Human indoleamine 2,3-dioxygenase (hIDO), a monomeric heme enzyme, catalyzes the oxidative degradation of L-tryptophan (L-Trp) and other indoleamine derivatives. Using Fourier transform infrared and optical absorption spectroscopy, we have investigated the interplay between ferrous hIDO, the ligand analog CO, and the physiological substrate L-Trp. These data provide the long-sought evidence for two distinct L-Trp binding sites. Upon photodissociation from the heme iron at T > 200 K, CO escapes into the solvent. Concomitantly, L-Trp exits the active site and, depending on the L-Trp concentration, migrates to a secondary binding site or into the solvent. Although L-Trp is spectroscopically silent at this site, it is still noticeable due to its pronounced effect on the CO association kinetics, which are significantly slower than those of L-Trp-free hIDO. L-Trp returns to its initial site only after CO has rebound to the heme iron.

Recent studies of hIDO have shown that its activity follows typical Michaelis-Menten behavior only for concentrations of L-Trp <50 μM; a further increase in the concentration of L-Trp caused a decrease in the activity (9). Substrate inhibition is well-known for rabbit IDO (rIDO) (10,11). It was generally believed that, at high concentrations of L-Trp, the substrate binds directly to the ferric heme iron, thereby inhibiting its reduction to the active ferrous state (11). This scenario, however, can be excluded on the basis of two new observations: (i) the dissociation constant of L-Trp from ferric hIDO (Kd = 0.9 mM) is significantly higher than the self-inhibition constant, Ksi (0.17 mM) (9), and (ii) the redox potential of L-Trp-bound ferric hIDO is ~46 mV higher than that of the substrate-free enzyme (12), indicating that L-Trp binding to the ferric enzyme...
does not prevent its reduction. On this basis, it has been proposed that substrate inhibition of hIDO is a result of the binding of a second L-Trp molecule in an inhibitory substrate binding site of the enzyme (9).

Simulation studies of substrate docking in hIDO have suggested that the secondary substrate site is located close to the initial binding site (13). Unfortunately, such computational studies may be problematic because residues 360 – 380, which could not be resolved in the crystal structure of hIDO, may play an important role in substrate binding. We have investigated the interplay between ligand and substrate (L-Trp) in ferrous hIDO by using UV/visible and Fourier transform infrared (FTIR) absorption spectroscopy over a wide temperature range, replacing the physiological ligand O₂ by carbon monoxide (CO). CO allows us to exploit its excellent properties as a spectroscopic probe. Moreover, CO is not reactive towards L-Trp, so that we can focus on ligand and substrate dynamics in the heme pocket in the absence of the ensuing enzymatic reaction.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**– Recombinant hIDO protein was prepared according to previously described procedures (14). The protein concentration was estimated based on the peak extinction coefficient in the Soret band, ε = 172 mM⁻¹ cm⁻¹, of the ferric form (12). The catalytic activity of hIDO was confirmed by monitoring formation of the product N-formylkynurenine, which has an optical absorbance band at 321 nm with a peak extinction coefficient of 3.75 mM⁻¹ cm⁻¹ (15). The protein was stored at −80 °C prior to use.

**FTIR Absorption Spectroscopy**– A solution of hIDO at a final concentration of ~ 2 mM in cryosolvent (glycerol / 0.1 M potassium phosphate buffer, pH 7) was stirred under a CO atmosphere for 1 h and reduced with a two-fold molar excess of an anaerobically prepared sodium dithionite solution. Subsequently, L-Trp was added and the mixture was stirred under CO for an additional 20 min. To remove undissolved and aggregated material, the sample was centrifuged for 15 min at 5,000 rpm (Eppendorf centrifuge).

The sample solution was sandwiched between two CaF₂ windows separated by a 75-µm Mylar spacer and enclosed by a sample holder made from oxygen-free high-conductivity copper. The holder was mounted to a cold finger of a closed-cycle helium refrigerator (model SRDK-205AW, Sumitomo, Tokyo, Japan). A silicon temperature sensor diode and a digital temperature controller (model 330, Lake Shore Cryotronics, Westerville, OH) enabled precise temperature regulation of the sample.

The sample was photolyzed with a continuous-wave, frequency-doubled Nd-YAG laser (model Forte 530-300, Laser Quantum, Manchester, UK) emitting 300 mW output power at 532 nm. The laser beam was split and focused onto the sample from both sides. FTIR transmission spectra were collected at a resolution of 2 cm⁻¹ with an IFS 66v/S FTIR spectrometer (Bruker, Karlsruhe, Germany) using an InSb detector (1700 – 2300 cm⁻¹). Photolysis difference spectra were calculated from transmission spectra taken before and after photolysis, ΔA = log(Iₜₐᵣₐ₉/Iₙₐₙ₉).

**Low Temperature UV/Visible Absorption Spectra**– Cryosolvent (glycerol / 0.4 M potassium phosphate buffer, pH 8) was equilibrated with 1 atm CO for 20 min. Subsequently, a two-fold molar excess (final concentration 20 µM) of sodium dithionite solution was added, followed by a few microliters of a concentrated stock solution of hIDO to reach a final protein concentration of ~ 10 µM. For data collection on substrate-bound protein, L-Trp was added to the cryosolvent before deaeration.

The sample solution was sealed in a plastic cuvette and placed within a copper block that was mounted on the cold finger of a closed-cycle helium cryostat (model 22, CTI Cryogenics, Mansfield, MA). Sample temperature was detected by a silicon temperature sensor diode and regulated by a digital temperature controller (model 330, Lake Shore Cryotronics, Westerville, OH). Spectra were measured with a modified Cary 14 spectrometer (On-Line Instrument Systems, Borgart, GA) with a resolution of 1 nm. Photolysis was achieved with a xenon lamp (75 W).

**RESULTS AND DISCUSSION**

**FTIR Spectroscopy: Monitoring the heme-bound CO.** To gain insight into the dynamics of the CO ligand following photodissociation, we performed FTIR absorption spectroscopy. The frequency of the strong CO stretching vibration is extremely sensitive to the local environment due to interactions of the CO dipole with the local electric field (16-18), both in the heme-bound state (19-22) and when the CO is trapped at transient docking sites in the protein (photoproduct bands).
(23-25). Consequently, CO has been used extensively as a local probe of heme protein conformation and dynamics (26).

The FTIR photolysis difference spectrum of hIDO-CO at 4 K shows two major absorption bands of heme-bound CO at 1925 and 1947 cm\(^{-1}\) and a third, weak band at 1937 cm\(^{-1}\) (Figure 1). These bands arise from slightly different protein conformations denoted as ‘taxonomic’ or ‘A’ substrates (27,28). At T > 200 K, the 1937-cm\(^{-1}\) band gains amplitude at the expense of the other two bands (Figure 1). We denote these bands with \(A_{\text{SF}}\) (subscript SF for ‘substrate free’). With substrate present (hIDO-CO/L-Trp), an \(A_{\text{S}}\) band (subscript S for ‘substrate’) at 1895 cm\(^{-1}\) dominates the spectrum at 4 K. It shifts to 1899 cm\(^{-1}\) at 290 K. Residual bands at 1925 and 1947 cm\(^{-1}\) indicate that hIDO was not fully saturated with L-Trp under the conditions employed. The \(A_{\text{SF}}\) and \(A_{\text{S}}\) bands have also been reported in earlier work using resonance Raman spectroscopy (14). The negative amplitudes of the \(A\) bands in the difference spectra indicate removal of the heme-bound CO. The CO photoproduct bands in the range 2100 – 2150 cm\(^{-1}\) emerge upon \(\Delta\) photolysis and are therefore plotted with positive amplitude (see below).

We performed isothermal kinetics experiments after CO photodissociation of both hIDO-CO and hIDO-CO/L-Trp between 200 and 260 K. Initially, a transmission spectrum, \(I_{\text{dark}}\), was collected. After illumination for 100 s, the sample was kept in the dark for 5,000 s to enable CO rebinding, while transmission spectra, \(I_{\text{light}}\), were recorded in logarithmically spaced time intervals. Photolysis difference spectra were calculated from transmission spectra taken before and after photolysis, \(I_{\text{dark}} = \log\left(I_{\text{dark}}/I_{\text{light}}\right)\) (Figure 2A – C). The temporal developments of the \(A_{\text{SF}}\) and \(A_{\text{S}}\) bands at the different temperatures, quantified by their normalized peak areas, are plotted in Figure 2D and E, respectively.

The photolysis difference spectra of hIDO-CO and hIDO-CO/L-Trp at 200 K are shown in Figure 2A and B, respectively. Arrows indicate the progression of the reaction with time. In the control experiment with the substrate-free hIDO-CO protein, the \(A_{\text{SF}}\) bands disappeared within ~300 s (Figure 2A and D). Essentially the same kinetics were observed for the \(A_{\text{SF}}\) bands in the hIDO-CO/L-Trp sample, demonstrating that all substrate-free hIDO molecules rebound their CO ligands within the time window examined (Figure 2B, D, step 1). In contrast, \(A_{\text{S}}\) remained constant (Figure 1B and E), indicating that no CO rebound to the substrate-bound enzyme to form hIDO-CO/L-Trp.

After illumination of hIDO-CO/L-Trp at 240 K, \(A_{\text{S}}\) remained essentially constant for the first 100 s, indicating that no CO rebound in L-Trp-bound hIDO (top panel in Figure 2C). In the same time period, however, \(A_{\text{SF}}\) changed significantly. Within less than 1 s, \(A_{\text{SF}}\) decayed to zero, implying that all substrate-free hIDO molecules quickly rebound CO (Figure 2C, step 1). Similar kinetics were obtained in a control experiment with the completely substrate-free protein (data not shown).

In a second step, the amplitude of \(A_{\text{SF}}\) turned positive with \(t_{1/2} \sim 15\) s (Figure 2C, step 2), indicating that additional, apparently L-Trp-free deoxy hIDO molecules had been generated by photolysis that subsequently rebound CO. Thus, CO photolysis clearly promotes escape of L-Trp from the active site. The CO association kinetics of the newly formed, deoxy hIDO molecules is significantly slower than that of the true L-Trp-free molecules, suggesting that, in the photoproduct, L-Trp occupies the inhibitory substrate binding site. There, it affects the CO rebinding kinetics but has no influence on the stretching band of heme-bound CO. In a final step (Figure 2C, step 3a), the absorption changes in both \(A_{\text{S}}\) and \(A_{\text{SF}}\) decayed to zero (bottom panel in Figure 2C). The crisp isosbestic point at 1917 cm\(^{-1}\) again suggests a two-state transition with \(t_{1/2} \sim 300\) s: L-Trp returned to its primary binding site at the fully CO-ligated hIDO active sites to restore the pre-photolysis state.

The schematic in Figure 3 summarizes the results. After photodissociation of CO, substrate-free deoxy hIDO rebinds its ligand within <1 s at 240 K (Figure 6, species 1 and 2). At low L-Trp concentration, a fraction of the sample is substrate-free, as seen from the spectrum in Figure 2B. The substrate-bound fraction contains, in principle, two conformations with one bound substrate molecule (species 3 and 4). However, the probability of forming the latter species in equilibrium is essentially zero because the affinity of the second site is expected to be significantly lower than that of the primary site (9). After photodissociation of species 3, with L-Trp bound at the active site, CO escapes into the solvent, resulting in species 6. Afterwards, species 7, which is spectroscopically indistinguishable from substrate-free deoxy hIDO, forms due to L-Trp migration to the secondary site. Its CO association is, however, significantly slower due to the presence of L-Trp in the secondary site (step 2). Finally, the initial species
3 is restored by migration of L-Trp back to the primary site (step 3a).

**FTIR Spectroscopy: Tracking the Photolyzed CO**—The temporal changes of the A state bands suggest that L-Trp leaves the immediate vicinity of the active site and possibly occupies the inhibitory docking site. The marked influence on the CO association kinetics implies that the L-Trp substrate is still located within the protein and modifies the CO migration pathway.

In hemoglobins, ligands are known to visit transient docking sites on their way into and out of the protein (26,29-33). Time-resolved x-ray crystallography experiments on CO-ligated myoglobin (MbCO) have visualized the journey of the CO molecule through the protein (34-39). We have developed experimental protocols to trap CO ligands selectively at these transient sites so that they can be detected by spectroscopy (29-31,40) and x-ray crystallography (39,41) at cryogenic temperatures. A CO molecule in a well-defined docking site typically displays a narrow IR stretching band in the range 2100 – 2150 cm–1, with the frequency fine-tuned by the interaction of the CO dipole with the local electric field at the transient site (23,24,26). Moreover, CO has been observed to adopt opposite orientations in the intermediate docking sites with different electrostatic coupling to its surroundings, causing a Stark splitting of the absorption band (16,42,43). If these ligand docking sites also exist in hIDO and the second L-Trp binding site is close to one of these sites or even blocks a site, we would, therefore, expect changes of the CO photoproduct spectrum.

The photolysis difference spectrum of hIDO-CO determined at 4 K after cooling the sample under continuous illumination from 160 to 4 K shows three CO photoproduct bands in the range 2100 – 2150 cm–1 (Figure 1, solid line). Their integrated absorbance is ~25× smaller than the one of the A bands. Sharp photoproduct bands confirm that CO ligands indeed accumulate in well-defined docking sites. In the photolysis difference spectrum of hIDO-CO/L-Trp, we identified an additional photoproduct band at 2112 cm–1 (Figure 1, dotted line), indicating that the presence of L-Trp at the active site either creates a new docking site or affects the electric field at an existing site.

To trap L-Trp at its secondary binding site, hIDO-CO/L-Trp was illuminated for 100 s at 230 K to photodissociate CO and to concomitantly prompt L-Trp to exit the active site. Afterwards, the sample was kept in the dark at 230 K for 1,000 s to ensure complete CO rebinding. During this time interval, L-Trp does not yet return to the active site (as is evident from Figure 2E). Subsequently, the sample was cooled to 4 K to trap the protein in this non-equilibrium conformation, with CO bound to the heme iron and the L-Trp substrate in the secondary binding site. At 4 K, the CO was photodissociated again. The A bands in the photolysis difference spectrum (Figure 1, dashed line) are similar to those of hIDO-CO (Figure 1, solid line), verifying that L-Trp is no longer present at the active site. The small ΔS band represents a minor fraction of hIDO molecules that have relaxed into the hIDO-CO/L-Trp state during cooling. The photoproduct spectrum (2100 – 2150 cm–1) is essentially indistinguishable from that of hIDO-CO (Figure 1, dashed line), indicating that the presence of L-Trp in the secondary binding site has no effect on the electric fields sensed by the CO ligands in the transient docking sites. Apparently, the secondary L-Trp site is not close to any of the CO docking sites. The distal pocket is, however, very spacious so that it is not surprising that L-Trp does not affect the vibrational bands of the photodissociated CO.

**UV/Visible Spectroscopy: Monitoring the Heme**—The FTIR experiments have shown that the stretching band of heme-bound CO can serve as a spectral signature of L-Trp at the primary site. In contrast, L-Trp at the secondary site has only a kinetic signature. The strong absorbance bands of the heme prosthetic group in the visible region of the spectrum are known to be sensitive to the surrounding polypeptide environment and reflect structural changes, oxidation states, and the binding of ligands, making visible spectroscopy an excellent tool for physical studies of protein function. Therefore, L-Trp within hIDO may influence these bands as well.

The UV/visible absorption spectrum of substrate-free hIDO-CO at 240 K (and also at 290 K, data not shown) displayed a Soret band at 420 nm (dash-dotted line in Figure 4A). If the sample solution was saturated with L-Trp (hIDO-CO/L-Trp), the Soret peak was shifted to 416 nm (solid line in Figure 4A). After illumination of hIDO-CO for 3 min at 240 K, bimolecular CO recombination was complete within 1 s (data not shown). The effect of light on hIDO-CO/L-Trp was markedly different from that on the substrate-free enzyme. Immediately after illumination at 240 K, the spectrum displayed two Soret bands at 438 and 420 nm, indicating a mixture of deoxy hIDO and
hIDO-CO, respectively (dashed line in Figure 4A). There was no indication of any residual hIDO-CO/L-Trp. Subsequently, the sample was kept in the dark and spectra were collected continuously. The intensity of the Soret band at 420 nm increased during the ensuing 30 min. Apparently, CO ligands rebound to an hIDO conformation with the spectral signature of the substrate-free protein (Figure 4A). The slow CO rebinding, however, may suggest that L-Trp was still present. The isosbestic point at 427 nm is consistent with a two-state transition between the deoxy hIDO and hIDO-CO states; a relaxation to the initial L-Trp-bound conformation, with its Soret at 416 nm, was absent over the entire time interval. At 245 K, the intensity increase of the Soret band at 420 nm was accompanied by a peak shift toward 416 nm, indicating that L-Trp migrated back to its initial position at the active site (Figure 4B). At 250 K, rebinding of CO and return of L-Trp occurred on similar time scales (Figure 4C), as both the intensity increase and the band shift to 416 nm happened concomitantly. These data confirm the conclusions drawn from the FTIR experiments that, under the chosen experimental conditions, both CO and L-Trp exit the active site after CO photodissociation from the heme iron. CO escapes into the solvent, and L-Trp populates the secondary site and thus slows the rebinding of CO. In a final step, L-Trp returns to its primary binding site.

At temperatures below the glass transition temperature of the cryosolvent, CO photolysis studies of hIDO-CO/L-Trp showed geminate CO rebinding within <1 s even at temperatures as low as 60 K (data not shown). Spectrally, recombination led to an increase of the Soret band at 416 nm, characteristic of hIDO-CO/L-Trp and a concomitant decrease of the deoxy Soret band. No peak shift from 416 to 420 nm was observed, implying that L-Trp did not escape from its original binding site at these temperatures. Apparently, escape of L-Trp requires large conformational changes that are not activated at low temperatures (27,44).

We obtained further insight from photolysis experiments at 240 K on samples prepared with different L-Trp concentrations. Select spectra for [L-Trp] = 0.64, 4.8 and 20 mM are displayed in Figure 5. Equilibrium spectra collected prior to photolysis (solid lines) showed a shift of the Soret peak as a function of L-Trp concentration. The dependence of the peak position, \(\lambda_{\text{Soret}}\) on [L-Trp] can be described by a simple bimolecular binding isotherm. The equilibrium dissociation coefficient, \(K_D = 1.5 \pm 0.5 \text{ mM}\) (Figure 6A) quantifies the affinity for binding of L-Trp in the primary site of hIDO-CO (in cryosolvent at 240 K). For comparison, \(K_D = 140 \pm 10 \mu\text{M}\) in aqueous solution at room temperature, also determined from the Soret peak shift. UV/visible spectra collected immediately after photolysis showed two Soret bands at 420 and 438 nm, independent on the L-Trp concentration (Figure 5, dashed lines). The amplitude of the Soret band at 420 nm decreased with increasing L-Trp concentration. Consequently, the fraction of hIDO molecules that rebound CO very fast decreases with increasing L-Trp concentration. The fast rebinding kinetics implied that these molecules are void of any L-Trp.

The spectra plotted with dotted lines were measured 40 min after photolysis at 240 K. The difference between the spectra plotted with dashed and dotted lines (shaded area in Figure 5A) represents the fraction of proteins that slowly rebind CO within the observation window. At low L-Trp concentrations, the difference spectra showed an absorption increase at 420 nm, indicating that CO rebound to molecules with L-Trp-free active sites. The slow kinetics showed that these molecules had L-Trp in their secondary sites. Up to L-Trp concentrations of ~3 mM, the fraction of these slow rebinders increased with \(K_D = 1.5 \text{ mM}\) (Figure 6B), suggesting that the occupancy of L-Trp at the secondary site is governed by intramolecular migration of L-Trp. In addition, the kinetics of the return of L-Trp to the primary site, as obtained from the Soret shift, were independent on the L-Trp concentration in the solvent, as expected for an internal process (Figure 6C, dotted line).

At L-Trp concentrations above ~5 mM, the fraction of slowly rebinding proteins was higher than predicted from the binding isotherm (Figure 6B), implying that the slow species (with L-Trp at the secondary site) is no longer exclusively generated by internal L-Trp migration. Instead, a certain fraction of the secondary sites was already occupied by L-Trp before photolysis. In these doubly occupied hIDO molecules, CO photolysis caused L-Trp from the primary site to exit into the solvent to yield the slowly rebinding species. The return of L-Trp to the primary site is a bimolecular process, as seen from the linear concentration dependence of the apparent rate coefficient, \(\lambda_{\text{kin}} = [\text{L-Trp}] \times k_{\text{kin}} + k_{\text{off}}\) (Figure 6C, solid line). The fit yields \(k_{\text{kin}} = 2 \times 10^{-5} \text{ mM}^{-1}\text{s}^{-1}\) and \(k_{\text{off}} = 7 \times 10^{-6} \text{ s}^{-1}\); \(K_D = k_{\text{off}}/k_{\text{kin}} = \sim 3 \text{ mM}\). This dissociation coefficient gives an estimate of the L-Trp affinity.
of the primary site of hIDO-CO if the secondary site is occupied by L-Trp.

These findings are also included in the schematic shown in Figure 3. At higher concentrations of L-Trp, the secondary site becomes populated under equilibrium conditions (species 5). After photodissociation, CO escapes into the solvent, so that species 8 forms and, subsequently, the L-Trp molecule in the primary site migrates into the solvent to yield species 7, which has L-Trp only at the secondary site and is spectroscopically indistinguishable from substrate-free deoxy hIDO (species 2). CO association is, however, significantly slower (step 2). Relaxation to the initial state occurs by bimolecular rebinding of L-Trp (step 3b).

CONCLUSIONS

The present study provides a glimpse at the rather complicated interplay between ligand, substrate and protein. Under anaerobic conditions, the deoxy enzyme has lower affinity for L-Trp as compared to the CO complex. Photodissociation of the CO ligand prompts L-Trp to exit its primary docking site at the active site of hIDO. This release requires large conformational changes of the protein matrix. Our results confirm the presence of an inhibitory substrate binding site in hIDO. The occupation of L-Trp in the inhibitory binding site hinders CO rebinding, suggesting that substrate inhibition of hIDO at high concentrations of L-Trp is due to the retardation of O₂ association (9). Recently, hIDO has emerged as a therapeutic target for cancer treatment, leading to an active search for potent inhibitors (45). The observation that the inhibitory substrate binding site is a target for potential uncompetitive inhibitors (9) underscores the importance of elucidating the structural properties of this inhibitory substrate binding site.

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7


FIGURE LEGENDS

FIGURE 1. 4-K photolysis difference spectra. Dotted line: hIDO-CO/L-Trp, L-Trp at the active site. Dashed line: hIDO-CO/L-Trp, L-Trp removed from the active site. Solid line: substrate-free hIDO-CO (Thin solid line: absorption spectrum of substrate-free hIDO-CO at 240 K).

FIGURE 2. (A – C) Temporal development of FTIR photolysis difference spectra. (A) hIDO-CO (200 K), (B, C) hIDO-CO/L-Trp at 200 (B) and 240 K (C). (D, E) Temporal development of the normalized \( A_{\text{SF}} \) and \( A_{\text{S}} \) band areas of hIDO-CO/L-Trp at ∇ 260 K, ○ 250 K, ● 240 K, △ 230 K, ◇ 220 K, □ 200 K, and hIDO-CO at ■ 200 K. Samples were prepared in 50%/50% glycerol / potassium phosphate buffer. Numbers refer to reaction steps described in Figure 3.

FIGURE 3. Schematic depiction of L-Trp migration in hIDO, initiated by photodissociation of CO.

FIGURE 4. UV/visible absorption spectra of hIDO-CO/L-Trp (in 75%/25% glycerol / potassium phosphate buffer) measured before (solid line) and after photolysis at (A) 240 K, (B) 245 K and (C) 250 K. Spectra plotted as dashed lines were recorded immediately after illumination. The dotted line represents the spectrum collected 30 min after photolysis. Dash-dotted line in (A) is a reference spectrum of L-Trp-free enzyme, hIDO-CO.

FIGURE 5. UV/visible absorption spectra of hIDO-CO/L-Trp (in 70%/30% glycerol / potassium phosphate buffer) measured before photolysis (solid line), immediately after photolysis (dashed line) and 40 min after photolysis (dotted line) at 240 K. (A): [L-Trp] = 20 mM. (B): [L-Trp] = 4.8 mM. (C): [L-Trp] = 0.64 mM. Numbers refer to reaction steps described in Figure 3.

FIGURE 6. (A) Peak position of the hIDO-CO Soret band as a function of [L-Trp]. (B) Fraction of hIDO molecules, which slowly rebind CO, as a function of [L-Trp]. (C) Apparent rate coefficient of L-Trp relaxation into the primary substrate binding site as a function of [L-Trp]. Solid line: Linear fit to the data points at [L-Trp] > 3 mM. Dotted line: average \( \lambda_{\text{kin}} \) for [L-Trp] < 5 mM. Data were taken at 240 K (cryosolvent: 70%/30% glycerol / potassium phosphate buffer).
FIGURE 2
Figure 3
FIGURE 4
FIGURE 6

Panel A: Variation of the Soret peak wavelength ($\lambda_{\text{Soret}}$) with concentration of L-Trp ([L-Trp]) for $K_D = 1.5$ mM. The plot shows a decrease in $\lambda_{\text{Soret}}$ as [L-Trp] increases.

Panel B: Slowly rebinding fraction plotted against [L-Trp]. The fraction increases with increasing [L-Trp] for $K_D = 1.5$ mM.

Panel C: Kinetic rate constant ($\lambda_{\text{kin}}$) plotted against [L-Trp]. The rate constant increases linearly with [L-Trp].
Ligand and substrate migration in human indoleamine 2,3-dioxygenase
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