Volume 267, number 2, 285–288 FEBS 08619 July 1990

Modelling heme d_1

The spectral properties of copper(II) porphyrindiones

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Received 16 March 1990; revised version received 18 May 1990

The heme d_1 macrocycle of *Ps. aeruginosa* dissimilatory nitrite reductase is an iron porphyrin-3,8-dione with a 17-acrylate substituent. We have compared the RR properties of Cu-d_1, the copper(II) TME of extracted heme d_1, with those of models that differ only with respect to the acrylate: Cu-17-acrylate-mesoporphyrin-3,8-dione (2) and Cu-mesoporphyrin-3,8-dione (3). The RR spectrum of Cu-d_1 is very similar to that of 2, including ν(C=O) at ~1720 cm^{-1}. Replacement of the acrylate with propionate changes the spectrum markedly. For example, the ν(C=O) mode of 3 shifts to 1712 cm^{-1}, and peaks of Cu-d_1 and 2 at ~1400 and ~1535 cm^{-1} are shifted or absent from the spectrum of 3. FTIR spectra of 2 and 3 also differ in their ν_{oxo}(C=O) frequencies. The acrylate thus has a surprisingly strong influence on the electronic structural and spectral properties of heme d_1. These data provide a foundation for studies of the novel biological porphyrindione macrocycles.

Nitrite reductase; *Pseudomonas aeruginosa*; Heme d_1; Resonance Raman; Porphyrin; Saturated porphyrin; Hydroporphyrin

1. INTRODUCTION

Pseudomonas aeruginosa exemplifies bacteria that catalyze 'dissimilatory' reduction of soil nitrites to N_2O (and ultimately to N_2) via cytochrome *cd*$_1$ [1]. Because the optical spectra of heme d_1 and the heme *d* chlorin of *Escherichia coli* are similar [2], d_1 was also presumed to be an iron chlorin [2–4]. However, Chang [5] recently proposed that heme d_1 was an iron porphyrindione (dione, **1**, Fig. 1). This structure is strongly supported by optical and NMR spectra of model diones [6] and confirmed by synthesis [7]. A new synthetic route to these macrocycles has recently been reported [8].

Redox potentials of diones are comparable to those of the 'parent' porphyrins [9], whereas siroheme iBCs from 'assimilatory' nitrite reductases [10] are much more easily oxidized than porphyrins [9]. X-Ray crystallography of diones [8,11] indicates a more planar macrocycle than occurs for S_4-ruffled iBCs. Thus, a porphyrindione is not simply an iBC with oxo substituents.

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Abbreviations: iBC, isobacteriochlorin; RR, resonance Raman; FTIR, Fourier transform infrared; TME, tetramethylester; Cu-d_1, the copper(II) TME of *P. aeruginosa* heme d_1; THP, tetrahydroporphyrin

Resonance Raman and FTIR spectroscopy are valuable methods for structural analysis of saturated porphyrins [12]. In this paper, we compare RR properties of Cu-d_1, the copper(II) TME of heme d_1 extracted from *P. aeruginosa*, with those of copper(II)dione models (Fig. 1): **2** [copper(II)-2,7-diethyl-3,8-dione-17-(2-methoxycarbonylethenyl)-2,7,12,18-tetramethyl-13-propionate methylester] and **3** [copper(II)-2,7-diethyl-3,8-dione-2,7,12,18-tetramethyl-13,17-dipropionate methylester]. RR spectra of Cu-d_1 and model complex **2** are very similar to one another but distinct from that of **3**. FTIR spectra of **2** and **3** also differ. These data illustrate the strong effect of the acrylate moiety on the electronic structural and spectral properties of heme d_1.

2. MATERIALS AND METHODS

Free base heme d_1 TME from purified *P. aeruginosa cd*$_1$ nitrite reductase was prepared as described previously [4]; synthesis of metal-free analogues of **2** and **3** followed published methods [5,6]. Copper(II) insertion was according to previous reports [12]. FTIR spectra of **2** and **3** were obtained on a Perkin-Elmer Model 1800. Raman spectra were obtained with a computer-controlled Jarrell-Ash scanning spectrophotometer [13] using a Spectra-Physics 164-05 Ar ion laser [12].

3. RESULTS AND DISCUSSION

FTIR and RR spectral features specific to chlorins primarily derive from decreased effective symmetry between porphyrins (D_{4h}) and chlorins (C_2) [12,14].

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00145793/90/$3.50

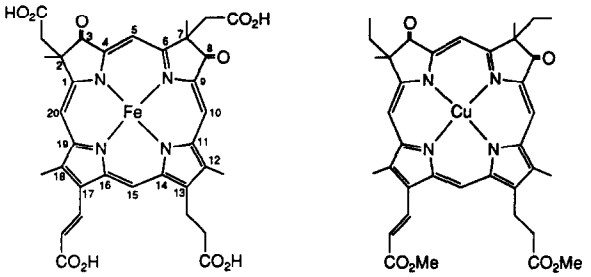

Fig. 1. **1**, heme d_1 of *P. aeruginosa*; **2**, Cu-acrylate-mesodione; **3** is the same as **2**, with propionate substituted for the 17-acrylate.

Much less is known about the vibrational properties of tetrahydroporphyrins (THP) such as iBCs or diones, although RR studies of sirohemes [15,16] and cytochrome cd_1 reductases [17,18] have been reported. For the latter, a 1715–1720-cm^{-1} band in the RR was proposed as ν(C=O) of a keto group on the (then) presumed chlorin of heme d_1 [17,18].

3.1. *Predicted vibrational features of diones*

Diones, like siroheme iBCs, maximally have C_2 symmetry. Their spectra should thus be analogous to those of chlorins and iBCs, yet distinct from those of porphyrins: e.g. a loss of mutual exclusion between RR and FTIR spectra is expected [12,14]. New RR and FTIR bands are expected from the 3,8-oxo groups and from the 17-acrylate of Cu-d_1 and **2**. The latter, known to be conjugated as judged from electronic absorption spectra [19], introduces both ester and olefin modes. An acrylate is not a common substituent of biological tetrapyrroles; its only other occurrence is on the D-ring of chlorophylls *c* [20].

3.2. *RR and FTIR spectra of Cu-*d_1 *and of model diones*

RR spectra of Cu-d_1, **2**, and **3** are shown in Fig. 2; FTIR spectra of **2** and **3** are shown in Fig. 3. Raman spectra of isolated d_1 or model diones have not been reported previously. A significant RR feature of both Cu-d_1 and **2** is the broad band at ~1720 cm^{-1}, assigned as the accidentally degenerate ν(C=O) mode of the two keto groups and the acrylate ester moiety. This band occurs at 1718 cm^{-1} in the FTIR spectrum of **2**, concurring with ν(C=O) of methyl acrylate or cinnamic acid ethyl ester [21]. Loss of the acrylate, as for **3**, results in a downshift of the RR ν(C=O) mode to 1712 cm^{-1}, and the FTIR spectrum of **3** (Fig. 3) now has a doublet at 1714 and 1704 cm^{-1}. The ~1740 cm^{-1} FTIR band of **2** and **3** is ν(C=O) of the non-conjugated propionate methyl ester group(s); these vibrations are silent in RR spectra.

The ν(C=C) of an acrylate is expected at ~1635 cm^{-1} [22] and may correspond with the weak

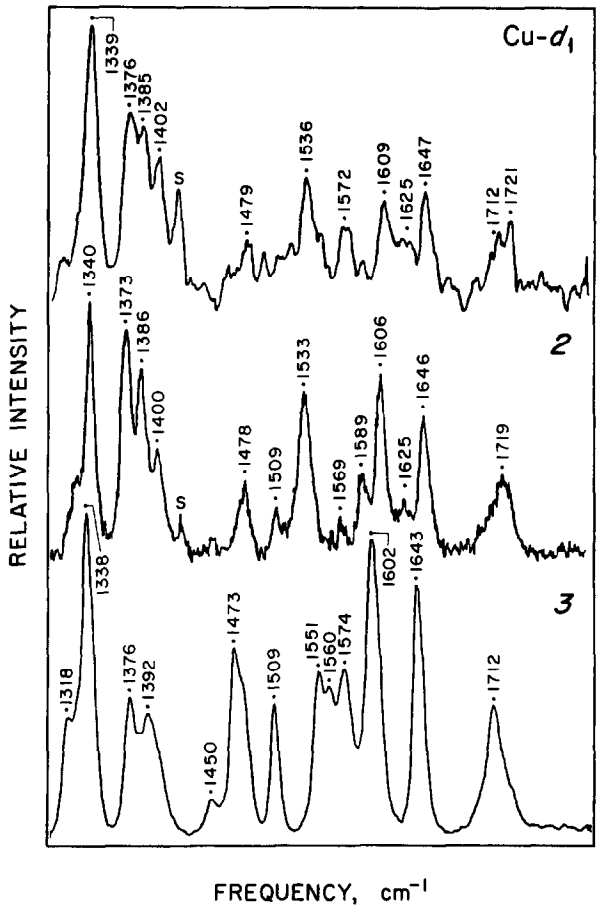

Fig. 2. RR spectra of Cu-d_1; **2**, Cu-acrylate-mesodione; and **3**, Cu-mesodione. Conditions: Cu-d_1, ~0.5 mg/ml in CH_2Cl_2; **2** and **3** ~1 mg/ml in CH_2Cl_2; anaerobic samples in melting-point capillaries, ~2°C, sample Dewar [11]; 457.9 nm excitation, 50 mW; backscattering; scan speed, 2 $cm \cdot s^{-1}$, repetitive scanning. The RR spectrum of **3** in a ~1:100 KBr matrix is identical to these data.

FTIR band of **2** at 1630 cm^{-1} and the surprisingly weak ~1625 cm^{-1} RR bands of Cu-d_1 and **2**. Conjugated substituents such as vinyls and formyls often exhibit low intensity. These features are absent from FTIR and RR spectra of **3**.

Other differences between the RR spectra of Cu-d_1 and **2**, and of complex **3**, occur in the ~1505–1550 cm^{-1} region. RR spectra of Cu-d_1 and **2** exhibit a 1536 cm^{-1} feature with a weak ~1509 cm^{-1} band. In contrast, for complex **3**, the 1536 cm^{-1} band is absent and there is a strong band at 1509 cm^{-1}, along with a new group of bands at ~1550 cm^{-1}.

The 1300–1410 cm^{-1} region of the RR spectra of Cu-d_1, **2**, and **3** has multiple bands dominated by a strong feature at ~1340 cm^{-1}. This pattern concurs with that of cytochrome cd_1 reductases [17,18]. (The ~1340 cm^{-1} band in the data of Ching et al. [18] is obscured by a plasma line.) Cotton et al. [17] suggested that the ~1342 cm^{-1} band of d_1 was the oxidation-state-sensitive band. Multiple RR bands in the

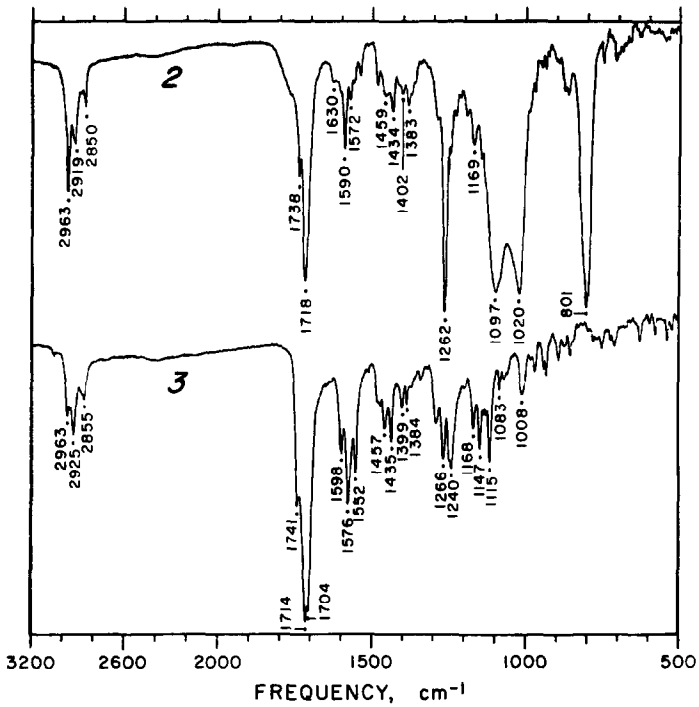

Fig. 3. FTIR spectra of the porphyrindiones in ~1:100 mg KBr pellets: 2, Cu-acrylate-mesodione; and 3 Cu-mesodione.

~1300–1410 cm^{-1} region are also seen for siroheme iBCs [15,16] and bacteriochlorophylls [23]. This complex pattern thus appears characteristic of THPs as well as chlorins [12,14]. However, the most intense band in this region is at ~1400 cm^{-1} for iBCs [15,16,24] vs ~1340 cm^{-1} for the diones in this study and for the cd_1 reductases [17,18]. We are investigating the diagnostic utility of this difference.

A strong ν(C–O) IR mode of an acrylate is expected at ~1310–1250 cm^{-1} [22], and is observed in the FTIR spectrum of **2** at 1262 cm^{-1} vs 1269 cm^{-1} for methyl acrylate and 1257 cm^{-1} for cinnamic acid methyl ester [22]. The FTIR band of **2** at 801 cm^{-1} is similar to that of methyl acrylate at 812 cm^{-1} [21], and is typical of a *cis* olefin conjugated with a carbonyl (~820 cm^{-1} for –CH=CH–COOR) [22]. These bands are also absent from the FTIR spectrum of **3**.

4. CONCLUSIONS

The 17-acrylate moiety of heme d_1 and model diones has a significant effect on their vibrational spectra, demonstrating participation of the acrylate in the electronic structural properties of the macrocycle. RR spectra of diones have multiple bands in the ~1300–1410 cm^{-1} region, as observed for siroheme iBCs and bacteriochlorophylls. This property of chlorin RR spectra is thus generalizable for THPs. Work is in progress to identify vibrational features that distinguish chlorins from tetrahydroporphyrins, and iBCs from diones.

How the keto groups of heme d_1 function in dissimilatory nitrite reduction is not yet known. Indeed, *Paracoccus halodenitrificans* cd_1 will catalyze both dissimilarity *and* assimilatory nitrite reduction [25]. This suggests the versatility of biological porphyrindiones and emphasizes the need for further studies of this novel macrocycle.

Acknowledgements: This work was supported by the National Institutes of Health (GM 34468 to T.M.L., C.K.C. and L.A.A., GM 36520 to C.K.C., and GM 36264 to R.T.).

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