centered at 282 and 289 mμ. Since they differ in relative magnitude in the two oxidative states, it may be assumed that they have different origins. Having found a 289 mμ absorption peak in these samples, we assume this and its attendant Cotton effect are due to the single tryptophan residue. Similarly the 282 mμ feature would be due to one or more of the four tyrosyl residues. The disparate behavior of the 282 and 289 mμ Cotton effects provides two additional parameters which, with the known sequence of cytochrome *c*,[13] should be useful in further detailing the conformations of this protein. Additional features of the ferrocytochrome *c* curve which may be noted are the multiple Cotton effects arising from the α- and β-bands. These bear striking resemblance to those reported using magnetic optical rotatory dispersion.[5]

Indeed the richness of the detail in the ORD curves for this material invites systematic studies of the influence of a number of variables on regions of the curves that are selectively responsive to helical content, heme environment, and aromatic residue environment.

Of course, the conformation change herein reported can only be functional in the mechanism of electron transport if its rate is at least as fast as that of electron transfer. Moreover, such an alteration in the protein may also have relevance to the "mechanochemical"[14] properties of mitochondrial membranes.

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(13) E. Margoliash, E. L. Smith, G. Kreil, and H. Tuppy, *Nature*, **192**, 1125 (1961).
(14) A. L. Lehninger and C. L. Wadkins, *Ann. Rev. Biochem.*, **31**, 47 (1962).

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Bonding in the Thiosulfatopentaamminecobalt(III) Complex

Sir:

We wish to report the results of some electron-transfer reactions and their relation to the structure of the complex ion, thiosulfatopentaamminecobalt(III).

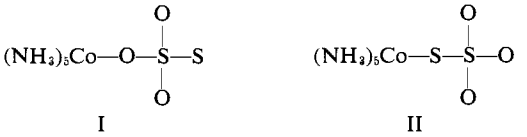
Thiosulfatopentaamminecobalt(III) chloride was first prepared by Ray,[1] who assigned to it the structure I, largely on the basis of its purple-red color, which is considered to be characteristic of a cobalt–oxygen bond.[2] However, the infrared spectrum of the complex has recently been compared with those of the thiosulfate and S-methyl thiosulfate ions, and on the basis of this comparison structure II has been assigned.[3]

We have re-examined the absorption spectrum of $(NH_3)_5CoS_2O_3^+$ as a mull and as a potassium bromide pellet of the chloride, using a Perkin-Elmer 421 infrared spectrophotometer equipped with a 2000- to 200-cm.$^{-1}$ grating interchange. Associated with the absorptions at 424 and 1000 cm.$^{-1}$, we find shoulders, not previously recorded in the literature (Table I). Furthermore, both of these shoulders are displaced from the main peaks in directions toward the corresponding absorptions in the spectra of the S-alkyl thiosulfates; that is, they are displaced toward the frequencies expected for an ion containing a bond between the metal center and the outer sulfur of the thiosulfate group. The magnitude of the shifts indicates that this bond in the cobalt(III) complex is half-ionic. The ν_e (S–O) splitting at 1168 and 1138 cm.$^{-1}$ previously observed was not explained satisfactorily in terms of structure II. If, instead, we attribute structure I to the predominant isomer, the splitting can be explained in terms of the nonequivalence of the S–O bonds. The percentage of isomer I can be obtained from the ratios of the relative integrated band intensities (assuming a Lorentzian shape for the absorptions[4]). The value obtained from the 424–414-cm.$^{-1}$ pair is 89 ± 3% and from the 997–1010-cm.$^{-1}$ pair the percentage is 92 ± 4.

Table I. Infrared Absorptions of Thiosulfate

Ion	ν (S–S), cm.$^{-1}$	ν_s (S–O), cm.$^{-1}$	ν_e (S–O), cm.$^{-1}$	Ref.
$S_2O_3^{2-}$	451	1002	1125	*a*
	...	997	1125	*b*
$CH_3SSO_3^-$	410–412	1026–1032	1203–1215	*c*
$(NH_3)_5CoS_2O_3^+$	424	997	1168	*a*
	...	1000	1138	*d*
	415 sh	997	1167	*b*
	424	1010 sh	1137	*b*

[a] Reference 3. [b] This work. [c] A. Simon and D. Kunath, *Chem. Ber.*, **94**, 1980 (1961). [d] E. P. Bertin, R. B. Penland, S. Mizushima, C. Curran, and J. V. Quagliano, *J. Am. Chem. Soc.*, **81**, 3818 (1959).



I II

An examination of the chromium(II) reduction of thiosulfatopentaamminecobalt(III) perchlorate (prepared from the chloride by precipitation with sodium perchlorate) shows that there are two different Co(III) complexes. One of these ("fast") is reduced 70 times more rapidly than the other ("slow"): the specific rates are listed in Table II, together with some rate constants for the reaction

$$(NH_3)_5CoX^+ + Cr^{2+} + 5H^+ \rightarrow 5NH_4^+ + Co^{2+} + CrX^+$$

$X = SO_4$, SO_3, or HO_2C–C$_6$H$_4$–S–C$_6$H$_4$–CO_2

Extrapolation of the rate plots to zero time shows that the fast isomer comprises 90 ± 1% of the mixture. Experiments using cation-exchange resins[5] and 0.15 *M* perchloric acid show that 99% of the complex ion carries a charge of +1; there is no evidence for dimer formation, so that the "fast" component can be identified with structure I. In this connection, it is interesting to note that the specific rate for its reduction with chromium(II) ion is comparable to those observed for the sulfato and sulfito complexes, while the reduction of the "slow" isomer is as slow as that of the 4-carboxythiodibenzene-4′-carboxylatopentaamminecobalt(III)

(1) P. R. Ray, *J. Indian Chem. Soc.*, **4**, 64 (1927).
(2) J. C. Bailar and D. H. Busch, Ed., "The Chemistry of Coordination Compounds," A.C.S. Monograph Series, No. 131, Reinhold Publishing Corp., New York, N. Y., 1956, p. 57.
(3) A. V. Babaeva, I. B. Baranovskii, and Yu. Ya. Kharitonov, *Russ. J. Inorg. Chem.*, **8**, 307 (1963).
(4) D. A. Ramsay, *J. Am. Chem. Soc.*, **74**, 72 (1952).
(5) E. L. King and E. B. Dismukes, *ibid.*, **74**, 1674 (1952).

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

Complex	k, M^{-1} sec.$^{-1}$	Temp., °C.	ΔH^*, kcal. mole^{-1}	ΔS^*, 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: $[Co(III)L]_0 = 4\text{–}6 \times 10^{-3}$ *M*, $[Cr(II)]_0 = 5\text{–}9.5 \times 10^{-2}$ *M*, $[H^+] = 0.2\text{–}0.6$ *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 ΔC_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.

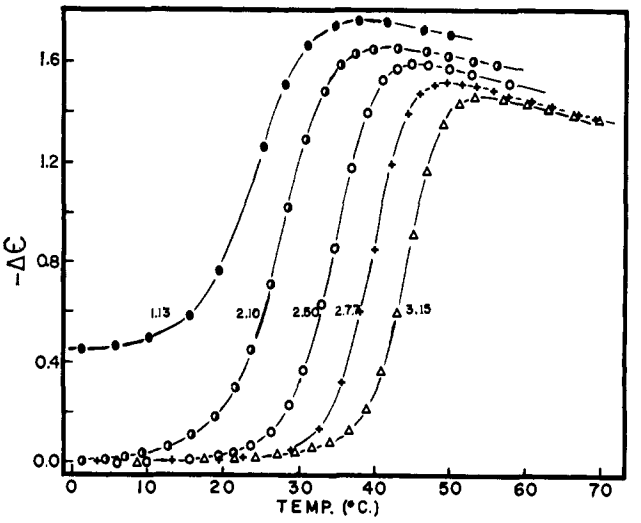

Figure 1. The changes in extinction coefficient at 287 mμ for ribonuclease A at five pH values. The samples at pH 2.77 and 3.15 were buffered with 0.04 *M* glycine. The other samples contain only HCl. Protein concentrations are 0.03–0.04 g./100 ml.

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.*

$$\underset{\text{(native)}}{N} \rightleftharpoons \underset{\text{(denatured)}}{D}$$

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).