Mössbauer Studies of Ferriheme–Quinidine Complexes

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Abstract

The system ferriprotoporphyrin IX–(+)-quinidine (FP–Qd) was investigated by Mössbauer spectroscopy at both 4.1 and 90 K. FP–Qd complexes were prepared by interaction of 10^{-2} to 10^{-3} M aqueous solutions of the components at pH 11–12 and 26 °C. Previous investigations of analogous complexes showed characteristic and unusually large circular dichroism bands near 400 nm at alkaline pH values. The present Mössbauer data obtained for FP either in the presence or absence of Qd at both pH 11–12 and 9 indicate identical isomeric shifts in all cases. Both free and complexed FP iron is in a high-spin state. The temperature dependence of the FP–Qd complex indicates slow spin–spin relaxation at 90 K and fast relaxation at 4.1 K. Qd appears to increase the iron–iron distance of FP in the complexes with reference to FP alone, in agreement with previous suggestions on the structure of the complex.

Introduction

Optical and hydrodynamic properties of ferriprotoporphyrin IX–quinidine complexes have been described in previous publications [1–3]. The complex aggregates formed under certain conditions in dilute aqueous solutions in the pH range 11.0–11.5 are characterized by unusually large molar ellipticities in the Soret region near 400 nm, where two adjacent CD† bands of opposite sign are observed. The large negative band centered at 418–420 nm, as well as a smaller positive band at 388–390 nm (complex **A**), both change their sign upon acidification of the solution to pH values below about 10 (complex **B**). Analogous changes in the signs of all bands are also observed in the visible region (see Fig. 1).

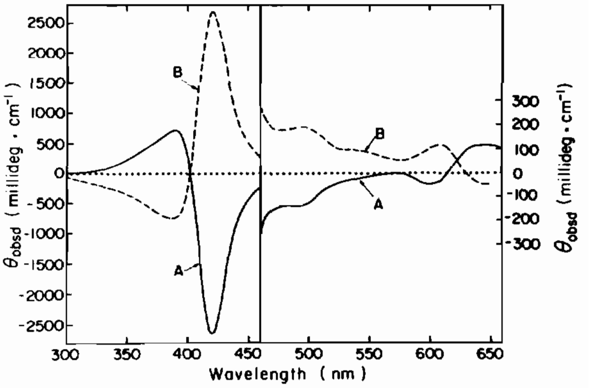

Fig. 1. CD spectra of FP in the presence of Qd at pH 11.5 (curve A) and pH 9 (curve B): FP, 1.0×10^{-4} M; Qd, 2.0×10^{-4} M; NaCl, 0.05 M; temperature of measurement, 26.5 ± 1 °C. Curve A (complex **A**): pH 11.5, measured 5 days after preparation of the above solution kept at 26 °C. Curve B (complex **B**): pH 9; the solution was prepared at pH 11.5 and kept at 26 °C for 7 days, after which it was brought to pH 9 and kept at 26 °C for 4 additional days (reproduced from ref. 3, with permission). Similar CD spectra were obtained upon dilution of the solutions used in the Mössbauer experiments (see Table I Experimental).

CD titrations indicated a predominant molar ratio of 1 between FP and Qd in the complex. Ultracentrifugation measurements of solutions of both complexes **A** and **B** indicate the formation of very large aggregates. An interpretation of the extremely large optical activity observed was based on optical interactions between molecules of FP arrayed chirally in macromolecular aggregates, the chirality being imparted by the Qd molecules [2, 3]. The data obtained in the present work are considered to support the proposed structure of the complex aggregate.

Experimental

Hemin (chloroferriprotoporphyrin IX) was obtained from Sigma (Type I). Stock solutions of FP (8×10^{-3} M) in 0.1 N aqueous sodium hydroxide were prepared. The light-absorption spectra in the visible and Soret regions were checked after dilution in 0.02 N sodium hydroxide.

(+)-Quinidine sulfate was purchased from Sigma. Stock solutions of 30–35 mg in 5 ml distilled water,

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†Abbreviations: FP, ferriprotoporphyrin IX (ferriheme); Qd, quinidine; FP 11, FP at pH 11–12, etc.; CD, circular dichroism; TPP, tetraphenylporphine.

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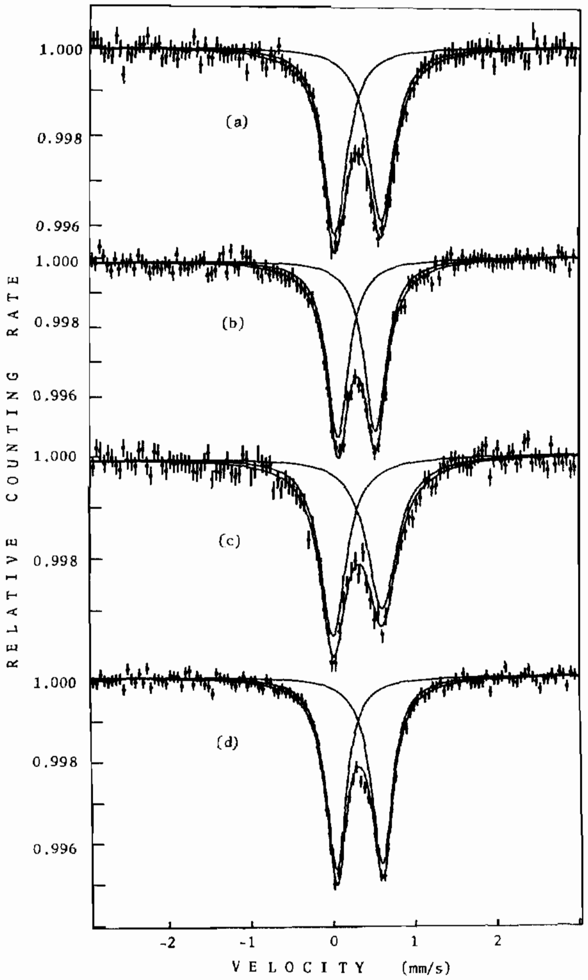

Fig. 2. Mössbauer spectra of FP both in the presence and absence of Qd at 4.1 K. Solutions were prepared as given for Table I. (a) FP only, pH 11.6–11.9; (b) FP–Qd, pH 11.8; (c) FP only, pH 9; (d) FP–Qd, pH 9.

containing 10 μl of 1 N hydrochloric acid were prepared. The light-absorption spectra in the near ultraviolet region were checked [2].

Circular dichroism was measured on a Cary Model 60 recording spectropolarimeter equipped with a Model 6002 accessory for CD measurements [2]. For the measurements, part of the solutions were diluted at constant pH and ionic strength to 1.0×10^{-4} M FP after completion of the reaction at times indicated in the caption to Table I.

Mössbauer measurements were performed using a conventional constant acceleration Mössbauer spectrometer [4] together with a 100 mCi ^{57}Co in rhodium source at room temperature and a Harwell proportional counter. Mössbauer spectra were obtained with the samples held in a liquid nitrogen cryostat at 90 K and in a liquid helium cryostat at 4.1 K.

Solutions of FP and FP–Qd were prepared as described elsewhere, using correspondingly higher concentrations [1–3]. The solutions were quickly transferred in dim light into lucite sample holders, sealed and immediately placed in liquid nitrogen. Some of the samples were transferred to the holders in a glove box in a nitrogen atmosphere. No difference between the samples prepared in either way could be detected.

Results and Discussion

The spectra obtained for the FP and FP–Qd samples at 4.1 K are shown in Fig. 2. All spectra at 4.1 K are composed of two well-defined, relatively narrow lines which constitute a quadrupole doublet. The FP–Qd 9 and FP–Qd 11 spectra are symmetric. The FP 9 spectrum displays an asymmetric doublet

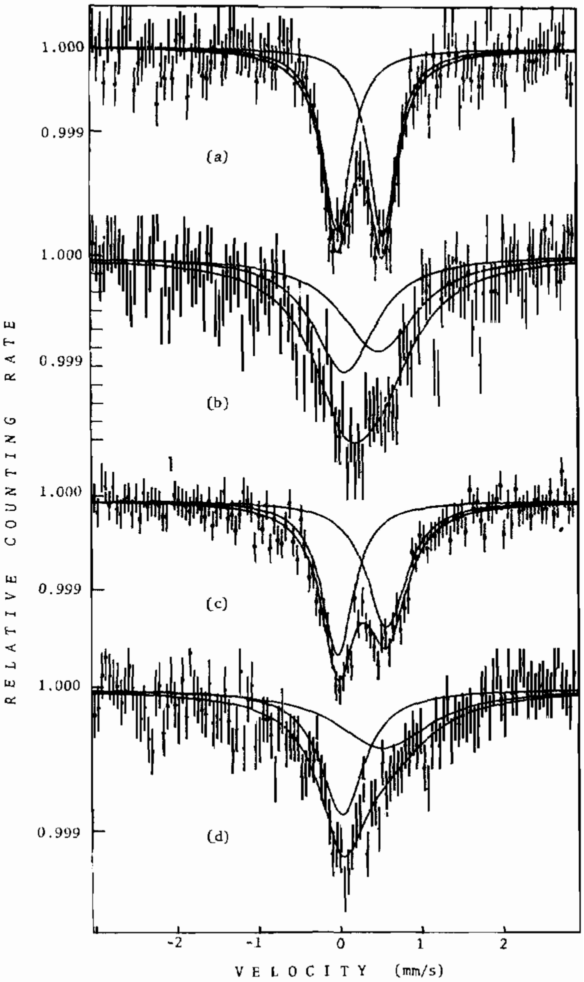

Fig. 3. Mössbauer spectra of FP both in the presence and absence of Qd at 90 K. Solutions prepared as given for Table I. (a) FP only, pH 11.6–11.9; (b) FP–Qd, pH 11.8; (c) FP only, pH 9; (d) FP–Qd, pH 9.

TABLE I. Mössbauer Parameters of FP both in the Presence and Absence of Qd at two Different Temperatures (4.1 and 90 K)[a]

System	pH	T (K)	Γ_1[b] (mm/s)	Γ_2[c] (mm/s)	Δ[d] (mm/s)	δ[e] (mm/s)
FP	11–12	90	0.41(2)	0.40(2)	0.56(1)	0.38(1)
FP	9	90	0.46(4)	0.58(7)	0.59(3)	0.38(3)
FP–Qd	11–12	90	0.9(1)	1.1(2)	0.45	0.38
FP–Qd	9	90	0.65(7)	1.4(2)	0.55	0.38
FP	11–12	4.1	0.34(1)	0.37(1)	0.56(1)	0.45(1)
FP	9	4.1	0.38(1)	0.44(1)	0.60(1)	0.44(1)
FP–Qd	11–12	4.1	0.32(1)	0.32(1)	0.46(1)	0.44(1)
FP–Qd	9	4.1	0.30(1)	0.31(1)	0.56(1)	0.44(1)

[a] Solutions: FP, 4.0×10^{-3} M; Qd, 8.0×10^{-3} M; NaCl, 0.05 M. For measurements at pH 9, 0.01 M sodium carbonate was included. The FP–Qd system was kept at 26 °C and pH 11.8 for 10 days until frozen in liquid nitrogen. Part of this solution was brought to pH 9 after 6 days from preparation and kept at this pH at 26 °C for another 4 days until frozen. By this procedure, complex B was formed [3]. FP in the absence of Qd was kept at 26.0 °C and pH 11.6–11.9 for 6 days until frozen. Part of this solution was brought to pH 9 after 3 days and kept at this pH at 26 °C for another 3 days until frozen. Another sample of FP only was kept at pH 11.5 and 26 °C for 15 days until frozen. Another series of analogous experiments at pH 11.3–11.5 and 9, respectively, gave similar results. [b] Γ_1: FWHM (full width at half maximum) of left line of the doublet. [c] Γ_2: FWHM of the right line of the doublet. [d] Δ: $eqQ/2$, the quadrupole splitting. [e] δ: the isomer shift relative to iron metal at room temperature. The numbers in brackets are the errors in the last figure; where no errors are given, the numbers were taken as fixed parameters as explained in the text.

and the FP 11 spectrum is slightly asymmetric. The Mössbauer parameters obtained from computer fits to the experimental spectra are summarized in Table I. The spectra were fitted with two independent lines. The width of each line, the distance between the lines (the quadrupole splitting), the isomer shift, and the ratio between the areas of the two lines were taken as free parameters. The isomer shifts obtained are identical for all samples. There are slight differences in the quadrupole splittings. The quadrupole splitting in FP–Qd 9 is slightly smaller than that in FP 9, whereas the splitting in FP–Qd 11 is significantly smaller than in all other samples. The splitting in both FP and FP–Qd at pH 11 is somewhat smaller than that in the respective samples at pH 9.

The spectra obtained at 90 K are shown in Fig. 3. At this temperature, the spectra of FP at both pH 9 and pH 11 are again composed of two well-defined, relatively narrow lines which constitute a quadrupole doublet. The FP 11 spectrum is symmetric, whereas the FP 9 spectrum again displays an asymmetric doublet, with somewhat broader lines than those observed at pH 11. No such well-defined doublets can be seen in the FP–Qd spectra. The observed FP–Qd spectra are composed of very broad lines and it is impossible to fit them with two lines in an unambiguous way. For fitting these spectra with two lines, the quadrupole splittings were assumed to be identical to those found at 4.1 K in the same samples and the isomer shifts were assumed to be the same as in the FP samples at 90 K (as the isomer shifts at 4.1 K were identical in the FP and FP–Qd samples); only the line width of the two lines, supposedly composing a doublet, as well as the ratio between the areas of the two lines, were left as free parameters. In this way the parameters summarized in Table I were obtained. Within the accuracy of the measurements, the areas of the two lines composing the doublet were equal for all samples.

The spectra obtained for the FP samples under the conditions given and the parameters derived from them are very similar to those found in oligomeric FP, which are considered to be of the μ-oxo type [5]. In this structure, two iron ions are antiferromagnetically coupled and thus do not display relaxation spectra, as found in monomeric hemin [6]. The asymmetry seen in the FP 9 spectra might be due to incomplete antiferromagnetic coupling between adjacent iron ions and the spectra thus display some temperature independent magnetic relaxation. The spectra obtained for the FP–Qd samples and their temperature dependence are very similar to those found in ferric protoporphyrin chloride (or rather Fe(TPP)Cl) [7]. These latter spectra have been interpreted as slow spin–spin relaxation at 90 K and fast relaxation at 4.1 K, where only the ±1/2 states are occupied [6, 7]. It thus seems that the quinidine disrupts the FP oligomer (dimer) and increases the iron–iron distance in the FP–Qd samples (complexes **A** and **B**). This rupture does not change the isomer shift or the s-electron density at the iron nuclei. It changes somewhat the symmetry around the iron nuclei, and thus the quadrupole splitting in both FP–Qd 11 and FP–Qd 9 (complexes **A** and **B**) is smaller than the quadrupole splitting in FP 9 and FP 11, respectively. In all samples the iron is in a high-spin trivalent state. The suggested increase in the iron–iron distance in the samples containing Qd is also consistent with regular alternation in 1:1 FP–Qd complex aggregates, postulated previously on the basis of optical (CD) data [1, 3]. Also, the high-spin ferric state in the complexes is in agreement with the absence of base coordination to the iron, as previously observed by light absorption [1, 2].

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