Crystals of Tryptophan Indole-Lyase and Tyrosine Phenol-Lyase Form Stable Quinonoid Complexes*

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The binding of substrates and inhibitors to wild-type *Proteus vulgaris* tryptophan indole-lyase and to wild type and Y71F *Citrobacter freundii* tyrosine phenol-lyase was investigated in the crystalline state by polarized absorption microspectrophotometry. Oxindolyl-L-alanine binds to tryptophan indole-lyase crystals to accumulate predominantly a stable quinonoid intermediate absorbing at 502 nm with a dissociation constant of 35 μM, approximately 10-fold higher than that in solution. L-Trp or L-Ser react with tryptophan indole-lyase crystals to give, as in solution, a mixture of external aldimine and quinonoid intermediates and *gem*-diamine and external aldimine intermediates, respectively. Different from previous solution studies (Phillips, R. S., Sundararju, B., & Faleev, N. G. (2000) *J. Am. Chem. Soc.* 122, 1008–1114), the reaction of benzimidazole and L-Trp or L-Ser with tryptophan indole-lyase crystals does not result in the formation of an α-aminoacrylate intermediate, suggesting that the crystal lattice might prevent a ligand-induced conformational change associated with this catalytic step. Wild-type tyrosine phenol-lyase crystals bind L-Met and L-Phe to form mixtures of external aldimine and quinonoid intermediates as in solution. A stable quinonoid intermediate with λ_{max} at 502 nm is accumulated in the reaction of crystals of Y71F tyrosine phenol-lyase, an inactive mutant, with 3-F-L-Tyr with a dissociation constant of 1 mM, approximately 10-fold higher than that in solution. The stability exhibited by the quinonoid intermediates formed both by wild-type tryptophan indole-lyase and by wild type and Y71F tyrosine phenol-lyase crystals demonstrates that they are suitable for structural determination by x-ray crystallography, thus allowing the elucidation of a key species of pyridoxal 5′-phosphate-dependent enzyme catalysis.
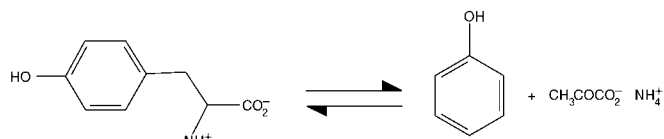
Tyrosine phenol-lyase (TPL)[1] (EC 4.1.99.2) and tryptophan indole-lyase (Trpase, EC 4.1.99.1) are pyridoxal 5′-phosphate

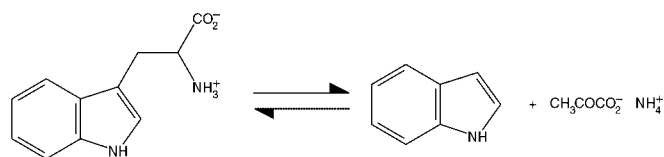(PLP)-dependent enzymes that catalyze β-elimination reactions to form phenol or indole and ammonium pyruvate from tyrosine and tryptophan, respectively (Schemes 1 and 2) (1, 2). The amino acid sequence alignment and crystallographic structures show that these two enzymes are very closely related (1, 2). Mechanistic studies (3–5) demonstrated that TPL and Trpase follow very similar catalytic mechanisms (Scheme 3). Both enzymes can catalyze the elimination reactions *in vitro* of a wide range of amino acids with suitable leaving groups on the β-carbon including *S*-(*o*-nitrophenyl)-L-cysteine (6, 7), *S*-alkyl-L-cysteines (8, 9), β-chloro-L-alanine (9, 10), L-Ser (8), and *O*-acyl-L-serines (7). However, *in vivo*, the enzymes are extremely specific for their respective physiological substrates. Both TPL and Trpase react with substrates and inhibitors to form equilibrating mixtures of external aldimine and quinonoid complexes (5, 10–14). We have previously reported the x-ray crystallographic structure of TPL complexed with the substrate analog 3-(4′-hydroxyphenyl)propionic acid (15). This complex resembles the Michaelis-Menten complex, because the analog lacks an amino group and is unable to form an external aldimine. We would like to obtain structures of quinonoid intermediates for both enzymes, because quinonoid intermediates are proposed to play a central role in the mechanisms of both enzymes (Scheme 3). In previous studies with Trpase and tryptophan synthase (16, 17), we prepared inhibitors oxindolyl-L-alanine (Scheme 4, *I*) and dihydro-L-tryptophan, which resemble the indolenine intermediate (Scheme 3), a proposed intermediate in the reactions of both enzymes. Trpase and tryptophan synthase are inhibited by different diastereomers of dihydro-L-tryptophan, suggesting that the indolenine intermediates in the reactions of the two enzymes exhibit opposite chirality (17). Oxindolyl-L-alanine was found previously to inhibit *Escherichia coli* Trpase with a K_i value of ~2.5–6 μM (3, 16, 18) and to form a prominent absorption band at 502 nm, demonstrating the predominant formation of a stable quinon-

SCHEME 1. **Reaction of TPL.**

SCHEME 2. **Reaction of Trpase.**
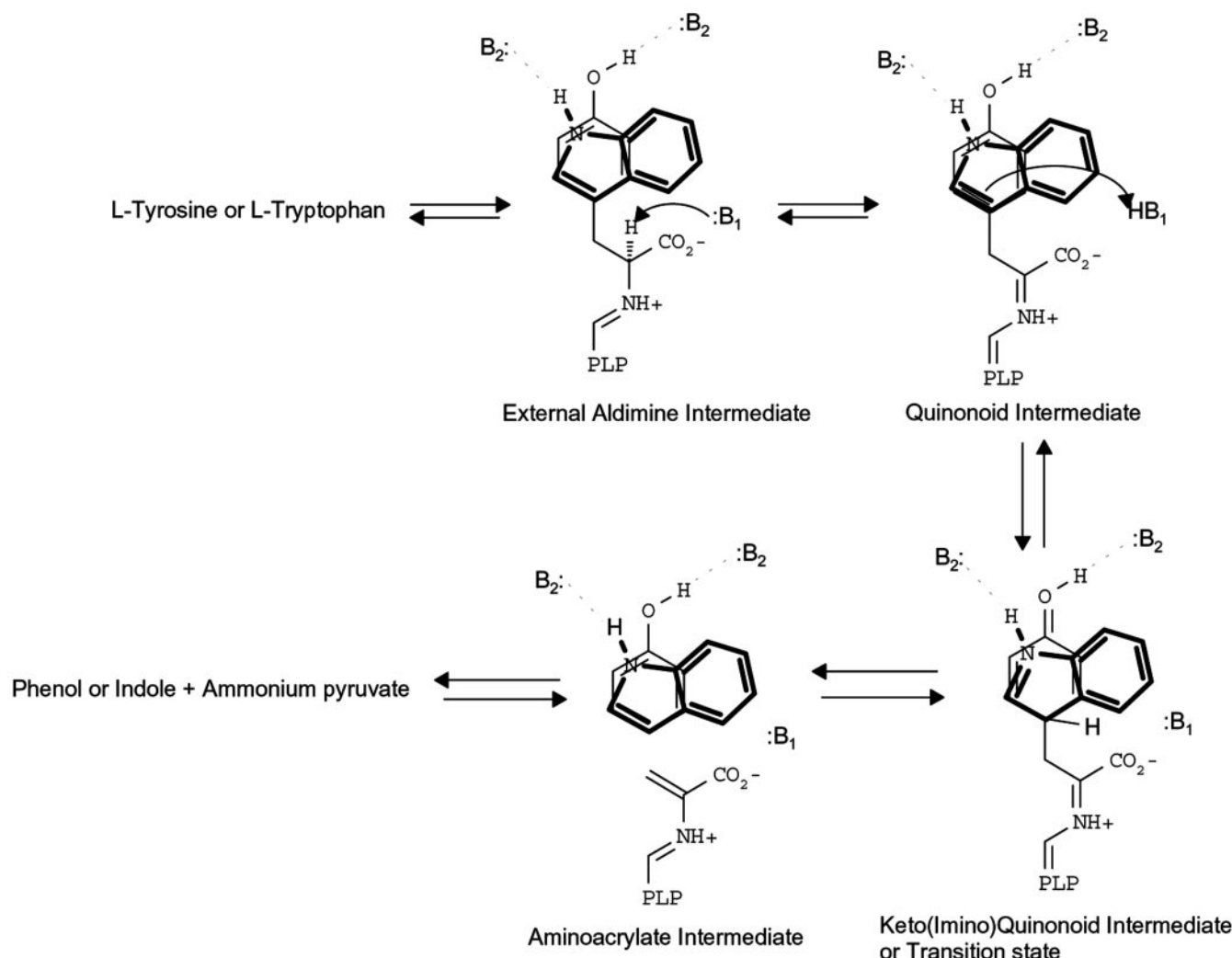
SCHEME 3. **Mechanism of Trpase and TPL.**

SCHEME 4. **Structures of oxindolyl-L-alanine (*I*) and 3-F-Tyr (*II*).**

oid intermediate. The Y71F mutant TPL was found to be inactive for the elimination of L-Tyr, but it binds L-Tyr and L-Phe tightly and forms stable quinonoid complexes with very strong absorption peaks at 502 nm (10). Presently, there is only one structure reported of a quinonoid intermediate of a PLP-dependent enzyme (19), indicating difficulties in the isolation and predominant accumulation of this species. This is at least partly because of quinonoid intermediate instability as a result of high reactivity and photosensitivity (20). By exploiting the capability of single crystal-polarized absorption microspectrophotometry in the characterization of enzyme reactivity in the crystalline state (21, 22) and particularly PLP-dependent enzymes (20, 23–25), we have investigated the catalytic comp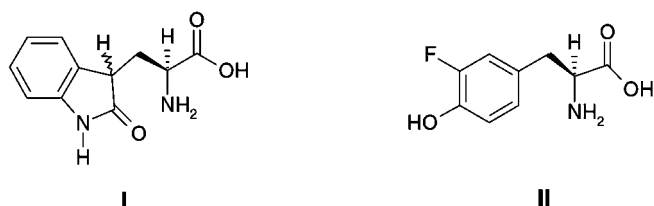etence of Trpase and TPL crystals. We found that stable quinonoid intermediates can be formed by soaking crystals of *Proteus vulgaris* Trpase with oxindolyl-L-alanine and the wild-type and Y71F mutant of *Citrobacter freundii* TPL with 3-fluoro-L-tyrosine (Scheme 4, *II*), L-Met, and L-Phe. These results provide a background for the future determination of the structures of these quinonoid intermediates by x-ray crystallography.

EXPERIMENTAL PROCEDURES

Materials—L-Tyr and L-Ser were obtained from Sigma. Benzimidazole and indole were purchased from Aldrich. 3-Fluoro-L-tyrosine was prepared from 2-fluorophenol and ammonium pyruvate using TPL as described previously (26). L-Trp and PLP were purchased from United States Biochemical Company. All other chemicals and reagents were of analytical grade.

Purification of Enzymes—*E. coli* SVS370 cells containing pTZTPL with wild type and the Y71F mutant *TPL* genes were grown, and the enzyme was purified as described previously (27). *P. vulgaris* Trpase was purified from cells of *E. coli* SVS370 containing the pAVK2 plasmid (28) with the *P. vulgaris tnaA* gene.[2]

Crystallization of TPL and Trpase—Crystallization of both wild-type TPL and Y71F mutant enzymes was performed with monomethyl ether polyethylene glycol 5000 (Fluka) by the hanging drop vapor diffusion technique using the conditions for the crystallization of the TPL complex with 3-(4′-hydroxyphenyl)propionic acid (15). Typically, 2 μl of a solution containing 20 mg/ml enzyme, 50 mM triethanolamine-HCl buffer, 0.2 mM PLP, 0.5 mM dithiothreitol, 0.1 M KCl, or 0.1 M CsCl, pH 8.0, were mixed with an equal volume of a reservoir solution. Crystallization was performed at 4 °C, and orthorhombic crystals appeared within 5 days. The best crystals were obtained using a solution containing 40–45% (w/v) monomethyl ether polyethylene glycol and either 0.1–0.4 M KCl or 0.1–0.4 M CsCl.

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[2] L. N. Zakomirdina and T. V. Demidkina, unpublished data.

Crystallization of Trpase was performed by a slightly modified procedure with respect to that described previously (29). Crystals were grown by the hanging drop technique at 4 °C. Polyethylene glycol 4000 (Fluka) was used as a precipitant. Two microliters of a solution containing 20 mg/ml enzyme, 0.1 M potassium phosphate buffer, 0.2 mM PLP, 5 mM dithiothreitol, pH 8.0, were mixed with an equal volume of a reservoir solution. The crystals were obtained within a week using a solution containing 20–30% (w/v) polyethylene glycol, 0.1–0.2 M KCl. As reported previously (29), there were two crystal forms in each drop. The orthorhombic crystals were used for spectral measurements.

Microspectrophotometric Measurements of TPL and Trpase Crystals—Polarized absorption spectra were recorded with a Zeiss MPM03 UV-Vis microspectrophotometer equipped with ×10 objectives. A Glan-Thompson polarizer is used to obtain linearly polarized light parallel to the extinction directions of the crystal. A second polarizer is used to properly orient the crystal. Depending on the orientation of the electronic transition dipole moments of the chromophores within the crystal asymmetric unit with respect to the directions of the electric vector, the absorption intensity along the perpendicular directions varies (30). Polarized absorption spectra were recorded on crystals suspended in solutions containing ligands or substrates and placed into a flow-cell (21, 25). The experiments were carried out at 20 °C.

RESULTS

Tryptophan Indole-Lyase Crystals—Polarized absorption spectra of the native *P. vulgaris* Trpase crystals exhibit peaks at 420 and 330 nm and a weak band at 490 nm (Fig. 1a, *solid line*). This spectrum is similar to that in solution with the exception that the relative intensity of the 330-nm band is much lower in solution.[3] The polarization ratio (*i.e.* the ratio of absorbance at each wavelength) is shown in Fig. 1, *top panel*, and values at selected wavelength are reported in Table I. An addition of oxindolyl-L-alanine (Scheme 4, *I*), a potent inhibitor of the *E. coli* enzyme (16), to crystals of *P. vulgaris* Trpase results in the formation of an intense absorbance peak at 502 nm that is attributed to a quinonoid intermediate (Fig. 1). This spectrum is essentially identical to that of the *E. coli* enzyme in solution in the presence of oxindolyl-L-alanine (18). The polarization ratio of the quinonoid species is 3-fold higher than that of the internal aldimine (Table I). The titration of the enzyme crystals with oxindolyl-L-alanine fits well to a binding isotherm with a calculated K_d value of 35 ± 5 μM (Fig. 1a, *inset*), approximately 10-fold higher than the K_i value of ~2.5–6 μM previously observed for *E. coli* Trpase in solution (3, 16, 18) (Table II). When *P. vulgaris* Trpase crystals are suspended in a solution containing 10 mM L-Trp, the polarized absorption spectra exhibit peaks at 425 and 505 nm with similar intensity corresponding to external aldimine and quinonoid intermediates, respectively (Fig. 2, *a* and *b*). This spectrum is also similar to that observed in solution for *E. coli* Trpase (14, 31). However, when benzimidazole, a non-nucleophilic analog of indole and an noncompetitive inhibitor of Trpase (14), is added together with L-Trp to the suspending medium, only a small decrease in the 505-nm absorption peak and a corresponding small increase in the peak at 345 nm were observed (Fig. 2, *a* and *b*). This result is quite different from the behavior of *E. coli* Trpase in solution in which benzimidazole binds to the aminoacrylate intermediate formed by the elimination of indole from L-Trp, resulting in a large decrease in the absorption peak at 505 nm and the formation of a corresponding absorption peak at 345 nm with a good isosbestic point (14, 31). The polarization ratio of the peak at 505 nm is significantly lower than unity (Table I), suggesting that either a different orientation of the PLP ring or a different direction of the transition dipole moment with respect to the quinonoid species formed in the presence of oxindolyl-L-alanine (Fig. 1b). An addition of 0.5 M L-Ser to Trpase crystals results in a decrease in the absorbance of the aldimine band at 420 nm and an absorbance increase at 340 nm with only a small ab-
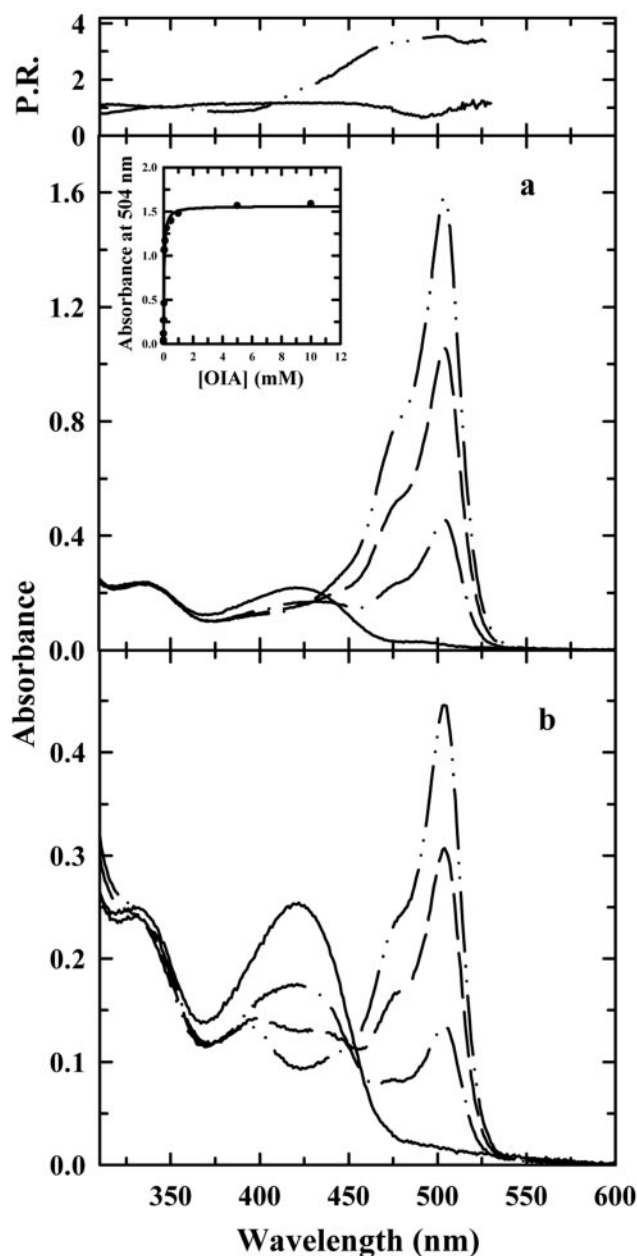
FIG. 1. **Polarized absorption spectra of wild-type *P. vulgaris* Trpase crystals.** Crystals of Trpase were suspended in a solution containing 25% polyethylene glycol 4000, 0.1 M potassium phosphate, 0.1 M KCl, pH 7.8. Spectra were recorded with the electric vector parallel to the diagonals of rhombic-shaped crystals in the absence (*solid line*) and in the presence of increasing concentrations of OIA. The structure of OIA is shown in Scheme 4. *Panel a* (*inset*), absorbance change at 504 as a function of OIA concentration. The *solid line* through data points is the fit to a binding isotherm with dissociation constant of 35 ± 5 μM. The same value was obtained along the other direction of polarization. The *top panel* is the polarization ratio for the spectra recorded in the absence (*solid line*) and presence (*dash-dot-dot-dash line*) of 10 mM OIA. The polarization ratio at each wavelength is calculated by dividing the absorption intensity of the spectrum recorded along a direction reported in *panel a* with the absorption intensity of the spectrum recorded along the perpendicular direction reported in *panel b*.

sorption peak at 490 nm (Fig. 2, *c* and *d*). The concomitant presence of 250 μM indole and 0.5 M L-Ser produces a small increase in the peak at 490 nm and a small absorbance decrease of the 340-nm peak (Fig. 2, *c* and *d*). Also, for this quinonoid species, the polarization ratio at 490 nm is lower than unity (Table I).

Tyrosine Phenol-Lyase Crystals—TPL binds a wide range of

TABLE I

Polarization ratio of absorption bands of Trpase and TPL crystals

Enzyme form	Polarization ratio	Figure
	$(\lambda\ nm)$	
Trpase	1.16 (420)	1, *a* and *b*
	0.81 (480)	1, *a* and *b*
Trpase + OIA	3.50 (502)	1, *a* and *b*
Trpase + L-Trp + benzimidazole	0.28 (505)	2, *a* and *b*
Trpase + L-Ser + indole	0.84 (340)	2, *c* and *d*
	0.42 (490)	2, *c* and *d*
TPL	1.25 (420)	3, *a* and *b*
TPL + L-Met	1.6 (425)	3, *a* and *b*
	0.40 (505)	3, *a* and *b*
Y71F-TPL + 3-F-L-Tyr	0.32 (502)	3, *c* and *d*

TABLE II

Comparison of dissociation constants for ligands of Trpase and TPL in the crystal and in solution

Enzyme + ligand	Crystal	Solution	Ref.
	K_d	K_i	
Trpase + OIA	$35 \pm 5\ \mu M^a$	2.5–6 μM	3, 16
TPL + L-Met	$0.5 \pm 0.1\ mM^b$	0.7 mM	5
	$1.5 \pm 0.5\ mM^c$		
TPL + L-Phe	$2.6 \pm 0.4\ mM^b$	1.7 mM	5
	$3.8 \pm 0.5\ mM^c$		
Y71F-TPL + 3-F-L-Tyr	$1.01 \pm 0.28\ mM^{b,d}$	0.1 mM (L-Tyr)	10
	$0.72 \pm 0.22\ mM^{c,d}$		
	$0.42 \pm 0.07\ mM^{b,e}$		
	$0.21 \pm 0.04\ mM^{c,e}$		

[a] The value of K_d is the same along both directions of polarization.

[b] The K_d was obtained from a titration monitored along the higher absorption intensity of enzyme crystals.

[c] The K_d was obtained from a titration monitored along the lower absorption intensity of enzyme crystals.

[d] Titrations were carried out on Y71F-TPL crystals grown in the presence of potassium chloride.

[e] Titrations were carried out on Y71F-TPL crystals grown in the presence of cesium chloride.

substrate and non-substrate amino acids in solution, forming equilibrating mixtures of external aldimine and quinonoid intermediates (5). Native TPL crystals exhibit polarized absorption spectra characterized by a peak centered at 420 nm and a weaker absorption at 340 nm (Fig. 3, *a* and *b*), similar to spectra in solution (32). The polarization ratio is 1.25 at 420 nm (Table I). When TPL crystals are suspended in a solution containing L-Met (Fig. 3, *a* and *b*) or L-Phe (data not shown), the resulting polarized absorption spectra are very similar to those observed in solution with absorption maxima at 425 and 505 nm. The polarization ratio at 505 nm is significantly lower than unity (Table I). The titrations of TPL crystals with either L-Met (Fig. 3*a*, *inset*) or L-Phe (data not shown) were fitted to a hyperbolic binding curve. The calculated dissociation constants are reported in Table II. The Y71F mutant TPL does not exhibit elimination activity with L-Tyr, although it binds L-Tyr, 3-F-L-Tyr and L-Phe and forms quasi-stable absorption spectra with intense peaks at 502 nm attributed to quinonoid species (10). Crystals of Y71F TPL, grown in the presence of K$^+$ and suspended in a solution containing increasing concentrations of 3-F-L-Tyr, exhibited absorption spectra characterized by a peak at 502 nm (Fig. 3, *c* and *d*). The titration was fitted to a single hyperbolic binding isotherm. The value of the dissociation constant is ∼1 mM as reported in Table II. This value is ∼10-fold higher than the K$_d$ of Y71F TPL is for L-Tyr of 110 μM (10). Y71F TPL crystals, grown in the presence of Cs$^+$, gave similar results (Table II) (data not shown).

DISCUSSION

The reaction mechanisms proposed for TPL and Trpase are very similar as shown in Scheme 3. Both enzymes initially form an external aldimine structure with an absorption peak at
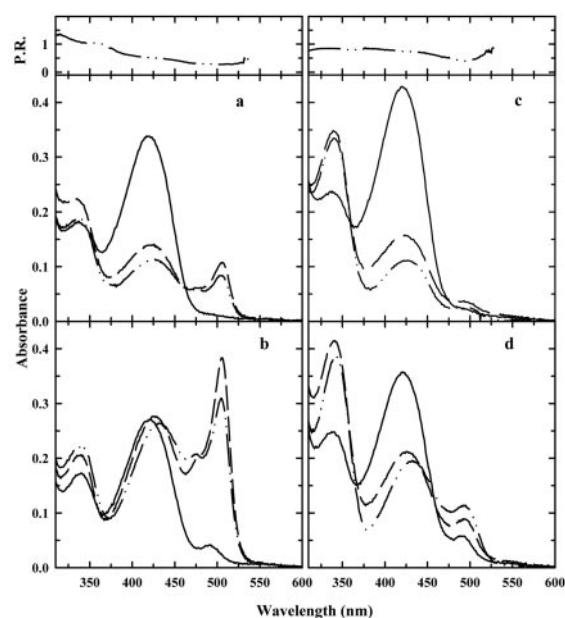

FIG. 2. **Polarized absorption spectra of wild-type *P. vulgaris* Trpase crystals.** Crystals of Trpase suspended in a solution containing 25% polyethylene glycol 4000, 0.1 M potassium phosphate, 0.1 M KCl, pH 8.05, in the absence (*solid line*). Panels *a* and *b*, in the presence of 10 mM L-Trp (*dashed line*), 10 mM L-Trp, and 5 mM benzimidazole (*dash-dot-dot-dash line*). Panels *c* and *d*, in the presence of 0.5 M L-Ser (*dashed line*) and 0.5 M L-Ser and 250 μM indole (*dash-dot-dot-dash line*). The *left top panel* is the polarization ratio for the spectra in the presence of L-Trp and benzimidazole (*dash-dot-dot-dash line*), and the *right top panel* is the polarization ratio for the spectra in the presence of L-Ser and indole (*dash-dot-dot-dash line*).

420–425 nm upon reaction with the substrate. The external aldimines are then deprotonated on the α-carbon to form quinonoid intermediates, which exhibit absorption maxima at ∼500–505 nm. The elimination of the aromatic leaving group takes place concomitant with or after tautomerization of the initial quinonoid intermediate (31). The strict reaction specificity of these enzymes has been proposed to be controlled by the position of a second catalytically essential base (shown as B_2 in Scheme 3) in the substrate binding site, which controls the tautomerization (3–5). Structural studies and kinetic analyses of R381A, R381I, and R381V TPL previously demonstrated that Arg-381 is required for the tyrosine substrate specificity of TPL (15), and thus it may be the proposed catalytic base. Recent studies of *E. coli* H463F Trpase suggest that His-463 is important in Trp specificity; hence it might be the proposed catalytic base either directly or through a hydrogen-bonded network with other residues and/or active site water (33). Both TPL and Trpase have an active site Tyr residue, which is essential for the elimination of the physiological substrates and which may serve as a general acid to transfer a proton to the leaving group (10, 33). Thus, to understand the molecular basis of the reaction specificity of these enzymes, it is critical to obtain three-dimensional structures of the quinonoid intermediates by x-ray crystallography.

We found that oxindolyl-L-alanine (Scheme 4, *I*), a potent inhibitor of Trpase, binds to *P. vulgaris* Trpase crystals and forms a quinonoid spectrum very similar to that seen in solution (Fig. 1). The spectral intensity remains constant for long periods, indicating that the quinonoid species is fairly stable. Furthermore, the crystals do not show any evidence of cracking. Therefore, the structural change responsible for the formation of the external aldimine and quinonoid intermediates is evidently not large enough to disrupt the crystal packing. A spectroscopic parameter particularly sensitive to crystal integ-

FIG. 3. **Polarized absorption spectra of *C. freundii* wild-type TPL (*panels a* and *b*) and Y71F TPL crystals (*panels c* and *d*).** Crystals of wild-type TPL and Y71F suspended in a solution containing 45% polyethylene glycol 5000 monomethyl ether, 50 mM triethanolamine-HCl, 0.2 M KCl, 0.2 mM PLP, pH 7.8, in the absence (*solid line*) and in the presence of increasing concentrations of L-Met (*panels a* and *b*) and increasing concentrations of 3-F-L-Tyr (*panels c* and *d*). The structure of 3-F-L-Tyr is shown in Scheme 4. *Panel a* (*inset*), absorption changes at 503 nm as a function of L-Met concentration. The *solid line* through data points is the best fitting to a binding isotherm with a dissociation constant of 0.5 ± 0.1 mM. *Panel c* (*inset*), absorption changes at 502 nm as a function of 3-F-L-Tyr. The *solid line* through data points is the best fitting to a binding isotherm with a dissociation constant of 0.72 ± 0.22 mM. The *left top panel* is the polarization ratio for the spectra recorded in the absence (*solid line*) and presence (*dash-dot-dot-dash line*) of 50 mM L-Met. The *right top panel* is the polarization ratio for the spectra recorded in the absence (*solid line*) and presence (*dash-dot-dot-dash line*) of 20 mM 3-F-L-Tyr.
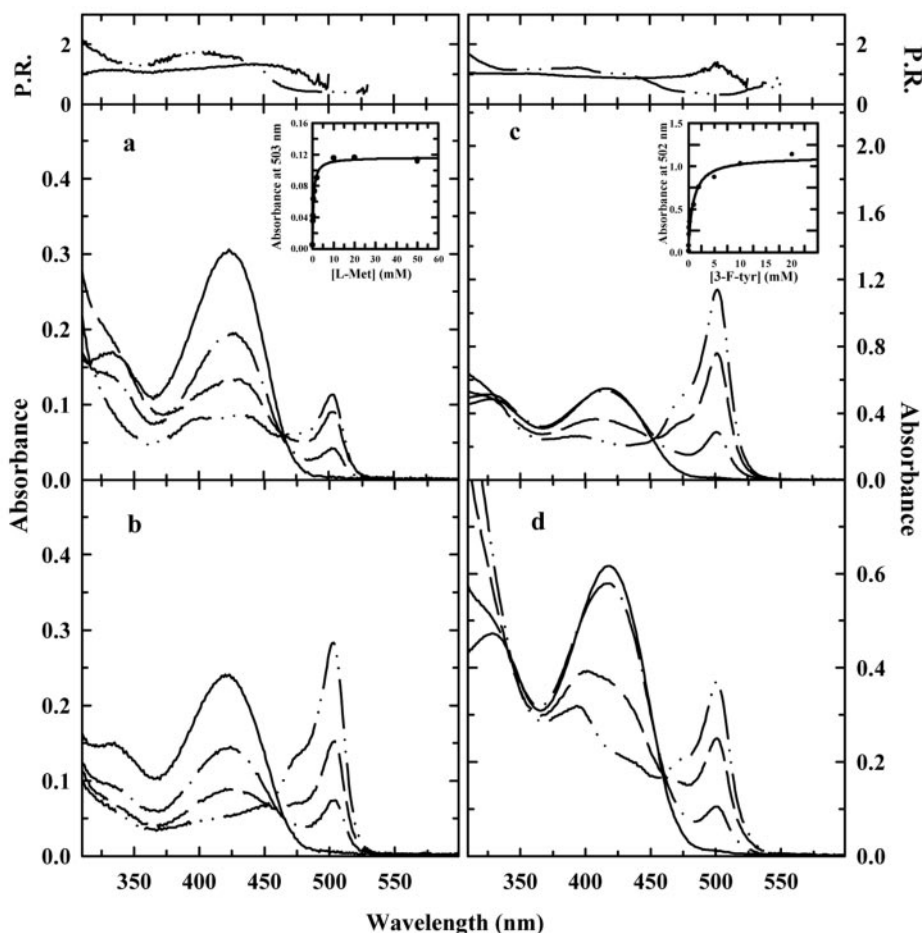
rity is the polarization ratio (*i.e.* the ratio of absorbance at each wavelength along perpendicular directions of polarization). This parameter is related to the projection of the electronic transition dipole moments of the chromophore along the direction of the electric vectors of the linearly polarized light. A value of the polarization ratio close to unity indicates either an equal projection or a disordered crystal. For Trpase as well as TPL crystals, the internal aldimine exhibits values of polarization ratio slightly higher than unity, whereas external aldimine and quinonoid species exhibit values significantly higher and lower than unity (Table I). The binding of oxindolyl-L-alanine to the crystals is somewhat weaker (10-fold) than in solution (Table II). Slightly higher dissociation constants have been usually observed in protein crystals (21, 23, 24), probably because of constraints caused by lattice forces on protein flexibility. The case of the reaction of *O*-acetyl-L-Ser sulfhydrylase crystals with *O*-acetyl-L-Ser to form an aminoacrylate species is a clear example of the extreme dependence of ligand binding on lattice effects (24). Three distinct *O*-acetyl-L-Ser sulfhydrylase crystal forms were grown under very similar experimental conditions. One form exhibits a dissociation constant that is ~500-fold higher than in solution. A second form is almost unable to bind *O*-acetyl-L-Ser, whereas the third form binds the substrate with a concomitant crystal breakage. These findings suggest that binding and catalysis are associated to ligand-induced conformational changes, some of which are hindered by lattice forces.

The reactivity of Trpase crystals toward L-Trp results in a steady-state mixture of external aldimine and quinonoid intermediates similar to that seen in solution (Fig. 2), and the addition of benzimidazole results in only a small decrease in the 505-nm absorbance. This finding is in contrast to solution in which benzimidazole causes the 505-nm band to decrease dramatically and form an α-aminoacrylate intermediate (13, 14). This finding suggests that a conformational change associated with the aminoacrylate intermediate formation might be prevented by lattice forces. This conclusion is supported by the reactivity of L-Ser to Trpase crystals, showing the disappearance of the 420-nm peak with the concomitant formation of a 340-nm intermediate (Fig. 2). This intermediate could be either an α-aminoacrylate or *gem*-diamine structure. The addition of indole plus L-Ser did not result in much change in the 340-nm peak, suggesting that it is a *gem*-diamine rather than an α-aminoacrylate structure. Thus, although the Trpase crystals seem to behave normally with respect to the formation of aldimine and quinonoid intermediates, they do not appear to readily form α-aminoacrylate intermediates. This finding suggests that there is a larger conformational change in the formation of the α-aminoacrylate intermediate than for the external aldimine and quinonoid intermediates. The data on the formation of the aminoacrylate intermediate in *O*-acetyl-L-Ser sulfhydrylase crystals as described above lead to a similar conclusion (21).

An intriguing finding is the different polarization ratios observed for the quinonoid intermediates formed upon reaction with OIA and with L-Trp or L-Ser (Table I). According to Vincent *et al.* (34), the direction of the transition dipole moments of quinonoid structures is rotated ~45° clockwise with respect to the those of the internal and external aldimine. The observation that for the OIA-quinonoid complex, the polarization ratio is ~3.5 and for the L-Trp and L-Ser-quinonoid complexes, the polarization ratio is ~0.3 suggests that the PLP ring might have different orientations in distinct quinonoid species. Either crystallographic studies or spectra along the third direction of

the crystal should allow solution of the uncertainty between ring rotation and distinct directions of transition dipole moments. Unfortunately, the crystal morphology prevented the collection of spectra in the third direction. Because oxindolyl-L-alanine is a potent transition-state analog inhibitor (3, 16, 18), it is not unexpected that its quinonoid structure has a different conformation than the ground-state quinonoid complex of L-Trp or L-Ser. It is also interesting that the Trpase as isolated exhibits a low intensity quinonoid peak at 490 nm, which shows strong polarization (Fig. 1*a*). Torchinsky and co-workers (35) observed a similar peak in native *E. coli* Trpase, which shows weak absorption but exhibits intense linear dichroism. This band was removed by the formation of the apoenzyme and reconstitution with PLP, so the absorption may be due to a ligand found in cell extracts that remains tightly bound through the isolation (35).

An addition of either L-Phe or L-Met to crystals of wild-type TPL results in equilibrium mixtures of external aldimine and quinonoid intermediates (Fig. 3), similar to those seen previously in solution (5, 10–12). The K_d of these amino acids is similar to that in solution (Table II) (5). Y71F TPL has no detectable activity for the elimination of L-Tyr, but it forms quasi-stable quinonoid complexes with intense absorption peaks at 502 nm in the presence of L-Phe, L-Tyr, and 3-F-L-Tyr (10). Soaking Y71F TPL crystals with 3-F-L-Tyr results in a similar intense absorption peak at 502 nm (Fig. 3, *c* and *d*). 3-F-L-Tyr was used in the titration experiments, because it has similar kinetic properties to L-Tyr with TPL (27); however, 3-F-L-Tyr has significantly higher solubility (>20 mM) in aqueous buffer solutions than L-Tyr (~4 mM). The binding constant for 3-F-L-Tyr of 1 mM for the crystalline enzyme is ~10-fold higher than that observed in solution for L-Tyr (Table II) (10). These results demonstrate that wild type and Y71F TPL crystals can readily form quinonoid complexes, once again suggesting that the conformational changes to form quinonoid structures do not disrupt the crystal packing of the enzyme. It should be noted that the polarization ratios for these spectra are close to those observed for the L-Trp and L-Ser quinonoid complexes of Trpase.

The observation that the crystals of Trpase and Y71F TPL can form stable complexes simply by soaking with oxindolyl-L-alanine and 3-F-L-Tyr, respectively, which are mostly if not exclusively composed of quinonoid species, will facilitate the future determination of the structures of these complexes by x-ray crystallography. Although quinonoid intermediates are central to the proposed mechanisms of Trpase, TPL and many other PLP-dependent enzymes, they are frequently unstable and do not accumulate in large quantity in the crystalline state. Indeed, only one structure has been reported for a quinonoid intermediate in a PLP-dependent enzyme to date (19), and the structure of the quinonoid complex formed in the reaction of indoline and L-Ser catalyzed by tryptophan synthase is in progress.[4]

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Crystals of Tryptophan Indole-Lyase and Tyrosine Phenol-Lyase Form Stable Quinonoid Complexes

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