CYTOCHROME c. I. EFFECT OF Cl^- ON THE CONFORMATIONAL TRANSITIONS OF FERRICYTOCHROME c AT ACID pH

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Received April 15, 1968

The visible absorption spectrum of ferricytochrome c exhibits over the pH range 0 - 14 five different types, with transitions occurring at pH 0.42, 2.5, 9.5 and 12.76 (Theorell and Åkesson, 1941). The transition from the type III, low spin ferricytochrome c present at neutral pH, to the high spin type II, occurs at pH 2.5. It is accompanied by profound changes in the visible absorption spectrum resulting from a disruption of the central coordination complex of ferricytochrome c. An appreciable conformational change accompanies this transition (Bull and Breese, 1966). Boeri et al. (1953) have shown that in the absence of any anion, the transition from type III to type II should occur at pH 2.12. Other conformational transitions below neutrality have recently been reported by Flatmark (1964, 1966). Their relationships to the transitions observed earlier remains unclear.

The weak absorption band present in the spectrum of ferricytochrome c at 695 mu is known to be sensitive to conformational changes in the molecule (Schejter et al., 1963). It disappears in the presence of denaturing agents, of ligands capable of subsituting for one of the two ligands in positions 5 and 6 of the central coordination complex

of ferricytochrome c, and on increase in temperature (Schejter and George, 1964). Recently, this band has been assigned to a charge transfer transition (Eaton and Hochstrasser, 1967).

We report below some preliminary results on the effect of low pH and extrinsic KCl on the intensities of the 695 mu and 620 mu absorption bands of horse ferricytochrome c.

Horse heart cytochrome c was Sigma type VI used without further purification, or type III, chromatographed on Amberlite CG-50 (Margoliash and Walasek, 1967). The cytochrome c was thoroughly dialyzed against distilled water prior to each experiment. The concentrations ranged from 0.5 to 2 x 10^{-4} M. The spectrophotometric titrations were performed on a Cary model 15 spectrophotometer and a glass apparatus consisting of a 2 cm quartz cell and a thermostatted reservoir fitted with a Radiometer glass-calomel electrode GK 220, and with microburettes for the addition of acid or base. The assembly was maintained at 25±0.1°C. The pH was measured by means of a Radiometer PHM 4 pH meter. The glass electrode was standardized before and after each experiment with a series of standard solutions, pH 1.68, 2.00, 3.00, 4.01 and 7.00 (Harleco Co.). The solution of the ferricytochrome c was circulated continuously through the cell placed in the sample compartment of the spectrophotometer, allowing simultaneous measurement of pH and absorption spectra. The solutions were titrated with concentrated HCl or H_3PO_4 and 10 N KOH. The titrations were performed starting at neutral pH and proceeding towards low pH.

Figure 1 shows a typical spectrophotometric titration of horse ferricytochrome c dissolved in water, with conc. HCl or H_3PO_4. The variation of absorbance at 695 mu and 620 mu with pH is shown in Figure 2. The reversibility of the titrations at 620 mu was acceptable whereas reverse titrations at 695 mu failed to reproduce exactly the forward titration. Figures 3 and 4 show the spectra and titration

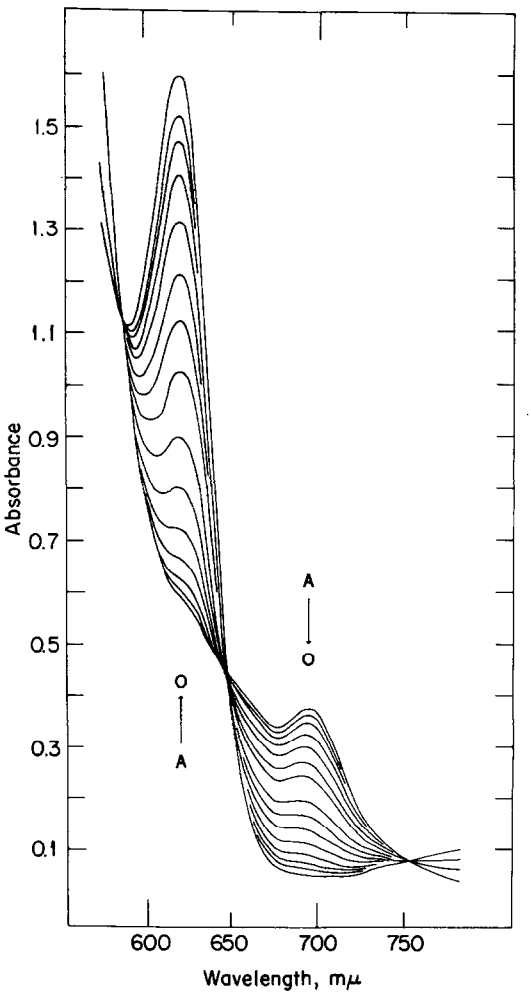

FIGURE 1. Forward titration of horse heart ferricytochrome c with concentrated H_3PO_4 in the absence of extrinsic KCl. Temperature 25° C. pH: A-4.01, B-3.27, C-3.04, D-2.87, E-2.76, F-2.69, G-2.63, H-2.54, I-2.49, J-2.43, K-2.36, L-2.28, M-2.20, N-2.02, O-1.80.

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curves, respectively, for horse ferricytochrome c titrated in the presence of 1 M KCl.

In the absence of extrinsic salt, the transition from low spin to high spin type ferricytochrome c as indicated by the increase in the

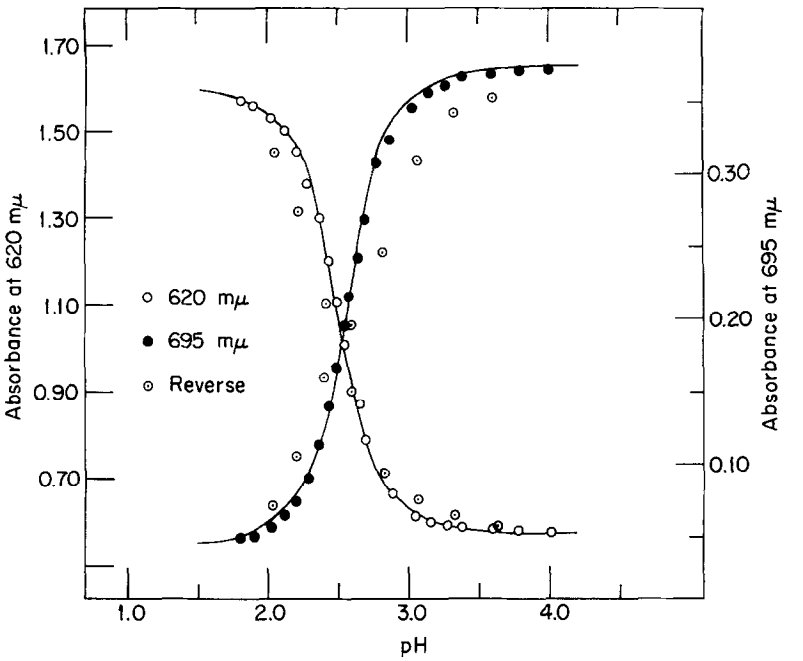

FIGURE 2. Plot of absorbance versus pH for the forward (data of Figure 1) and reverse titrations.

intensity of the 620 mu band, is effectively completed by pH 1.8 - 2.0. The midpoint of this transition from type III to type II occurs at pH 2.4 - 2.6. The isosbestic point at 755 - 760 mu remains constant over the entire pH range spanning the transition (Figure 1), while the isosbestic point at 645 mu remains constant over a more restricted pH range. Assuming the transition to be a simple protonation equilibrium involving only two types of ferricytochrome c molecules

$$C_1 + nH^+ \rightleftharpoons C_2$$

the experimental titration curves were rectified using the relationship

$$\log (A_2 - A)/(A - A_1) = pK' - npH$$

In the presence of KCl the single transition exhibited by the variation of the intensities of the 695 mu and 620 mu absorption peaks with decreasing pH, gives way to two transitions, one at a higher and

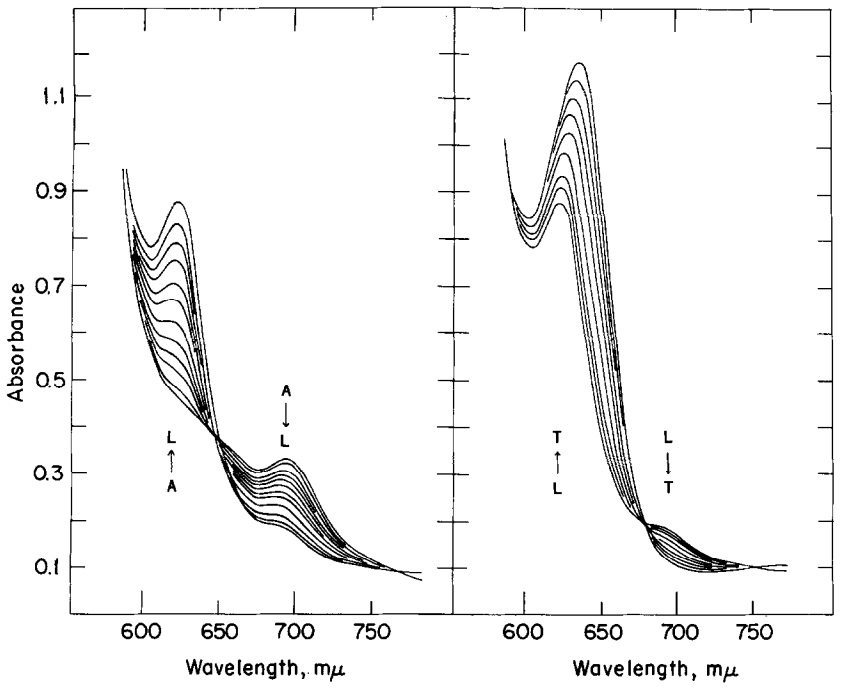

FIGURE 3. Forward titration of horse heart ferricytochrome c with concentrated H_3PO_4 in the presence of 1 M KCl. Temperature 25° C; pH: A-4.48, B-4.11, C-3.85, D-3.73, E-3.64, F-3.54, G-3.40, H-3.30, I-3.10, J-2.86, K-2.53, L-1.86, M-1.47, N-1.27, O-1.02, P-0.84, Q-0.68, R-0.56, S-0.41, T-0.22.

the other at a lower pH than previously (Figures 3 and 4). In 1 M KCl the first transition occurs at pH 3.4 - 3.6, while the second transition occurs at a pH of about 0.8 based on 695 mu data and at ca. 1.0 based on 620 mu data. The maximum of the high-spin absorption band at 620 mu begins to shift gradually towards higher wavelengths with increasing acidity below pH 2. The isosbestic point at 645 mu begins to shift below pH 2.5 to higher wavelengths and then remains constant at 675 mu below pH 1.9. The results of the rectification of the spectrophotometric titration curves are given in Table 1.

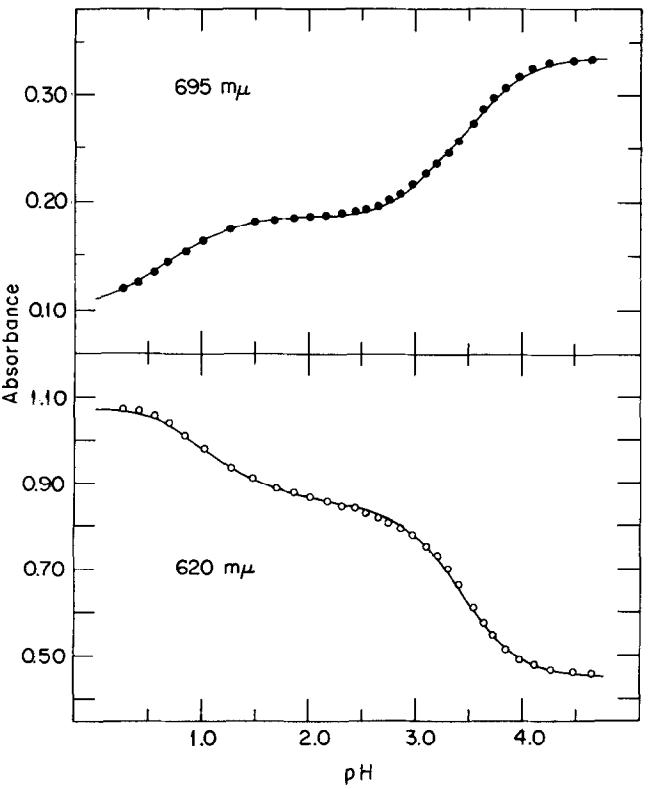

FIGURE 4. Plot of absorbance versus pH for the forward titration (data of Figure 4).

TABLE 1

Effect of extrinsic Cl^- on the conformational transition of horse ferricytochrome c at acid pH

	Decrease in absorbance at 695 mu		Increase in absorbance at 620 mu	
	pH midpoint[a]	n	pH midpoint[a]	n
In water	2.58	2.7	2.50	2.6
In 1 M KCl	3.58	1.5	3.51	1.5
	0.78	1.5	0.95	1.6

[a] Obtained graphically.

The transition type III ⟶ type II has n = 1.14, and the transition type II ⟶ type I which occurs at pH 0.42, has n = 1.53 (Theorell and Åkesson, 1941). The values of n obtained spectrophotometrically for the conformational transitions of ferricytochrome c at acid pH apparently vary with the wavelength at which the absorbance data is obtained. The simplest interpretation at present of the effect of extrinsic KCl is to relate the two observed transitions, at pH 3.5 and at ca. pH 0.8, to the transitions type III ⟶ type II and type II ⟶ type I, respectively. If this interpretation is accepted, the conclusion then is that the presence of an excess of extrinsic Cl^- ions stabilizes the type I and II forms of ferricytochrome c relative to type III.

This work was supported in part by Research Grant HE 11620-01 from the National Institutes of Health, U. S. Public Health Service, and by a grant from the Michigan Heart Association.

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