EXCLUDED VOLUME AS A DETERMINANT OF PROTEIN STRUCTURE AND STABILITY

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INTRODUCTION

Classical analyses of the structure and stability of proteins in dilute solution (Kauzmann, 1959; Tanford, 1970; Von Hippel and Schleich, 1969) have emphasized the role of electrostatic and hydrophobic interactions as structural and energetic determinants. The purpose of the present work is to demonstrate that in highly volume-occupied media (i.e., media containing a large volume fraction of mobile macromolecular species), excluded volume effects play an energetic role in the determination of the tertiary and quaternary structures of proteins, and in the determination of their stability with respect to denaturation, which may be comparable to or greater than those of electrostatic and hydrophobic interactions.

NONIDEAL CONTRIBUTION TO STANDARD STATE FREE ENERGY CHANGE

For the general equilibrium scheme

$$n_1X_1 + n_2X_2 + \cdots + n_jX_j \rightleftharpoons n_{j+1}X_{j+1} + n_{j+2}X_{j+2} + \cdots + n_NX_N, \tag{1}$$

it may be readily shown that the standard state free energy change as normally defined, which is in fact an ideal standard state free energy change, may be written as the difference between a total standard state free energy change, ΔG°_T, and a nonideal contribution ΔG°_{NI}:

$$\Delta G^0 = \Delta G^0_T - \Delta G^0_{NI}. \tag{2}$$

These free energy terms are defined by

$$\Delta G^0_T = -RT \ln K_c \qquad K_c \equiv \prod_{i=j+1}^{N} c_i^{n_i} \Big/ \prod_{c=1}^{j} c_i^{n_i} \tag{3}$$

$$\Delta G^0_{NI} = RT \ln \Gamma \qquad \Gamma \equiv \prod_{i=1}^{j} \gamma_i^{n_i} \Big/ \prod_{i=j+1}^{N} \gamma_i^{n_i} \tag{4}$$

where c_i is the molar or w/v concentration of the ith species, and γ_i is the activity coefficient, or ratio between the thermodynamic activity and concentration of the ith species. It follows

that

$$K_c = \exp(-\Delta G^0/RT)\Gamma = K_c^0\Gamma, \quad (5)$$

where K_c^0 represents the thermodynamic equilibrium constant in the ideal limit. The studies outlined here are based upon the observation that, because of excluded volume, the activity coefficients of any macromolecular species appearing in equilibrium scheme 1, and hence Γ and K_c, will be functions of the concentrations of all macromolecular species present in the medium, not just those participating in scheme 1.

METHODS OF CALCULATION

It has been shown earlier (Ross and Minton, 1977; Ross et al., 1978; Ross and Minton, 1979) that excluded volume interactions between globular proteins in solutions of moderate ionic strength may be quantitatively described by the approximation that the protein molecules behave as hard particles with no long-range interactions between them. Hard particle models were therefore employed to calculate approximate activity coefficients arising from volume exclusion in protein solutions. Two complementary theoretical methods were employed. The scaled particle theory for mixtures of hard spheres (Lebowitz et al., 1965) as generalized by Gibbons (1969) may be used to calculate approximately the activity coefficient of a convex hard particle in an environment of similarly shaped convex hard particles of arbitrary size. Because this approach does not permit the calculation of activity coefficients in mixtures of dissimilarly shaped particles, a lattice model was devised which enables one to calculate approximately the activity coefficient of any particle which can be represented by a rectangular parallelopiped (i.e., cube, rod of square or rectangular cross-section, square or rectangular plate) in an environment of cubes of arbitrary size.

RESULTS

The effect of excluded volume upon protein conformation, stability, and self-association was studied via model equilibria in which reactants and products are represented by equivalent hard particles. For example, one way of modeling a dimerization reaction is to represent the monomer by a cube of volume v and the dimer by a rectangular parallelopiped of volume $2v$ with edge ratio 1:1:2. The results of these studies are qualitatively summarized as follows: (*a*) As volume occupancy increases, compact quasispherical protein conformations become increasingly energetically favored over extended anisometric conformations of equal volume. (*b*) As volume occupancy increases, the native conformation of a protein becomes more stable relative to any less compact denatured conformation. (*c*) As volume occupany increases, self-association processes are enhanced, particularly those leading to the formation of compact, quasispherical aggregates. (*d*) A given degree of volume occupancy by larger macromolecules has less effect upon the structure and self-association of smaller macromolecules than the same degree of volume occupancy by smaller macromolecules has upon the structure and self-association of larger macromolecules.

The model calculations have indicated that for some types of reactions or structural changes, the excluded volume contribution to the standard state free energy change may amount to several tens of kilocalories per mole at levels of volume occupancy comparable to those found in certain physiological environments. The significance of these results is thus twofold: (*a*) The effect of excluded volume upon conformational or chemical equilibria in protein solutions should be readily observable if the experimental system is properly chosen. (*b*) In the absence of information as to the nature and/or magnitude of excluded volume effects upon a particular protein species, extreme caution is required when attempting to

extrapolate the results of experiments performed in dilute solution to conditions similar to those found in vivo.

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THERMODYNAMICS OF MACROMOLECULAR ASSOCIATION REACTIONS

Analysis of Forces Contributing to Stabilization

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We have been interested in the correlation between thermodynamics and structure regarding the molecular details of interactions involving biological macromolecules. In particular we are concerned with the sources of the enthalpic and entropic contributions to the free energy of protein-ligand and macromolecular associations. The results of our analysis necessitate a reexamination of the emphasis that has been placed upon hydrophobic interactions in attempts to explain these processes.

In Table I we have assembled the thermodynamic parameters, in order of increasing exothermic enthalpy change (calorimetrically determined), for protein association complexes whose structures have been determined (in 6 cases) by x-ray crystallography.

Table II lists the thermodynamic parameters for the binding of NAD and NAD analogs to rabbit muscle lactic dehydrogenase. Similar results are obtained for these same ligands and six other dehydrogenases whose coenzyme binding site has been shown to be closely identical by x-ray crystallography. These data illustrate the effects of charge (NAD^+ vs. NADH), polarizable groups (iodosalicylic acid), and the presence or absence of stacking interactions (NADH vs. ADP-ribose) upon the thermodynamic parameters.

From Tables I and II we note that: (*a*) The values of ΔG° are all negative, favoring association, but no discernible pattern in the magnitude of ΔG° is evident. (*b*) The values of ΔH° and ΔS° range from positive to negative, but in most cases these association processes are enthalpically controlled. (*c*) The values of ΔC_P° are large and negative in all instances,