SOME WAYS TO USE THERMODYNAMIC INFORMATION TO CHARACTERIZE LINKAGE SYSTEMS

Rufus Lumry, *Chemistry Department, University of Minnesota, Minneapolis, Minnesota 55455 U.S.A.*
Carmel Jolicoeur and Ezio Battistel, *Chemistry and Biochemistry Departments, University de Sherbrooke, Sherbrooke, Quebec J1K 2R1, Canada*

In systems of coupled reactions, processes linked to the measured process whether they be free-swinging equilibria such as ionization of protein groups, or vehicles for site to site linkage such as conformation changes, are detectable and made available for quantitative study through compensation relationships between enthalpy and entropy often surprisingly linear. In protein systems thus far studied the most common effector of such behavior is a change of pH. This perturbs a measured protein process with only small net change in chemical potentials of the ionizing groups and the proton buffer species involved since cratic and unitary parts nearly balance. However, enthalpy and entropy changes can be large and will tend toward a linear relationship on pH variation with a proportionality constant near the mean experimental temperature. The ionized and un-ionized states of each acidic or basic group form a two-state system linked to the measured process. Other free-swinging processes such as the change in state populations of water produce similar results but the water process is complicated by the fact that the standard chemical potentials (unitary parts) of the macro states of bulk water are equal near 25°C, the usual mean experimental temperature. Coupled multistate systems such as these are a common source of enthalpy-entropy compensation behavior, but a more fundamental source lies in the second-law requirement that those parts of ΔH and ΔS reserved to accommodate enthalpy and entropy fluctuations between system and surroundings at constant T and thus not able to contribute to ΔG in isothermal, isobaric processes, must cancel each other through the relationship $\Delta H_{\text{compensation}} = T_{\text{mean}}\,\Delta S_{\text{compensation}}$. The remaining parts contribute to ΔG, thus $\Delta G = \Delta H(0) - kT\ln\Delta'$, in which the prime indicates that the terms in the Guggenheim partition function are of the form $\omega_{ij}\exp[-\{\epsilon_f-\epsilon(o)\}/kT_e-p\{v_f-v(o)\}/kT]$. Both kind of compensation behavior appear in hydrophobic hydration in water exemplified in Fig. 1 which describes the solubility of argon as hydrophobic solute in water and in hydrazine. H. Frank (personal communication) has suggested that hydrazine is a very useful "nonaqueous water" in the sense that it has values for those parts of $\Delta H°$ and $\Delta S°$ contributing to the free energy of solvation nearly identical to those of water but it lacks the multi-state behavior of water responsible in the latter for between-states ("relaxation") effects and it also has relatively small within-states enthalpy and entropy fluctuations, that is low intrinsic heat capacities. Thus to what appears now to be an excellent first approximation the difference between $\Delta H°$ or $\Delta S°$ for solubility of argon in hydrazine and water is a measure of the fluctuation behavior of water appearing thermodynamically in both the between-states and the within-state contributions to the standard heat-capacity of solution or transfer of a hydrophobic solute from a favorable solvent to water. The two solvents are equivalent insofar as they manifest the characteristic unfavorable free energy of solution of hydrophobic solutes. Frank and Lumry (1) have previously shown with this reasoning that hydrophobic hydration is poor because of a positive $\Delta H°(0)$ of solvation; $k\ln\Delta'°$ is in fact negligible. The experimental totals of $\Delta H°$ and $\Delta S°$ are thus misleading

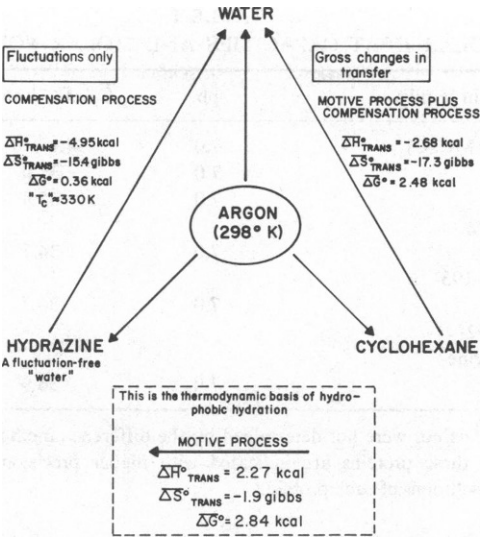

Figure 1 Mystery of hydrophobic hydration unveiled. Solubility of argon in representative solvents. From the free-energy point of view, the poor solubility of argon in water is entirely normal, i.e., it is due to an unfavorable enthalpy of solvation. (1) Data from references 2, 3, and 4.

because the large negative enthalpy and entropy changes ultimately due to entropy fluctuations make only small contributions to $\Delta G°$.

In proteins and proteins systems free energy introduced, for example by ligand binding, is stored, transmitted, and generally redistributed by linkage processes which may be of several types. These processes form the machinery which makes function possible. Depending on the size of the free energies coupling them to the processes they link, on their own standard enthalpy and entropy changes and thus on their intrinsic transition temperatures, these linkage processes are reflected in the two parts of the measured $\Delta H°$ and $\Delta S°$ values and, of course, in different ways depending on which of the more obvious processes of the protein is measured. If the measurement is carried out at some temperature distant from the intrinsic transition temperature, the area enclosed by the enthalpy distribution function, that is the total heat, may be large as it will be if a linkage process involves large changes in H and S of the conformation or ionizing groups. The distribution width which is measured to the first order by the heat capacity may not be unusually wide. In such cases the linkage process is "off resonance" so the between-states contribution to the standard heat capacity is small. On the other hand when the experimental temperature is near a transition temperature, the enthalpy distribution function will tend toward two or more peaked regions which may be well separated. In such cases the between-states part of the heat capacity will be large. To provide a quantitative basis of thermodynamic information with which hypotheses of molecular mechanism can be developed and tested for consistency it is necessary to separate the measured $\Delta H°$ into its two parts and it is necessary to measure $\Delta C_p°$. The former task is not possible in a rigorous way for complex systems so methods, such as the use of hydrazine in analysing hydrophobic hydration, must be developed to give reliable estimates of the separation, a task which is only now beginning to be explored. The measurement of $\Delta C_p°$ to quite high precision, now a straight-forward undertaking, is available to measure the width of the enthalpy distribution function so as to determine whether or not the linkage processes are near resonance or well removed. The variations of C_p and $\Delta C_p°$ with changes in the

TABLE I
MOLAR HEAT CAPACITIES AND MOLAR VOLUMES

Chymotrypsin family	ph	$\overline{C}_p$(κJK^{-1}mol^{-1})	$\overline{C}_p/\overline{V}$(JK^{-1}cm^{-3})
α-Chymotrypsin* +0.01MCaCl$_2$	7.0	34.1 ± 0.1	1.903 ± 0.001
α-Chmotrypsin*	7.0	35.0	1.953
Chymotrypsinogen A*	7.0	35.8	1.949
Methionine sulfoxide-192-α-chymotrypsin*	7.0	34.7	1.948
Methane sulfonyl serine-195-α-chymotrypsin*	7.0	34.7	1.906
Methionine sulfoxide-192–methane sulphonyl serine-195-α-chymotrypsin*	7.0	36.9	2.023

*25°; pH 7. Since $\overline{C}_p$ and $\overline{V}$ values were not determined by the difference method, concentration errors are large: ~0.4%. Differences among these proteins are indicated with higher precision in the ratios $\overline{C}_p/\overline{V}$ determined simultaneously on the same solutions of each protein.[1]

independent variables carried out systemically can provide considerable information not only about the linkage processes but also about the entire linkage system especially when models for some part processes are available, e.g., model heme complexes for ligand binding by heme proteins.

The apparent molar heat capacities and molar volumes of representative members of the chymotrypsinogen A family of proteins[1] are given in Table I. The pH dependence is small and similar for these proteins except at pH values above 8 where large effects due to the active-to-inactive transition are found with all but chymotrypsinogen A itself.

The pH dependence of the apparent molar heat capacity and molar volume of fully

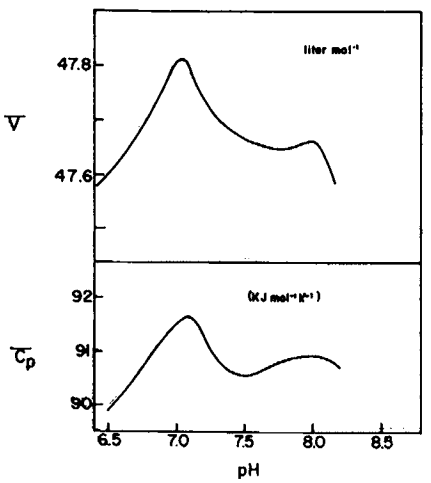

Figure 2 Dependence of apparent molar volume and molar heat capacity of fully oxygenated hemoglobin A on pH at 25°C.[2] Absolute scale errors are ±0.1%. Preliminary data for unliganded hemoglobin show it to have a molar volume 100 ml mol^{-1} higher than Hb(O$_2$)$_4$ and a molar heat capacity higher by 1,000 J mol^{-1}K^{-1} at pH 7.05 but there is no heat-capacity spike.

[1]Jolicoeur, C., R. Lumry, J. Boileau, P.-A. Leduc, and E. DeMedicis. Manuscript submitted for publication.

oxygenated hemoglobin A[2] is shown in Fig. 2. In contrast to the large relaxation heat capacity of this Hb form deoxygenated hemoglobin A and $Hb(CO)_4$ pH dependencies give no evidence for significant relaxation contributions. With $Hb(O_2)_4$ maximum at pH 7.05 in the molar volume, a first-derivative of free energy, correlates well with the relaxation peak of the molar heat capacity, a second derivative of free energy, to establish a resonance-relaxation process (between-states fluctuations) at 25°C. The existence of a coupled process of this sort has been suggested by numerous reports of enthalpy-entropy compensation behavior in a variety of hemoglobin processes. Quantitative comparisons with published data must be deferred to a longer publication.

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