OPTICAL ACTIVITY OF HEMATIN a

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Received February 27, 1967

Subsequent to the classical prediction by Warburg in 1924, the structure of hematin a has been diligently studied in a number of laboratories. Although the structure depicted in the reviews, for example by Lemberg (1) and more recently by others (2), has been generally considered correct with only minor reservations, the fact of its optical inactivity remains a great obstacle to accepting it. The incompatibility of the proposed structure and the optical behavior has greatly taxed investigators in the field as witnessed by the discussions of symposia and reviews (1-3). Considerations of this serious, apparent disparity has prompted us to communicate our recent findings at the present time in this preliminary communication.

We have found that fresh hematin a is optically active. The results from optical rotatory dispersion (ORD) experiments further suggest that the main contribution of optical activity is from the conformation rather than the asymmetric carbon atoms.

Experimental--Hematin a was isolated from purified cytochrome oxidase by our method as reported previously (4, 5) with the exception that three additional extractions by methanol-chloroform (1 : 2) were introduced prior to acetone extraction. ORD experiments were conducted at 23° in a

Cary 60 spectropolarimeter. The slit-width was programmed for a band width of 15A° to give a constant light intensity over the region 600 to 185 mμ.

Results and Discussion--A protocol of the ORD spectra of hematin a is given in Fig. 1. The main Cotton effect is centered at 410 mμ with an amplitude of 14,000° expressed as molar rotation. Upon reduction, it shifts to 397 mμ with an amplitude of 59,000°. That the inflection points do not exactly coincide with the absorption maxima may be due to their broad band widths (cf. Fig. 2) as well as to the perturbation of the second Cotton effect in the 370 mμ region (cf. Fig. 1). It must also be pointed out that the absorption spectra (also cf. Ref. 5) are different in the presence and the absence of detergents, such as Emasol, a nonionic detergent. The absolute values of ORD data have been found to be somewhat dependent upon the experimental manipulations,* but the Cotton effects are still several times smaller than

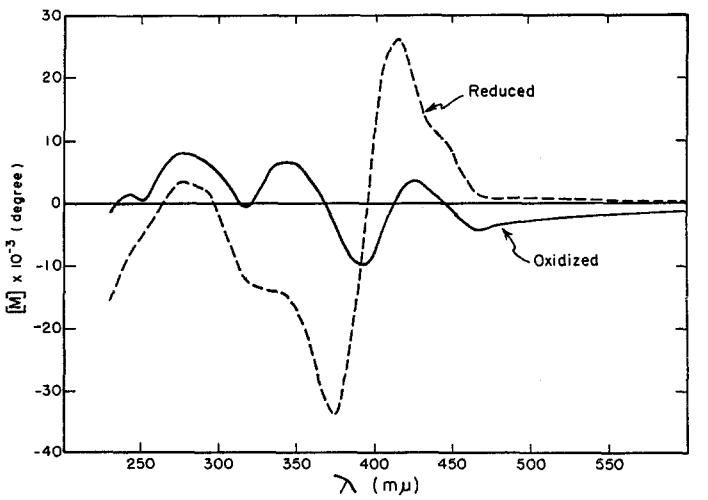

Figure 1. ORD spectra of oxidized and reduced hematin a in 0.05 M phosphate buffer, pH 7.8. Hematin a was 191.2 μM and cell path was 0.01 dm. The ordinate is in the unit of molar rotation.

*Whether the variation is due to more complicated reactions, such as effect of pH and concentration-dependent aggregation and dissociation, is being investigated.

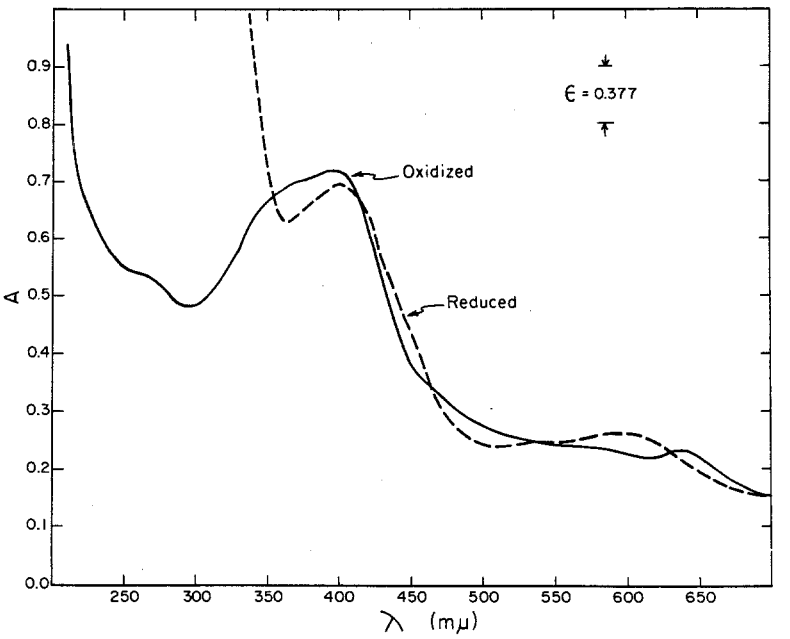

Figure 2. Absorption spectra of oxidized and reduced hematin a in 0.05 M phosphate buffer, pH 7.8. These spectra were obtained by scanning the samples used in ORD experiments as described in Fig. 1.

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those found in cytochrome oxidase (6, 7). In other words, the optical activity of the native enzyme in this region is mainly due to the interactions of the asymmetric environment of the protein moiety with the prosthetic groups.

The Cotton effects as shown in Fig. 1 are abolished by aqueous pyridine or Emasol and greatly diminished in systems with pH higher than 11 or lower than 2. The optical inactivity reported in earlier studies (1, 2, 7), including those conducted in this laboratory, ** was apparently due to the

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**In collaboration with Dr. John A. Schellman, we previously also found that hematin a was optically inactive in 0.5% Emasol-50 mM phosphate buffer, pH 7.8 (presented in Discussion of a recent symposium (2)). However, the amplitude of the main Cotton effect of cytochrome oxidase in the same solvent system was more than 200,000° (6, 7).

solvents employed in the measurements. Hematin a is relatively insoluble in aqueous media despite its two propionic acid groups. Consequently, workers usually use alkali, pyridine or detergents to facilitate solution.

Although samples of hematin a employed in our work were not subjected to the characterizations prescribed by classical organic chemistry, it is inconceivable, nevertheless, that the rotations or the Cotton effects are from traces of impurities. This conclusion is reached inter alia from (a) the degree of purity previously discussed (4, 5), (b) the relatively satisfactory coincidence of the centers of the Cotton effects with the absorption maxima, and (c) the great dependence of the ORD behavior upon the oxidation state of the hematin. Consequently, the present finding may remove an important obstacle to the acceptance of the main structure proposed for hematin a as discussed, for example, by Lemberg (1).

Acknowledgement--The work described was supported by grants from the National Institutes of Health, the Life Insurance Medical Research Fund, the American Heart Association and the National Science Foundation.

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