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Iso-Cytochrome *c* Species from Baker's Yeast

ANALYSIS OF THEIR CIRCULAR-DICHROISM SPECTRA

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The circular-dichroism spectra of baker's-yeast iso-1- (methylated and unmethylated forms) and iso-2-cytochrome *c* species were examined between 200 and 600nm. In the visible region the yeast haemoproteins have characteristics nearly indistinguishable from those of horse heart cytochrome *c*. From the spectra in the u.v. region the latter appears, however, to be more helical. It is proposed that the likely element of non-helical structure in iso-1-cytochrome *c* is residues 62–70.

The two baker's-yeast iso-cytochrome *c* species differ from mammalian cytochrome *c* species in several important respects, including amino acid sequence. Baker's-yeast iso-cytochrome *c* species have five extra amino acid residues in place of the acetyl group found at the *N*-terminal end of mammalian cytochromes (Narita *et al.*, 1963; Stewart *et al.*, 1966). They also have a single cysteine residue near the *C*-terminal end of the protein. This residue makes them susceptible to dimerization (Margoliash & Schejter, 1966). In addition, in the yeast haemoproteins, the presence of one residue of ε-*N*-trimethyllysine has been found. In iso-1-cytochrome *c* this residue was located at position 72 of the molecule (Delange *et al.*, 1970). The methylated and unmethylated forms of iso-1-cytochrome *c* from *Saccharomyces cerevisiae* have also been shown to coexist *in vivo* (Foucher *et al.*, 1972).

From the physicochemical point of view, baker's-yeast iso-cytochrome *c* species appear to be relatively unstable to alkali, particularly at low ionic strength (Margoliash & Schejter, 1966; Mirsky & George, 1967). We have also shown that they are less resistant than horse heart ferricytochrome *c* to thermal and guanidinium chloride denaturations (Polastro *et al.*, 1976).

To obtain a better understanding of these differences in stability between cytochrome *c* species from yeast and horse heart, we have undertaken analysis of their circular-dichroism (c.d.) spectra.

Experimental

Horse heart cytochrome *c* (type VI, lot 72C-7160) was from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and used without further purification.

Baker's-yeast iso-cytochrome *c* species (iso-1, methylated and unmethylated forms, and iso-2) were prepared by the procedure outlined by Foucher *et al.* (1972).

Ferrocytochrome *c* and ferricytochrome *c* species were prepared by treatment with sodium dithionite and potassium ferricyanide respectively. In both cases the excess of reagent was removed on a Sephadex G-25 column. Solutions of ferrocytochrome *c* species were freshly prepared before use.

The c.d. curves were obtained with a Cary 61 spectropolarimeter. Measurements were made at 20°C in quartz cells with a path length of 1cm in the region above 250nm and of 1mm below this wavelength. The protein concentration never exceeded 0.25mg/ml. All the solutions were prepared in 50mM-Tris/HCl buffer, pH7. Concentration of yeast and horse heart cytochrome *c* species were determined spectrophotometrically after reduction with sodium dithionite. A molar extinction coefficient at 550nm of 29800litre·mol^{-1}·cm^{-1} was used (Margalit & Schejter, 1970). Absorbances were measured with either a Zeiss PMQ II spectrophotometer or a Cary 15 spectrophotometer.

Results and Discussion

C.d. spectra in the range 250–600nm

In the oxidized form, the yeast cytochrome *c* species as well as horse heart cytochrome *c* give complex c.d. spectra, with most of the bands overlapping. The spectra of the baker's-yeast haemoproteins possess, in the range 250–600nm, all the characteristics of the spectrum of mammalian cytochromes as typified by horse heart cytochrome *c*. In particular, the signs and positions of the extrema at 250, 263, 283, 290, 330, 375, 405, 420, 495, 530 and 550nm are conserved, although the ellipticity of the different bands between 250 and 380nm vary somewhat from one haemoprotein to another.

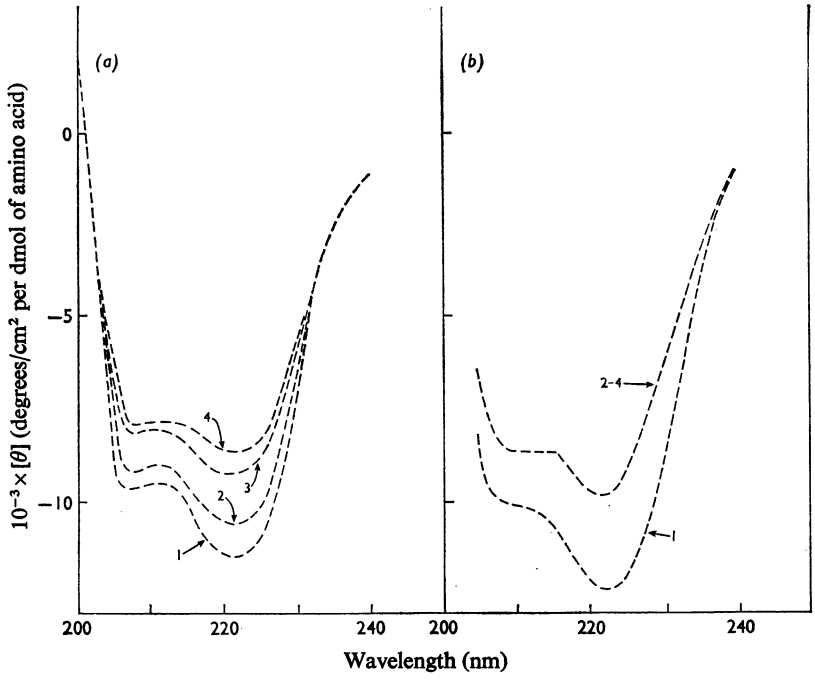

Fig. 1. *C.d. spectra of oxidized (a) and reduced (b) cytochrome c species from horse heart and baker's yeast in 50mM-Tris/HCl buffer, pH7, at 20°C*

The reduced molar ellipticity is expressed as degrees/cm² per dmol of amino acid. Curve 1 refers to horse heart cytochrome *c*, curves 2, 3, and 4 refer to baker's-yeast iso-cytochrome *c* species, iso-2 and iso-1 methylated and unmethylated forms respectively.

In the reduced form, the close similarity between the yeast iso-cytochrome *c* species and horse heart cytochrome *c* is yet more striking. Here, the position as well as the ellipticity of the different bands (270, 335, 375, 425, 510, 530 and 550nm) are identical for any of the cytochrome *c* species that we investigated in the present study.

Such a similarity, in both the reduced and the oxidized states, demonstrates that the haem environment in the yeast proteins is very close to if not identical with that found in horse heart cytochrome *c*.

C.d. spectra in the range 200–240nm

These c.d. spectra are shown in Fig. 1; Fig. 1(*a*) refers to the cytochrome *c* species in the oxidized state and Fig. 1(*b*) to the proteins in the reduced state. For all four cytochrome *c* species, the two extrema centred at 222 and 207nm are clearly visible. From the extremum at 222nm, a helical content may be estimated (Chen & Yang, 1971).

In the oxidized state horse heart cytochrome *c* and iso-2-cytochrome *c* from yeast, with helical contents of 30 and 27% respectively, clearly appear more ordered than the methylated and unmethylated forms of the iso-1-cytochrome *c* (helical contents of 21–22%) from baker's yeast.

These conclusions are in excellent agreement with the results of Mirsky & George (1967), who studied the optical-rotatory-dispersion properties of these haemoproteins and estimated their helical contents from the depth of the 233nm trough.

The c.d. spectra of all four proteins can be explained without any β-conformation contribution (Chen *et al.*, 1972).

From X-ray-diffraction studies, it is now well established that horse heart ferricytochrome *c* has three segments of α-helix at residues 1–13, 62–70 and 88–102 (Dickerson *et al.*, 1971; Takano *et al.*, 1973). With its 104 residues, this would correspond to a helical content of about 34%, in good agreement with our results.

To get a better understanding of the differences in helical content between horse heart ferricytochrome *c* on the one hand and the methylated and unmethylated forms of baker's-yeast iso-1-ferricytochrome *c* on the other hand, we have referred to

the published amino acid sequences of these haemoproteins (Narita *et al.*, 1963; Margoliash & Smith, 1961).

The amino acid sequences in the helical regions discussed above are more or less well conserved. There is only one clear exception. It concerns the helical segment from residue 62 to residue 70. In horse heart cytochrome *c*, this sequence is largely composed of helix-promoting amino acids (Chou & Fasman, 1974). The corresponding segment in the iso-1-protein from baker's yeast, Asn-Asn-Met-Ser-Glu-Tyr-Leu-Thr-Asn, may be surmised to be completely non-helical, since it contains a very high proportion of helix-breaking amino acids. Evidence has been presented for a weaker predisposition for ordered structure in the *C*-terminal region of yeast iso-1-cytochrome *c* compared with the corresponding region in horse cytochrome *c* (Moroder *et al.*, 1975). However, this evidence comes from a study on synthetic peptides and may not be regarded as definitive.

Unfortunately, the amino acid sequence of iso-2-cytochrome *c* from baker's yeast is only known up to residue 26 (Stewart *et al.*, 1966), so an analysis cannot be made now for this protein.

Also, in the reduced state, horse heart cytochrome *c* shows a higher degree of helicity than do the yeast iso-proteins. On reduction, however, the differences that we observed between iso-1- and iso-2-ferricytochrome *c* species are now completely abolished. In good agreement with earlier c.d. studies and with X-ray diffraction studies (Takano *et al.*, 1973, and references therein), we find that the oxidation of horse heart cytochrome *c* does not lead to dramatic changes in the helical contents of the protein. This is also true for the methylated and unmethylated forms of iso-1-cytochrome from baker's yeast. The reason why the helix contents of iso-2-cytochrome *c* (from yeast) becomes lower on reduction remains obscure.

Several points of interest are relevant to this work. For yeast iso-1-cytochrome, when compared with horse heart cytochrome *c*, a loss of helical content may be correlated to a lower stability. Also, the biological methylation of lysine-72 of iso-1-cytochrome *c* from baker's yeast does not change the helical content of this protein, nor does it afford a greater resistance to thermal, acidic and guanidinium chloride denaturation (Polastro *et al.*, 1976).

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