Analysis of the optical absorption and magnetic-circular-dichroism spectra of peanut peroxidase: electronic structure of a peroxidase with biochemical properties similar to those of horseradish peroxidase

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The electronic structures of the cationic isoenzyme of peanut peroxidase, horseradish peroxidase (isoenzyme C) and bovine liver catalase are compared through analysis of their optical absorption and magnetic c.d. (m.c.d.) spectral properties. The spectral data for the native resting states and compounds I and II of peanut peroxidase (PeP) are reported. The absorption and m.c.d. data for the native PeP exhibit bands characteristic of the high-spin ferric haem. The absorption spectrum of PeP compound I closely resembles that observed for the HRP compound I species. The m.c.d. data for PeP I clearly identifies that ring oxidation has occurred. One-electron reduction forms the PeP compound II species. The absorption and m.c.d. spectra recorded for PeP II exhibit the well-resolved spectral characteristics previously observed for both HRP compound II and catalase compound II. The spectral data of PeP with HRP and catalase

are compared. The data clearly indicate that the m.c.d. spectral patterns of both plant peroxidases (PeP and HRP) are very similar and, therefore, the electronic structures of their resting states, and as well their primary and secondary compounds, must be similar. The m.c.d. data suggest that, while the compound I species of PeP and HRP belong to one electronic class, catalase compound I belongs to a different class. These data emphasize how the ground states of these two classes of oxidized haem, may be characterized as predominantly $^2A_{2u}$ (PeP I and HRP I) or $^2A_{1u}$ (catalase I). Peanut peroxidase is the second plant peroxidase for which the electronic structure of the compound I intermediate has been studied using the m.c.d. technique. The similarities with horseradish peroxidase allow us to suggest that plant peroxidases may operate by the same general mechanism, in spite of the low degree of sequence similarity between their polypeptide chains.

INTRODUCTION

The catalases and peroxidases are enzymes that contain non-covalently bound iron protoporphyrin IX as their prosthetic group (Dawson, 1988). Both enzymes react with H_2O_2 to form the catalytic intermediate, compound I (Dunford and Stillman, 1976). This green intermediate is the highest oxidation state available for the ferric haem group and contains two electrons less than the resting ferric haem. The chemical, biochemical and spectroscopic properties of the compound I species of both catalases and peroxidases has long been the subject of detailed studies (Dawson and Dooley, 1989). For catalase, the catalytic reaction proceeds in two single steps, first oxidation of the ferric haem by H_2O_2 , then reduction of the haem by a second molecule of H_2O_2 to form water and O_2 , according to the following scheme:

The enzymically significant reactions of horseradish peroxidase follow a similar path for the first step. Compound I is formed by reaction with one molecule of H_2O_2 . However, compound I in peroxidases undergoes a one-electron reduction to generate compound II, which further catalyses the oxidation of a number of organic substrates (primarily amines and phenols) to regenerate the resting enzyme, according to the following scheme:

The spectral properties of these species have been studied extensively in order to interpret the role of the electronic structure of the iron and the porphyrin ring in the observed enzymatic chemistry (Strickland et al., 1968; Fajer et al., 1973; Stillman et al., 1975, 1976; Dunford and Stillman, 1976; Morishima and Ogawa, 1978; Browett and Stillman, 1979, 1980, 1981a; Dolphin, 1981; Hanson et al., 1981; Teraoka et al., 1982; Browett et al., 1983a,b; Sievers et al., 1983; Fujita et al., 1983; Ohlsson et al., 1984; Oertling and Babcock, 1985; van Wart and Zimmer, 1985; Penner-Hahn et al., 1986; Foote et al., 1987; Oertling et al., 1987; Ogura and Kitagawa, 1987; Browett et al., 1988; Gasyna et al., 1988a; Dawson et al., 1991; Loew et al., 1991; Du et al., 1991; Du and Loew, 1992). The absorption spectra of HRP I and catalase I have long puzzled spectroscopists, as these spectra are quite unique. Slight differences in the absorption spectra between HRP I and catalase I were interpreted (Dolphin et al., 1971) in terms of differences in the orbital ordering for the π -cation radicals that comprise electronic configurations of the compound I haem groups. Dolphin's (1971) assignment of the electronic structure of compound I of HRP and catalase involves oxidation of both the ring and the metal to a species that can be written as $[Fe(IV)P(-1)]^{3+}$ (using current nomenclature) to describe the porphyrin charge, where $P(-2)$ is the dianion and $P(-1)$ is the radical cation. Compound II then involves reduction of the ring to $[Fe(IV)P(-2)]^{2+}$. The m.c.d. spectra recorded for these intermediate species unambiguously confirmed that the site of oxidation included the ring by comparison of the m.c.d. spectra

Abbreviations used: PeP, peanut peroxidase; PeP I, PeP compound I; PeP II, PeP compound II; HRP, horseradish peroxidase; HRP I, HRP compound I; HRP II, HRP compound II; m.c.d., magnetic c.d.; OEP, octaethylporphyrin; TPP, tetraphenylporphyrin.

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Figure 1 Scheme of the proposed energies of the top two filled molecular orbitals in porphyrin cation radical species

Orbitals for five compounds are shown. MgOEP(-1) and ZnTPP(-1) exhibit m.c.d. spectra characteristic of $^2A_{1u}$ and $^2A_{2u}$ ground states respectively (Gasyna and Stillman, 1990). In both cases the two top-filled orbitals that are essentially degenerate for the neutral ZnTPP(-2) split apart in the radical cations. Transitions between the highest occupied and lowest unoccupied molecular orbitals in the cations are overlaid by the partially filled, highest occupied orbitals, shown by arrows. The orbital ordering for the compound I species of catalase and HRP/PeP is based on the characterization of the m.c.d. spectral features discussed in the text.

Figure 2 Nodal-plane patterns for the $^2A_{1u}$ and $^2A_{2u}$ ground-state configurations of the porphyrin π cation radical species

M, methine-bridge substitution as in TPP; P, substitution at the periphery, as in OEP. Regions with dark shading indicate phases opposite those regions with light shading. Data taken from Gouterman (1978).

of a number of model compounds with those of the enzyme intermediates (Browett and Stillman, 1980, 1981b; Browett et al., 1988).

Many techniques have been applied to these remarkable enzymes in attempts to define the electronic structure more completely. Together, the results from these studies have sup-

ported the electronic configuration as a combination of an oxidized porphyrin ring, P(-1), and ferryl iron, Fe(IV), for the compound I species of HRP and catalase. In the peroxidatic reaction that forms compound II, the absorption and m.c.d. spectra clearly and unambiguously show that the reduction takes place on the ring leaving the Fe(IV) to be reduced in the last step (Browett and Stillman, 1980). Porphyrins with a different pattern of substitution have been synthesized as models for these proposed structures; these include, most commonly, tetraphenylporphyrin (TPP) and octaethylporphyrin (OEP). From studies of the spectral data of these model compounds, it appears that there is indeed a difference in the ordering of the highest occupied molecular orbitals for the π -cation radical species. Optical data for TPP and OEP complexes can be clearly interpreted in terms of either a $^2A_{1u}$ or a $^2A_{2u}$ ground state (Dolphin, 1971; Browett and Stillman, 1981a). While the m.c.d. data for different ring-oxidized porphyrins easily allow characterization of either a $^2A_{1u}$ or $^2A_{2u}$ ground state (Gasyna and Stillman, 1990), the differences observed for HRP I and catalase I are not nearly as well marked (Browett et al., 1988). We have most recently concluded that the $^2A_{2u}$ and $^2A_{1u}$ states must lie close in energy so that a greater component of either $^2A_{2u}$ for HRP I or $^2A_{1u}$ for catalase I is actually seen in the optical spectrum (Browett et al., 1988). A scheme explaining the proposed energies of these two states is shown in Figure 1. Semiempirical molecular-orbital calculations (Fajer and Davis, 1979; Loew and Herman, 1980; Hanson et al., 1981; Edwards and Zerner, 1985; Loew et al., 1991) and e.p.r. experiments performed on the enzymes and on porphyrin model compounds (Peisach et al., 1968; McIntosh and Stillman, 1977) indicate that, in the $^2A_{2u}$ state, the spin density is placed on the *meso* positions, while in the $^2A_{1u}$ state the spin density is primarily located on the pyrrole α C atoms (Fajer and Davis, 1979). These differences are illustrated in Figure 2.

In the present study we analysed the u.v.-visible absorption and m.c.d. spectra of two plant peroxidases and compare them with the electronic structure of catalase. Up to now, no other peroxidase for which detailed biochemical and structural data are available has been studied by absorption and m.c.d. spectroscopy to allow an estimation of how significant are the differences between compounds I of catalase and peroxidase first described by Dolphin et al. (1971). Our results show that the electronic structure of the peanut peroxidase compound I can be explained as a ring-oxidized Fe(IV) π -cation radical with a predominantly $^2A_{2u}$ ground state.

MATERIALS AND METHODS

Purity index of horseradish peroxidase

Horseradish peroxidase, isoenzyme C (HRP, Boehringer Mannheim, lyophilized, grade 1) was dissolved in triply distilled water. The A_{403}/A_{280} ratio for this solution was 3.3.

Isolation and purification of peanut peroxidase cationic isoenzyme

Peanut cells, derived from cotyledonary slices, were cultured in a liquid medium biweekly for over a decade. The macromolecular components of the spent culture medium at the end of cell growth were precipitated by 70% (v/v) acetone. The pellet was resuspended in 0.05 M phosphate buffer, pH 7.0, and the insoluble material removed by centrifugation. The 15000 g supernatant was adjusted to 80%-satd. $(\text{NH}_4)_2\text{SO}_4$ and the precipitate resolubilized in the phosphate buffer. The protein solution was dialysed against 3×250 ml of 0.01 M acetate buffer,

pH 5.0, for at least 24 h, following which the flocculated material was removed by centrifugation (15000 *g*, 10 min).

Approx. 20 mg of the dialysed protein was placed on a 0.01 M acetate (pH 5.0)-equilibrated 2 cm × 15 cm column of carboxymethylcellulose (CM). The sample was washed on the column with 100 ml of 0.01 M acetate buffer, pH 5.0. Then a continuous gradient from 0.01 to 0.25 M acetate, pH 5.0, was used to isolate the peanut peroxidase cationic isoenzyme from the column. The isoenzyme C was eluted at 0.05 M acetate buffer (van Huystee, 1991). The purity of the cationic isoenzyme was determined by SDS/7.5%-PAGE. The A_{405}/A_{280} ratio for the purified protein solution was 3.0.

The peroxidase solutions were pretreated with H_2O_2 (1.2:1 molar ratio of H_2O_2 to protein) and left at room temperature for 12–24 h before use. A second 1.2:1 molar ratio aliquot of H_2O_2 was added to the solution at 273 K to form the compound I species. HRP compound II was prepared by the addition of 2 molar equivalents of H_2O_2 to a solution of HRP already containing 2 mol.-equiv. of *p*-cresol at pH 10.

Purity index of bovine catalase

Bovine liver catalase was obtained from Boehringer Mannheim as a crystalline suspension in 0.01% alkylbenzyltrimethylammonium chloride and prepared in an 0.05 (pH 7) phosphate buffer as previously reported (Browett and Stillman, 1979). The purity of the native catalase samples was measured in terms of the ratio of the Soret and band absorbance of the protein ($A_{405}/A_{276} = 0.88$). Compound I of catalase was obtained by adding a 50-fold excess of peroxyacetic acid to a solution cooled to 2 °C. Conversion of compound I into compound II was achieved by the addition of a second 50-fold excess of peroxyacetic acid after recording the spectrum of compound I (Browett and Stillman, 1979).

Spectroscopy

The compound I and II intermediates were formed by rapid mixing of solutions held at ice temperature. The absorption spectra were obtained by placing the cuvette into a thermostatically controlled cell holder held at 1 °C inside a Cary 219 spectrometer. M.c.d. spectra were obtained by placing a second sample into a cooled liquid sample cell within the room-temperature bore of an Oxford Instruments SM2 magnet that was mounted in a c.d. spectrophotometer built in this laboratory and based on a Cary 14 monochromator. The m.c.d. spectra were recorded at a nominal temperature of 10 °C. All spectra shown here were automatically digitized as they were recorded, and have had the appropriate c.d. baselines subtracted.

RESULTS

We show here the absorption and m.c.d. spectra of peanut peroxidase at pH 7 (Figure 3), the compound I and II intermediates (Figures 4–7), HRP compound I (Figure 8) and catalase compound I (Figure 9). Native peanut peroxidase at pH 7 exhibits a typical high-spin ferric haem spectrum (Figure 3). The appearance of bands at 640 nm and the weak m.c.d. signal under the Soret band at 403 nm are characteristics of high-spin ferric haems (Browett and Stillman, 1979; Eglinton et al., 1983; Dawson and Dooley, 1989). Peanut peroxidase binds the usual range of ligands to give absorption and m.c.d. spectra typical for

(i) low-spin ferrous haem, (CO)Fe(II)PeP, (ii) low-spin ferric haem, (CN⁻)Fe(III)PeP and (iii) high-spin ferric haem with fluoride at pH 5, (F⁻)Fe(III)PeP (results not shown; M. J. Rodríguez Marañón, D. M. Mercier, R. B. van Huystee and M. J. Stillman, unpublished work). Addition of 1.2 mol.-equiv. of H_2O_2 at 0 °C rapidly converts the brown native enzyme into the olive-green compound I intermediate. At 0 °C the intermediate is reasonably stable over periods of 30–45 min. Figure 4 shows three spectra recorded over a 2 h period. As with HRP I, even in the absence of a specific electron donor, these compound I species will scavenge electrons and slowly reduce to compound II, and subsequently to the native enzyme. This conversion takes over 24 h under the conditions used to record these spectra. The isosbestic points and band maxima seen in Figure 4 are typical for the compound-I-to-II change, being observed also for HRP (Browett et al., 1983a). The m.c.d. spectra were recorded within the first 30 min following preparation, so we anticipate the formation of some compound II impurity.

Figure 3 Absorption and m.c.d. spectra of peanut native Fe(III) peroxidase at pH 7.0

Figure 4 Absorption spectra recorded at 0, 32 and 135 min for the decay of the compound I intermediate of PeP at 0 °C following formation with 1.2 mol.-equiv. of H_2O_2

Figure 5 Absorption and m.c.d. spectra of compound I of PeP at 0 °C formed by the addition of 1.2 mol-equiv. of H_2O_2 to native PeP

The numbers 1, 2, 3 and 4 indicate specific spectral features used in comparing the spectral data with that of HRP I and catalase I. The shaded region indicates spectral intensity assigned to PeP compound II that formed during the measurement.

Figure 6 Absorption and m.c.d. spectra of compound II of PeP recorded at 0 °C

Figure 5 shows the absorption and m.c.d. spectra for compound I of PeP. The absorption starts at 710 nm and continues until the peptide absorption below 250 nm. Analysis of the absorption and m.c.d. spectra show that a great many transitions make up the absorption envelope (Browett et al., 1988). Significant features are, starting at 700 nm: the negative band centred on 660 nm that leads to a series of overlapping bands to 450 nm, then the weak bands under the Soret band at 400 nm,

Figure 7 Absorption spectra of native, compound I and compound II of peanut peroxidase recorded at 0 °C

and finally the sharp bands associated with the aromatic amino acids in the peptide chain between 350 and 250 nm. The spectral feature of the negative band at 660 nm followed by the continuous, featureless series of positive bands to 450 nm is characteristic of ring oxidation (Fuhrhop and Mauzerall, 1968, 1969; Dolphin et al., 1973; Browett and Stillman, 1980, 1981a, 1981b; Dolphin, 1981; Calderwood et al., 1985; Gasyna et al., 1988b; Gasyna and Stillman, 1990; Mandon et al., 1992). In the presence of 1 mol-equiv. of reducing agent, compound II is formed following the addition of H_2O_2 . Figure 6 shows how reduction of the P(-1) ring back to P(-2) results in formation of a well-resolved spectrum. The m.c.d. spectrum provides the necessary evidence that a low spin iron species has formed with little interaction with the ring. The presence of A terms, derivative spectral features that are centred on the band maximum in the absorption spectrum, identifies the degeneracy in the α and Soret bands, as expected for a haem porphyrin with little interaction with the central iron(IV). Bands between 600 and 700 nm are assigned as charge transfer.

The absorption spectra for native PeP, compound I and compound II are assembled in Figure 7. The characteristic features observed for HRP are seen here too. In particular, the broad absorption band that stretches to 710 nm and very weak absorption at 400 nm for compound I, and the resolved pair of α and β bands, together with the red-shifted Soret band at 418 nm for compound II.

In Figures 8 and 9, we show the absorption and m.c.d. spectra for the two other enzymes for which these data are available, HRP I and catalase I (Browett and Stillman, 1980; Browett et al., 1983c). The significant feature in these spectra is the broadness of all the bands and the lack of any really resolved maxima, except for the Soret bands near 398 nm and the lowest-energy band near 660 nm. The m.c.d. spectrum for HRP I closely resembles that of PeP I. The set of weak overlapping bands starting at 460 nm culminates in the resolved negative signal at 650 nm. This spectral feature is observed generally for porphyrin-ring oxidation. The m.c.d. bands under the Soret band at 400 nm are weak, but well resolved. The m.c.d. spectrum for catalase compound I (Figure 8) is significantly different from the data of PeP I and HRP I in that the m.c.d. bands under the Soret band are so broad and featureless. The weakness in signal, the lack of resolution between 400 and 500 nm and the increased resolution and red-shift of the first band near 660 nm are characteristic of catalase compound I.

Figure 8 Absorption and m.c.d. spectra of compound I of HRP recorded at 0 °C

The numbers 1, 2, 3 and 4 indicate specific spectral features used in comparing the spectral data with that of PeP I and catalase I.

Figure 9 Absorption and m.c.d. spectra of compound I of bovine liver catalase recorded at 0 °C

The numbers 1, 2, 3 and 4 indicate specific spectral features used in comparing the spectral data with that of HRP I and PeP I. The asterisk (*) indicates spectral bands assigned to catalase compound II that formed during the measurement.

Similarly, these features are also typical of a radical cation with a $^2A_{1u}$ ground state.

DISCUSSION

The visible and u.v. regions in the electronic spectra of many neutral metalloporphyrins have been successfully described in terms of Gouterman's (1978) 'Four-Orbital' model. The two

highest filled π -molecular orbitals of metalloporphyrins are essentially degenerate and have a_{1u} and a_{2u} symmetries. Removal of an electron from either of these orbitals generates a π -cation radical with either the $^2A_{1u}$ or $^2A_{2u}$ ground state (Figure 1). In the neutral porphyrin complexes of diamagnetic metals, $^2A_{2u}$ is the ground state, the $a_{2u} \rightarrow e_g$ transition is responsible for the Q-band absorption near 560 nm and the $a_{1u} \rightarrow e_g$ transition gives rise to the B or Soret band absorption found near 410 nm. Transitions to these states will exhibit positive A terms in the m.c.d. spectrum. As a result of the 'accidental' degeneracy of the a_{1u} and a_{2u} molecular orbitals, configuration interaction is significant allowing intensity to be coupled from the 'allowed' B band to the formally 'forbidden' Q band. The ground state is $^2A_{1u}$ for tetra-azatetrabenzoporphyrins, the phthalocyanines, where the $a_{1u} \rightarrow e_g$ transition is responsible for the Q band absorption near 670 nm and the $a_{2u} \rightarrow e_g$ transition gives rise to the B band absorption found near 350 nm (Stillman and Nyokong, 1989). Very different spectral properties are found because of the different geometrical distribution of electron charge between these two orbitals.

Ring oxidation of synthetic metalloporphyrins based on the Phe₂P and OEP rings, clearly results in large differences in the optical spectrum (Browett and Stillman, 1981b). When $a_{1u} \rightarrow e_g$ is lowest in energy the spectrum is red-shifted and major changes are expected *throughout* the spectral region as the configuration interaction between the Q and B states will also change. When the lowest energy transition is $a_{2u} \rightarrow e_g$, this ordering reflects the usual porphyrin spectrum and we anticipate absorption spectra that differ mainly in the visible region due to the loss of the electron and from transitions into this half-filled orbital. The $a_{1u} \rightarrow e_g$ transition responsible for the Soret or B band is largely unaffected by the oxidation, and we anticipate that this band will be relatively unchanged. Thus the spectrum of the radical cations should reflect quite clearly which orbital is highest. The m.c.d. spectra of MTPP(-1) and MOEP(-1) complexes are dramatically different and can be interpreted as described above (Gasyana and Stillman, 1990).

Compound I, an iron-oxo (Fe=O) porphyrin complex, is the common catalytic intermediate in the biochemical cycles of peroxidases and catalases, both of which require 1 mol of H₂O₂ for its formation. Because the reaction involves a two-electron oxidation from a ferric haem, there has long been interest in the electronic structure of such a highly oxidized species that can be supported in aqueous solution. The overall shape of the spectral envelope in the u.v.-visible region has been taken as the most suitable discriminating criterion for determining which of the two possible electronic ground states of the π -cation radical species is present. However, it is the m.c.d. spectrum that best reflects the differences in the ground states of these two π -cation radical species. In previous work (Browett and Stillman, 1981a; Browett et al., 1988), we have noted that the m.c.d. spectra of neither catalase I nor HRP I are represented closely by either the TPP(-1) or OEP(-1) radical cation models. Indeed, the two enzyme intermediates appear to straddle the midpoint line between the two possible ground states, as shown in Figure 1.

Nevertheless, the optical spectra of the $^2A_{1u}$ and $^2A_{2u}$ ground states of simple ring-oxidized metalloporphyrins and those of the catalase and peroxidase primary compounds exhibit many similar features. In the m.c.d. spectra of all ring-oxidized porphyrins reported to date, a negative band at near 660 nm is followed by a large number of overlapping broad bands between 640 and 450 nm (Browett and Stillman, 1981a; Gasyana and Stillman, 1990). The m.c.d. envelope under the B or Soret band of the models is an excellent market for the $^2A_{2u}$ or $^2A_{1u}$ ground states. This means that the m.c.d. spectra unambiguously confirm that the electronic configurations of catalase and peroxidase in their

highest oxidation state are those of π -cation radicals [Fe(IV)-protoporphyrin]³⁺ species, where the overall charge is balanced by ligands provided by the protein. However, clearly, assignment of the m.c.d. spectral envelope in the B region is not easy. The availability of the m.c.d. spectrum for a second plant peroxidase, PeP, allows us to conclude that the compound I spectra of HRP and PeP are very similar and both significantly different from the m.c.d. spectrum of catalase I. Comparison of all of the data described above suggests that the HRP I and PeP I spectra more closely resemble the spectrum of MTPP(−1), which has the ²A_{2u} ground state. The catalase I spectrum exhibits the broad, weak Soret m.c.d. band and the red-shifted Q band associated with the ²A_{1u} MOEP(−1).

A number of attempts have been made to understand if this orbital ordering influences the stability of the radical cation once formed. In this way the orbital ordering in the compound I species would affect the reaction profile with substrates that reduce the HRP and PeP compound I to the compound II species or to the native enzyme from catalase I.

In the present paper, we have described m.c.d. spectral data that show that the environment of the haem in PeP I is essentially the same as for HRP I. Both horseradish peroxidase isoenzyme C and the cationic isoenzyme of peanut peroxidase have been isolated and purified. PeP has been extensively studied (van Huystee, 1991). The complete amino acid and nucleotide sequences are known for both peroxidases, and recently preliminary results of the X-ray-diffraction pattern to 0.28 nm (2.8 Å) resolution for the cationic isoenzyme of peanut peroxidase have been published (Ban et al., 1992). Even though the amino acid sequences belonging to peroxidases of higher plants are less than 50% similar (Welinder, 1985), a comparison of the amino acid sequence for HRP and PeP shows two regions of sequence similarity: the region for the acid-base catalysis and the fifth ligand of the haem (Buffard et al., 1990). A secondary-structure prediction shows a striking similarity in the relative distribution of the predicted helices of the peroxidases. These predicted secondary structures are in good agreement with the tertiary-structure model constructed by Welinder (1985) for cytochrome *c* peroxidase. The rates of compound I formation are remarkably similar for both HRP and PeP; in fact the differences are of the same order as variation between isoenzymes of the same species. It appears, therefore, that, in spite of some differences of reactivity between HRP and PeP, the active site of peroxidases has to fulfil very strict requirements to form compound I (Lambeir et al., 1985).

The three-dimensional structure of the catalase isolated from bovine liver has been described, and its X-ray-diffraction pattern has been studied to 0.25 nm (2.5 Å) (Reid et al., 1981). A tyrosine residue is found in the proximal side of the haem, its phenolic group occupying the fifth ligand position, while a small isolated region of electron density on the haem's distal side might be interpreted as a water molecule.

The absorption, c.d. and m.c.d. spectra of bovine liver catalase have been studied in detail (Browett and Stillman, 1980). Its primary compound is clearly predominantly ²A_{1u} in character and essentially different from the two plant peroxidases studied here: horseradish peroxidase isoenzyme C and peanut peroxidase cationic isoenzyme.

In conclusion, HRP and PeP compounds I possess spectra assigned to an ²A_{2u} electronic ground state characterized by the broad visible absorption between 530 and 650 nm and the well-resolved m.c.d. bands under the Soret band. Catalase compound I is assigned to an ²A_{1u} ground state π -cation radical, characterized for the intense long-wavelength peak and less intense peak at shorter wavelength.

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