INTERACTION OF HEME a AND POLYLYSINE

Tsoo E. King, Fook Choy Yong and S. Takemori
Oregon State University, Corvallis

Received February 7, 1966

In cytochrome chemistry, knowledge concerning the particular amino acids of the protein moiety which coordinate with the heme is meager. Indeed, the methodology available is very limited. Recently we have analyzed (1) the spectral behavior, borohydride reaction, and enzymic activity of cytochrome oxidase at pH 11.6. This study coupled with that on the synthetic complexes formed from heme a and polylysine as well as globin described here has led us to suggest that lysine may be possibly the amino acid in the protein which coordinates with heme a in cytochrome oxidase at pH 11.6. The preliminary note reports some circumstantial evidence.

Cytochrome oxidase was prepared from the Keilin-Hartree preparation of beef heart (2). Hematin a was isolated from purified soluble cytochrome oxidase (1). Protohematin and the native globin were obtained from freshly prepared beef hemoglobin (3). The "nativeness" of the isolated globin was ascertained by the reconstitution technique with protohematin (3) regarding the spectral behavior and spectrophotometric titration. One sample of poly-L-histidine with a molecular weight of 8,000, a product of the biophysics department of the Weizmann Institute, was used. Three samples of poly-L-lysine with an average degree of polymerization ($\bar{p}$) of 480, 586, and 1,640 and a molecular weight of 70,000, 75,000, and 210,000, respectively, and other poly-L-amino acids were procured from Mann Research Laboratories. Cytochrome oxidase (4) and tetrachlorohydroquinone oxidase (5) activities were determined polarographically at 23-25° according to the published methods.

In this paper, the terms protohematin and hematin a refer to their oxidized state, and protoheme and heme a to the reduced state. When in general description the oxidation state is not a point of significance, hematin and heme are used interchangeably.

Hematin a interacted with polylysine in alkaline media and the spectrum of the reduced complex showed maxima at 428 and 574 mμ (Fig. 1).

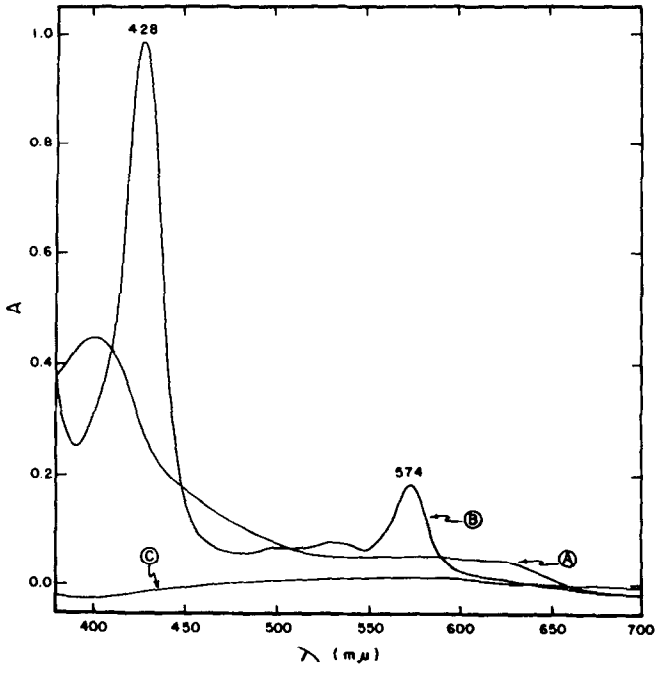

Figure 1. Absolute spectra of the heme a-polylysine complex at pH 11.6. The solution contained 9.2 μM heme a and 6.5 μM polylysine in 10 mM phosphate buffer, pH 11.6, 20°. Curve A, oxidized; Curve B, dithionite-reduced; Curve C, the base line.

Although hematin a also reacted with polylysine in neutral solution the spectra of the resulting complex were completely different from those formed in alkaline pH. The pH profile for the absorbance of the Soret maximum of the reduced complex approximated that for the helicity of polylysine; the absorbance increased rapidly as soon as the pH of the medium exceeded 9.5. Protoheme and polylysine reacted in both neutral and alkaline media; the spectra were, however, different from those of the heme a complex (Table I).

Table I. Comparison of spectral behavior of cytochrome oxidase and heme-complexes at pH 11.6.

Treatments (I), as such, i.e. oxidized; (II) reduced with a few grains of dithionite; (III) the reduced sample was saturated with carbon monoxide; (IV) the complex in solution was treated by borohydride at 0-4° for 3 hours, then reduced by dithionite; and (V), the same as (IV) but further saturated with carbon monoxide. See legends of Table II for further details. All solutions were in 50 mM phosphate buffer except cytochrome oxidase which was in 10 mM phosphate and 0.1% Emasol. A, cytochrome oxidase; B, hematin a-polylysine; C, hematin a-globin; and D, protohematin-polylysine. (*) broad peak.

	α-band maximum				γ-band maximum			
Treatment	A	B	C	D	A	B	C	D
I. None	*	*	*	545	407	403	406	420
II. $Na_2S_2O_4$	574	574	575	558	428	428	428	424
III. $Na_2S_2O_4$ + CO	586	584	586	567	424	424	424	418
IV. Borohydride + $Na_2S_2O_4$	560	558	558	557	422	418	420	421
V. Borohydride + $Na_2S_2O_4$ + CO	565	564	566	563	418	416	416	413

Our spectral values for protohematin-polylysine complex are in agreement with Blauer's data (6); the latter author did not study the reduced form.

Under various conditions, many α-amino acids, poly-L-glutamate, poly-L-proline, poly-L-tyrosine, poly-L-sarcosine and poly-L-histidine did not apparently react or reacted differently with heme a as polylysine did. The details of this aspect will be described elsewhere.

Heme a also interacted with the native globin from bovine hemoglobin. The heme a-globin complex at pH 11.6 exhibited maxima at 428 and 575 mμ; upon the reaction of carbon monoxide, the peaks shifted to 424 and 586 mμ. These values are similar to the figures reported by Morrell et al. (7), who have used denatured globin and heme a at pH 13. A comparison of these artificial complexes and cytochrome oxidase regarding their spectral behavior is summarized in Table I.

When a constant amount of heme a was titrated with varying amounts of polylysine, the absorbance at 428 mμ rapidly increased with the increase of the polypeptide concentration. It was found that on the average of the

samples with different $\bar{p}$, 13 ϵ-amino groups of polylysine were required for 1 mole of heme a at pH 11.6. Titration data derived by a similar technique showed that the stoichiometry was 4.7 moles of hematin a per mole of globin at pH 7.0. This value compared favorably to 4.0 for the protohematin. The titration curves in both systems, polylysine and globin, were clear-cut.

Borohydride treatment of the heme a-polylysine and the heme a-globin complexes changed their spectra (Table I). Like cytochrome oxidase, the heme a was fixed to the protein moiety and became no longer extractable by acid-acetone. This finding was further confirmed by direct chemical analysis for iron. The protoheme-polylysine complex or the complex formed from polylysine and heme a which had been pre-reacted with borohydride, did not share the same property as clearly shown in Table II.

Neither the heme a-polylysine nor the heme a-globin complexes exhibited activity either in the oxidation of reduced cytochrome c or tetrachlorohydroquinone (TCHQ). We recall that pH 11.6-treatment of cytochrome oxidase abolishes its enzymic activity (1). On the other hand, prolonged digestion of cytochrome oxidase by trypsin does not impair the oxidase in TCHQ oxidation (5).

A few salient points, *inter alia*, may be deduced from these observations. (i) Heme a can form a complex with either globin or polylysine. The spectral behavior of the complexes is dependent upon the conformation of the protein. It is a well-known fact that the helicity of polylysine is dependent upon the pH of the solution (8). (ii) These complexes may be formed as a result of the coordination between the heme iron and the lysine residue. But a formyl group is not required for the coordination. (iii) Results of this study, especially when supported by those described previously (1), indicate that a Schiff's base is formed between the formyl group of heme a and a non-protonated ϵ-amino group of a lysine residue in alkali media. The Schiff's base is reduced by borohydride to a stable covalent bond and thus the heme a

Table II. Direct iron analysis of acid-acetone extract and residue of some heme protein complexes.

System I contained 0.1 mM hematin a and 0.0115 mM polylysine ($\bar{p}$ = 586); total volume, 5 ml. System II contained 0.119 mM hematin a and 0.011 mM polylysine; total volume, 7.5 ml. System III contained 0.086 mM hematin a, which had been pre-reduced by borohydride for 3 hours, and 0.0095 mM polylysine; total volume, 7.5 ml. System IV contained 0.208 mM protohematin and 0.0114 mM polylysine; total volume, 5 ml. System V contained 0.075 mM hematin a and 27.5 mg globin; total volume 15 ml. System I, II, III, and IV were in 0.1 M phosphate buffer, pH 11.6 and the reaction time was about 10 minutes. System V was in 0.02 M phosphate, pH 7.0; after 1 hour, the solution was adjusted to pH 11.6. Each mixture was then mixed with about 10 mg sodium borohydride and allowed to stand at 0-4° for 3 hours. Subsequent extractions and iron analysis were the same as previously described (1).

Complex	System	Treatment	Distribution of iron	
			µatoms	Percent
Heme a-polylysine	I	Borohydride reduced		
		1. Acid-acetone extract	0.12	25
		2. Residue	0.35	70
	II	No borohydride reduction		
		1. Acid-acetone extract	0.79	89
		2. Residue	0.07	8
Borohydride-pre-reduced heme a-polylysine	III	Borohydride reduced		
		1. Acid-acetone extract	0.36	71
		2. Residue	0.15	29
Protoheme-polylysine	IV	Borohydride reduced		
		1. Acid-acetone extract	0.94	90
		2. Residue	0.13	12
Heme a-globin	V	Borohydride reduced		
		1. Acid-acetone extract	--	--
		2. Residue	1.02	91

is fixed to the protein moiety. (iv) More striking perhaps is the close resemblance in spectral behavior between cytochrome oxidase on one hand and the heme a-polylysine and heme a-globin complexes on the other (see Table I). It is tempted to conclude that at least one linkage between heme a and the protein in cytochrome oxidase, in alkaline media, is in the coordination through a lysine residue. But the biological activity is dependent upon much more than merely this coordination. Although spectra of cytochrome oxidase

at pH 11.6 have been somewhat simulated by the synthetic complexes, no normal spectra of the oxidase, however, are known other than in its native state concurrent with biological activity. Thus, the question of whether the same coordination with lysine exists in the native cytochrome oxidase remains to be investigated.

Acknowledgement -- This work was supported by grants from the Life Insurance Medical Research Fund, the National Institutes of Health, the National Science Foundation, and the American Heart Association.

References

(1) Takemori, S., and King, T. E., J. Biol. Chem., 240, 504 (1965); and bibliography cited for earlier references.

(2) King, T. E., Kuboyama, M., and Takemori, S., in T. E. King, H. S. Mason, and M. Morrison (Eds.), Oxidases and related redox systems, Wiley, New York (1965), Vol. 2, p. 707.

(3) Rossi Fanelli, A., Antonini, E., and Caputo, A., Biochim. Biophys. Acta, 30, 608 (1958).

(4) Camerino, P. W., and King, T. E., Biochim. Biophys. Acta, 96, 18 (1965).

(5) Machinist, J. M., Crane, F. L., and Jacobs, E. E., J. Biol. Chem., 240, 1788 (1965).

(6) Blauer, G., Biochim. Biophys. Acta, 79, 547 (1964).

(7) Morrell, D. B., Barrett, J., Clezy, P., and Lemberg, R., in J. E. Falk, R. Lemberg, and R. K. Morton (Eds.), Hematin enzymes, Pergamon, Oxford (1961), Vol. 1, p. 320.

(8) Applequist, J., and Doty, P., in M. Stahmann (Ed.), Polyamino acids, University of Wisconsin Press, Madison (1962), p. 161.