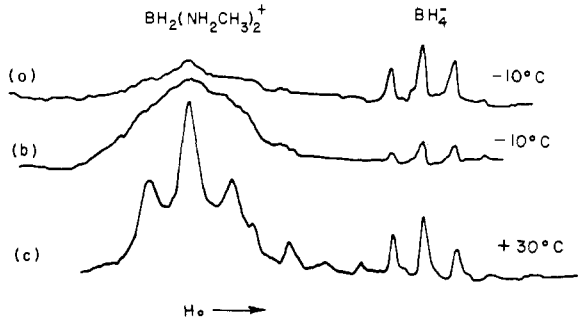


Figure 2. Effect of radiofrequency power temperature on $B^{11}$ n.m.r. spectra of cleavage products whose normal spectra are shown in Figure 1a: (a) radiofrequency power increased to produce noticeable saturation of $BH_4^-$: (b) additional increase in radiofrequency power; (c) increased temperature at the same radiofrequency power as (a).

high radiofrequency power, sufficient to produce significant attenuation (saturation) of the $BH_4^-$ signal, was the cation signal detectable, but as a broad band rather than the expected triplet (Figure 2a,b). However, at ambient temperature, a well-defined triplet was observed. Figure 2c is the exaggerated spectrum at high radiofrequency power and Figure 1a is the normal spectrum. The temperature dependence of the $H_2B(NH_2CH_3)_2^+$ spectrum indicates that nuclear quadrupole spin-lattice relaxation[9] is responsible for the absence of a detectable triplet at low temperature. This appears to be the first such example which has been identified for $B^{11}$. However, unsymmetrical cleavage of $B_2H_6$ by $(CH_3)_2SO$[10] and $B_4H_{10}$ by tetrahydrofuran[11] have been reported recently, and the characteristic triplets of the cations were not detected in the $B^{11}$ n.m.r. spectra. Conceivably, quadrupole relaxation occurs in these systems also.

Discrepancies in reported[3,4,7,12] properties of $H_3BNH_2CH_3$ and $H_3BNH(CH_3)_2$ are resolved by the present investigation. Authentic samples[7] of these compounds are crystalline solids at room temperature which show no tendency to decompose *in vacuo*. On the other hand, the products of direct addition of diborane to amine, unsymmetrical cleavage products, which are reported as symmetrical cleavage products in investigations prior to this one,[3,4] are liquids at room temperature which evolve hydrogen *in vacuo*. The only evidence for symmetrical cleavage in the products of direct reaction in earlier work was based upon molecular weight studies of the $CH_3NH_2$ and $(CH_3)_2NH$ adducts in liquid ammonia by vapor pressure depression.[4] We have found, however, that the molecular weight of the product of direct reaction of $B_2H_6$ with $CH_3NH_2$ by cryoscopy in liquid $CH_3NH_2$ is consistent with the formula of the unsymmetrical cleavage product (theory for $H_2B(NH_2CH_3)_2^+BH_4^-$: 89; found: 100).

Results of this study and other recent investigations[10,11] indicate that unsymmetrical cleavage of the bridge system of diborane by Lewis bases may be more prevalent than previously suspected. One of the factors which determines the course of cleavage is most likely steric, considering the change in type of cleavage observed with progressive methyl substitution on nitrogen in the methylamines.[13] However, more subtle factors are probably involved also. Work on new examples of unsymmetrical cleavage and factors which determine the course of cleavage is continuing in this laboratory.

*Acknowledgment.* Support of this work by the National Science Foundation is gratefully acknowledged. S. G. S. thanks The Ohio State University for assigned research duty.

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## On the Conformation of Horse Heart Ferri- and Ferrocytochrome *c*

*Sir:*

Much indirect evidence has accumulated suggesting a difference in the conformations of ferri- and ferrocytochrome *c*, *e.g.* separation on cation-exchange resins,[1] differing susceptibility to digestion by bacterial protinase,[2] and differing crystal forms.[3] This preliminary report on the optical rotatory dispersion (ORD) of cytochrome *c* in the range 195–600 mμ presents relatively detailed information as to the nature of these differences. Earlier work[4,5] was neither of sufficient spectral range nor of the resolution required to make the observations reported here.

Horse heart cytochrome *c* (Sigma Chemical Co. Type III) in 0.05 *M* phosphate at pH 7 was treated with β-diphosphopyridine nucleotide and phenazine methosulfate[6] or sodium dithionite (in the absence of oxygen) to effect reduction or with potassium ferricyanide to complete oxidation. The mixture was then passed through a Sephadex G-75 column to free the protein of reductant or oxidant and their products and to be assured of the monomeric form.[7] The ORD curves of each sample, initially free of oxidant or reductant, were determined on a Cary Model 60 spectropolarimeter. Solid dithionite was then added to the oxidized protein or crystalline potassium ferricyanide to the reduced protein and the curves were rerun so that the same molecules were studied in both states without a significant change in concentration. This procedure also allowed comparison of the curves in the absence of oxidant or reductant. The concentration of each sample was spectrophotometrically determined in both states of oxidation on a Cary Model 14 spectrophotometer under conditions of sufficiently narrow spectral band widths. Using the extinction coefficients of Van Gelder and Slater,[6] the calculated concentrations of the same sample in both

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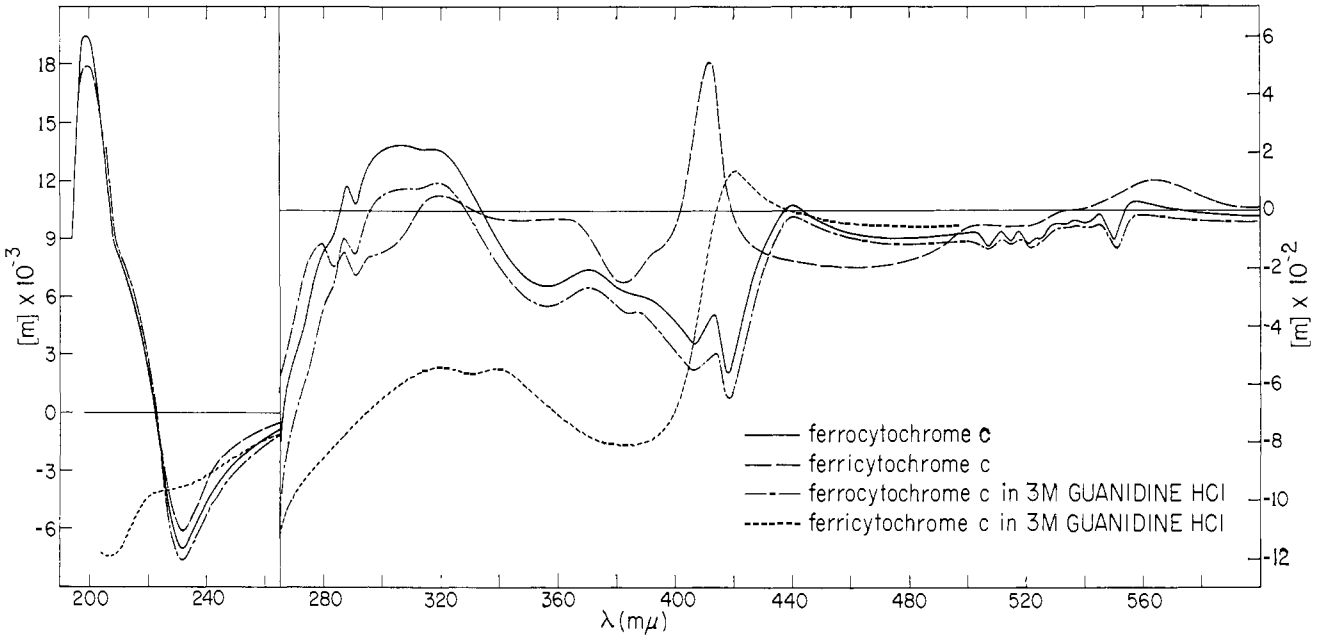


Figure 1. Optical rotatory dispersion curves for ferri- and ferrocytochrome *c*. Data are not corrected for index of refraction of the medium.

states of oxidation agreed to better than ±2%. For determinations in *3 M* guanidine hydrochloride at pH 7.2, $8 \times 10^{-4}$ *M* ferricytochrome *c* was placed in a 0.1-mm. cell. The dispersion was recorded; dithionite was then added and the curve rerun.

The dispersion results for ferri- and ferrocytochrome *c* in the absence and presence of guanidine hydrochloride are displayed in Figure 1. A most interesting difference is that the magnitudes of the extreme characteristic of helical proteins, the trough in the vicinity of 233 mμ and the peak near 198 mμ, are greater in the reduced forms. In 3 *M* guanidine hydrochloride, ferricytochrome *c* gives a characteristically nonhelical or random coil curve, but upon reduction *in the presence of* 3 *M* guanidine hydrochloride there results a curve which contains pronounced helical Cotton effects. Quite unequivocally one can say that there is an increase in helical content upon reduction under these conditions. It should be mentioned that, while the mean residue rotations show the trough to be greatest for ferrocytochrome *c* in the presence of denaturing agent, the difference between this and the absence of guanidine hydrochloride is essentially removed when refractive index corrections are made.

Although all procedures for estimating helical content become less accurate with diminishing amounts of helix the present case is probably best estimated by applying the relation[8]

$$[m']_\lambda^{\text{obsd}} = [m']_\lambda^{\text{D}} + f_{\text{H}}[m']_\lambda^{\text{H-D}}$$

where $[m']_\lambda^{\text{D}}$ and $[m']_\lambda^{\text{H}}$ are taken to be −1800 and −12,700 respectively.[9] From this, values of 27 and 34% are calculated for the helical content of ferri- and ferrocytochrome *c*. The increase in helical content upon reduction in guanidine hydrochloride may be approximated without knowledge of the refractive index of the medium by noting that the rotation at the trough for the reduced protein is twice that of the denatured, oxidized protein. Taking the denatured value to be −1800, the increase is found to be 17%. An alternate estimate using the parameters of Yang and Samejima[10] gives 14%. Such calculations are expected to give low estimates of helical content. *Thus, as there are 104 amino acid residues in this molecule it appears that a minimum of 15 amino acid residues shift into helical conformation when ferricytochrome c is reduced in the presence of 3 M guanidine hydrochloride.* This conclusion increases the confidence with which the 7% change in the phosphate buffer can be interpreted as an increase in helical content upon reduction. More importantly, the qualitatively similar behavior in these two different media suggests that a similar transition may be operative for the lipid bound cytochrome *c* in mitochondria.

Much may also be inferred by considering the anomalous dispersion in the spectral region of the Soret band. Upon reduction in phosphate buffer the complex Cotton effects change markedly in form indicating that the average environment of the heme has been altered. Significant too is the observation that ferricytochrome *c* in guanidine hydrochloride gives a simpler Cotton effect whereas the reduced species yields a dispersion curve of the same form as native ferrocytochrome, though on a slightly more negative background. The oxidized and reduced forms of myoglobin[11] on the other hand have Soret Cotton effects of the same form with only a red shift upon reduction. A reasonable interpretation of these observations is that the reduced protein has a strong requirement for a particular ligand. The work of Hettinger and Harbury[12] indicates that the ligand introduced upon reduction is likely an imidazole of histidine. In this regard it is of interest to note that the third histidine residue is 16 residues from the cysteine which is bound to the heme by a thioether linkage; hence this may be the region undergoing the change.

A further point of interest is the two Cotton effects

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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.

*Acknowledgment.* The authors express appreciation to the National Science Foundation under Grant GB-1328 for financial support of this research and to P. Urnes for many helpful and stimulating discussions.

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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,

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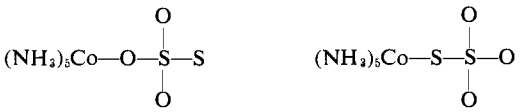
**Table I.** Infrared Absorptions of Thiosulfate

| Ion | ν (S–S), cm.$^{-1}$ | $\nu_s$ (S–O), cm.$^{-1}$ | $\nu_e$ (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).

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 fre-



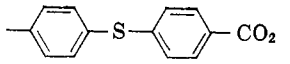
I II

quencies 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 $\nu_e$ (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.

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



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)

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