THE SORET BAND OF MONOMERIC HEMATIN AND ITS CHANGES ON POLYMERIZATION

Yuji Inada and Kazuo Shibata

The Tokugawa Institute for Biological Research, Tokyo,* and Tokyo Institute of Technology, Japan.

Received September 25, 1962

In the spectroscopic investigations of hemoproteins or hematin complexes with various chelating reagents, we often deal with the spectra of aqueous solutions of free hematin (ferriprotoporphyrin IX). Complications arise in interpreting the data because the spectra generally show a flat Soret band of hematin dimer or polymers around 390-400 mμ; in addition, the band is flattened further by the formation of higher polymers when the solution is left standing at room temperature. By spectroscopic or potentiometric titration, Shack and Clark (1947), Walter(1952) and Smith(1958,1959) studied the complexing of either cyanide or pyridine with heme, hematin and deuteroporphyrin dimethyl ester disulfonic acid. These authors revealed that the titration curves are well accounted for by assuming a dimeric form of these hematins in solution before chelation, although ultracentrifugal and diffusion analyses made by Shack and Clark(1947) showed that aqueous solutions of hematin contain aggregates of the dimers interacting with each other very weakly. The dimer hypothesis was successfully applied in our previous study(Tohjo et al., 1962) to explain

* mailing address

the changes of the peroxidase activity and absorption spectrum in the complexing of hematin with a variety of chelating reagents. In this study, because of the polymerization of the dimer, caution had to be exercised so that hematin solutions were not left standing for more than 30 minutes before addition of reagents. Trials were made in the present study to obtain the spectrum of monomeric hematin in aqueous solutions, and the success led us to the kinetic study of its polymerization. Spectroscopic observations were made at 18°C with a Cary recording spectrophotometer 14.

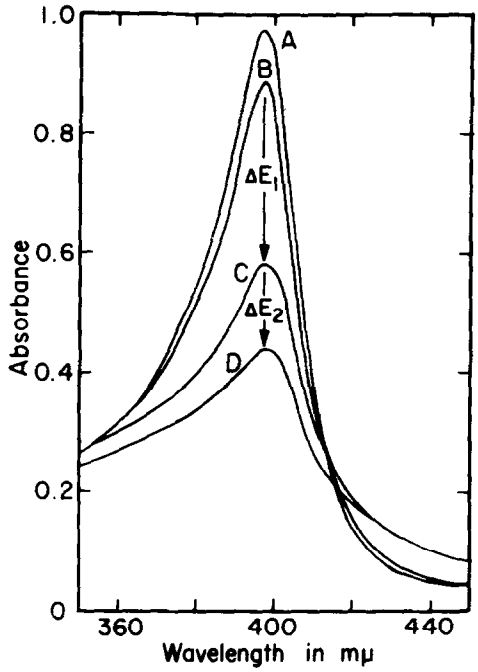

Fig. 1. The Soret bands of aqueous solutions of hematin(See text for details).

Hemin crystals(Tohjo et al., 1962) of 65.2 mg. were dissolved in 1.0 ml. of 0.1 N KOH solution, and then diluted to 100 ml. with distilled water. To obtain a solution of monomeric hematin free from polymers, the alkaline stock solution had to be

diluted more than 1000 times with distilled water to give a hematin concentration less than 1.0×10^{-6} M. The alkaline solution became neutral or a little acidic by the dilution. Curve A in Fig. 1 is the spectrum of 0.8×10^{-6} M hematin observed with a 10.0 cm. cell at pH 6.8, and shows a sharp band at 398 mμ with ε_{mM} (milli-molar extinction coefficient) = 122 ± 3. This band is thought to be the band of monomeric hematin for the following reasons. i) The sharp band contrasts with the flat and lower band of hematin dimer or polymers commonly observable for more concentrated hematin. ii) The addition of buffers or salts transformed the band into a flat band. iii) A similar experiment with hemin bromide in place of hemin chloride showed an identical Soret band with ε_{mM} = 120 ± 3. iv) The ε_{mM} value of 120-122 is similar to the values obtained for the Soret bands of various monomeric hematin complexes; e.g. 110 at 408 mμ for the hematin-pyridine and -histidine complexes, 120 at 401 mμ for the hematin-formamide complex (Tohjo et al.,1962), and 81 at 400 mμ and pH 7.0 and 131 at 398 mμ and pH 1.0 for the hematin-ethanol complex(Maehly,1958). v) Beer's law is obeyed below 1.0×10^{-6} M hematin, provided that the measurements are completed within 5 minutes after the dilution. This is because the band was lowered gradually and the drop of the band height was negligibly small within 5 minutes but was appreciable after the time. vi) The lowering obeys the mechanism of dimerization as described below.

Above 1.0×10^{-6} M hematin, polymerization proceeds with an appreciate rate. Curve B in Fig. 1, which was observed for 1.60×10^{-6} M hematin with a 5.0 cm. cell at 3 minutes after dilution, should be identical with curve A if the polymerization does not occur, but the observed curve is lower than

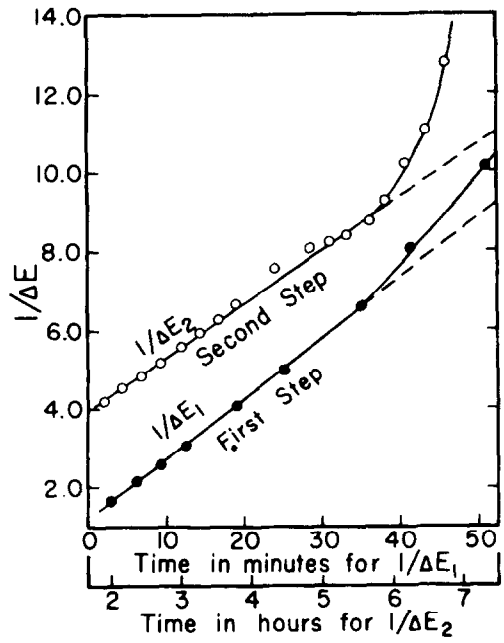

Fig. 2. The relation between $1/\Delta E$ and incubation time observed at 18°C.

curve A. During incubation at 18°C for 115 minutes, curve B is transformed into curve C with a clear isobestic point at 414 mμ(denoted as the first step). During the further lowering of the band from curve C to curve D between 115 minutes and 10 hours, the absorbance value decreases over the entire range of wavelength with no isobestic point(the second step). The reciprocal of the difference(ΔE_1) between the absorbance value at 398 mμ of a band in the first step and that of curve C is plotted against time in Fig. 2. The linear relationship thus obtained suggests that the lowering is due to the dimerization of the monomeric hematin(rate constant = 1.29×10^3 M^{-1} $sec.^{-1}$). A slight deviation from the linear relationship after 35 minutes of incubation suggests that the second step starts before the first step proceeds to completion. Curve C is, therefore, the band of dimeric hematin(ε_{mM} = 146 per mole

of the dimer) with a little contamination with polymers. A similar plot of $1/\Delta E_2$ against time for the second step gave us also a linear relationship until 6 hours of incubation(rate constant = 6.6×10 $\underline{M}^{-1}\text{sec.}^{-1}$), suggesting the formation of a tetramer from the dimer. However, the change in absorbance by this time is only 37 % of the total absorbance change in the second step, and a great deviation from the linearity occurs after the time. This implies that curve D is a composite of the bands of polymers.

REFERENCES

Maehly, A. C., and Åkeson, Å., Acta. Chem. Scand., 12, 1259 (1958)

Shack, J., and Clark, W. M., J. Biol. Chem., 171, 143(1947).

Smith, M. H., Biochem. J., 68, 27(1958).

Smith, M. H., Biochem. J., 73, 90(1959).

Tohjo, M., Nakamura, Y., Kurihara, K., Samejima, T., Hachimori, Y., and Shibata, K., Arch. Biochem. Biophys., in press.

Walter, R. I., J. Biol. Chem., 196, 151(1952).