon attempts to calculate stable conformations of proteins and polypeptides, as well as other macromolecules, starting from fundamental physico-chemical principles.[7-9] The details of such computations as well as results obtained so far are discussed in several reviews.[10-11]

Most calculations are based on the fundamental hypothesis that the sequence of the protein is sufficient to determine (given the specifications of the environment, such as temperature and solvent composition) the unique most stable conformation (or group of closely related conformations) of the macromolecule, i.e. the molecule takes up the conformation of lowest free energy. If the potential energy of the macromolecule is represented as an energy surface over a space of all the conformational parameters (torsional angles), then the most stable conformation ought to correspond to the point of lowest energy on the map (Fig. 2). In the past, most calculations were carried out by searching for a minimum of the potential energy. The existence of metastable minima, with energies above that of the most stable conformation, may lead to false results. However, it must also be recognized that the most stable conformation may not correspond to that with the lowest potential energy.[12] Vibration around an equilibrium conformation, or equivalently, the entropy contribution from a loosely held conformation may result in the stabilization of a conformation above the one having lowest potential energy (Fig. 3). Thus the overall free energy rather than the potential energy must be minimized.[12]

The important interactions contributing to the conformational free energy are listed in Table I. The classification in the table is convenient for practical purposes of description or the subdivision of computations. In terms of fundamental principles, the classes overlap. For example, all of the effects listed as group B in the table involve combinations of van der Waals and electrostatic forces.

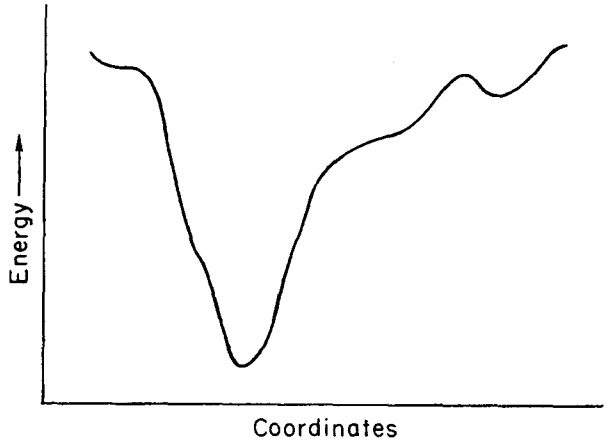

Figure 2 Schematic representation of the conformational potential energy of a protein as function of conformational parameters (atomic coordinates or torsional angles of internal rotation). While these parameters actually describe a multidimensional space, here they are symbolically represented by a single dimension on the abscissa. Theoretical calculations of stable protein conformations are directed towards the determination of the lowest point of the curve

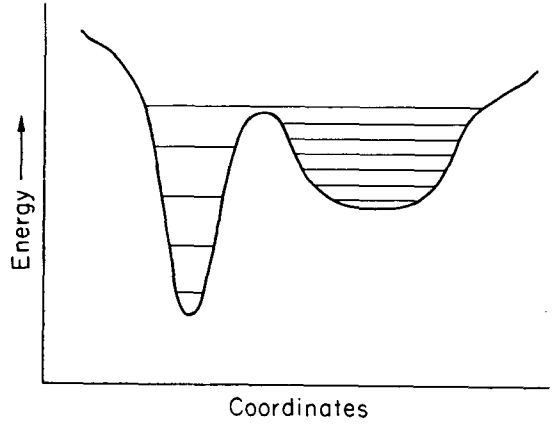

Figure 3 Comparison of a conformation of lowest potential energy but little internal freedom (narrow potential well on left) with a conformation of higher potential energy and high internal freedom (wide potential well on right). The spacing of vibrational energy levels is indicated schematically by the horizontal lines. A large vibrational partition function may stabilize the second conformation over the first one. Schematic representation as in Figure 1

Table I Factors influencing the conformational stability of proteins

A. Effects intrinsic to the macromolecule
1. Internal rotation about single bonds
2. Distortion of covalent bond geometry
   (a) Bond angle bending
   (b) Torsion about the peptide bond
3. Van der Waals ("nonbonded") interactions
   (a) Repulsion at short range ("steric" or "excluded volume" effects)
   (b) Attraction at close approach of atoms (London forces)

B. Effects depending strongly upon interactions with the solvent
4. Hydrogen bonds
5. Hydrophobic interactions in aqueous solution
5a. Differential van der Waals interactions in nonaqueous solution
6. Intra- and intermolecular dipole interactions
7. Electrostatic charge interactions
8. Preferential interaction with solvent components ("solvent binding")
9. Specific binding of ligand molecules

In most of the computations performed so far, only the effects grouped under A, as well as *intramolecular* hydrogen bonds and dipole interactions, were considered. These are the interactions which can be described exactly as functions of the conformational parameters (torsional angles). Details of the potential functions used and of the mathematical techniques are found in the reviews cited.[10,11]

While the effects listed under B in Table I are of fundamental importance in the selection of stable conformations, their contributions are more difficult to handle. For some of the effects, theoretical models are available which correlate macroscopic thermodynamics with molecular parameters, using statistical thermodynamics. However, such an approach is not adaptable as directly to the energy calculations as are the formulations of the effects in Class A. For an exact calculation, a detailed knowledge of local liquid structure would be necessary. In the only attempt of including solvent interactions in the computations,[13] empirical functions based on averaged molecular interactions of the solvent were used.

A statistical thermodynamic model for hydrophobic interactions was developed several years ago[14], based on model theories for liquid water[15] and for the solution of hydrocarbons in water.[16] In the theory for water,[15] the presence of hydrogen bonds in the liquid was considered as the most important characteristic feature. The liquid was described in terms of a distribu-

tion of water molecules among states with various number of hydrogen bonds, assuming that the hydrogen-bonded molecules form compact clusters.*

In the application of the water model to solutions of hydrocarbons,[16] it was shown that the presence of the hydrocarbon causes a shift in the energy levels of neighboring water molecules. As a result, the equilibrium distribution of water molecules over the energy levels changes, resulting in an increase of hydrogen bonding and of ordering of the water. This is the source of the unfavorable free energy of solution. When hydrophobic interactions are established between nonpolar groups, the solution process is reversed. The source of the free energy of stabilization of such interactions is the decrease in ordering of water.

The theory of water structure was applied recently to an explanation of the thermodynamics of alcohol-water solutions.[18] The main features of the new theoretical model are summarized in Section III.

III The thermodynamics of alcohols in aqueous and hydrocarbon solutions

In order to understand the thermodynamics of hydrogen bond formation between polar groups "buried" in the nonpolar interior of a protein molecule, it is necessary to describe the thermodynamics of transfer of a polar group from solution in a nonpolar solvent into aqueous solution. An empirical model for this process is given by the solution of aliphatic alcohols in hydrocarbons and in water, respectively.

We have derived a very simple model for the description of dilute solutions of alcohols in water,[18] based on the theories of water structure[15] and hydrocarbon solutions in water.[16] In the derivation of the model, consistency with that for water was maintained, with the introduction of the least possible number of new assumptions and parameters. Details of the derivation are published elsewhere.[18] The main physical features are summarized here.

The calculations were performed for alcohol solutions in water and in a hydrocarbon solvent at infinite dilution. Thus, any association of the solute can be disregarded. In the hydrocarbon solution, only van der Waals interactions operate between solvent molecules and the alkyl chain and hydroxyl

* Work is in progress (A. T. Hagler, G. Némethy, and H. A. Scheraga) on an improvement of the theory, eliminating some of the restrictive physical assumptions and avoiding some of the problems of the statistical mechanical formulation which came under criticism recently.[17]

group of the alcohol. The total interaction energy can be determined by summing over all pairwise contacts the interaction energies, using the standard formulae[10] for van der Waals interactions. A partition function is written for this single energy state, with the proper statistical weights for external and internal degrees of freedom.

In aqueous solution, the hydroxyl group can form hydrogen bonds with neighboring water molecules. A partition function for the alcohol molecule can thus be constructed in analogy with that derived earlier[15] for water.

It is assumed that G^{aq}_{ROH}, the total free energy of the alcohol in aqueous solution can be written as the sum of three contributions:

$$G^{aq}_{ROH} = G^{aq}_{OH} + G_R + \Delta G_W \tag{1}$$

Here G_{OH} is obtained from the partition function of the alcohol when its hydroxyl group interacts with water, G_R represents the interaction energy of the alkyl chain with the water molecules surrounding it, and ΔG_W is the change in the free energy of the layer of water surrounding the solute, due to structural changes occurring upon introduction of the solute molecule.[16] It should be noted that G_{OH} depends upon the nature and size of the alcohol carrying the OH group, so that eq. (1) should not be construed as representing the division of the total free energy into additive contributions by each functional group on the solute.

A hydroxyl group can form maximally three hydrogen bonds: two involving the lone pair electrons of the oxygen, and one involving the hydrogen. Thus, in analogy with the model for liquid water, one can consider the alcohol molecules as being distributed over four energy levels, E_i, where $i = 3, 2, 1,$ or 0 indicates the number of hydrogen bonds formed with neighboring water molecules. In all states (except the tri-bonded one) there will be also some water neighbors to which the hydroxyl group is not hydrogen-bonded. For the completely non-hydrogen-bonded state, the average number of nearest neighbor water molecules (z_u) has been estimated as five, in analogy with the corresponding number ($z_u = 8$) for water.[15]

For the completely hydrogen-bonded state, $z_3 = 3$; z_i is assumed to change linearly for the intermediate states. (Fig. 4, water molecules indicated as b and c.) We assume that the intrinsic strength of the hydrogen bond and the van der Waals energy gained upon increasing by one the number of non-hydrogen-bonded neighbors are the same as for water,[15] namely, $\mathscr{E}_H = 3.57$ and $E_w = 2.25$ kcal/mole, respectively. Then the net energy of breaking a hydrogen bond in the liquid is $E_H = 2.07$ kcal/mole for the hydroxyl group (for water,[15] $E_H = 1.32$ kcal/mole). Thus E_H is derived from