Indoleamine 2,3-dioxygenase (IDO) catalyzes the oxidative degradation of L-Trp and other indoleamines. We have used resonance Raman spectroscopy to characterize the heme environment of purified recombinant human indoleamine 2,3-dioxygenase (hIDO). In the absence of L-Trp, the spectrum of the Fe^{II} form displayed six-coordinate, mixed high and low spin character. Addition of L-Trp triggered a transition to predominantly low spin with two Fe-OH^- stretching modes identified at 546 and 496 cm^{-1}, suggesting H-bonding between the NH group of the pyrrole ring of L-Trp and heme-bound OH^- . The distal pocket of Fe^{II} hIDO was explored further by an exogenous heme ligand, CN^- ; again, binding of L-Trp introduced strong H-bonding and/or steric interactions to the heme-bound CN^- . On the other hand, the spectrum of Fe^{III} hIDO revealed a five-coordinate and high spin heme with or without L-Trp bound. The proximal Fe-His stretching mode, identified at 236 cm^{-1}, did not shift upon L-Trp addition, indicating that the proximal Fe-His bond strength is not affected by binding of the substrate. The high Fe-His stretching frequency suggests that Fe^{III} hIDO has a strong "peroxidase-like" Fe-His bond. Using CO as a structural probe for the distal environment of Fe^{III} hIDO revealed that binding of L-Trp in the distal pocket converted IDO to a peroxidase-like enzyme. Binding of L-Trp also caused conformational changes to the heme vinyl groups, which were independent of changes of the spin and coordination state of the heme iron. Together these data indicate that the strong proximal Fe-His bond and the strong H-bonding and/or steric interactions between L-Trp and dioxygen in the distal pocket are likely crucial for the enzymatic activity of hIDO.
To investigate the structural features underlying the chemical reactivity of IDO in more detail, we have measured the resonance Raman spectra of recombinant human indoleamine 2,3-dioxygenase (hIDO) and its cyanide, carbon monoxide, and L-Trp complexes. The data show that the distal and proximal heme environments of hIDO are distinctly different from that of conventional Mb and that L-Trp binds closely but not directly to the distal side of the heme iron. Together they suggest that the strong proximal Fe–His bond and the strong H-bonding and/or steric interactions imposed by L-Trp on dioxygen in the heme pocket are likely to play crucial roles in the catalytic reaction of hIDO.

**EXPERIMENTAL PROCEDURES**

**Materials**—CO, 13C18O, K13C15N, K13CN, and D2 18O were purchased from Icon (Mt. Marion, NV), and KC15N was from Cambridge Isotopes. All other materials were purchased from Sigma and were of the highest available purity.

**Expression and Purification of hIDO**—hIDO was expressed and purified as a fusion protein to a hexahistidyl tag as detailed elsewhere (18). Previous studies established that the hexahistidyl tag does not affect the physical properties of the enzyme (18). Before use, hIDO was gel-filtered through a Sephadex G-25 column eluted with 100 mM phosphate buffer (pH 7.4) containing 100 mM EDTA. The heme content of hIDO, assessed by the ratio of absorbance at 406 to 280 nm, was 1.6 ± 0.2, and the specific activity, measured by the methylene blue/ascorbate assay (11), was 90 ± 0.2 mol/min/mol of enzyme. This activity is comparable to that of native human (89 mol/min/mol; Ref. 18) and rabbit IDO (~108 mol/min/mol; Ref. 11). Measurement of hIDO activity after resonance Raman experiments showed <10% loss of activity due to laser-induced damage to the protein.

**Preparation of Sample Complexes**—Concentrated hIDO was diluted to a final concentration of ~30 µM in phosphate buffer, and ~100 µl was used for resonance Raman measurements in a septum-sealed cell. Ferrous and Fe(II)CO hIDO were prepared by anaerobic reduction with sodium dithionite and introduction of CO gas by syringe injection. Ferric cyanide complexes were prepared by addition of a small amount of solid potassium cyanide (CN −) to the Fe2+ sample. Stocks of L-Trp (50 mM) were prepared in phosphate buffer and added to samples as indicated. Spectra recorded in D2 18O were prepared by dilution of concentrated hIDO in an appropriate volume of D2 18O (80–90% final volume of D2 18O). The electronic absorption spectrum of the samples was recorded before and after every experiment to confirm sample purity and stability.

**Resonance Raman Spectroscopy**—All resonance Raman measurements were made using the instrumentation described previously (19). Briefly, the output at 413.1 nm from a krypton ion laser (Spectra Physics, Mountain View, CA) (~5 milliwatts) was focused to a ~30-µm spot on a cell rotating at ~1000 rpm. For Fe(II)CO complexes, laser power was kept at <2 milliwatts to avoid ligand photodissociation. The acquisition time was typically 10–15 min. The scattered light was collected at right angles to the incident beam and focused on the entrance slit (100 µm) of a 1.25 m Spex spectrometer (Jobin Yvon, Edison, NJ) where it was dispersed and then detected by a CCD camera (Roper Scientific, Princeton, NJ). For calibration, the lines of indene in the 170–1750 cm −1 region were recorded daily. All measurements were done at room temperature.

**RESULTS**

**Ferric hIDO**—We used 413.1 nm laser light in resonance with the strong Soret electronic transition of heme. This yielded a resonance Raman spectrum of Fe3+ hIDO with several intense bands that can be assigned to totally symmetric vibrational modes of the porphyrin ring system (Fig. 1). For the substrate-free enzyme (Fig. 1B, top), the strongest band was the oxidation-state marker band v4 at 1370 cm −1, typical for Fe3+ heme. The high frequency region also showed a heme peripheral vinyl group stretching band at 1621 cm −1. The porphyrin core-size marker bands v2 and v3 were observed at 1483 and 1563 cm −1, respectively, characteristic of a six-coordinate high spin heme with shoulders at 1505 and ~1578 cm −1, respectively, characteristic of a six-coordinate low spin heme (20, 21). The mixed spin character of Fe3+ hIDO is similar to mammalian globins (22) in which the distal side of the heme iron is coordinated by a solvent water molecule.

In the low frequency region of the substrate-free enzyme (Fig. 1A, top), the most intense band was the characteristic heme marker band v5 at 676 cm −1. The 250–450 cm −1 region of heme proteins displayed several bands that are assigned to porphyrin out-of-plane vibrations, Fe-porphyrin stretching, and peripheral vinyl and propionate group in-plane bending vibrations (23). For hIDO the bands in this region were not resolved well and displayed differences in frequencies and relative intensities compared with sperm whale Mb (23). Nevertheless, using this comparison the intense 335 cm −1 band was assigned to the Fe-porphyrin nitrogen stretching mode v15 with vinyl bending character. The 384 and 416 cm −1 bands were assigned to propionate and vinyl in-plane bending modes, respectively, the frequency and intensity of which are sensitive to...
conformational changes of these heme peripheral groups (24).

Addition of l-Trp to Fe\(^{3+}\) hIDO triggered a pronounced transition of the heme iron to six-coordinate and low spin as indicated by the increased intensities of \(v_3\) and \(v_2\) at 1500 and 1578 cm\(^{-1}\) (Fig. 1B, bottom). To identify the distal ligand of the Fe\(^{2+}\)-Trp complex, the H\(_2\)O-D\(_2\)O difference spectrum was obtained. In the isotope difference spectrum, all the heme modes are canceled out except those associated with the heme-bound hydroxide or water. Two vibrational bands at 546 and 496 cm\(^{-1}\) were observed (Fig. 2). They are similar to the \(-550\) and \(-490\) cm\(^{-1}\) bands observed in the alkaline form of globins (Table I), which were assigned to the Fe-OH stretching modes (\(v_{Fe-OH}\)) of the low spin and high spin heme, respectively (19, 22). Similarly we assigned the two bands in the hIDO Fe\(^{3+}\) difference spectrum to \(v_{Fe-OH}\), although both bands may correspond to the same low spin species as a high spin species was not seen in the high frequency region of the spectrum (Fig. 1B, bottom). In the absence of l-Trp \(v_{Fe-OH}\) bands were not observed for Fe\(^{2+}\) hIDO (Fig. 2) even at pH 10 when the distal water molecule was partially deprotonated and the contribution from the six-coordinate low spin component was increased (data not shown). These data suggest that l-Trp binds closely to the heme iron, and the heme-bound hydroxide interacts with the NH group of the pyrrole ring of the L-Trp through an H-bond (Scheme 2, II), characteristic of a five-coordinate and high spin Fe\(^{2+}\) heme with His as a proximal ligand. In the low frequency region, a band at 236 \(\pm 1\) cm\(^{-1}\) (Fig. 3A, top) was assigned as the proximal iron-His stretching mode \(v_{Fe-His}\). This frequency is significantly higher than that observed for globins (\(-220\) cm\(^{-1}\)), although it occurred in the 230–245 cm\(^{-1}\) range commonly observed for peroxidases (21, 25, 26) (Table I). In contrast to the Fe\(^{3+}\) form, addition of l-Trp did not induce a spin state change to Fe\(^{2+}\) hIDO as the \(v_3\) band remained characteristic of five-coordinate and high spin heme (Fig. 3B, bottom), supporting the notion that the substrate does not bind directly to the heme iron. In addition, the \(v_{Fe-His}\) (Fig. 3A) remained the same at 236 cm\(^{-1}\), indicating that the substrate does not affect the strength of the proximal Fe–His bond.

The data suggest that the distal coordination position remains free in the catalytically active hIDO, allowing for the binding of dioxygen during the catalytic reaction cycle.

**Cyano-bound Complex**—It was not possible to study the interaction of O\(_2\) and l-Trp using the present techniques as the O\(_2\) complexes were too unstable. Instead we studied the resonance Raman spectra of the CN\(^-\) and CO complexes of Fe\(^{3+}\) and Fe\(^{2+}\) hIDO, respectively, to obtain information on the interactions between the substrate and distal ligands. The high frequency region for the CN\(^-\) complex displayed characteristics of a six-coordinate, low spin complex (Fig. 4). Addition of l-Trp caused pronounced changes to vinyl-associated bands, such as an increased frequency and intensity of the stretch at 1630 cm\(^{-1}\) (Fig. 4B, bottom), an up-shift and sharpening of the bend at 422 cm\(^{-1}\), and sharpening of \(v_3\) at 328 cm\(^{-1}\) (Fig. 4A). The shoulder at 1639 cm\(^{-1}\) (Fig. 4B, top) was assigned to the porphyrin band \(v_{10}\), which was buried in the strongly enhanced 1630 cm\(^{-1}\) band in the presence of l-Trp.

To further obtain the structural information of the heme bound CN\(^-\) and thereby infer the chemical environment of the distal pocket, various isotopes, including \(^{13}\)C\(_{14}\)N, \(^{12}\)C\(_{15}\)N, \(^{13}\)C\(_{14}\)N, and \(^{12}\)C\(_{14}\)N, were used. In the low frequency region of the isotope difference spectra, at least two major isotope-sensitive bands were detected (Fig. 5A). We assigned the bands at 450 and 410 cm\(^{-1}\) to the Fe-CN stretching (\(v_{Fe-CN}\)) and bending mode (\(b_{Fe-CN}\)), respectively, associated with a linear but slightly tilted conformation of the CN\(^-\) ligand (27, 28). The assignment of the stretch and bend is supported by their respective disappearance in the C\(_{15}\)N\(_{14}\)CN and C\(_{13}\)N\(_{14}\)C\(_{15}\)N difference spectra (Fig. 5A). We also noted another, weaker bending mode at 440 cm\(^{-1}\) that was assigned to a bent Fe-C-N conformation (27, 28). In the presence of l-Trp, similar stretching and bending modes were seen at 455 and 410 cm\(^{-1}\), respectively (Fig. 5B); the bending mode corresponding to a bent Fe-C-N linkage appeared at 444 cm\(^{-1}\). The enhancement of this mode in the presence of l-Trp suggests a steric and/or H-bonding interaction between the ligand and l-Trp. The other low frequency bands, which were not canceled out in the isotope difference spectra, are likely porphyrin modes coupled to the motion of the CN\(^-\) ligand (28).

**CO-bound Complex**—The high frequency region of the spectrum of the Fe\(^{2+}\)-CO complex of hIDO (Fig. 6B, top) indicated a six-coordinate and low spin complex. Addition of l-Trp caused similar changes to vinyl-associated bands as described above for the Fe\(^{3+}\)-CN\(^-\) complex (Fig. 6B, bottom). The Fe-CO stretching (\(v_{Fe-CO}\)) and bending (\(b_{Fe-CO}\)) modes appeared prominently (Fig. 6A) at 518 and 577 cm\(^{-1}\), respectively. This assignment is supported by the appearance of two isotope-sensitive bands at 518 and 577 cm\(^{-1}\) in the CO\(_{13}\)C\(_{15}\)O difference spectrum (Fig. 7A, top). In the presence of l-Trp, \(v_{Fe-CO}\) and \(b_{Fe-CO}\) shifted to 542 and 589 cm\(^{-1}\) (Fig. 6A, bottom), respectively, as reflected by the associated strong bands in the CO\(_{13}\)C\(_{15}\)O difference spectrum (Fig. 7A, bottom). The presence of l-Trp also caused the \(b_{Fe-CO}\) mode to be narrowed and intensified (Fig. 6A), indicating a steric and/or H-bonding interaction imposed by l-Trp to the Fe-C-O moiety. The internal C-O stretch \(v_{C-O}\) can sometimes be seen around 1900–1950 cm\(^{-1}\) for CO-heme complexes. In the case of hIDO, \(v_{C-O}\) was not observed in the absence of l-Trp.
Iron-ligand vibrational frequencies (cm$^{-1}$) for hIDO and other heme proteins.

<table>
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<th>Species</th>
<th>Fe$^{3+}$</th>
<th>Fe$^{2+}$</th>
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<tbody>
<tr>
<td>hIDO - Trp</td>
<td>$\nu_{Fe-His}$</td>
<td>$\nu_{Fe-CO}$</td>
</tr>
<tr>
<td>hIDO + Trp</td>
<td>236</td>
<td>518</td>
</tr>
<tr>
<td>Mb</td>
<td>220</td>
<td>512</td>
</tr>
<tr>
<td>Hb</td>
<td>215</td>
<td>507</td>
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<tr>
<td>HRP</td>
<td>241, 244</td>
<td>531, 541</td>
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<tr>
<td>CCP</td>
<td>227, 248</td>
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$^a$ Ref. 29.
$^b$ Refs. 22, 23, 28, 30, and 40.
$^c$ Refs. 22, 28, and 40.
$^d$ Refs. 27, 40, and 41.
$^e$ Refs. 27 and 40.

**Scheme 2. Cartoon of the hIDO heme pocket.** The proposed heme pocket of hIDO is shown. The proximal and distal His residues are depicted with the latter engaged in H-bonding with distal water (I). Binding of Trp to the Fe$^{2+}$ enzyme promotes distal hydroxide binding by stabilization through H-bonding (II). The Fe$^{3+}$-CO (III) and Fe$^{2+}$-CO-Trp (IV) complexes are also depicted. The analogous CN complexes would look similar to that depicted for CO. For the Fe$^{2+}$ species, the proximal His is engaged in H-bonding with a neighboring amino acid residue ($X^*$), which gives the imidazole ring of His imidazole character that strengthens the proximal Fe–His bond.

**Fig. 3. Resonance Raman spectra of ferrous hIDO in the absence and presence of L-Trp.** Experimental conditions were as described in Fig. 1 legend. Ferrous hIDO was prepared by mild dithionite reduction as described under “Experimental Procedures,” and the final concentration of L-Trp in the bottom spectrum was 5 mM.

(Fig. 7B, top). However, in the presence of L-Trp, $\nu_{Fe-OH}$ appeared at 1901 cm$^{-1}$ (Fig. 7B, bottom), a value close to that determined by IR spectroscopy for rIDO (29).

**Fig. 4. Resonance Raman spectra of the Fe$^{2+}$-CN$^{-}$ complex of hIDO in the absence and presence of L-Trp.** Panel A, low frequency region. Panel B, high frequency region. Experimental conditions were as described in Fig. 1 caption and the final concentration of L-Trp in the bottom spectrum was 5 mM.

Relating the frequencies of $\nu_{Fe-CO}$ and $\nu_{Fe-OH}$ can give valuable information on the nature of the proximal ligand and the polarity of the distal pocket (see e.g. Refs. 25 and 30). Thus, separate correlation curves can be discerned for five-coordinate complexes and six-coordinate complexes with imidazole versus thiolate as proximal ligand (Fig. 8). The position along a curve is determined by either steric interactions or a local electric field generated by polar interactions. Positive dipoles originating from H-bonding interactions typically generate low $\nu_{Fe-CO}$ and high $\nu_{Fe-CO}$; whereas negative dipoles do the opposite (30). For example, in sperm whale Mb, the frequencies of the $\nu_{Fe-CO}$ and $\nu_{Fe-OH}$ bands are 512 and 1944 cm$^{-1}$, respectively (30). As the distal His is mutated to a nonpolar residue, $\nu_{Fe-CO}$ becomes lower, and $\nu_{Fe-CO}$ becomes higher as shown in Fig. 8 (31). Similar effects are observed for peroxidases (30, 32). The distal pockets of peroxidases are typically more polar than that in Mb; the data points therefore fall on the upper left corner of the imidazole correlation curve.

Assuming that the hIDO data point falls on the correlation curve 2 (Fig. 8), the $\nu_{Fe-CO}$ for the substrate-free hIDO is predicted to be $\sim$1930 cm$^{-1}$ based on the $\nu_{Fe-CO}$ mode observed at 518 cm$^{-1}$. This predicted value corresponds closely with one of the two $\nu_{Fe-CO}$ bands at 1933 and 1953 cm$^{-1}$ observed in rIDO with the intensity of the 1933 cm$^{-1}$ band less than half that of the 1953 cm$^{-1}$ band (29). The fact that only the weaker isomer
of rIDO was seen in hIDO suggests that the distal heme environment is slightly different in IDO from the two species. The position of the predicted hIDO data point in the correlation curve (Fig. 8) indicates further that the distal pocket of hIDO is slightly more polar than that in wild-type Mb although less polar than that in peroxidases. Binding of L-Trp caused the data point to shift to the upper left corner, corresponding to a more polar and peroxidase-like distal environment presumably due to the presence of a strong H-bond between L-Trp and CO.

**DISCUSSION**

The present resonance Raman characterization of the active site of hIDO revealed a blend of structural features common to globins and peroxidases. In the Fe$^{3+}$ state hIDO is six-coordinate with a distal water/hydroxide ligand similar to globins, whereas the Fe$^{2+}$ species displays a peroxidase-like proximal Fe–His bond. The polarity of the distal pocket of hIDO is intermediate between that of globins and peroxidases. Binding of L-Trp reconditions the distal pocket to a peroxidase-like and sterically congested polar environment, which imposes strong steric and/or H-bonding interactions to the heme-bound ligands, although the proximal Fe–His bond strength is not affected. The strong proximal Fe–His bond and the unique distal environment provided by L-Trp likely play a crucial role in the enzymatic activity of hIDO.

**Proximal Heme Environment**—The present study on hIDO and previous spectroscopic work limited to rIDO (17) establish His as the proximal (fifth) ligand. This is indicated e.g. by $v_\text{CN}$ at 1353 cm$^{-1}$ in Fe$^{2+}$ hIDO (Fig. 3B) and the $v_\text{Fe-CO}/v_\text{H-C-O}$ data points falling on the His correlation curve (Fig. 8). Two highly conserved His residues have been identified in mammalian IDO and the IDO-like Mb of archaeogastropods (7) that correspond to His$^{346}$ and His$^{303}$ in hIDO (33). Replacement of His$^{346}$...
with Ala by site-directed mutation prevents heme binding and decreases dioxygenase activity, whereas replacement of His³⁰³ with Ala has no effect on heme content or activity,² suggesting strongly that His³⁴⁶ is the proximal ligand for hIDO.

The Fe²⁺ hIDO spectrum displayed a νFe-His band at 236 cm⁻¹ (Fig. 3), suggestive of a relatively strong Fe–His bond similar to that seen in peroxidases. The latter has been linked to an H-bond between the NH group in heme His and the carboxylate group of a nearby Asp residue (26), giving the His an imidazolate character that strengthens the Fe bond. In some cases, such as dehaloperoxidase, the peptide C=O group of a proximal residue can also interact with the proximal His to produce a strong Fe–His bond (34). Our data therefore suggest that the proximal His residue in Fe²⁺ hIDO may similarly be engaged in H-bonding with a presently undetermined, neighboring amino acid residue (indicated by X in Schemes 2 and 3).

**Distal Heme Environment**—The distal binding site in most ferric mammalian globins is occupied by a water molecule at neutral pH, and as a result, the electronic configuration of the heme iron is normally a six-coordinate high spin and low spin mixture. On the other hand, the strong proximal Fe–His bond in peroxidases, such as cytochrome-c peroxidase, forces the iron to move out of the porphyrin plane and thereby prevents the coordination of a weak distal water ligand to the heme. The ferric protein in most peroxidases thus favors a five-coordinate structure (35). The six-coordinate configuration of the Fe³⁺ hIDO therefore suggests that the proximal Fe–His bond is not as strong as in the Fe²⁺ hIDO. This may be due to differences in protein conformation between Fe³⁺ and Fe²⁺ hIDO similar to those reported for rIDO based on CD spectroscopic studies (17). A weaker Fe–His bond would be expected for Fe³⁺ hIDO if its conformation disfavors the H-bonding interaction between the proximal His ligand and the neighboring residue X⁻.

Like mammalian globins, the Fe³⁺ hIDO is predominantly high spin at neutral pH, and the weak low spin signal increased at alkaline pH (data not shown), suggesting that the weak field ligand water is partially replaced by a strong field ligand OH⁻ due to protonation. It is plausible that the heme-bound water is stabilized by the distal His³⁰³ through H-bonding as illustrated in Scheme 2f based on amino acid alignment and spectroscopic evidence reported by others (6). The six-coordinate mixed spin character of IDO was also observed in Fe³⁺ rIDO using EPR spectroscopy (17). However, in that study, Sono (17) suggested the a His residue, instead of a water molecule, is the sixth ligand coordinated to the heme iron. Histidine is a strong field heme ligand, which is typically associated with a low spin configuration. The mixed spin character of rIDO was thus attributed to a sterically hindered His (17). Based on the present data, we consider water to be a more likely distal ligand in Fe³⁺ hIDO.

The binding of L-Trp in the distal pocket introduces significant conformational changes to the heme peripheral groups as suggested by the changes in the vibrational modes associated with the vinyl groups. The L-Trp also modified the chemical environment of the distal pocket as reflected by the pronounced transition from a six-coordinate mixed spin configuration to a low spin configuration (Fig. 1B) and the appearance of the two νC-O modes in the Raman spectrum, which are attributed to the H-bonding interaction between the OH⁻ ligand and the NH group of the pyrrole ring of the L-Trp (Scheme 2II).

In the absence of L-Trp, the putative distal His³⁰³ may also impose a steric influence and/or H-bonding interaction on other exogenous distal ligands such as CN⁻ and CO for the ferric and ferrous protein, respectively. This is supported by the presence of a bent conformation for the Fe-CN⁻ complex and the position of the data point for the Fe-CO complex on the νC-O versus νFe-H data point to shift to the upper left end of the correlation curve in Fig. 8, indicative of strong H-bonding between L-Trp and CO (Scheme 2IV). The frequency and intensity of δνLCO also increased markedly in the presence of L-Trp as predicted for a greater steric distortion of the Fe-C-O moiety. Similarly in the ferric protein, the intensity of the bending mode associated with a bent Fe-C-N form was increased by L-Trp, and the frequency of the stretch ννLCO was 5 cm⁻¹ higher in the presence than in the absence of L-Trp (Table I). Together these data indicate that the substrate is placed in very close proximity to the distal ligands, which allows for mutual H-bonding and causes some steric distortion of the ligand coordination geometry. We suggest that such an interaction between L-Trp (or other substrates) and O₂ is one of the principal driving forces for the reaction catalyzed by IDO (see below). The fact that His³⁰³ appears to have no catalytic significance according to mutation studies (see above) suggests that in the catalytic reaction L-Trp occupies and overwhelmingly determines the distal heme pocket environment experienced by a distal ligand as concluded from our data.

**Substrate-Heme Interactions**—Throughout this work we noted binding of L-Trp in the distal pocket substantially affects the configuration of the heme vinyl groups. Ferric hIDO displayed a single vinyl stretching band at 1621 cm⁻¹, indicating similar orientation (relative to the heme plane) for both heme vinyl groups (35). Upon binding of L-Trp, the frequency of the stretching band increased, suggesting that the binding of the substrate forces the vinyl groups further out of the heme plane. This effect is independent of the spin and coordination state of the heme iron (e.g. Figs. 3, 4, and 6), suggesting that they were due to either direct interaction between L-Trp and the vinyl group(s) or due to L-Trp-induced protein conformational changes that modulate the configuration of the vinyl groups through direct contact between the vinyl groups and the surrounding protein matrix. L-Trp-induced protein conformational changes have been proposed for rIDO (17). The functional

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with the distal heme iron coordination position open for bind-
tron-withdrawing groups (parallel orientation may be possible.
perpendicular to the heme plane for simplicity, although a
parallel orientation may be possible.

Implications for Catalytic Reaction—We showed that Fe$^{2+}$
hIDO has a very strong proximal Fe–His bond like peroxidases.
Binding of L-Trp in the distal pocket does not affect the Fe–His
bond strength; however, it reconditions the distal pocket to a
more polar and sterically congested chemical environment.
In peroxidases, the cleavage of the peroxide O–O bond isfacili-
tated by a combination of the strong proximal Fe–His bond and
the polar distal environment providing necessary H-bonding
interactions based on the “push-pull” mechanism (36). The
reaction catalyzed byIDO is inherently different from that of
peroxidases. Whereas peroxidases carry out a heme iron-cata-
yzed cleavage of the O–O bond, IDO catalyzes the electrophilic
addition of O$_2$ into the pyrrole ring of L-Trp, leading to the
likely formation of a 3-hydroperoxyindolenine intermediate
(37). The O–O bond breakage occurs later, which may not
require the catalysis by the heme iron. Despite the differences
in the overall nature of the catalytic mechanisms, the similar-
ity in the heme pockets of IDO and peroxidases suggest that
hIDO utilizes similar mechanisms to modulate the chemical
properties of dioxygen.

Scheme 3 outlines a possible catalytic reaction mechanism.
Accordingly the catalytically active Fe$^{2+}$ IDO is five-coordinate
with the distal heme iron coordination position open for bind-
ing of substrates. L-Trp binds first in close proximity (but not
directly) to the distal heme iron coordination position. It is
followed by the binding of dioxygen to the sixth coordination
position of the heme iron. The heme-bound dioxygen interacts
with L-Trp through an H-bond between its proximal oxygen
atom and the NH group of the pyrrole ring of L-Trp. The
electronic effect imposed by this H-bond and the imidazolate
character of the strong proximal Fe–His bond facilitates the
electrophilic addition of the dioxygen to the pyrrole ring. We
note that N-methyl-Trp and the thiophene and furan analogs
(in which the pyrrole nitrogen atom is replaced by a sulfur or
oxygen atom, respectively), which cannot form an H-bond, bind
to but are not catabolized by IDO (16). Also, addition of elec-
donating groups (e.g. methyl) at the five or six position of
L-Trp enhances the reactivity of the substrate, whereas elec-
tron- withdrawing groups (e.g. -NO$_2$) have the opposite effect
(38). Together these data strongly suggest an electrophilic re-
ation that involves O$_2$ attacking the double bond between C-2
and C-3 in indole (Scheme 3). The resulting 3-hydroperoxy-
denile is strongly implicated as intermediate from previous
chemical studies (37). We speculate, based on known, nonen-
zymatic oxidative chemistry of Trp (39), that the primary prod-
uct of the catalytic reaction of IDO, N-formylkynurenine, is
then formed via an endoperoxide intermediate. Further studies
are required to establish the mechanism outlined in Scheme 3,