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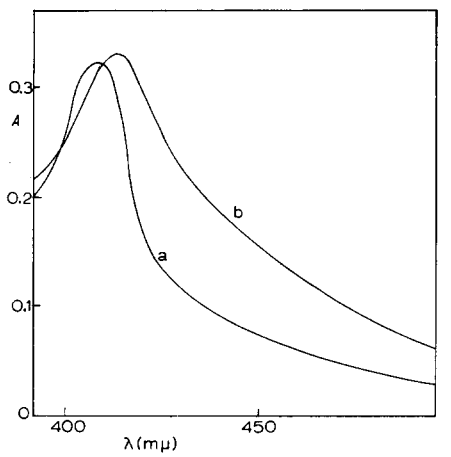
The reactions of cytochrome c_1 with ligands

Cytochrome c_1 is very similar to cytochrome c in its spectrum, typical of an iron porphyrin coordinated by two strong field protein ligands, in what is usually called a closed crevice structure[1], and typical structurally, by having the haem group covalently bound to the protein through thioether bonds[2]. These similarities suggest that cytochrome c_1 should react with cyanide in reactions similar to those of cytochrome c. However, it has been reported that cytochrome c_1 does not react at all with cyanide[3].

The reactivity with cyanide of a preparation of cytochrome c_1 made from beef heart mitochondria by the procedure of BOMSTEIN, GOLDBERGER AND TISDALE[2] was examined spectroscopically. Fig. 1 shows the Soret region of the spectrum of ferricytochrome c_1, and the spectrum of the enzyme after standing 10 min in the presence of 0.1 M KCN, neutralized to pH 7.1. The formation of a complex is evidenced by a red shift of the Soret peak of about 6 mμ, similar to that observed in the analogous reaction of cytochrome c (ref. 4).



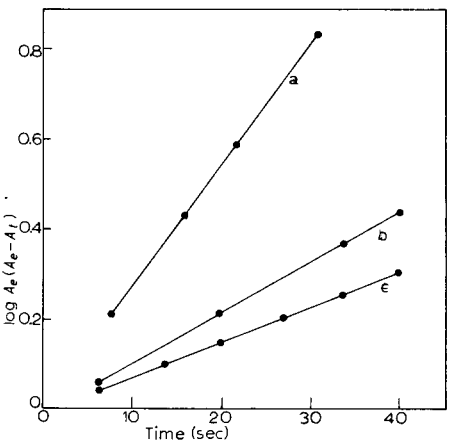


Fig. 1. Spectra of ferric cytochrome c_1 (a) and its cyanide complex (b) in the Soret region; 24°, pH 7.1, 0.1 M KCN.

Fig. 2. Kinetics of the reaction of ferricytochrome c_1 with cyanide at pH 7.1, 24°, and various concentrations of the ligand. A_t: absorbance at time t; A_e: absorbance at equilibrium. Wavelength: 427 mμ. [KCN]: (a) $16.7 \cdot 10^{-3}$ M, (b) $3.3 \cdot 10^{-3}$ M, (c) $1.67 \cdot 10^{-3}$ M.

The difference in the absorption is maximal at 427 mμ. At this wavelength, the rate of the reaction was measured at 24° and pH 7.1 (0.06 M phosphate buffer), using cyanide concentrations between 1.6 and 16 mM. Under these conditions, the reaction did not go to completion, but reached equilibrium in a few minutes. From the absorbances at equilibrium, A_e, and those measured at different times, A_t, the values of $\log A_e/A_e - A_t$ were calculated, and found to depend linearly on the time (Fig. 2). The observed rate constants, k_{obs}, estimated from the slopes of the lines in Fig. 2 increased linearly with increasing cyanide concentrations. For a ligand

binding reaction reaching equilibrium in excess of the ligand, the expression $k_{obs} = k_f \cdot [KCN] + k_b$ applies, with k_f and k_b representing the time formation and dissociation rate constants. Thus, a plot of k_{obs} *versus* [KCN] permits us to estimate k_f and k_b, from which K_{obs}, the equilibrium constant, may be calculated. The values obtained are presented in Table I, together with the values observed for the analogous reaction of ferricytochrome *c* (ref. 5). It can be observed that, under similar conditions, ferricytochrome c_1 cyanide is formed distinctly faster than its cytochrome *c* analog, although the stability of the latter is 20 times larger.

TABLE I

OBSERVED RATE CONSTANTS AND EQUILIBRIUM CONSTANTS FOR THE REACTIONS OF FERRI CYTOCHROMES *c* AND c_1 WITH CYANIDE, AT 24° AND pH 7.1

Data for cytochrome *c* estimated from ref. 5.

	$k_{forward}$ $(M^{-1} \cdot sec^{-1})$	k_{back} (sec^{-1})	K_{eq} (M^{-1})
Cytochrome c_1	3.2	$1.4 \cdot 10^{-2}$	$2.3 \cdot 10^{2}$
Cytochrome *c*	$7.6 \cdot 10^{-2}$	$1.8 \cdot 10^{-5}$	$4.3 \cdot 10^{3}$

Ferricytochrome *c* cyanide can be reduced by dithionite, yielding a reduced cyanide complex that decomposes readily[6]. Ferricytochrome c_1 cyanide was also reducible with dithionite. The resulting spectrum (Fig. 3), compared to that of free ferrocytochrome c_1, showed a small but distinct red shift, with the α and β bands appearing at 555 and 525 mμ, as in ferrocytochrome *c* cyanide[6]. In contrast to the latter, however, ferrocytochrome c_1 cyanide was stable, and did not decompose for several hours. Another reduced complex, ferrocytochrome c_1–CO (Fig. 3), was formed by bubbling CO through a solution of the reduced enzyme at pH 13. Its spectrum is very similar to that of ferrocytochrome *c*–CO (ref. 7). Upon neutralization it also proved to be very stable; after 24 h less than 10 % decomposition could be observed.

Thus, although spectroscopically similar to the complexes of reduced cytochrome *c*, those of reduced cytochrome c_1 decompose with extremely slow rates. It is of special interest to notice that, while the dissociation of the complex of ferric cytochrome c_1 is faster than that of ferricytochrome *c* cyanide, the situation is inverted in the case of the reduced complexes. Since the rate of these decompositions appears

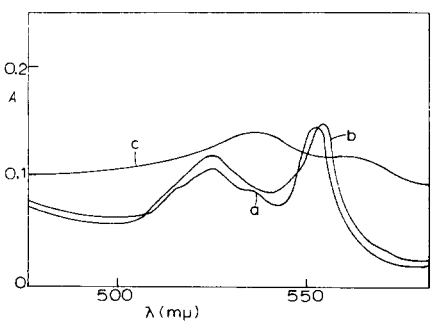


Fig. 3. Visible region spectra of ferrocytochrome c_1 (a) and its cyanide (b) and carbon monoxide (c) complexes, obtained as described in the text.

to be governed by conformation changes of the molecule[6], while spectra of haem proteins arise from electronic transitions of the porphyrin, these results suggest that although cytochrome *c* and cytochrome c_1 may have very similar structures in the haem region, they differ widely in their conformations.

The reactions of ferric and ferrous cytochrome c_1 with ligands are similar to those of cytochrome *c*. The quantitative differences are probably an indication of the different strengths of their crevices.

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