L-Tryptophan 2',3'-Oxidase from Chromobacterium violaceum

SUBSTRATE SPECIFICITY AND MECHANISTIC IMPLICATIONS*

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L-Tryptophan 2',3'-oxidase, an amino acid α,β-dehydrogenase isolated from Chromobacterium violaceum, catalyzes the formation of a double bond between the Cα and Cβ carbons of various tryptophan derivatives provided that they possess: (i) a L-enantiomeric configuration, (ii) an α-carboxyl group, and (iii) an unsubstituted and unmodified indole nucleus. Kinetic parameters were evaluated for a series of tryptophan analogues, providing information on the contribution of each chemical group to substrate binding. The stereochemistry of the dehydro product was determined to be a Z-configuration from proton nuclear magnetic resonance assignments. No reaction can be observed in the presence of other aromatic β-substituted alanyl residues which behave neither as substrates nor as inhibitors and therefore do not compete against this reaction. The enzymatic synthesis of α,β-dehydrotryptophanyl peptides from 5 to 24 residues was successfully achieved without side product formation, irrespective of the position of the tryptophan residue in the amino acid sequence. A reactional mechanism involving a direct α,β-dehydrogenation of the tryptophan side chain is proposed.

Amino acid oxidases and dehydrogenases are widely distributed in living cells where they play a key role in the metabolic fate of amino acids, generating α-keto acids by oxidative deamination or dehydration reactions (1-3).

In this context, we recently isolated a novel enzyme from Chromobacterium violaceum (ATCC 12472), and showed that it catalyzes the conversion of N-acetyl-L-tryptophanamide (NATA) into N-acetyl-α,β-dehydrotryptophanamide (ΔNATA) (4). We named this enzyme L-tryptophan 2',3'-oxidase (or L-tryptophan: oxygen 2',3'-oxidoreductase). L-Tryptophan 2',3'-oxidase, therefore, might contribute to the conversion of L-tryptophan into indole-3-pyruvic acid, its α-keto derivative, by catalyzing the first step of the reaction shown in the Scheme 1. Indeed, Dakin (5) first demonstrated in 1926 that an α,β-dehydroamino acid exists in a tautomeric equilibrium with its imino acid form and subsequently, this latter species was shown to be readily hydrolyzed into the β-substituted corresponding pyruvic acid (α-keto acid) (6). Hence, as it was originally suspected (7, 8), the transient formation of α,β-dehydroamino acids could account for a possible mechanism in the course of the amino acid catabolism. L-Tryptophan 2',3'-oxidase is a novel hemoprotein exhibiting a number of interesting features (4). First, it seems to directly catalyze the conversion of NATA into ΔNATA and in a stoichiometric manner the reduction of molecular O2 to generate H2O2. Second, it possesses a high molecular weight heteropolymetric structure (Mr = 680,000) consisting of two subunits: α (Mr = 14,000) and β (Mr = 74,000), presumably organized in an (αβ)8 manner with one heme molecule per αβ protomer. Third, L-tryptophan 2',3'-oxidase was shown to be active at high temperature (up to 80°C) and under conditions that are frequently denaturing for proteins, i.e. 0.2 M dithiothreitol, 1% SDS, or 4.5 M urea. At present, however, little is known as to the substrate specificity of the enzyme and to a possible reaction mechanism.

With the view to shed light on these questions, we investigated the capacity of L-tryptophan 2',3'-oxidase to react with a variety of tryptophan derivatives. Here we report steady-state kinetic parameters determined for these reactions and specify the stereochemistry of the dehydro product. Together, the data obtained suggest that L-tryptophan 2',3'-oxidase proceeds to a direct α,β-dehydrogenation of the tryptophan side chain via a mechanism closely related to that proposed for fatty acyl-CoA dehydrogenases (9), in sharp contrast with the complex pH-dependent dichotomous mechanism postulated for the Pseudomonas tryptophan side chain oxidase (EC 1.13.99.3) (10, 11). Finally, we demonstrate that L-tryptophan 2',3'-oxidase can also proceed to the α,β-dehydrogenation of L-tryptophan side chains included in a number of peptides. This finding offers new enzymatic issues as to the modification and labeling of this peculiar side chain in proteins.

EXPERIMENTAL PROCEDURES

Materials—Amino acids and derivatives were all purchased from Sigma. N,N'-Formyl-L-tryptophan was prepared according to Previero et al. (12). Pentagastrin, glucagon fragment-(22–29) (human), and luteinizing hormone-releasing hormone were purchased from Bachem AG (Switzerland). Adenocorticotropic hormone fragment (ACTH 1-24, human) was obtained from Ciba Pharmaceutical Co. The substance P analogue was a generous gift of Dr. G. Chassaing (Laboratoire de chimie organique biologique,URA CNRS 493, France). The analogue of the fragment-(α185–199) of the α-subunit of the nicotinic acetylcholine receptor was synthesized in our laboratory by Dr. G. Mourier. Catalase (beef liver, EC 1.11.1.6) was purchased from Sigma. All other chemicals were purchased from Merck Chemical Co. (Germany) and Prolabo (France).

L-Tryptophan 2',3'-oxidase was purified from C. violaceum (ATCC 12472) according to Genet et al. (4), and stored at -80°C in 0.1 M bis-Tris buffer, pH 7, in the presence of 5 mM EDTA and 0.58 mM phenylmethylsulfonyl fluoride. Working solutions, prepared by dilution in the same buffer, were not used for more than a day. Using NATA as a model substrate, the molar activity of the purified enzyme, as defined with respect to 1 mol of ΔNATA produced per s per mol of enzyme protomer (mole of heme), was estimated to be equal to 44.4 ± 4.1 s⁻¹.

Methods—Steady-state kinetics were carried out under standard conditions. The standard assay mixture comprised 1 mM NATA in 50

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mm succinate buffer, pH 5.5. Catalase was added to the reaction mixture at a final concentration of 10 μg ml⁻¹ in order to prevent enzyme inactivation by the produced H₂O₂. The reaction was initiated by adding the enzyme, and the increase in absorbance at 330 nm due to the formation of α-NA (ε₃₃₀ = 18.8 mM⁻¹ cm⁻¹) (4)) was monitored at 30 °C, using a Beckman DU-70 spectrophotometer. Kₚ, and k_cat were determined using the KaleidaGraph (version 2.1.3) software (Abelbeck Software), either by nonlinear regression from the direct plot or by linear regression using a double-reciprocal plot; both methods gave self-consistent results. Kₚ were measured by kinetic competition experiments against α-NA. Chromatographic separations and purifications of dehydro amino acids and peptides were carried out using a Waters 600 chromatographic system (column, reverse-phase C18 (Vydac); eluant, gradient from 0 to 40% acetonitrile in 0.1% trifluoroacetic acid; flow rate, 1 ml min⁻¹) supplied with a Waters 996 photodiode array detector. Dehydro products were analyzed either by chemical ionization-mass spectrometry (CI-MS) or by fast atom bombardment. Proton nuclear magnetic resonance (¹H NMR) spectra were recorded using a Bruker WM250 spectrometer (250 MHz).

RESULTS

Enzymatic Activity of L-Tryptophan 2',3'-Oxidase toward Various Tryptophan Derivatives—In a previous study (4), we showed that the enzymatic conversion of α-NA into α-L-tryptophan 2',3'-oxidase activity could thus be directly quantified spectrophotometrically and the steady-state kinetic parameters of the reaction were determined to be k_cat = 45.2 ± 3.2 s⁻¹ and K_m = 19.5 ± 2.8 μM under standard conditions. Then we demonstrated the absence of any side products under various experimental conditions, including a wide pH range varying from 3 to 8. Assuming that formation of a double bond conjugated with the indole ring would produce a similar characteristic spectral modification, we examined the behavior of L-tryptophan 2',3'-oxidase toward a variety of tryptophan derivatives.

A number of tryptophan derivatives behaved as substrates. These are L-tryptophan, L-tryptophanamide, N-acetyl-L-tryptophan, and indole-3-propionate whose conversion exhibited an absorbance increase at characteristic wavelengths (Table 1). The intensity of these absorption bands varied from one product to another. Surprisingly, however, in the case of L-tryptophan and L-tryptophanamide, the absorbance increase was followed after 1 to 3 min of reaction by a reverse effect, i.e. an absorbance decrease at 320 or 326 nm, respectively. This phenomenon suggested the instability of the reaction products and the inhibition of the enzyme by one of the associated side products. Such an inhibition was reversible since the enzyme was still fully active upon dilution in the presence of α-NA (5 mM).

In all cases, therefore, the steady-state kinetic parameters were accurately determined according to classical methods. The initial velocities were evaluated during the first 10 s of the reactions at various substrate concentrations. The resulting conventional hyperbolic curves (Fig. 1) allowed us to estimate the kinetic parameters by nonlinear regression analyses. Note that in these calculations, we assumed that all products whose instability sometimes made their isolation difficult had the same molar absorption coefficient as α-NA (ε₃₃₀ = 18.8 mM⁻¹ cm⁻¹) (4)).

The kinetic parameters of the reactions performed with the different substrates are summarized in the Table 1. One may note that L-tryptophan 2',3'-oxidase displays the highest efficiency for L-tryptophan, which has been proposed to be the most probable natural substrate of the enzyme (4). Otherwise, most substrates did not exhibit major kinetic differences. In summary therefore, modifications of L-tryptophan which do not abolish the enzymatic activity of L-tryptophan 2',3'-oxidase are blockage of the α-amino group (N-acetyl-L-tryptophan), the α-carboxylic group (L-tryptophanamide), or both (α-NA), as well as deamination (indole-3-propionate).

Identification of the Reaction Products—The nature and role of the products of the reactions were also tentatively analyzed. Thus, experiments of isolation of the reaction products were carried out using reverse-phase chromatography under conditions described above. No clear cut results were obtained in the case of L-tryptophan due to the fact that more than 95% of the substrate remained in the reaction medium after several hours of incubation. More clearly, conversion of L-tryptophanamide yielded 3 resolved peaks (Fig. 2). The major one represented the nonconverted substrate (retention time, 21.5 min; λ_max = 281 nm), whereas the two others (retention times, 30.2 and 37.2 min), characterized by an absorption band at (λ_max) 326 nm, were identified, respectively, as the α,β-dehydrotryptophanamide product (M_r = 201.2) and its rearrangement as indole-3-pyruvic amide (M_r = 202.2), as judged from mass spectrometry (CI-MS) analyses (m/z = 202 and 203; [M+H]+). This side reaction was not unexpected due to the fact that α,β-dehydro derivatives possessing a free amino group are unstable species (see Scheme 1).

Unambiguously, the dehydro products of N-acetyl-L-tryptophan and indole-3-propionate (Fig. 1) were, respectively, identified as N-acetyl α,β-dehydrotryptophan and indole-3-acrylate, characterized by an absorption band at (λ_max) 318 nm (ε_318 = 14.8 mM⁻¹ cm⁻¹) and 326 nm (ε_326 = 19.6 mM⁻¹ cm⁻¹) (6), and by mass spectrometry (CI-MS) (m/z = 245 and 188; [M+H]+). Mass spectrometry analyses also revealed the presence of an additional component whose mass was correspondingly 44 units lower. The same molecular species is also detected in the commercially available indole-3-acrylic acid, suggesting that these additional components were in fact generated during the mass spectrometry experiments, resulting probably from unwanted fragmentation (decarboxylation) associated with the mode of ionization.

Configurational Assignment of the α,β-Dehydro Product—An important aspect associated with the enzymatic conversion concerned the geometric stereochemistry of the dehydro products. It is known that an α,β-dehydrotryptophan component can adopt either E- or Z-configurations (Scheme 2) and these species can be readily identified from assignment of the vinyl proton by ¹H NMR spectroscopy (13). The reference compounds
Substrate Specificity of L-Tryptophan 2',3'-Oxidase from C. violaceum

| Substrates                        | $K_m$ (μM) | $k_{cat}$ (s⁻¹) | $k_{cat}/K_m$ (s⁻¹/μM) | $\lambda_{max}$ (nm) | Ref.     |
|-----------------------------------|-----------|----------------|------------------------|----------------------|---------|
| L-Tryptophan                      | 5.8 ± 0.3 | 39.6 ± 0.5     | $7 \times 10^6$         | 320                  | This work |
| L-Tryptophanamide                 | 9.1 ± 0.9 | 35.6 ± 1.1     | $4 \times 10^6$         | 326                  | This work |
| N-Acetyl-L-tryptophanamide        | 19.5 ± 2.8| 45.2 ± 3.2     | $2 \times 10^5$         | 333                  | 4       |
| N-Acetyl-L-tryptophan             | 79.1 ± 9.9| 47.4 ± 2.4     | $6 \times 10^4$         | 318                  | This work |
| Indole-3-propionate               | 20.0 ± 2.8| 47.1 ± 3.1     | $2 \times 10^5$         | 326                  | This work |

**Table I** Substrate specificity of L-tryptophan 2',3'-oxidase from C. violaceum

Fig. 1. Determination of steady-state kinetic parameters of various substrates for L-tryptophan 2',3'-oxidase. Experimental conditions are given under “Experimental Procedures.” The total enzyme concentration, $E_c$, was 6.6 μM (mole of heme).

Fig. 2. Analysis of the reaction of L-tryptophanamide with L-tryptophan 2',3'-oxidase. The general experimental conditions are given under “Experimental Procedures.” 1 mM L-Tryptophanamide was incubated in 50 mM succinate buffer, pH 5.6, containing 40 μg ml⁻¹ catalase, in the presence of 6.6 mM enzyme (mole of heme), for 15 h at 30 °C. The components of the reaction medium were separated by reverse-phase chromatography on a Vydac C18 column, eluted with a 30-min gradient from 0 to 40% acetonitrile in 0.1% trifluoroacetic acid, at a flow rate of 1 ml min⁻¹. The elution was followed at 275 (---) and 330 (-----) nm. The peaks were identified by mass spectrometry (CI-MS).

N-acetyl-(Z,E)-α,β-dehydrotryptophan ethyl ester used in our study were made available by chemical synthesis according to the method previously described by Hengartner et al. (14). One should emphasize that this procedure requires a careful control of the temperature during the different steps of the synthesis in order to reach an appropriate 1:1 ratio of the two forms, the E-isomer being readily converted into the Z derivative upon heating. NMR analyses of the purified isomers confirmed that they can be easily identified from the singlet signal of their vinyl group (Fig. 3). The chemical shifts of this peculiar proton was 6.90 and 7.60 ppm for the Z-isomer being readily converted into the E-isomer, respectively, in good agreement with previous data (14).

To identify the stereochemistry of the dehydro product generated by L-tryptophan 2',3'-oxidase, we incubated the enzyme (0.8 μg ml⁻¹; heme concentration, ~9.1 nm) with N-acetyl-L-tryptophan ethyl ester (2.2 mM) and purified the resulting dehydro product on reverse-phase chromatography under conditions described above. N-Acetyl-α,β-dehydrotryptophan ethyl ester was characterized by an absorption band at ($\lambda_{max}$) 340 nm and by mass spectrometry (m/z 273; [M+H]+). As shown in Fig. 3, 1H NMR spectrum of this compound showed the vinyl proton to be shifted downfield (δ = 7.60 ppm) as previously observed in the case of the Z-isomer. No signal was found corresponding to the E-isomeric form. Therefore, the steric configuration of the α,β-dehydrotryptophanyl moiety produced by enzymatic dehydrogenation was confirmed unambiguously to have a Z-geometry.

Inhibition of the L-Tryptophan 2',3'-Oxidase Activity by Other Tryptophan Derivatives—In contrast to the previous tryptophan derivatives, no spectral perturbation happened when L-tryptophan 2',3'-oxidase was incubated in the presence of methyl-3-indole (skatole), tryptamine, 5-hydroxy-L-tryptophan, in-formyl-L-tryptophan, D-tryptophan, or N-acetyl-L-phenyl-alaninamide, and other aromatic or pseudoaromatic β-substituted alanyl residues, i.e. N-acetyl-L-phenyl-alaninamide, N-acetyl-L-tyrosinamide, and L-histidine, incubated at concentrations up to 1.2 mM under competitive inhibition of L-tryptophan 2',3'-oxidase (Fig. 4). From Dixon plots (Fig. 4) we showed these compounds to act as competitive inhibitors of NATA. $K_i$ values determined for the inhibitors are summarized in Table II.

Therefore, the modifications that make a tryptophan derivative an inhibitor of L-tryptophan 2',3'-oxidase are: (i) alteration of the indole ring (5-hydroxy-L-tryptophan, N^⁶-formyl-L-tryptophan, α-tryptophan, or N-acetyl-α-tryptophan). From Dixon plots (Fig. 4) we showed these compounds to act as competitive inhibitors of NATA. $K_i$ values determined for the inhibitors are summarized in Table II.

Contribution of the Indole Nucleus to Substrate Binding—The fact that, in our hands, skatole exhibited the more potent competitive inhibition of L-tryptophan 2',3'-oxidase suggested that the indole moiety could play a key role in the recognition and accommodation of ligands to the active site. Indeed, other aromatic or pseudoaromatic β-substituted alanyl residues, i.e. N-acetyl-L-phenyl-alaninamide, N-acetyl-L-tyrosinamide, and L-histidine, incubated at concentrations up to 1.2 mM under competitive inhibition of L-tryptophan 2',3'-oxidase (Fig. 4). From Dixon plots (Fig. 4) we showed these compounds to act as competitive inhibitors of NATA. $K_i$ values determined for the inhibitors are summarized in Table II.

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**Table II** Substrate specificity of L-tryptophan 2',3'-oxidase from C. violaceum

**Scheme 2. N-Acetyl(Z,E)-α,β-dehydrotryptophan ethyl ester.** Ac, acetyl; OEt, ethyl ester.
Dehydrogenation of Tryptophan Side Chain in Peptides—From the above results, no indication emerged suggesting that L-tryptophan 2',3'-oxidase would not be able to recognize a tryptophan side chain in a peptide or in a protein. We therefore investigated the possibility of forming dehydropeptides using six different peptides comprising 5 to 24 amino acid residues. As an example, Fig. 5 shows the reaction of L-tryptophan 2',3'-oxidase with pentagastrin (a), luteinizing-hormone releasing-hormone (b), and adenocorticotropic hormone (ACTH 1–24, human) (c). In each case, an absorption band occurred around 335 nm, as expected for the formation of a dehydro product (Table III). The corresponding dehydrotryptophanyl products were purified on reverse-phase chromatography (see “Experimental Procedures”) and characterized by mass spectrometry (fast atom bombardment) analyses. No side product was found to occur during this reaction. Steady-state kinetic parameters were estimated for all peptides supplied in sufficient quantity (Table III). Considering the ratio of \( k_{cat}/K_m \) that can be used as an index for the enzyme preference for a series of substrates, it seems that, in a first approximation, the enzyme efficiency tends to decrease with respect to the peptide chain length.

**DISCUSSION**

Minimal Requirements for Enzymic Catalysis—In the present study, we confirmed our previous conclusion that L-tryptophan 2',3'-oxidase specifically catalyzes the dehydrogenation of tryptophan side chain (4). However, we also found that not any tryptophan derivatives can be recognized as a substrate by the enzyme, allowing us to identify key elements for the recog-
Spectral changes associated with the dehydrogenation of tryptophan side chain in (a) pentagastrin, (b) luteinizing hormone-releasing hormone (LH-RH), and (c) adenocorticotropic hormone (ACTH 1–24). Experiments were performed under standard kinetic conditions at 30 °C in the presence of catalase (30 μM ml⁻¹) as described under “Experimental Procedures.” Peptide and enzyme (expressed in mole of heme) concentrations were: a, 38 μM and 11.8 nm; b, 39 μM and 11.8 nm; c, 35 μM and 20.4 nm. Spectra were recorded after the addition of L-tryptophan 2,3'-oxidase at regular intervals of: a, 1.3 min; b, 4 min; c, 12 min.

Table III

| Peptidic substrates | Km (μM) | kcat (s⁻¹) | kcat/Km (M⁻¹s⁻¹) | kmax dehydro (mm) |
|---------------------|---------|------------|------------------|------------------|
| Pentagastrin        | 26      | 32         | 10⁶              | 337              |
| Glucagon fragment   | ND      | ~10        |                  |                  |
| Luteinizing hormone-releasing hormone (LH-RH) | 71 | 20 | 3×10⁵ | 338 |
| Luteinizing hormone-releasing hormone (LH-RH) (N-glutaryl-trp-leu-met-asn-thr) | ND | ~10 | | 334 |
| Substance P analogue (Arg-Phe-Lys-Pro-Gln-Glu-His-Phe) | ND | ~10 | | 334 |
| Fragment [Lys₁₈₅₋₁₉₉] α₁(185–199) of the acetylcholine nicotinic receptor (Lys-Lys-Lys-His-Trp-Val-Tyr-Tyr-Thr-Cys-Cys-Pro-Asp-Thr-Pro-Tyr-Leu) | 272 | 2 | 7×10² | 333.5 |
| Adrenocorticotropic hormone (ACTH 1–24) (Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Val-Glu-Lys-Lys-Glu-Arg-Pro-Lys-Gly-Lys-Val-Tyr-Pro) | 930 | 17 | 2×10⁴ | 338 |

We also unambiguously showed, on the basis of ¹H NMR analyses, that α,β-dehydrotryptophanyl products that are generated by L-tryptophan 2,3'-oxidase adopt a pure Z-geometry (Scheme 2). This result is in good agreement with that previously reported by Gustafson et al. (15), who proposed that the carboxybenzoyl-α,β-dehydrotryptophan, which is directly produced in growing cultures of C. violaceum, results from the syn elimination of the H₂ and pro-S-H₂ hydrogen atoms. This mechanism appears, however, all the more striking as it is unfavorable in peptide structure, due to steric hindrance that prevents formation of an eclipsed conformation of the Cα, Cβ substituents.

Does L-Tryptophan 2,3'-Oxidase Catalyze a Direct Removal of the α and β Hydrogens from the Tryptophan Side Chain?—Davis et al. (6) reported that conversion of N-benzoyl-L-tryptophan into its α,β-dehydro derivative in C. violaceum cultures requires the presence of molecular oxygen or, under anaerobiosis, the addition of phenazine methosulfate as an electron acceptor. The concomitant reduction of the phenazine dye indicated that the reaction proceeded by a direct dehydrogenation rather than by an alternative mechanism involving coupled hydroxylation or oxygenation/dehydration reactions (6).

Two pieces of evidence suggest that L-tryptophan 2,3'-oxidase may proceed by a direct dehydrogenation process, involving the direct removal of two reducing equivalents from the Cα and Cβ carbon atoms of a tryptophan residue. First, no component other than the α,β-dehydro products themselves could ever be identified upon reaction with L-tryptophan 2,3'-oxidase. Second, we did not detect any lag period before the formation of the dehydro product, in contrast to what is observed with other enzymes such as Pseudomonas tryptophan side chain oxidase (see below) which proceeds via the formation of a cyclic intermediate (10). Therefore, we wish to suggest that L-tryptophan 2,3'-oxidase proceeds by a direct dehydrogenation mechanism such as those that are often mediated by flavin prosthetic groups. One should note, however, that if the presence of a flavin has been disproved in the case of L-tryptophan 2,3'-oxidase (4), the nature of the dehydrogenase cofactor and the mode of electron transfer from the substrate to molecular oxygen remain to be elucidated.

Previous observations now deserve to be recalled to provide a basis for a mechanistic model that could account for L-tryptophan 2,3'-oxidase. In 1926 Dakin et al. (5) suggested that the initial stages of amino acid and fatty acid metabolism may be essentially similar, and it was shown later that mitochondrial flavoenzymes, such as acyl-CoA dehydrogenases, catalyze the α,β-dehydrogenation of fatty acyl-CoA thioesters in the initial step of fatty acid β-oxidation. Among the acyl-CoA dehydrogenase superfamily, the medium chain fatty acyl-CoA dehydrogenase exhibits such a broad substrate specificity that it can tolerate aromatic group substitution at the ω position of the fatty acyl-CoA substrate. In this context, 3-indolepropionyl-CoA was
The mechanism of this reaction and the role of EpID in the decarboxylation reaction remain to be elucidated. However, the reaction mechanism of EpID is also clearly distinct from that of L-tryptophan 2',3'-oxidase in that it has been recently demonstrated to exhibit a strict substrate specificity towards peptides of at least 4 residues possessing close sequence analogies to the C-terminal epidermin tetrapeptide (18). Presumably, a variety of still unknown enzymes may cause dehydrogenation of amino acids, hence contributing to their metabolic fate.

L-Tryptophan 2',3'-Oxidase, A Tool for the Directed Modification of Tryptophan Residues in Peptides and Proteins—We investigated the capacity of L-tryptophan 2',3'-oxidase to catalyze the modification of tryptophan side chains in peptides and proteins. In all cases reported here, the dehydrogenation of the tryptophanyl residue catalyzed by L-tryptophan 2',3'-oxidase resulted in the formation of a single α,β-dehydrotryptophanyl product, characterized by an absorption band centered at 335 nm. This band, however, can be shifted between 330 and 340 nm (Table III), an effect that could be due to a change in the electronic environment of the double bond and to its partial delocalization between the carbonyl and the indole amino group. In a first approximation, the smaller peptides appear to be the most efficient substrates for L-tryptophan 2',3'-oxidase, irrespective of the position of the tryptophan residue in the sequence. The enzyme efficiency was clearly diminished with high molecular weight peptides, probably due to steric hindrance effects. Nevertheless, considering the enzyme's peculiar properties, especially its stability at high temperature and in the presence of reducing and/or denaturing agents (4), L-tryptophan 2',3'-oxidase can be used under conditions that are reversibly denaturing for a protein, yielding tryptophan residues accessible to the enzymatic modification. From these results, we propose that the name of the C. violaceum's enzyme should be extended to "peptidyl L-tryptophanyl 2',3'-oxidase."

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