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 $\Delta G^\circ$ are all negative, favoring association, but no discernible pattern in the magnitude of $\Delta G^\circ$ is evident. (*b*) The values of $\Delta H^\circ$ and $\Delta S^\circ$ range from positive to negative, but in most cases these association processes are enthalpically controlled. (*c*) The values of $\Delta C_P^\circ$ are large and negative in all instances,

TABLE I

THERMODYNAMICS OF PROTEIN ASSOCIATION*

| Association process | $\Delta G^0_u$ | $\Delta H^0$ | $\Delta S^0_u$ | $\Delta C^0_p$ |
|---|---|---|---|---|
| | (*kcal mol*$^{-1}$) | (*kcal mol*$^{-1}$) | (*cal K*$^{-1}$*mol*$^{-1}$) | (*cal K*$^{-1}$*mol*$^{-1}$) |
| Trypsin (bovine) + inhibitor (soybean) | −14.6 | 8.6 | 78 | −440 |
| Deoxyhemoglobin S gelation | −3.4 | 2.0 | 18 | −200 |
| Lysozyme self-association (indefinite) | −3.9 | −6.4 | −8.3 | — |
| Deoxyhemoglobin $2\alpha\beta$ = $\alpha_2\beta_2$ | −16.7 | −29 | −41 | — |
| Glucagon trimerization | −12.1 | −31 | −64 | −430 |
| Hemoglobin + haptoglobin | −11.5 | −33 | −73 | −940 |
| α-Chymotrypsin dimerization | −7.1 | −35 | −95 | — |
| S peptide + S protein (ribonuclease) | −13 | −40 | −90 | −1,100 |

*All thermodynamic parameters expressed per mole of complex formed, except in the indefinite association cases of hemoglobin S and lysozyme, for which the mole refers to the monomeric protein reacted. Unitary entropy and free energy are given for processes of defined stoichiometry. Standard states taken to be 1 M protein, pH at which reaction was measured. All pH's were close to 7 except for trypsin (pH = 5), haptoglobin (pH = 5.5), and glucagon (pH = 10.5). All data for 25° except glucagon, where T = 30°.

ensuring that the enthalpic contribution to $\Delta G^0$ will become more predominant at higher temperature. (*d*) The magnitude of $\Delta H^0$ and $\Delta C^0_p$ for protein-protein association are severalfold larger than those found for the protein-small molecule interactions, reflecting a multiplicity of interactions and large areas of subunit contact in the former case.

From the thermochemistry of small molecules we know the signs and magnitude of the

TABLE II

PROTEIN-LIGAND ASSOCIATION, LACTIC DEHYDROGENASE (RABBIT MUSCLE), 25°, PH = 7.6

| Ligand | $\Delta G^0$ | $\Delta H^0$ | $\Delta S^0$ |
|---|---|---|---|
| | (*kcal mol*$^{-1}$) | (*kcal mol*$^{-1}$) | (*cal K*$^{-1}$*mol*$^{-1}$) |
| $NAD^+$ | −3.8 | −6.3 | −8.3 |
| NADH | −7.4 | −6.9 | +1.7 |
| ADP-ribose | −4.6 | −7.6 | −10.0 |
| Iodosalicylic acid | −3.5 | −21.7 | −61.0 |

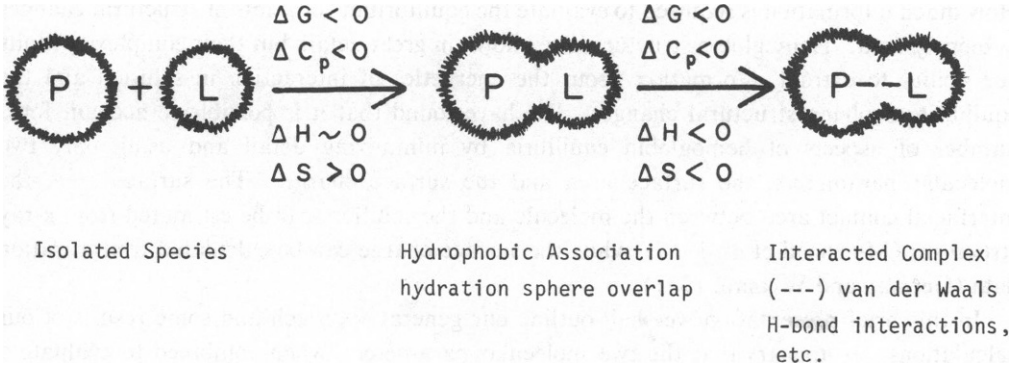
TABLE III

SIGN OF CONTRIBUTIONS TO $\Delta H$ AND $\Delta S$

| Process | $\Delta H$ | $\Delta S$ |
|---|---|---|
| Hydrophobic interaction (burial of nonpolar group) | slightly positive | positive |
| van der Waals' | negative | negative |
| H-bond formation in low dielectric medium | negative | negative |
| Ionic (charge neutralization) | slightly negative | positive |
| Protonation | negative | negative |

thermodynamic parameters for the different types of interactions that occur in these systems (Table III). It may be seen that hydrophobic interactions alone ($\Delta H^0 \sim 0$, $\Delta S^0 > 0$) cannot account for the negative values of $\Delta H^0$ and $\Delta S^0$ observed for the protein association processes in Tables I and II.

For clarity in this discussion, we present a schematic drawing of an association between protein *P*, and species *L*, which may represent either the same protein, another protein, peptide, or ligand molecule. The fuzzy outline surrounding reacting species represents domain of water that is "more ordered" than bulk solvent. Signs of thermodynamic parameters shown for each step:




Hydrophobic interactions (step 1) *prima facie* constitute the driving force for association reactions in aqueous media. However, they may compensate only marginally over the translational and rotational entropy losses incurred in many macromolecular association reactions. The major sources of contribution to the observed negative free energies of predominantly enthalpy-controlled macromolecular association phenomena are the following: (*a*) the strengthening of hydrogen bonds in the low dielectric macromolecular interior, (*b*) protonation incidental in the binding reactions, and (*c*) most importantly, van der Waals' interactions coming into play as a direct result of hydrophobic interactions (Table III). These three kinds of interactions (represented by *P--L* in the schematic) all provide large negative enthalpy changes (partially offset by negative entropy changes) and are thus ultimately responsible for the stability of the formed complexes.

Finally, from examination of x-ray crystallographic results we have found interatomic distances and amino acid side chain orientations that would be consistent with nonbonded stacking interactions among aromatic residues and hydrogen bond formation in the apparently nonaqueous contact regions. Detailed examples will be presented.