ALKALINE ISOMERIZATION OF FERRICYTOCHROME c FROM EUGLENA GRACILIS

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SUMMARY

Euglena gracilis ferricytochrome c has a small absorption maximum at about 700 nm having an extinction of 850 ± 10 $M^{-1}cm^{-1}$. This absorption band is analogous to the more commonly found maximum at 695 nm which is observed in ferricytochromes from other sources and which is characteristic of ligation of methionine 80 with the heme ion. The 700 nm band disappears upon raising the pH to 11 giving a transition involving a single proton having an apparent pK of about 10. These results demonstrate that the phenolic ionization of tyrosine 67 is not required to trigger the alkaline isomerization of ferricytochromes c since Euglena cytochrome has a phenylalanine residue at position 67.

INTRODUCTION

Horse heart ferricytochrome c undergoes a pH dependent isomerization reaction having an apparent pK which ranges from 8.9 to 9.3 depending on the ionic strength of the solvent (1). This alkaline isomerization produces a modest conformational change (2) which precedes the gross unfolding of the protein structure, a transition having an apparent pK of 12.6 (3). The alkaline isomerization principally involves replacement of the methionine 80 sulfur ligand for the heme ion by an alternative protein ligand which has an electron paramagnetic resonance spectrum characteristic of a primary amine (4). This replacement lowers the redox potential of the heme (5) and prevents its reduction by ascorbate or ferrocyanide (6, 7). Recent circular dichroic studies of ferricytochrome c-cardiolipin complexes (8) suggest that the methionine ligand may be replaced at neutral pH when ferricytochrome is located in the inner mitochondrial membrane.

Specific nitration of tyrosine 67 (9), which lies adjacent to the methionine 80 ligand in the neutral isomer, or iodination of both tyrosine 67 and the

surface tyrosine 74 (10) lowers the apparent pK of the alkaline isomerization to pH 5.9. These results have lead several investigators (10-12) to suggest that the phenolic ionization of tyrosine 67 triggers the alkaline isomerization.

Cytochrome c obtained from Euglena gracilis is unique in that position 67 is occupied by a phenylalanine residue (13, 14) in place of the usual tyrosine residue. If the alkaline isomerization characteristic of cytochrome c is indeed triggered by the phenolic ionization of tyrosine 67, Euglena gracilis ferricytochrome c should not exhibit an alkaline isomerization.

EXPERIMENTAL

Euglena gracilis was cultured and its cytochrome c purified as described by Lin, et al. (14). The protein eluted from DEAE-cellulose as a single symmetrical peak and had the absorption spectral properties characteristic of the purified protein. Spectral titrations were obtained using a Radiometer pH meter and a Gilford spectrophotometer. Protein concentrations were calculated from the absorbance of the reduced cytochrome measured at 558 nm.

RESULTS AND DISCUSSION

As shown in Fig 1, the absorption spectrum of Euglena gracilis ferricytochrome c has a small maximum located at about 700 nm. Since the positions of all the visible absorption maxima of Euglena ferricytochrome c-558 are red shifted relative to the corresponding spectra of mammalian cytochromes c, it is very likely that the absorption band centered at 700 nm corresponds to the band centered at 695 nm for mammalian cytochromes c, which is characteristic of the ligation of methionine 80. The extinction of the maximum at 700 nm, 850 $\pm$ 10 $M^{-1}cm^{-1}$, is comparable with that measured for mammalian cytochromes c at 695 nm.

Increasing the pH of solutions of Euglena ferricytochrome c causes a progressive diminuation in the extinction of the 700 nm as shown in Fig 1. Above pH 10.5, however, the isosbestic point at 610 nm is lost and a new absorption band having a maximum at about 620 nm appears. The presence of a maximum at 620 nm is characteristic of ligation of a weak field ligand, such

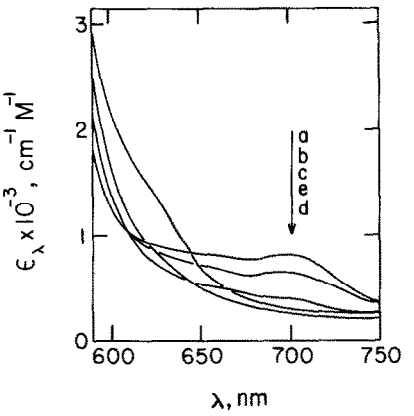

Fig 1. Visible spectra of Euglena gracilis ferricytochrome c-558. All spectra were obtained at 25° using a 0.19 mM protein solution in 0.38 M NaCl. The pH was changed by addition of 1 N NaOH. a. pH 8.10; b. pH 9.90; c. pH 10.47; d. pH 10.97; e. pH 11.17.

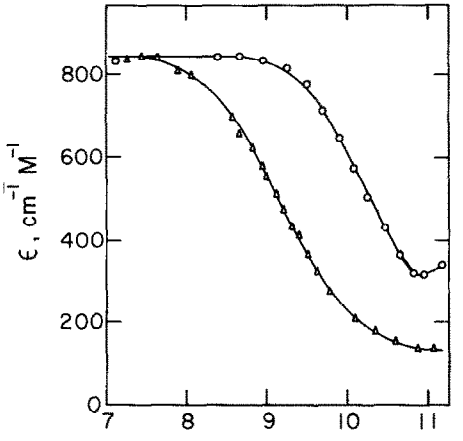

Fig 2. Alkaline isomerization of solutions of horse heart and Euglena gracilis ferricytochromes c. All spectra were obtained at 25° by addition of increments of 1 N NaOH to solutions of each protein dissolved in 0.38 N NaCl. o, Euglena gracilis cytochrome c-558; Δ, horse heart cytochrome c.

as the hydroxide anion, in the sixth coordination position of the heme ion (15). The changes in the extinction of horse heart and Euglena gracilis ferricytochrome c at 695 and 700 nm, respectively, with increasing pH are compared in Fig 2. The two proteins exhibit parallel transitions offset by one pH unit. However, above pH 10.5, the extinction of the Euglena, but not the horse pro-

tein, increases owing to the onset of ligation of a weak field ligand with the heme ion. The spectral titration curves for both proteins were analyzed using an expression for a two state transition,

$$\log \frac{\varepsilon_A - \varepsilon}{\varepsilon - \varepsilon_N} = pK - npH,$$

where ε_A is the extinction of the alkaline isomer, ε_N is the extinction of the neutral isomer, and n is the number of protons involved in the transition. In using this relationship, it was assumed that the ε_A for the Euglena protein would be identical to that of the horse protein in the absence of the competing hydroxide ligand. This analysis then indicates that the alkaline isomerization of Euglena ferricytochrome c involves the dissociation of 1.0 protons having an apparent pK of 10.3. The single proton involved in the isomerization of the horse heart protein has an apparent pK of 9.2. The apparent pK values for the isomerization of both proteins are lowered by decreasing the ionic strength of the solvent. Spectral titrations of the Euglena and horse proteins in 0.05 M NaCl each involve a single proton having apparent pK values of 9.9 and 8.9, respectively.

These spectral titrations of Euglena ferricytochrome c clearly demonstrate that the phenolic ionization of tyrosine 67 is not required to trigger the alkaline isomerization. Humicola lanuginosa ferricytochrome c also exhibits a normal alkaline isomerization (16) which has a phenylalanine residue at position 74 in place of the usual tyrosine residue. Since the remaining two tyrosine residues located at positions 48 and 74 in horse heart cytochrome c are distant from the methionine ligand, it is further unlikely that the phenolic ionization of any tyrosyl residues triggers the alkaline isomerization. The difference of one pH unit in the apparent pK values for the alkaline isomerization of horse and Euglena ferricytochrome c most likely reflects differences in the equilibrium between their respective neutral and alkaline isomers. In this context, it should be noted that the apparent pK values for the alkaline isomerization of horse and yeast ferricytochrome c also differ by almost one

pH unit (11) even though both of these proteins have tyrosyl residues at positions 48, 67, 74 and 97.

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