CIRCULAR DICHROISM OF FERRICYTOCHROME C

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SUMMARY: The circular dichroism (CD) spectra (650 - 220 mu) of horse heart ferricytochrome c, Pseudomonas aeruginosa ferricytochrome c and ferriheme octapeptide from horse heart cytochrome c have been measured at pH 7. The CD spectra were compared to the optical rotatory dispersion (ORD) spectra and it was found that some of the optically active transitions responsible for the multiple extrinsic Cotton effect observed previously in the ORD spectra of cytochrome c, could be resolved. Of special interest was the observation that the band shapes of the two proteins in the 480 - 410 mu region, appear to be approximate mirror images of one another. This phenomenon is most likely an indication of the arrangement of ligands in positions 5 and 6 of the heme group.

INTRODUCTION

The optical rotatory dispersion (ORD) of cytochrome c has recently been investigated in several laboratories (Urry and Doty, 1965; Ulmer, 1965; Urry, 1965; Myer and Harbury, 1965; Mirsky and George, 1966). The ORD spectra of both the oxidized and reduced forms of the protein are characterized by a number of complex Cotton effects throughout the visible and ultraviolet

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regions. Furthermore, the effect of pH on the ORD spectrum of ferricytochrome c roughly parallels the changes observed in its absorption spectrum (Mirsky and George, 1966). No detailed explanation has been provided for the occurrence of the multiple extrinsic Cotton effects in the ORD spectra of cytochrome c.

Circular dichroism (CD) is, potentially, a more useful technique than ORD, for distinguishing optically active transitions responsible for the complexity of the ORD of "mammalian type" cytochrome c*. We present below some preliminary results on the CD of horse heart ferricytochrome c, the ferriheme octapeptide derived from it, and of the "non-mammalian type" ferricytochrome c of Pseudomonas aeruginosa** The ORD spectra of Ps. cyt. c were reported earlier by Myer et al. (1966); those of H8, H8Im and other complexes of H8 were investigated by Myer and Harbury (1966). The ORD of H8 is similar to that of the heme undecapeptide from horse heart cyt. c (Ulmer, 1966).

MATERIALS AND METHODS

Horse heart cytochrome c was Sigma type VI. The heme octapeptide was prepared from Sigma type III cytochrome c (Tuppy and Paleus, 1955; Harbury and Loach, 1960). The bacterial cytochrome c was isolated and purified as described by Ambler (1963). The ORD and CD spectra were recorded on a Jasco ORD/UV/CD-5 spectropolarimeter, at 25°C. The slit-width was programmed to give a constant light intensity over the region 650 to 220 mu.

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* The term "mammalian type" cytochrome c is used in the same sense as defined by Margoliash and Schejter (1966).

** Abbreviations used: HH cyt. c - horse heart ferricytochrome c; Ps. cyt. c - Pseudomonas ferricytochrome c; H8 - ferriheme octapeptide from horse heart cytochrome c (amino acid residues 14-21); H8Im - ferri heme octapeptide in the presence of extrinsic imidazole.

Cells with fused quartz windows (Opticell Co.), having path lengths ranging from 0.1 to 10 mm were used. The oxidized form of the proteins was maintained by the addition of a minimal quantity of potassium ferricyanide. The concentrations were calculated from the absorption spectra of the solutions obtained with a Cary model 15M spectrophotometer and the known absorptivity of the cytochrome c (Margoliash and Frohwirt, 1959; Horio et al., 1960). The ORD spectra are plotted as molar rotations vs. wavelength and the CD spectra, as ellipticity vs. wavelength. No corrections for refractive index dispersion were made.

RESULTS AND DISCUSSION

Figures 1 and 2 show the ORD and CD spectra, respectively, of HH and Ps cyt. c and of H8 and H81 m. Comparison of the spectra, shows that although

FIG. 1

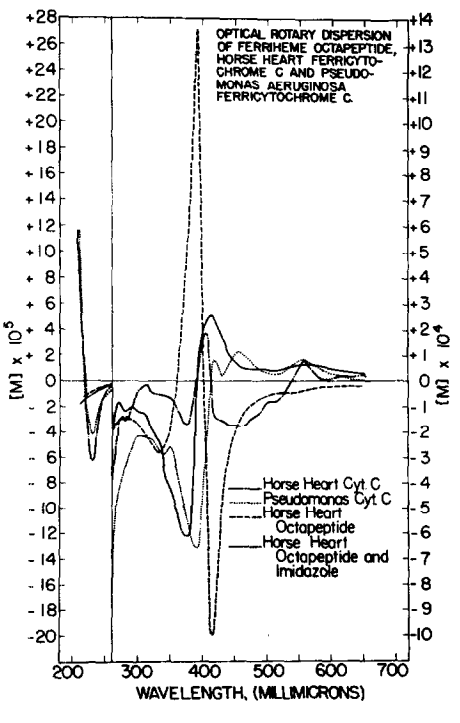
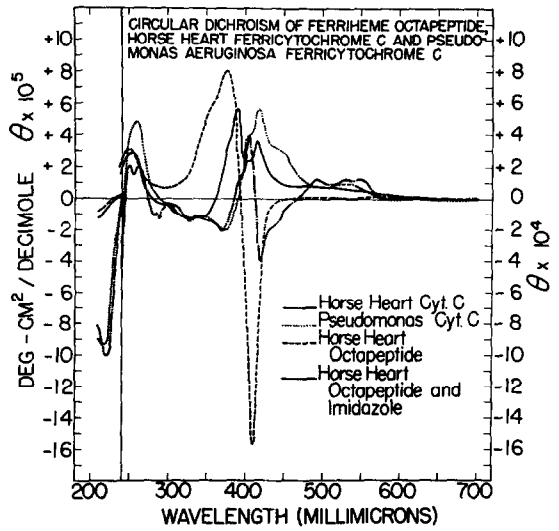


FIG. 2



the complexity of the CD spectrum is not much less than that of the ORD, some unraveling of the positions of optically active transitions can be achieved.

In the region of wavelengths above 580 mu, the ellipticities of both proteins and the heme peptide systems are very small. From 580 to 480 mu, the ellipticities are positive, complex and have an intensity about an order of magnitude, greater than in the previous wavelength region. The only exception is H8, whose ellipticity becomes appreciable only below 450 mu. HH cyt. c shows peaks at around 548, 528, and 494 mu, of approximately equal intensity, whereas Ps cyt. c shows a single broad peak at about 536 mu. In both cases, weak bands may be still hidden under the total band envelope.

In the Soret region, H8 exhibits the simplest and most intense CD spectrum, with a negative trough at 410 mu and a positive extremum at 376 mu. H8Im possesses in this region, two maxima, both positive, at about 415 and 390 mu. The HH and Ps cyt. c exhibit much more complicated contours in the Soret region. HH cyt. c shows a minimum at 420 mu and an inflection at 445 mu, both at

negative values of ellipticity, and a positive extremum at about 404 mu. Ps cyt. c shows positive ellipticity with a peak at 420 mu and inflections at approximately 450 and 400 mu. The band contours of the two proteins in the 480-410 mu region, appear to be approximate mirror images of one another. This phenomenon is perhaps an indication of the relative arrangement of ligands in positions 5 and 6 of the heme group.

The CD spectra of the two peptide systems from 360 to 290 mu are different, H8Im generally exhibiting negative ellipticity. HH and Ps cyt. c have negative ellipticities in this region with troughs at 370 and 330 mu. Below 290 mu, HH cyt. c exhibits negative, extrinsic ellipticity with troughs at 282 and 288 mu, probably due to the interaction between the heme group and the aromatic side-chain groups. All four systems possess positive ellipticity in the 250-260 mu region, with extrema at 250 mu for H8, 253 mu for H8Im, 258 mu for Ps cyt. c and at 250 and 260 mu for HH cyt. c. Below 240 to 230 mu the ellipticities of all four systems become negative. Those of the proteins are comparable in intensity, and are about an order of magnitude greater than those of the peptides. The latter result is to be expected in view of the absence of appreciable secondary structure in the heme peptide systems.

The central coordination complex of H8 and H8Im at neutral pH is probably His - Fe - H_2O and His - Fe - Im, respectively. The ORD of H8 is known to exhibit aggregation effects (Myer and Harbury, 1966) and thus only the gross features of its CD spectrum can be compared to the ellipticities of the other systems. The coordination in Ps cyt. c was shown to be His - Fe - Met (Harbury et al., 1965; Vinogradov and Harbury, 1967; Fanger, Hettinger, Vinogradov and Harbury, 1967). The nature of the central coordination complex

of HH cyt. c, as in other "mammalian type" cytochromes c, remains unknown. It is evident from previous work by Myer and Harbury (1965; 1966) that the extrinsic Cotton effect attributable to the heme group in the ORD of various "mammalian type" cytochromes c and complexes of H8 with a number of ligands, is very complex.

Our preliminary results indicate that the CD spectra of H8, H8Im, HH cyt. c and Ps. cyt. c, do provide means for the resolution and location of optically active transitions of the heme group, particularly in the Soret region. Furthermore, the spectra of the two proteins are quite different from those of the two peptide systems. Thus it is likely that the remaining polypeptide chain in cytochrome c and its conformation in the vicinity of the heme group exerts a considerable influence on the positions and intensities of the optically active transitions in the visible spectrum of the heme group. The differences between the CD spectra of the two proteins is probably due either to the presence of different side-chain groups in positions 5 and 6, or to the differences in the environment of the heme group caused by the tertiary structure. Further analyses of the CD spectra should provide greater insight into the nature of the central coordination complex and its relationship to the tertiary structure of cytochrome c of different origins.

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