IONIZATION OF CYTOCHROME C

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The titration of cytochrome c acid to its isoionic point is reversible; the heat of ionization at pH 3.6 is about -2,900 calories per mole. Viscosity measurements indicate a conformational change in the molecule acid to a pH of about 4.5.

Experimental: Horse heart cytochrome c, type III, lots 124B-7082 and 124B-7083, was from Sigma Chemical Company, 5% solutions of which were poured, as needed, through an ion exchange column consisting of Amberlite CG-120 in the hydrogen form in the lower part above which was Rexyn 201 in the hydroxide form. After passage through the column, lot 124B-7082 contained 0.7% and lot 124B-7083 3.8% autoxidizable component according to the method of Tsou (1951). The absorption spectra were in satisfactory agreement with those of Margoliash and Frohwirt (1959) for ferri cytochrome c from 250 to 570 mμ; protein concentrations were estimated from the optical densities.

Discontinuous titrations were conducted at 30° at an ionic strength of 0.20; the ionic strength was assumed equal to the sum of HCl and KCl present. The protein concentration was about 0.5% except in the extreme acid range where the concentration was increased to about 2%. Samples above pH 5 were protected from CO_2 by setting the beakers in weighing bottles containing a thin layer of concentrated NaOH; the weighing bottles were swept with washed N_2. The pH determinations were made with a Radiometer Model 25. Blank corrections were estimated from the activity coefficients of HCl in KCl solutions. Buffers for calibration were prepared according to Bates (1964).

Short segments of the titration curve were done at several ionic strengths at 30°. Segments were also measured at several temperatures at an ionic strength of 0.20. The pH of the resin treated protein was measured in the absence of HCl as a function of the KCl concentration.

A sample of cytochrome c was brought to a pH of about 2 by the addition of HCl and to an ionic strength of 0.20 by the addition of KCl. Standard KOH containing KCl, the sum of the two being 0.20 molar, was added with a microburette and the pH measured. A blank titration containing the same quantity of HCl as the above was conducted. The results of the titrations were plotted on a large scale graph and the differences between the curves interpolated at 0.5 pH units and the moles of protons bound per mole of protein calculated.

Viscosities were measured in an Ostwald viscometer at 30°. The protein solutions were brought to the desired pH at an ionic strength of 0.20 by the addition of HCl and of KCl. The 0.78% protein solutions were filtered through hard, washed and dried filter paper, the first portion of the solution through the filter being discarded.

Results and Discussion: The amino acid composition of cytochrome c (Margoliash, Kimmel, Hill, and Schmidt, 1962) requires that 24 groups be titrated acid to the isoionic point. It appears from Figure 1 that the titration curve is approaching a limit of 24 groups at low pH and, further, the curve is very nearly reversible.

The binding of hydrogen ions at pH 3.6 is very nearly a linear function of temperature from 30° to 80°, the total increase being 2.56 moles per mole of protein. At 30° and at h equals 16.5, the apparent heat of ionization is about -2,900 calories per mole which appears too large to represent that which could be assigned to carboxyl ionization. It seems likely that the heat is, in part, a reflection of a conformational change taking place in the protein as the temperature is raised.

The increase of the isoionic point of cytochrome c with increasing KCl

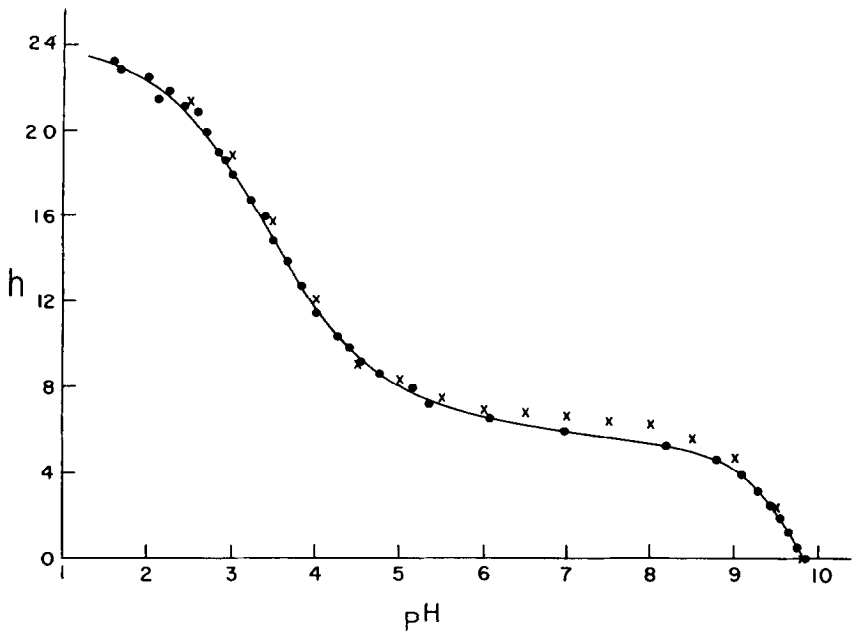

Figure 1. Moles of hydrogen ions (h) bound per mole of cytochrome c as a function of pH. Ionic strength 0.20; 30°. Filled circles, discontinuous titration. Crosses, back titration.

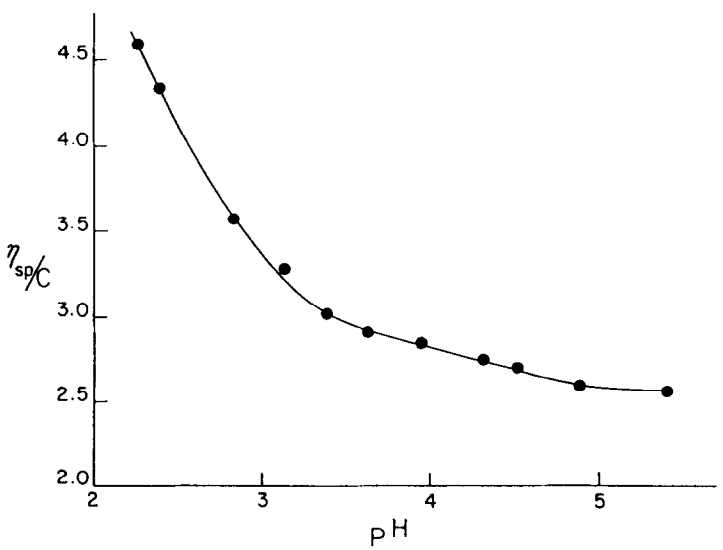

Figure 2. Reduced viscosity η_{sp}/C as a function of pH. Ionic strength 0.20; 30°.

concentration (see Table 1) arises, no doubt from binding of chloride ions by the protein; it is estimated that about one mole of chloride ions is bound per mole of protein in 0.20 M KCl.

The variation of the reduced viscosities, η_{sp}/c, of cytochrome c with pH (Figure 2) can best be interpreted as a swelling of the molecule beginning about pH 4.5 and increasing as the pH is lowered. The increase in viscosity is much too small to be consistent with an extensive unfolding of the cytochrome c molecule and probably considerable specific structure still remains even in the more acid solutions.

The variation of the slopes of the titration curve as a function of ionic strength (Table 1) appears to reflect a complex relation between the effect of the electrostatic field of the protein ion and a pH-dependent conformational change on the titration behavior of cytochrome c.

Theorell and Akesson (1941) have titrated cow heart cytochrome c, but their experimental procedure differed significantly from ours and comparison between the results is not justified.

TABLE 1

Slopes (dh/dpH) of titration curves of cytochrome c at h equals 16.5. Also the pH of an isoionic solution of cytochrome c. Both as functions of the ionic strength at 30°.

Ionic Strength	-dh/dpH	pH
0.00	-	9.80
0.05	7.5	-
0.10	7.4	9.88
0.20	6.7	9.90
0.50	7.7	9.92

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