Magnetic and Natural Circular Dichroism of L-Tryptophan 2,3-Dioxygenases and Indoleamine 2,3-Dioxygenase

II. SPECTRA OF THEIR FERRIC CYANIDE AND FERROUS CARBON MONOXIDE COMPLEXES AND AN OXYGENATED FORM*

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Both ferric cyanide and ferrous CO complexes of L-tryptophan 2,3-dioxygenases and an indoleamine 2,3-dioxygenase showed magnetic CD spectra similar to those of corresponding low spin complexes of myoglobin. However, intensities of their Soret magnetic CD were often unusually high as compared to those of other hemoproteins such as myoglobin and horseradish peroxidase. Upon addition of L-tryptophan, the high intensities decreased to an ordinary level, indicating that the substrate binding affects the electronic configuration of these low spin complexes. On the basis of these findings, we suggest that both the Fe-C-N and the Fe-C-O bonds, which are rather close to a linear configuration in the absence of L-tryptophan, became bent upon binding with the substrate. An oxygen complex of ferrous indoleamine 2,3-dioxygenase in the absence of L-tryptophan showed a magnetic CD spectrum analogous to those of oxyhemoglobin and compound III of horseradish peroxidase.

In the CD spectra, both cyanide and CO complexes of L-tryptophan 2,3-dioxygenases exhibited a strong negative extremum in the Soret region. Their intensities were not significantly affected by the addition of L-tryptophan except for a CO complex of the rat liver enzyme. On the other hand, CO and cyanide complexes of indoleamine 2,3-dioxygenase showed a couple of weak extrema which were converted to a single distinct extremum in the absence of L-tryptophan, became bent upon binding with the substrate. An oxygen complex of ferrous indoleamine 2,3-dioxygenase in the absence of L-tryptophan showed a magnetic CD spectrum analogous to those of oxyhemoglobin and compound III of horseradish peroxidase.

In the present study, we examined the MCD1 and CD spectra of ferric and ferrous low spin complexes of Pseudomonas and rat liver L-tryptophan 2,3-dioxygenases and of an indoleamine 2,3-dioxygenase from rabbit intestine. Effects of the organic substrate L-tryptophan on the spectra of these complexes were also studied. Each of three enzymes has been shown to form a ternary complex with L-tryptophan and oxygen as an obligatory intermediate of the reaction (2-5), in which the enzyme-heme is in a low spin ferrous state. The electronic structures of the low spin complexes in the presence and absence of L-tryptophan are therefore important for the elucidation of the reaction mechanisms. Our results suggest that their electronic structures are not greatly different from those complexes of myoglobin, but also that the binding of L-tryptophan to the enzyme significantly affects the bonding character between the heme and its ligands.

EXPERIMENTAL PROCEDURES

L-Tryptophan 2,3-dioxygenases were prepared from Pseudomonas acidovorans (ATCC 11299b) and from livers of male Wistar rat, and indoleamine 2,3-dioxygenase was prepared from rabbit intestine as described elsewhere (1, 6). The specific activities and the turnover numbers per heme of the enzymes were essentially the same as those described in the preceding paper (1). Ferric cyanide complexes were prepared by the addition of potassium cyanide to the ferric enzymes. Ferrous carbon monoxide complexes of the enzymes were obtained by reducing the ferric enzymes in CO-saturated phosphate buffer with sodium dithionate. The oxygenated form of indoleamine 2,3-dioxygenase was obtained by bubbling oxygen through the ferrous enzyme in the presence of 3.8 μM catalase. Experimental details for MCD and CD measurements were also described in the preceding paper (1).

1 The abbreviations used are MCD, magnetic circular dichroism; Hb, hemoglobin; Mb, myoglobin.
2 Figs. 1-6 for the results on rat liver L-tryptophan 2,3-dioxygenase are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-533, cite authors, and include a check or money order for $10.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverley Press.
Bovine liver catalase was purchased from Sigma Chemical Co. and dialyzed overnight at 4 °C against 5 mM potassium phosphate buffer (pH 7.0) to remove thymol.

RESULTS

Cyanide Complexes of the Ferric Enzymes—Figs. 1 and 2 show MCD and CD spectra of ferric cyanide complexes of Pseudomonas L-tryptophan 2,3-dioxygenase and rabbit intestinal indoleamine 2,3-dioxygenase with their electronic absorption spectra in the absence of L-tryptophan at a neutral pH. The spectra of rat liver L-tryptophan 2,3-dioxygenase are shown in Fig. 5 of the Miniprint. In each case, the Soret MCD spectrum resembles the first derivative of the corresponding absorption spectrum shown in the lower parts of the figures. In the visible region, a broad plateau from 440 to 560 nm and a trough around 575 nm were observed. These MCD patterns resembled that of ferric cyanide complexes of metMb and horseradish peroxidase (7). The intensities ($\theta_{\text{MCD}}$) of the Soret MCD were, however, considerably higher than those of metMb and horseradish peroxidase (Table I). On addition of L-tryptophan to the cyanide complex of either ferric Pseudomonas enzyme or ferric indoleamine 2,3-dioxygenase, a significant decrease in the intensity was observed giving a value close to those of the metMb and the horseradish peroxidase complexes. Such a decrease in the Soret MCD intensity by L-tryptophan was not observed with the cyanide complex of the liver enzyme, of which MCD intensity was comparable to that of metMb cyanide in the absence of L-tryptophan.

In CD spectra, the cyanide complexes of both Pseudomonas and liver enzymes showed distinct negative Cotton effects around 420 nm ($\theta < 0$), while the complex of indoleamine 2,3-dioxygenase had weak negative and positive CD extrema at 407 nm ($\theta = -2.3 \times 10^3$) and 428 nm ($\theta = +0.9 \times 10^3$), respectively, in the absence of L-tryptophan at pH 7.3 (Table II). The intensity of the CD spectrum of the Pseudomonas enzyme complex does not change significantly by the addition of L-tryptophan at the concentration between 0.05 and 3 mM. As for the liver enzyme, the CD intensity increased slightly by the addition of L-tryptophan. On the other hand, an addition of a small amount of L-tryptophan to the cyanide complex of indoleamine 2,3-dioxygenase gave a distinct negative extremum around 422 nm which became more evident as L-tryptophan concentration increased (Fig. 3A). These results suggest that, in the absence of L-tryptophan, the electronic states of the heme and its vicinity in the cyanide complex of indoleamine 2,3-dioxygenase were quite different from those of L-tryptophan 2,3-dioxygenases, but the binding of L-tryptophan made them very similar to one another. L-Tryptophan concentrations required for a half-maximal conversion of the L-tryptophan-free cyanide form to the bound form at 4 °C and pH 7.3 were less than 0.01 mM as judged by the Soret MCD measurements. It should be recalled that the dissociation constant of the ferric indoleamine 2,3-dioxygenase L-tryptophan complex (in the absence of cyanide) was 8.4 and 0.52 mM for the high and low spin forms, respectively (1).

Carbon Monoxide Complexes of Ferrous Enzymes—Fig. 4 shows MCD and CD spectra of carbon monoxide complexes of ferrous Pseudomonas L-tryptophan 2,3-dioxygenase with their electronic absorption spectra both in the presence and absence of L-tryptophan at pH 7.5. Because of an extremely low affinity of the enzyme for CO in the absence of L-tryptophan (2), the spectrum in the absence of L-tryptophan (Fig. 4A) was that of a mixture of CO-bound and free forms of the ferrous enzyme. Extrema in the MCD spectra at 415, 424, 519, 558, and 573 nm were assignable to the ferrous high spin form of the enzyme. The assignments were made on the basis of MCD spectra of the ferrous high spin form before the addition

\[ \theta_{\text{MCD}} = \frac{1}{3} \cdot \Delta \varepsilon / T; \theta = \frac{1}{3} \varepsilon \Delta \varepsilon. \]
of CO (1) and of ferrous CO complex of the enzyme obtained in the presence of L-tryptophan (Fig. 4C). It should be noted that such a small amount of residual ferrous high spin form was easily detected in the MCD spectrum (Fig. 4A). A CD spectrum of the enzyme in the absence of L-tryptophan showed a broad peak around 430 nm which appeared to have two extrema at 425 and 433 nm and the latter extremum disappeared upon addition of L-tryptophan (Fig. 4, B and D).

Soret MCD spectrum of the ferrous CO complex of the Pseudomonas enzyme obtained in the presence of L-tryptophan (Fig. 4C) was like the first derivative of the absorption spectrum (Fig. 4E) and, as a whole, was very similar to that of CO-Mb (8). A distinct difference in their intensities was again observed between the Soret spectra of the two CO complexes. MCD intensity ([θ]μ) of the peak or the trough in the Soret region was about 20 in myoglobin, while that of the Pseudomonas enzyme was over 30. Additional minor differences were found also in the visible region.

### Table 1

<table>
<thead>
<tr>
<th>Hemoproteins</th>
<th>[θ]μ (band width)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyanide complexes</strong></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas enzyme</td>
<td>40.8 (16)</td>
</tr>
<tr>
<td>Rat liver enzyme</td>
<td>34.6 (15)</td>
</tr>
<tr>
<td>Intestinal enzyme</td>
<td>40.5 (16)</td>
</tr>
<tr>
<td>MetMb</td>
<td>30 (16)</td>
</tr>
<tr>
<td>Horseradish peroxidase</td>
<td>30 (14)</td>
</tr>
<tr>
<td><strong>CO complexes</strong></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas enzyme</td>
<td>-d</td>
</tr>
<tr>
<td>Rat liver enzyme</td>
<td>29.2 (11)</td>
</tr>
<tr>
<td>Intestinal enzyme</td>
<td>29.0 (9)</td>
</tr>
<tr>
<td>Mb</td>
<td>21 (10)</td>
</tr>
<tr>
<td>Horseradish peroxidase</td>
<td>17 (9)</td>
</tr>
</tbody>
</table>

- d = Intensity of the Soret MCD trough was expressed by molar ellipticity per G. [θ]μ (degree cm²/dmol G) on the basis of molar concentration of the heme.
- *d = The band width was expressed by the wavelength difference (in nanometers) between the peak and the trough of the Soret MCD.
- *a = From Ref. 7.
- *b = The values were not determined because of low affinity of the ferrous Pseudomonas enzyme for CO.
- *c = Values in the presence of 10 mM l-tryptophan.

Fig. 4. Spectra of ferrous CO complexes of Pseudomonas enzyme at pH 7.5. A, B, and dotted line in E are the spectra in the absence of L-tryptophan which contained a small amount of CO-free ferrous form. C, D, and solid line in E are MCD, CD, and electronic absorption spectra in the presence of 0.3 mM l-tryptophan, respectively. The enzyme concentration employed was 6.0 μM in terms of the heme. The measurements were done in 0.1 M potassium phosphate buffer, pH 7.5, at 4 °C. Solid lines at the bottom of the electronic absorption spectrum (Abs.) represent the base-lines for the Soret and visible regions.

The CO complex of the liver enzyme exhibited an analogous MCD spectrum (Fig. 6 in the Miniprint) to that of the Pseudomonas enzyme. Additions of l-tryptophan to the complex of liver enzyme caused an increase in the negative Soret MCD extremum from [θ]μ = -29 (in the absence of L-tryptophan) to [θ]μ = -34 (in the presence of L-tryptophan). A similar increase in the apparent intensities was also observed for the positive extremum. However, when the intensities were corrected for the width of the bands, the values for the real intensities were almost unchanged or rather decreased by the binding of L-tryptophan. In CD spectra, the CO complex of the liver enzyme showed a negative Cotton effect at 423 nm. Upon addition of L-tryptophan, the intensity increased as L-tryptophan concentration increased: [θ]μ = -7 × 10⁴ without L-tryptophan and -10 × 10⁴ and -12 × 10⁴ with 0.05 and 3 mM L-tryptophan, respectively.

Additions of L-tryptophan to the complex of liver enzyme caused an increase in the negative Soret MCD extremum from [θ]μ = -29 (in the absence of L-tryptophan) to [θ]μ = -34 (in the presence of L-tryptophan). A similar increase in the apparent intensities was also observed for the positive extremum. However, when the intensities were corrected for the width of the bands, the values for the real intensities were almost unchanged or rather decreased by the binding of L-tryptophan. In CD spectra, the CO complex of the liver enzyme showed a negative Cotton effect at 423 nm. Upon addition of L-tryptophan, the intensity increased as L-tryptophan concentration increased: [θ]μ = -7 × 10⁴ without L-tryptophan and -10 × 10⁴ and -12 × 10⁴ with 0.05 and 3 mM L-tryptophan, respectively.

Fig. 5 shows MCD, CD, and electronic absorption spectra of the CO complex of ferrous indoleamine 2,3-dioxygenase in the absence of L-tryptophan at pH 7.3. The MCD spectrum pattern was also ascribable to a typical ferrous low spin type of a hemoprotein. The intensities ([θ]μ) of the Soret extrema at 415 and 424 nm were about +30 and -30, respectively, which were almost the same as the values for the CO complex of the Pseudomonas enzyme with L-tryptophan and those of the liver enzyme with or without L-tryptophan. Upon addition of L-tryptophan, the intensities of both peak and trough of the Soret MCD decreased to [θ]μ = +21 and -19, respectively, which were comparable to those for ferrous CO complexes of Mb and horseradish peroxidase (7, 8). It should be noted that the widths of the MCD band were almost invariable among the liver enzyme with or without L-tryptophan. Upon addition of L-tryptophan, the intensities of both peak and trough of the Soret MCD decreased to [θ]μ = +21 and -19, respectively, which were comparable to those for ferrous CO complexes of Mb and horseradish peroxidase (7, 8). It should be noted that the widths of the MCD band were almost invariable among
The complex was prepared by the addition of sodium dithionite to the ferric enzyme in the absence of CO. The enzyme concentration employed was 0.01 mM tryptophan at pH 7.3, which was very similar to that of liver enzyme with or without L-tryptophan. When the changes in the intensities of both the trough of MCD at 424 nm and the negative extremum of CD at 420 nm were plotted against the logarithm of L-tryptophan concentration, the apparent dissociation constant of the ferrous enzyme for L-tryptophan described in the previous section.

Oxygenated Form of Ferrous Indoleamine 2,3-Dioxygenase—Fig. 6 shows the MCD and CD spectra of the oxygenated form of indoleamine 2,3-dioxygenase in the absence of L-tryptophan at pH 8.0 and 4 °C. The complex was formed by the addition of oxygen to the ferrous enzyme which had been prepared by the addition of sodium dithionite to the ferric enzyme in the presence of catalase. The complex was very sensitive to hydrogen peroxide under the experimental conditions. In the absence of catalase, however, at least 95% of the complex initially formed was maintained for more than 1 h.

FIG. 5. Spectra of ferrous CO complex of indoleamine 2,3-dioxygenase at pH 7.3 in the absence of L-tryptophan. The enzyme concentration employed was 6.0 μM in terms of the heme. The measurements were done in 0.1 M potassium phosphate buffer, pH 7.3, at 4 °C. Solid lines at the bottom of the electronic absorption spectrum (Abs.) represent the base-lines for the Soret and visible regions.

The shape of the MCD spectrum of the oxygen complex in both the visible and the Soret regions was similar to that of oxy-Mb (8) and compound III of horseradish peroxidase (11) (Table II). Especially, our spectrum at 4 °C was very similar to that of horseradish peroxidase at -146 °C (11). On the other hand, it was distinct from that of cytochrome P-450, a protoheme-containing monooxygenase (12, 13). Essentially no change was observed in either MCD or CD spectra by changing the pH from 8 to 7.3. Although the Soret MCD of the oxygen complex was somewhat different from that of oxy-Mb in its intensity, the visible MCD was very much the same. We were not able to obtain the MCD and CD spectra of these hemoproteins and therefore the peak intensity reflected the real intensity of the MCD.

The shape and the intensity of CD spectrum of the CO complex of indoleamine 2,3-dioxygenase in the absence of L-tryptophan (Fig. 5) were different from those of Pseudomonas and liver enzymes in the presence and absence of L-tryptophan. The spectrum showed two weak negative troughs at 417 and 436 nm with approximate [δ] values of -5 × 10⁴ and -3 × 10⁴, respectively. By the addition of L-tryptophan, the weak CD extrema became a distinct negative extremum around 420 nm (Fig. 5B), which was very similar to that for the CO complex of Pseudomonas enzyme with L-tryptophan and also that of liver enzyme with or without L-tryptophan. When the changes in the intensities of both the trough of MCD at 424 nm and the negative extremum of CD at 420 nm were plotted against the logarithm of L-tryptophan concentration, the apparent dissociation constant of 0.4 mM for L-tryptophan was obtained in good agreement with the value, 0.35 mM, obtained by electronic absorption spectrophotometry at pH 7.0 and 24 °C (10). The apparent dissociation constant of the ferrous enzyme in the absence of CO has been shown to be 0.01 mM (Fig. 5 in Ref. 10). Thus, CO reduces the affinity of the ferrous enzyme toward L-tryptophan in contrast to the effect of cyanide on the affinity of the ferric enzyme for L-tryptophan described in the previous section.

**TABLE II**

<table>
<thead>
<tr>
<th>Hemoproteins</th>
<th>Extremum position (intensity)</th>
<th>Cyanide complexes</th>
<th>CO complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extinction (nm) (mM L-tryptophan)</td>
<td>+L-Tryptophan (0 mM)</td>
<td></td>
</tr>
<tr>
<td><strong>Cyanide complexes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas enzyme</td>
<td>420 (-10.5)</td>
<td>422 (-9.7)</td>
<td></td>
</tr>
<tr>
<td>Rat liver enzyme</td>
<td>420 (-7.2)</td>
<td>422 (-8.4)</td>
<td></td>
</tr>
<tr>
<td>Intestinal enzyme</td>
<td>407 (-5.3)</td>
<td>424 (-5.9)</td>
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<tr>
<td>MetMb*</td>
<td>428 (+0.9)</td>
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<tr>
<td>MetHb*</td>
<td>425 (+6.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horseradish peroxidase*</td>
<td>426 (+7)</td>
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<td></td>
</tr>
<tr>
<td><strong>CO complexes</strong></td>
<td></td>
<td></td>
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<tr>
<td>Pseudomonas enzyme</td>
<td>425 (-6.7)</td>
<td>425 (-9.4)</td>
<td></td>
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<tr>
<td>Rat liver enzyme</td>
<td>423 (-7.4)</td>
<td>420 (-12.0)</td>
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<tr>
<td>Intestinal enzyme</td>
<td>417 (-4.6)</td>
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<td>Mb*</td>
<td>436 (-3.3)</td>
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<tr>
<td>Hb*</td>
<td>420 (+25)</td>
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<tr>
<td>Horseradish peroxidase*</td>
<td>421 (+17.0)</td>
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</table>

* From Ref. 24.
* From Ref. 25.
+ Due to the CO-free ferrous form.
* A value in the presence of 10 mM L-tryptophan.
oxindoleamine 2,3-dioxygenase in the presence of L-tryptophan, because its half-life was too short to measure the spectra even at 4°C. For the same reason, MCD and CD of oxy-L-tryptophan 2,3-dioxygenase were not measurable.

**DISCUSSION**

We demonstrated in this study that MCD spectra of both cyanide and CO complexes of L-tryptophan 2,3-dioxygenases and indoleamine 2,3-dioxygenase were similar to those of corresponding complexes of Mb and horseradish peroxidase. It has been shown that the MCD spectra sensitively reflect the electronic state of the heme in hemoproteins (14,15). The results thus suggest that the electronic structures of these low spin complexes are not greatly different from one another. However, the Soret MCD intensities of some low spin complexes of the dioxygenases were significantly higher than those of Mb and horseradish peroxidase complexes, especially in the absence of L-tryptophan (Table I). Furthermore, the high intensity tended to decrease upon binding of L-tryptophan to a level closer to that of Mb and horseradish peroxidase complexes. Such decreases in the intensity were most clearly seen with the complexes of indoleamine 2,3-dioxygenase but was ambiguous in those of the rat liver enzyme.

In synthetic heme models, the intensities of the Soret MCD of carbon monoxide complexes were much higher than those of ordinary hemoproteins. The intensity (|\(\Delta \mu\)|) of the Soret MCD in the CO complex of iron tetrathenylporphyrin with N-methylimidazole as the trans axial base is about 75, and that in the CO complex of meso-tri(a,a,a-o-pivalamidophenyl)beta-o-4(-N-imidazolyl) butyramidophenylporphyrin is about 40 (16). These values are 2–4 times greater than those of Mb and horseradish peroxidase. One of the structural differences between hemoproteins and these model complexes was found in their bond angles of Fe-C-O. A reported bond angle of Fe-C-O is 135° in CO-Mb (17), while the bonding is linear in the CO complex of iron "picket fence" porphyrin (18). These findings suggest that the Soret MCD intensities of linear Fe-C-O complexes is higher than those of bent Fe-C-O complexes. The bond angle of Fe-C-O is essentially linear by itself (19) and, therefore, the linear bond angle of the Fe-C-O observed in the model complexes is forced to bend in CO-Mb due to the steric hindrance caused by the amino acid residues of the protein (20). This interpretation is in accord with the concept that the Soret MCD intensity of a hemoprotein is dependent on the strength of the ligand field of the heme iron (8). The symmetry around the heme iron thus influences the intensity of the Soret MCD of a hemoprotein. Based on these discussions, one can speculate that the bond angle of Fe-C-O is closer to a linear configuration in the dioxygenase with the high MCD intensity than in that with the lower intensity, and that the bond angle is significantly affected by the binding of L-tryptophan with the enzyme.

The intensities of the Soret MCD of the cyanide complexes of these dioxygenases were also significantly higher than those of other ordinary hemoproteins, and two of the three dioxygenases again decreased on the addition of L-tryptophan (Table I). Deatherage et al. (21) demonstrated in their x-ray crystallographic study that the cyanide molecule bound to iron in horse metHb lies off the line which is vertical to the heme plane and passes the center of the porphyrin nucleus, due to the steric effect of distal histidine (E7) and valine (E11). On the other hand, the Fe-C-N bond without steric hindrance is considered to be essentially linear from the findings on Fe(CN)₄⁺⁺ (22). From these and foregoing discussions on the MCD intensity of the CO complexes, the decrease in the Soret MCD intensities of the cyanide complexes by L-tryptophan might also be correlated with the changes in bonding character between the iron and cyanide. If such an interpretation is applicable to the endogenous sixth ligand of ferric indoleamine 2,3-dioxygenase, the observed shift in the spin equilibrium (1) may also be related to the changes in bonding character.

As discussed in the preceding paper (1), the Soret CD spectrum of a hemoprotein is attributable to the interaction between the heme and aromatic residues in the heme pocket (23). The observed changes in CD spectra of the low spin complexes of the dioxygenases by L-tryptophan can therefore be related to the changes in interaction between the heme and nearby aromatic compound(s). Although it is not possible to assign the aromatic moiety concerned, two alternative possibilities may be raised: (a) the direct interaction between the heme and the indole ring of the added L-tryptophan or, (b) the interaction between the heme and nearby aromatic amino acid from the protein whose position was changed as a result of the substrate binding. It is interesting to note that the CD spectra of the low spin complexes differ greatly from one another in the absence of L-tryptophan, but become very similar upon binding of L-tryptophan with the enzyme.

In conclusion of the findings of these two papers, the MCD and CD spectra of the three heme-containing dioxygenases were on the whole similar to those of Mb in their various redox and spin states; not many differences were found between the spectra of the dioxygenases and the oxygen carrier. However, the spectra sensitively reflected the differences in their fine structures and the substrate-induced changes in the spin states of the dioxygenases. The results led us to propose that the sixth ligands of the ferric forms are different between L-tryptophan 2,3-dioxygenase (H₂O) and indoleamine 2,3-dioxygenase (nitrogen) and also that the binding of L-tryptophan caused a change in the bonding character between the heme and the sixth ligand in these enzymes. Further studies on the interaction between the heme-prosthetic group and the bound substrate L-tryptophan are necessary for the elucidation of the catalytic mechanisms.

**Acknowledgments**—We are greatly indebted to Dr. Kazuo Kuratsuka and his associates, National Institute of Health, Japan, for their kind supply of fresh rabbit intestine. Skillful technical assistances of Y. Tanizaki and R. Kobayashi are also acknowledged.
REFERENCES

Supplemental Materials to Magnetic and Natural Circular Dichroism of L-Tryptophan 2,3-Dioxygenase and Indoleamine 2,3-Dioxygenase I and II

Fig. 1. MCD, CD, and electronic absorption spectra of ferric liver L-tryptophan 2,3-dioxygenase at pH 7.5 in the absence of L-tryptophan. The enzyme concentrations employed were 0.0 and 15.7, in units of the heme for the Soret and visible spectrums, respectively. The measurements were done at 4°C in 0.1 M potassium phosphate buffer. Solid lines at the bottom of the electronic absorption spectrum represent the base-line for the Soret and the visible regions.

Fig. 2. Visible MCD spectra of ferric liver L-tryptophan 2,3-dioxygenase at various pH values with or without L-tryptophan at 4°C. A, in 0.09 M potassium phosphate buffer at pH 6.8 in the absence of L-tryptophan; B, in 0.07 M potassium phosphate buffer at pH 4.8 in the presence of 10 mM L-tryptophan; C, in 0.08 M sodium borate-boric acid buffer at pH 8.3 in the presence of 10 mM L-tryptophan. The enzyme concentrations employed were 15.7, 12.8 and 10.6, in units of the heme for A, B, and C, respectively.

Fig. 3. MCD, CD, and electronic absorption spectra of ferrous liver L-tryptophan 2,3-dioxygenase at pH 7.5 and 4°C. The enzyme concentration employed was 5.3, in units of the heme. The medium used was 0.1 M potassium phosphate buffer. A, in the absence of L-tryptophan; B, in the presence of 0.1 mM L-tryptophan; C, in the presence of 3 mM L-tryptophan.
Fig. 5. MCD, CD, and electronic absorption spectra of ferric cyanide complex of liver L-tryptophan 2,3-dioxygenase. The experiments were carried out in 0.1 M potassium phosphate buffer at pH 7.5 and 4°C in the presence of 1 mM potassium cyanide without L-tryptophan. The enzyme heme concentration employed was 50 μM.

Fig. 6. MCD, CD, and electronic absorption spectra of ferrous CO complex of liver L-tryptophan 2,3-dioxygenase. The experiments were carried out in 0.1 M potassium phosphate buffer at pH 7.5 and 4°C in the presence of 1 mM potassium cyanide without L-tryptophan.
Magnetic and natural circular dichroism of L-tryptophan 2,3-dioxygenases and indoleamine 2,3-dioxygenase. II. Spectra of their ferric cyanide and ferrous carbon monoxide complexes and an oxygenated form.

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