Table II. Chromium(II) Reduction of Pentaamminecobalt(III) Complexes

| Complex | $k$, $M^{-1}$ sec.$^{-1}$ | Temp., °C. | $\Delta H^*$, kcal. mole$^{-1}$ | $\Delta S^*$, e.u. | Ref. |
|---|---|---|---|---|---|
| Sulfato[a] | 10.7 | 9 | 8.3 | −25 | *b* |
| | 17 | 19 | | | |
| | 31.6 | 30 | | | |
| | 18 | 25 | | | *c* |
| Sulfito | 10.3 | 15 | 8.3 | −26 | *d* |
| | 18.6 | 25 | | | |
| | 28.6 | 35 | | | |
| Thiosulfato | | | | | |
| "fast" | 7.7 | 5 | 4.2 | −39 | *d* |
| | 9.1 | 12 | | | |
| | 10.8 | 18 | | | |
| | 13.3 | 25 | | | |
| "slow" | 0.18 | 25 | 24.6 | 3 | *d* |

[a] Concentrations used: $[Co(III)L]_0$ = 4–6 × $10^{-3}$ $M$, $[Cr(II)]_0$ = 5–9.5 × $10^{-2}$ $M$, $[H^+]$ = 0.2–0.6 $M$. [b] R. T. M. Fraser, *Inorg. Chem.*, **2**, 954 (1963). [c] J. P. Candlin, J. Halpern, and D. L. Trimm, *J. Am. Chem. Soc.*, **86**, 1019 (1964). [d] This work.

ion,[6] a complex which contains a divalent sulfur in the ligand.

Thus, while it appears that both isomers of the thiosulfatopentaamminecobalt(III) ion are stable, the usual methods of preparation yield mainly the oxygen bonded species.

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(6) E. S. Gould and H. Taube, *J. Am. Chem. Soc.*, **86**, 1318 (1964).
(7) Alfred P. Sloan Foundation Research Fellow.

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## The Nature of the Complexities in the Ribonuclease Conformational Transition and the Implications Regarding Clathrating

*Sir:*

It has been suggested on at least four occasions[1-4] that the thermal conformational transition of ribonuclease may be complicated due to the existence of thermodynamically stable conformational states intermediate between the low temperature native form and the high temperature denatured form. The primary thermodynamic evidence bearing on this conclusion is the existence of a definite asymmetry in the transition curves such that the van't Hoff plots, based on the assumption of an equilibrium between only two states, are extremely nonlinear. Finally it was shown[2] that "it is now possible to resolve each of the transition curves into two symmetrical transitions which have linear van't Hoff plots," and this formed the basis for the conclusion that the thermal transition involves the independent unfolding of two distinct regions of the ribonuclease molecule.

It is the purpose of this paper to point out that the thermodynamic complexities apparent in the ribonuclease transition may not result from the existence of intermediate conformational states. It has been shown[5,6] that transfer of hydrophobic side chains from protein interior in the native state to a predominantly aqueous environment in the denatured state will give rise to an extremely large and temperature-dependent $\Delta C_p$ term which will lead to marked curvature of van't Hoff plots even for a simple two-state conformational transition. This heat capacity term in all likelihood arises from the melting of clathrate structures about the exposed hydrophobic groups in the denatured state. Therefore, even in the absence of complexities introduced by intermediate conformational states, protein denaturation reactions must be thought of as consisting of *two independent order–disorder transitions:* one concerned with the normal unfolding of the polypeptide chain itself and the other resulting from a solvent transition associated with the accommodation of nonpolar side chains in the denatured state.

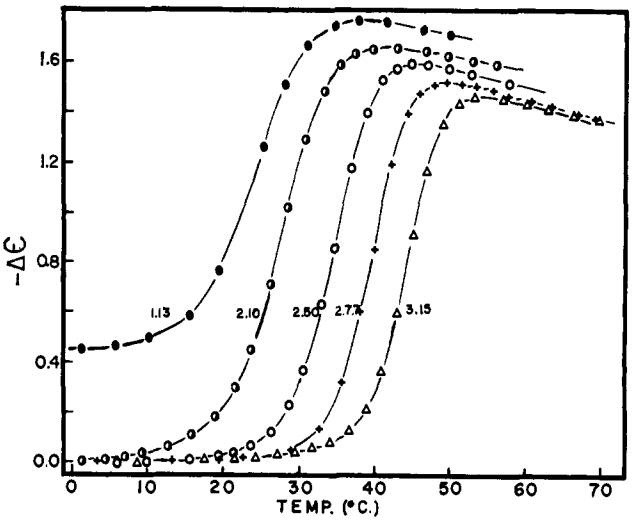


Figure 1. The changes in extinction coefficient at 287 mμ for ribonuclease A at five pH values. The samples at pH 2.77 and 3.15 were buffered with 0.04 $M$ glycine. The other samples contain only HCl. Protein concentrations are 0.03–0.04 g./100 ml.

To test this idea, we have made very careful spectrophotometric measurements on the ribonuclease transition under conditions where reversibility is complete. Our results are shown in Figure 1. These data have been analyzed assuming a simple two-state transition, *i.e.*

$$N \rightleftharpoons D$$
(native) (denatured)

In keeping with the above discussion, the free-energy difference between the two conformational states can be expressed as a power series involving four terms to specify enthalpy, entropy, and the temperature-dependent heat capacity contribution arising from clathrate melting, *i.e.*

$$\Delta F^\circ = E + FT + GT^2 - 0.00155GT^3 \qquad (1)$$

where $E$, $F$, and $G$ are temperature-independent parameters. The ratio of the coefficients of the squared and cubed terms (*i.e.*, −0.00155) has been estimated *a priori* from the amino acid composition of ribonuclease and appropriate model compound data in the same manner as previously done for chymotrypsinogen.[6]

(1) K. Beck, S. J. Gill, and M. Downing, *J. Am. Chem. Soc.*, **87**, 901 (1965).
(2) R. A. Scott and H. A. Scheraga, *ibid.*, **85**, 3866 (1963).
(3) D. N. Holcomb and K. E. Van Holde, *J. Phys. Chem.*, **66**, 1999 (1962).
(4) C. Tanford, *J. Am. Chem. Soc.*, **86**, 2050 (1964).
(5) J. F. Brandts, *ibid.*, **86**, 4291 (1964).
(6) J. F. Brandts, *ibid.*, **86**, 4302 (1964).

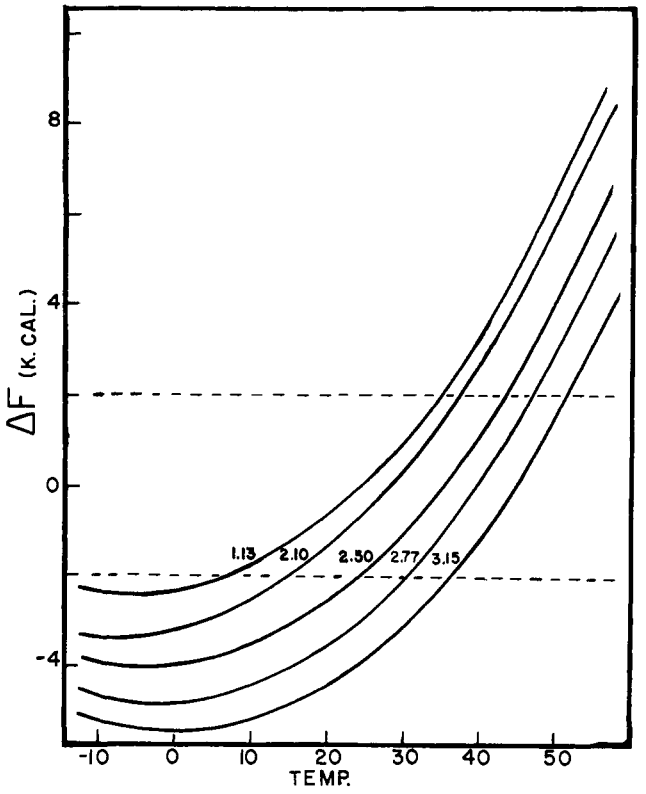


Figure 2. Free energy profiles for the ribonuclease transition assuming an equilibrium between two conformational states. These continuous curves are derived from the experimental data of Figure 1 at each pH (see text).

The best values of the three parameters at each pH were obtained by fitting eq. 1 to the experimental data by least-squares treatment. The average deviation of the experimental values of $\Delta F°$ from the calculated values using the best parameters corresponds to spectrophotometric errors of less than $2 \times 10^{-4}$ optical density unit. Thus, the data of Figure 1 are completely consistent with the two-state transition as outlined above. The smooth curves in Figure 2 are the free-energy values obtained from eq. 1 at each pH. Accurate values of $\Delta F°$ can be measured directly only within the limits of $+2000$ to $-2000$ cal. as indicated by the dashed lines. The extension of the curves outside this range is based on the assumption that the $E$, $F$, and $G$ parameters are temperature independent. The qualitative features of these stability curves for ribonuclease are seen to be extremely similar to those for chymotrypsinogen.[5]

The $\Delta C_p$ values in Table I are nearly independent of pH but strongly dependent on temperature. On a weight basis, these values of $\Delta C_p$ are within 10% of the values determined for the serum albumin[7] and the ferrihemoglobin[8] transitions by direct calorimetric measurements, and this provides strong support for the validity of the two-state analysis.

In conclusion, we wish to suggest that the ribonuclease transition is consistent with the assumption of an equilibrium between only two conformational states. The apparent complexity in the thermodynamics, which had previously been interpreted as an indication of intermediate conformational states, is predictable from model compound data and likely results from a structural transition in the solvent phase as the temperature is increased. The thermodynamics associated with this clathrate transition are superimposed upon the thermodynamics of the normal polypeptide unfolding transition and are most clearly manifested in the large positive $\Delta C_p$. It is anticipated that the essential features of the ribonuclease transition will be found to be characteristic of most reversible conformational transitions when examined closely.

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(8) W. W. Forrest and J. M. Sturtevant, *ibid.*, **82**, 585 (1960).

Table I. Values of the Enthalpy, Entropy, and Heat Capacity Changes for the Ribonuclease A Transition Assuming a Two-State Reaction

| | 0° | | | 60° | | |
|---|---|---|---|---|---|---|
| pH | $\Delta H°$, kcal. | $\Delta S°$, e.u. | $\Delta C_p$, cal. | $\Delta H°$, kcal. | $\Delta S°$, e.u. | $\Delta C_p$, cal. |
| 1.13 | 11 | 32 | 1230 | 137 | 442 | 3050 |
| 2.10 | 15 | 44 | 1175 | 135 | 436 | 2920 |
| 2.50 | 10 | 23 | 1180 | 131 | 416 | 2925 |
| 2.77 | 8 | 12 | 1210 | 132 | 416 | 3005 |
| 3.15 | 6 | 1 | 1180 | 126 | 395 | 2930 |

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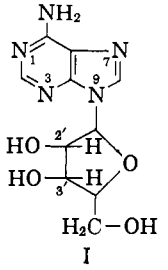
## Proton Ionization from Adenosine

*Sir:*

Adenosine (I) is known to have at least one acidic site ($pK \sim 12.5$) in aqueous solution.[1] This would be




I

expected to be a reactive site and would likely be involved in hydrogen bonding. Zamecnik has recently[2] postulated an intramolecular hydrogen bond between the C-2′ and N-3 positions of adenosine to explain the biosynthesis of proteins. Broom and Robins[3] have shown that in a homogeneous solution of water and 1,2-dimethoxyethane methylation of adenosine by diazomethane occurs at the 2′-position, suggesting this as the acidic site. However, McLaughlin and Ingram[4] and Wolfenden, *et al.*,[5] have shown that a rapid equilibration exists between the 2′- and 3′-aminoacyl isomers in neutral and basic solution, suggesting a close similarity of the 2′- and 3′-positions.

The purpose of this communication is to report a

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(2) P. C. Zamecnik, *Biochem. J.*, **85**, 257 (1962).
(3) A. D. Broom and R. K. Robins, *J. Am. Chem. Soc.*, **87**, 1145 (1965).
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