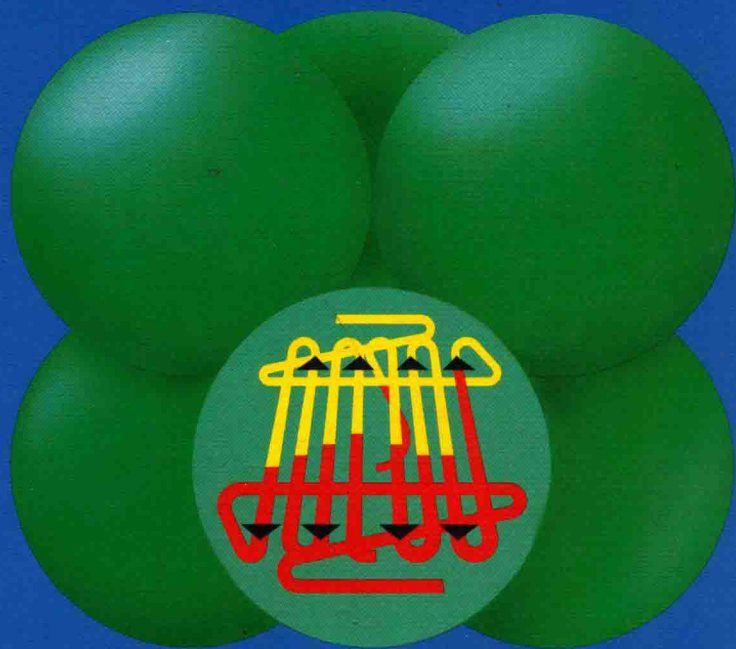# Protein Interactions

Edited by Hans Visser

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Developed from the Symposium on Protein Interactions, held at the 201st annual meeting of the American Chemical Society, Atlanta, GA, USA, April 15–17, 1991

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VCH

Preface

When Professor Carel Jan van Oss, State University of New York, at Buffalo, USA, approached me early in 1989 and invited me to organize a symposium on Protein Interactions as part of the 201st Annual Meeting of the American Chemical Society at Atlanta, in April 1991, I immediately said yes, not realizing the complexity of organizing such a meeting. Starting with one contribution, my own, I succeeded after a lot of writing and consulting other people including Dr. van Oss, the initiator of a series of related symposia, in bringing together 19 speakers. In total they were to present 26 papers.

In order to cope with such a large number of contributions in a limited amount of time – two full conference days – a well balanced and well timed meeting was required. For this reason I combined papers on similar subjects, e.g. on whey proteins, as far as possible in either a morning or an afternoon session. Looking back I think this was a good idea. Listeners did not have to switch from one subject to the other and were able to choose sessions devoted to their particular fields of interest.

It was a pity that a number of people from Germany and Russia had difficulties in obtaining funding for travelling to Atlanta. In particular, the absence of Professor Tolstogusov, from the Institute of Organometallic Compounds, Moscow, was clearly felt. At the last moment Dr. Paulsson, University of Lund, Sweden, was, to our great pleasure, able to attend, and replaced one of the speakers who was unable to come. After a successful meeting it was possible to interest the majority of the speakers in having their papers published in a conference proceedings book. We are very grateful to VCH, who gave us all the opportunity to do so. The preparation of a book has also given those speakers who were unable to attend the opportunity to bring their work to the attention of their colleagues. We are very pleased to be able to include papers by Dr. Plock and Dr. Kopperschläger.

The subject of protein-protein interactions in aqueous solutions is of great interest to every protein chemist. It is an integral part of our work. Bringing together such a larger number of papers coming from different areas shows the complexity and diversity of the subject. A unifying theory, however, is clearly lacking, although there is mutual agreement on the majority of the various types of interaction. Controversy exists mainly on the so-called hydrophobic interactions between proteins. This was actually a general conclusion of the meeting. It is interesting to compare the different theories presented in this book. The different views clearly suggest that a general topic for the next meeting on protein interactions should be focused on this particular aspect.

I would like to express my hope that this book will encourage other people, both the contributors as well as the readers, to organize a symposium on the issue of hydrophobic interactions in the not too distant future.

February 1992   Hans Visser
Vlaardingen

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An Overview

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1.   Introduction

When reviewing protein interactions we have to be aware that such an excercise implies discussing a broad range of subjects, which will become clear in particular when scanning through the contents of this book.

On the one hand specific interactions between proteins and ligands are encountered in molecular biology and these are of vital importance for the functioning of cells and organisms. For example, immunology is determined by antibody-antigen interaction, enzymes often only exhibit activity after substrate binding and repressor-operator interactions control gene regulation.

On the other hand proteins can aggregate and form large assemblies. Aggregation, for example, is induced by a change in the conditions which can be translated into a change in the interactions. Changing the temperature of a protein solution often invokes denaturation followed by gelation. Another intriguing protein assembly is the casein micelle which, being an aggregate itself can aggregate further to products such as cheese.

In this article we will focus on the various types of basic interactions which are occurring in protein containing systems. The basic forces will serve as a starting point for the discussion. The more specific interactions arising from these forces are divided into two classes. Molecular interactions will be discussed separately from interactions in colloidal systems. In our view this facilitates the understanding of phenomena occurring when proteins start to interact.

Finally, the gelation of proteins, with emphasis on globular proteins, will be used to illustrate the approach we take in discussing protein interactions.

2.   Forces and their interactions. Definition and magnitude.

In a recent article (*1*), Van Oss has tried to summarize the primary forces which are acting between biological, e.g. proteins, and polar entities in water. Although the content of his article and the choice of definitions are open to discussion, a good classification of the real fundamental forces and their interactions is given. The necessity to do so is essential for any future work on protein interactions as well as for a proper understanding of what is really going on in solution.

In our opinion, which is in agreement with the statements made by van Oss, the principal interaction forces are the Van der Waals force and the electrostatic force of interaction.

As already said in the introduction, for reasons of clarity, in the following sections we will make a distinction between interactions operating at the molecular level and interactions which are acting between protein aggregates.

2.1   Basic forces

Van der Waals forces
The presence of attractive forces between nonpolar entities, such as atoms and molecules, has already been identified by Van der Waals (*2*). The physical description of these forces in terms of fluctuating dipoles caused by motion of electrons around a nucleus was given by London (*3*). Hence, this force is often referred to as the London-Van der Waals attraction. According to theoretical equations the potential energy, ΔG, for the Van der Waals attraction between two atoms is proportional to the sixth power of their distance, r (*4*):

$$\Delta G = -\frac{A}{r^6} \tag{1}$$

The constant A can be related to the polarizability of the atoms or to the main frequency of the fluctuation of the dipoles which the atoms actually are.

Repulsion occurs when the atoms come too close together and their electron clouds start to interpenetrate. This is the so-called Born repulsion. Often the Van der Waals attraction and the Born repulsion are taken together in one potential energy function:

This potential function is the so called Lennard-Jones potential (*5*) where the

$$\Delta G = -\frac{A}{r^6} + \frac{B}{r^{12}} \tag{2}$$

second term is a description of the repulsion and has no real physical background. Although the constant A has a real physical meaning, both A and B are often determined semi-empirically. For an atom pair at a distance of 5 Å, the Van der Waals energy typically amounts to a few kcal/mole.

electrostatic forces

The second principal force is the electrostatic force which is a consequence of the charge distribution of electrons on the molecule. Depending on the sign of the charges the electrostatic or Coulomb force can either be attractive for oppositely charged entities, or repulsive for equally charged entities.

The potential energy depends on the charges, q_i, of the atoms, their distance, r, and the dielectric constant, ϵ, of the medium in between the charges in the following way:

$$\Delta G = -\frac{q_1 q_2}{er} \tag{3}$$

The interaction energy due to Coulomb forces is somewhat larger than the one arising from van der Waals force, i.e. is typically of the order of 20 kcal/mole and much further reaching.

covalent forces

The most important covalent bond playing a role in protein interactions is the S-S bridge between two cysteines. These covalent bonds are relatively strong with a potential energy of about 50 kcal/mole. The S-S bridges can form intra- and intermolecular cross-links and stabilize the protein structure to a large extent.

kinetic energy

Finally, one has to set these interactions forces against the kinetic energy of a molecule, which is driven by the thermal motion of the atoms. Each atom has three degrees of freedom and for each one the thermal energy is:

This thermal energy allows a molecule to undergo conformational changes in particular upon heating.

$$\Delta G = -\frac{1}{2}kT \qquad (4)$$

Through the kinetic energy term it is possible to calculate the average speed of atoms or particles, where the heavier ones have a lower average speed. This phenomenon extends to Brownian motion where dancing heavy particles and the surrounding lighter ones have the same average kinetic energy.

2.2   Molecular interactions

The total interaction energy between molecules and the resulting type of interaction, either attractive or repulsive, is the sum of the four above mentioned forces. A schematic overview of the different types of interactions in proteins is given in Figure 1.
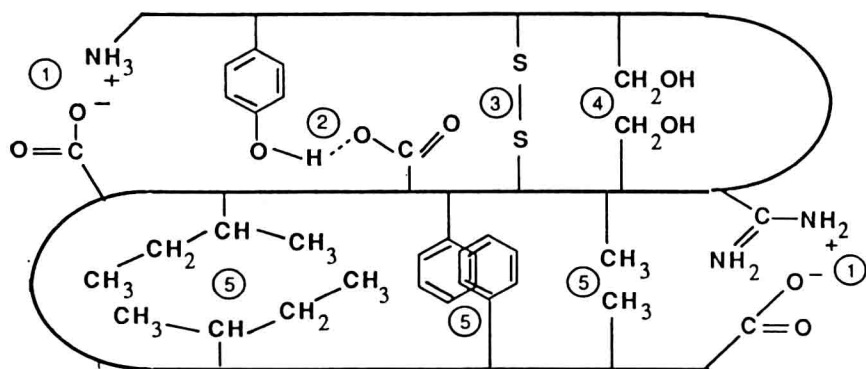

Figure 1. Schematic presentation of the stabilizing interactions in proteins. 1) ion-pair interactións; 2) hydrogen bonds; 3) disulfide bonds; 4) dipole-dipole interactions; 5) hydrophobic interactions

ion-pair interaction

Positively charged and negatively charged ions or ionized groups always attract each other due to their mutual attraction inherent to electrostatic forces. As a result, there is a driving force for oppositely charged ions to attach themselves to each other and to form a stable configuration. In proteins one can distinguish two types of ion-pair interaction. Firstly, when a negatively charged carboxylic group comes in the vicinity of a positively charged lysine or arginine group under pH conditions where these groups are ionized. This type of interaction is called salt bridging.

Secondly, binding of typical metal ions to specific protein sites can be considered as another type of ion pair interaction. This phenomenon is encountered in many proteins and enzymes. Enzymes often only function when specific metal ions are bound to the protein. Ion-pair interactions are observed in many of the proteins for which the three dimensional molecular structure has been resolved with the use of single crystal X-ray analysis (6).

An interesting example of ion pair interaction is the casein micelle which is held together by amorphous Ca-phosphate whereby interactions between the phosphoserines in the backbone of the casein and Ca-ions play a role (7,8).

Ion-pair interactions are interfered by polar water molecules which tend to interact with charged species. By surrounding the charged group the water molecules will screen the charge. Mostly then the water molecules around a charged group will be highly ordered. However, when a stronger salt-bridge can be formed the screening water molecules are expelled resulting in an increase in entropy of the system.

dipole-dipole interaction

This type of interaction is comparable to the ion-pair interaction in the sense that the partial charge of a dipole either attracts or repels a neighbouring dipole. Since the amide bond in the protein has a relatively large dipole moment, it is expected that the backbone of a protein is largely stabilized through dipolar interactions with the amide bonds. As shown in Figure 1, also other dipolar groups can give rise to an interaction which stabilizes the conformation of a protein. Since this interaction is electrostatic in nature, the Coulomb energy term, Eq. (3), can be used to calculate the potential energy of this type of interaction. However, in that case often semi-empirically determined partial charges are used to represent the atoms

in the dipole.

When dealing with the above mentioned electrostatic interactions in the interior of a protein the effect of the dielectric medium has to be taken into account. Often, a dielectric constant of the order of $\epsilon = 1\text{-}5$ instead of $\epsilon = 80$ for an aqueous medium is taken when the potential energy term of Eq. (3) is evaluated. This assumption is acceptable when the watermolecules are excluded from the interior of the protein.

hydrogen bonding

Due to the large dipole of e.g. an amide or a hydroxyl group on the surface of a protein their attraction can be rather large and can even overcome the electrostatic repulsion. Hydrogen bond formation is therefore often considered as a polar interaction. Although it is recognized that a sophisticated quantum mechanical description is required for hydrogen bond interaction to explain all observed phenomena, often a Coulomb energy term with partial charges is used to describe the energy contribution of the hydrogen bond. Since hydrogen bond formation in the interior of proteins can induce more significant enthalpy and entropy changes compared to an aqueous medium, these bonds play an important role in the stabilization of proteins in the form of e.g. both β-sheets and α-helices (6).

When a hydrogen bond between two dipoles is formed in principle no water is found at this site. However, since water is a dipole, it can also form hydrogen bonds and thus in principle this molecule will compete with the hydrogen bonding in proteins. Upon hydrogen bond formation in proteins the subsequent changes in enthalpy and entropy are negative (9,10).

Hydrogen bonds can be clearly identified using proton NMR spectroscopy (11). Also shifts in IR and Raman spectra can indicate hydrogen bond formation. Macroscopic thermodynamic parameters can also point to the existence of this type of interaction. For example, the melting and boiling point of molecules depends to a large extent on their ability to form hydrogen bonds.

hydrophobic interactions

As far as hydrophobic interactions are concerned many different theories have been porposed in literature (12). Structuring of water, entropic effects, solvation of non-polar groups and Van der Waals forces are amongst the list of explanations

given for the hydrophobic effect. The explanation we prefer to use in this article is based on the following: at so-called 'hydrophobic' sites of a protein molecule no polar or charged groups can be found. Since the interaction of polar water molecules with non-polar groups is less favourable than with polar ones, the water molecules tend to detach themselves from non-polar sites and associate with themselves. Formation of hydrogen bonds in the bulk water can accompany this change leading to a restructuring of water in the vicinity of such a hydrophobic site. In addition this non-polar site will interact favourably with another non-polar site predominantly through Van der Waals attraction, since the electrostatic and dipolar forces between non-polar sites are small, although the contribution of this effect to the overall interaction will be small. For a schematic drawing of this situation see Figure 2.
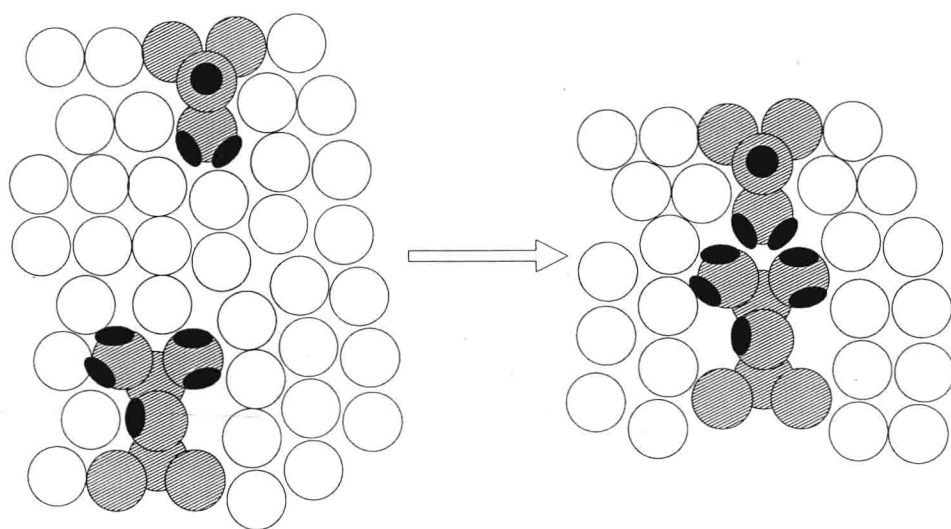
Figure 2. Schematic presentation of the formation of hydrophobic bonds between isolated side-chains. water molecules are shown schematically.

Since both the water molecules and the non-polar sites will rearrange to a more structured organization, an increase in entropy of the whole system may be expected. In addition, the change in enthalpy of formation of hydrophobic "bonds" will be positive. The importance of water molecules in the explanation of

hydrophobic associations of proteins has also been stressed by others (*13,14*). Analysis of the thermodynamic parameters may indicate whether hydrophobic interactions play a role in stabilizing a protein. Also some experimental techniques for the determination of the degree of surface hydrophobicity of proteins are proposed in literature. Fluorescence spectroscopy, for example, can be used for this purpose, i.e. fluorescent dyes exist which react specifically with 'hydrophobic' (non-polar) amino acids on the surface of a protein and consequently the degree of fluorescence can be taken as a measure for the degree of the overall hydrophobicity of a protein (*15*). It should be noted, however, that different dyes colour different amino acids and that possibly also internal hydrophobic residues within the protein can react with the dye, especially when dealing with open structures. Other attempts to measure hydrophobicity of proteins have been tried. Some of these are: *i*) reverse phase chromatography (*16*), *ii*) binding of hydrocarbons to proteins (*17*) and *iii*) contact angle measurements (*18*).

2.3   Factors specifically affecting molecular interactions

Apart from the typical molecular interactions between protein moelcules discussed in the last section, also other factors contribute to the overall process of protein-protein interactions. For sake of clarity, it is appropriate to deal with these factors separately, since in practice they are directly responsible for the changes encountered when the environmental conditions are altered.

temperature
Thermal or Brownian motion are very much dependent on the temperature of the system as the name indicates. An increase in temperature leads to an increase in mobility of molecules. Since the corresponding re-arrangement of (parts of) a molecule and the water molecules determines the overall entropy change expected for hydrophobic interactions, it is not surprising that this interaction is temperature dependent. At low temperatures (between 0°C and 50°C) the entropy term is dominant. By increasing the temperature an increase in hydrophobic association will be noticed. At higher temperatures the gain in entropy becomes smaller compared to the changes in enthalpy and the hydrophobic interaction will decrease again (see also *13,14,19*).

For hydrogen bonding within a protein a decrease in interaction is observed when the temperature is raised (*20*). A possible explanation for this effect can be found in a higher rate of opening of hydrogen bonds with the increase in temperature. As a consequence water molecules can more easily interfere with this bond through the formation of a hydrogen bond with the protein. This explanation is supported by the explanation used for the observed melting of the DNA double helix upon heating. Possibly the melting of the collagen triple helix can also be explained in this way.

solvent

The solvent, through its polarizability, also has a major influence on the interaction forces indicated above. The dielectric constant and the dipole moment, occasionally in combination indicated as the polarizability, mainly affect the interaction between polar and charged groups. Ion-pair interactions, for example, will be strengthened upon lowering the dielectric constant of the solvent. Changing the dipole moment of the solvent also has a clear influence on the hydrogen bond formation in proteins. This can be seen in Table 1, where a decrease in the formation of hydrogen bonds is shown upon an increase in dipole moment of the solvent.

Table 1. Thermodynamics of amide hydrogen bond formation by N-ethyl acetamide at 25°C in various solvents.

Solvent	ΔH (kcal/mole)	ΔS (kcal/mole)	ΔG (kcal/mole)
CCl_4	-4.2	-11.0	-0.92
Dioxane	-0.8	-4.0	0.39
H_2O	0.0	-10.0	3.1

pH, ionic strength and salts

Both pH and ionic strength of a solution phase determine the overall charge of a molecule. Electrostatic interactions, therefore, are very much dependent on these parameters. At pHs corresponding to the isoelectric point of proteins the net Van der Waals interaction dominates over the electrostatic interaction and in most cases proteins will precipitate.