Reaction of Lysyl Oxidase with trans-2-Phenylcyclopropylamine*

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trans-2-Phenylcyclopropylamine hydrochloride (tranylcypromine; TCP) was found to be both an inhibitor and a substrate of lysyl oxidase, the enzyme which oxidizes peptidyl lysine in elastin and collagen to initiate cross-linking in these proteins. The reaction of TCP with this enzyme was further characterized in view of the potential interference that chronic administration of this antidepressant compound may exert on the development and repair of connective tissues. In contrast to the irreversible and/or competitive inhibitors of lysyl oxidase previously described, TCP noncompetitively and reversibly inhibited the oxidation of both alkylamine and elastin substrates with $K_i$ values of 386 and 375 $\mu M$, respectively. The noncompetitive mode of interaction affected the accessibility of the active site to productive amine substrates since the reductive trapping of $n$-hexylamine to lysyl oxidase was largely prevented by the presence of TCP. It was of additional interest that lysyl oxidase catalyzed a limited degree of conversion of TCP to cinnamaldehyde accompanied by the production of hydrogen peroxide. The lack of significant incorporation of protein-bound tritium accompanying reduction of the enzyme-TCP complex with $[\text{3H}]$NaBH$_4$ argued against the formation of a Schiff base between the enzyme and the cinnamaldehyde product as the basis of the inhibitory effect. Spectral evidence was also obtained for an additional interaction between TCP and lysyl oxidase that was independent of the inhibitory effect of TCP. Cyclopropylamine, lacking the benzene moiety of TCP, inhibited lysyl oxidase irreversibly and competitively, and was not a substrate, pointing toward a defining role for the benzene moiety in the interaction of TCP with lysyl oxidase.

Lysyl oxidase is unique among the mammalian copper-dependent amine oxidases by virtue of its catalysis of the oxidation of peptidyl lysine to peptidyl $\alpha$-aminoisodic-6-semialdehyde in elastin and collagen substrates, thus initiating covalent cross-link formation within these proteins. In addition to copper, these amine oxidases also contain covalently bound carbonyl prosthetic groups, which are essential to catalytic function. Although early studies pointed to the identity of the carbonyl cofactor as pyrroloquinoline quinone (4), more recent studies strongly support the identity of the carbonyl cofactor in bovine plasma amine oxidase (2) and porcine kidney diamine oxidase (3) as the quinone of a trihydroxyphenylalanine residue. Although correspondingly detailed structural analyses of the functional carbonyl remain to be accomplished with lysyl oxidase, the irreversible inactivation of this enzyme by 1,2-alkyl diamines is consistent with the reactivity expected of an o-quinone such as pyrroloquinoline quinone or of the o-quinone tautomer of trihydroxyphenylalanine quinone (4).

Inhibitors directed at lysyl oxidase have importance because they may have chemotherapeutic potential as anti-fibrotic agents (5), whereas adventitious inhibition of this enzyme by agents directed at other enzymes may have deleterious effects on the development and/or repair of connective tissues. Among several such inhibitors of amine oxidases, cyclopropylamine has been shown to be an effective inhibitor of plasma amine oxidase (6, 7), whereas a structurally related compound, trans-2-phenylcyclopropylamine hydrochloride (tranylcypromine), noncompetitively inhibits diamine oxidase (8), both of which contain copper and carbonyl cofactors. Tranylcypromine is also a potent inhibitor of the mechanistically distinct, flavin-containing monoamine oxidase (9) and, on this basis, has been employed clinically as an anti-depressant (10). A recent report describes the oxidative cleavage of cyclopropylamine and its 1-phenyl analogue by a model o-quinone in a manner that can derivatize and thus inactivate an o-quinone acting as a cofactor (11). In view of its inhibitory effect on other amine oxidases, the potential of tranylcypromine to inhibit lysyl oxidase is investigated in this report.

MATERIALS AND METHODS

Enzyme Purification and Assay—Lysyl oxidase was purified to apparent homogeneity from bovine aorta as previously described (12). Purity was assessed by the presence of a single band migrating at a position equivalent to 32,000 Da by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (13). The purified preparations of lysyl oxidase consist of copurified mixtures of four ionic variants (14, 15), each of which has a molecular weight of 32,000 in sodium dodecyl sulfate. These variants exhibit a high degree of structural similarity and appear to operate by the same catalytic mechanism (14, 15).

Lysyl oxidase was assayed against an insoluble elastin substrate prepared from chick embryo aortas that had been pulsed in organ culture with l-[4,5-3H]lysine, as described (16, 17). Enzyme assays included 125,000 cpm (0.13 mg) of the elastin substrate in 0.1 M sodium borate, 0.15 M sodium chloride at pH 8.0 in a total volume of 750 $\mu L$ and were incubated for 2 h at 37°C. Tritiated water formed during the incubation was isolated by vacuum distillation and quantified by liquid scintillation spectrometry of 0.5-mL aliquots of the distillates. All activities were corrected for enzyme-free controls and were within the linear range of this assay (100–1200 cpm released/2 h). One enzyme unit was defined as 1 cpm of $^3$H released by enzyme action in 2 h, determined at a tritium counting efficiency of 30%. Specific activities of the purified enzyme preparations used here varied from 400,000 to 600,000 units mg$^{-1}$. Functional active site content was quantified by comparing the specific activity of each
enzyme preparation against the elastin substrate to the theoretical, maximum value of \(4 \times 10^6\) units mg\(^{-1}\) previously estimated as the value for the fully functional enzyme (18). The enzyme was also assayed against \(n\)-hexylamine as substrate by a peroxidase-coupled, fluorometric method (19). Lysyl oxidase (2–4 \(\mu\)g) was added to initiate the reaction, and fluorescence emission was continuously monitored at 425 nm.

**Assay for Cinnamaldehyde Production**—Lysyl oxidase (400 \(\mu\)g) was incubated with 5.9 \(m\)M TCP\(^1\) (Aldrich), a concentration that inhibits activity by 90%, in 0.05 \(M\) sodium borate, pH 8.2, for 90 min at 37 °C. The inhibited enzyme mixture was then reduced with 1 \(m\)C sodium borohydride (16 \(m\)M; 67.3 \(m\)Ci/mmol) for 1 h at 37 °C. The reduction was terminated and excess tritium dissipated by addition of glacial acetic acid to pH 2–3. Tritiated cinnamaldehyde was produced by the reduction of a putative cinnamaldehyde product of enzyme action was diluted with 5 \(m\) sodium borate as carrier and then extracted into ethyl acetate. The pooled ethylacetate extracts were extracted with 1% NaHC\(_0\)\(_3\) and further washed with 50% ethanol.

**Fluorescence Assay**—The n-hexylamine assay was performed to determine the activity of the enzyme. Lysyl oxidase (2–4 \(\mu\)g) was incubated with 5.9 \(m\)M TCP, a concentration which reduced rates of activity by 90%, in 0.05 \(M\) sodium borate, pH 8.2, for 60 min at 37 °C. The reaction mixtures were then reduced by incubating with 0.5 \(m\)g of sodium borohydride for an additional 30 min at 37 °C. The samples were separately dialyzed overnight against several changes of 16 \(m\)M potassium phosphate buffer, pH 7.8, and protein concentrations of the dialyzed samples were determined (20). Remaining enzyme activity was assayed by the peroxidase-coupled fluorescence method. Enzyme and enzyme-tranylcypromine complexes were also reduced under these conditions with \([3H]\)NaBH\(_4\) to assess for potentially reducible enzyme-tranylcypromine linkages. For this purpose, lysyl oxidase (64 \(\mu\)g) was incubated in the presence of or absence of 53 \(m\)M TCP, 5 \(m\)M \(n\)-hexylamine or their combination. One of each set of incubations was reduced with 0.5 \(m\)g of sodium borohydride for an additional 30 min at 37 °C. The samples were separately dialyzed overnight against several changes of 16 \(m\)M potassium phosphate buffer, pH 7.8, and protein concentrations of the dialyzed samples were determined (20). Remaining enzyme activity was assayed by the peroxidase-coupled fluorescence method. Enzyme and enzyme-tranylcypromine complexes were also reduced under these conditions with \([3H]\)NaBH\(_4\) to assess for potentially reducible enzyme-tranylcypromine linkages. For this purpose, lysyl oxidase (64 \(\mu\)g) was incubated in the presence of or absence of 53 \(m\)M TCP, 5 \(m\)M \(n\)-hexylamine or their combination. One of each set of incubations was reduced with 0.5 \(m\)g of sodium borohydride for an additional 30 min at 37 °C. The reaction mixtures were then reduced by incubating with 0.5 \(m\)g of sodium borohydride for an additional 30 min at 37 °C, and the reaction mixtures were then exhaustively dialyzed against 6 \(m\)M urea, 16 \(m\)M potassium phosphate, pH 7.8, and finally dialyzed against water in the absence of urea. Tritium incorporated into the protein was determined by liquid scintillation spectrometry of aliquots of the dialyzed samples.

**RESULTS**

**Inhibition of Lysyl Oxidase by Tranylcypromine**—Lineweaver-Burk plots of the inhibition of n-hexylamine oxidation by 177 and 265 \(\mu\)M TCP yielded lines that intersect at the 1/\([S]\) axis, indicative of noncompetitive inhibition (Fig. 1A). The \(K_i\) for TCP was calculated to be 386 ± 22 \(m\)M from these data. The inhibition of elastin oxidation was also found to be noncompetitive with a \(K_i\) of 375 ± 125 \(m\)M (Fig. 1B). The similarity of the \(K_i\) values is consistent with substrate-independent inhibitory interactions between TCP and lysyl oxidase.

To assess the reversibility of the inhibition by TCP, enzyme was incubated for 1 h at 37 °C in the presence or absence of 2.5 \(m\)M TCP, a concentration which reduced rates of activity to 11% of control. The enzyme-TCP mixture was assayed either after dialysis for 24 h against 16 \(m\)M potassium phosphate, pH 7.8, or after immediate dilution into the peroxidase-coupled assay mixture to lower the TCP concentration. Following dialysis, activity against the \(n\)-hexylamine substrate returned to 94% of the uninhibited control. Similarly, rapid dilution of the preincubated enzyme restored activity to 93% of the control, thus indicating that the inhibition is mostly and rapidly reversible. Moreover, the enzyme from which TCP had been removed by dialysis had the same \(K_i\) for \(n\)-hexylamine (2.97 \(m\)M) as that obtained with the control enzyme (not previously exposed to the inhibitor). Similarly, after dialysis of the TCP-treated enzyme, the \(k_{inact}\) for \(n\)-hexylamine oxidation (2.88 \(m\)M\(^{-1}\) minute\(^{-1}\)) was 91% of that of the native enzyme (3.18 \(m\)M\(^{-1}\) minute\(^{-1}\)). These results indicate the recovery of normal catalytic function after removal of the reversibly bound inhibitor. SDS-polyacrylamide gel electrophoresis revealed that the molecular weight of the enzyme incubated with 5.4 \(m\)M TCP at 37 °C for 1 h was not changed from the 32-kDa value for the enzyme incubated in the absence of the inhibitor, unlike the recent finding that similar incubation of methylamine dehydrogenase with cyclopropylamine resulted in the covalent polymerization of that enzyme (21). The aromatic substituent of tranylcypromine enhanced the inhibition since cyclopropylamine yielded linear plots intersecting at the 1/\([S]\) axis and a \(K_i\) of 516 \(m\)M. Furthermore, dialysis of lysyl oxidase which had been fully inhibited by preincubation with 5 \(m\)M cyclopropylamine for 1 h at 37 °C did not restore enzyme activity (data not shown). Thus, cyclopropylamine is a competitive, irreversible inhibitor of lysyl oxidase, in contrast to the noncompetitive, reversible pattern seen with TCP. Moreover, tranylcypromine enhanced the inhibition since cyclopropylamine yielded linear plots intersecting at the 1/\([S]\) axis and a \(K_i\) of 516 \(m\)M. Furthermore, dialysis of lysyl oxidase which had been fully inhibited by preincubation with 5 \(m\)M cyclopropylamine for 1 h at 37 °C did not restore enzyme activity (data not shown). Thus, cyclopropylamine is a competitive, irreversible inhibitor of lysyl oxidase, in contrast to the noncompetitive, reversible pattern seen with TCP. More-

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\(^1\)The abbreviations used are: TCP, tranylcypromine hydrochloride; DACH, 1,2-diaminocyclohexane.
**TABLE I**

<table>
<thead>
<tr>
<th>Amino</th>
<th>$K_{m}$</th>
<th>$k_{cat}$</th>
<th>$k_{cat}/K_{m}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexylamine</td>
<td>2.85</td>
<td>3.25</td>
<td>1.14</td>
</tr>
<tr>
<td>Phenethylamine</td>
<td>0.66</td>
<td>2.33</td>
<td>3.55</td>
</tr>
<tr>
<td>Tranylcypromine</td>
<td>0.037*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Determined at 353 μM TCP.

Moreover, hydrogen peroxide release was not detected in incubations of lysyl oxidase with cyclopropylamine, indicating that it is not a substrate for lysyl oxidase, in additional contrast to the results with TCP.

Since TCP is an amine, it was of interest to assess whether it served as a substrate for lysyl oxidase. Using a relatively large quantity of enzyme (14,700 units versus 2600 units in assays of n-hexylamine oxidation), a very slow rate of enzyme-TCP-dependent production of $H_2O_2$ was detected (Table I). This activity was completely inhibited by the presence in the assay of 50 μM β-aminopropionitrile, an active site-directed inhibitor of lysyl oxidase (22). Because of the negligible activity and the increasing inhibition with increasing TCP concentration, it was not feasible to assess a true $k_{cat}$ for this activity. However, the turnover was estimated as 0.037 min$^{-1}$ at the level of TCP used (353 μM; causes 80% inhibition of activity against n-hexylamine). This value was markedly less than the $k_{cat}$ values for n-hexylamine as an unsubstituted, primary alkyamine substrate or phenethylamine, a linear alkyamine substituted with an aromatic ring (Table I). Indeed, it appears that the presence of an aromatic moiety two carbons removed from the amino function favors oxidation as seen by comparison of the kinetic data for the catalytic oxidation of n-hexylamine and phenethylamine (Table I). Thus, it seemed unlikely that the limited substrate potential of TCP was largely due to its aromatic substituent.

NMR and mass spectroscopic analyses of the intermediates and products of the aerobic reaction of TCP with 9,10-phenanthrenequinone, the latter taken as a model of the o-quinonelike cofactor of lysyl oxidase, were consistent with the release of cinnamaldehyde as a reaction product. The potential for the catalytic processing of tranylcypromine to cinnamaldehyde has been previously observed with flavin-dependent monooxidase oxidase (23). This possibility was examined in the present study by incubation of lysyl oxidase with TCP followed by reduction of the mixture with $[PH]NaBH_4$ to label and stabilize a putative cinnamaldehyde product to the corresponding alcohol. Incubations of TCP in the absence of enzyme and of authentic cinnamaldehyde were separately reduced and correspondingly analyzed as controls. The reaction mixtures were extracted and analyzed by high performance liquid chromatography as described under "Materials and Methods." It was determined that 0.035 mol of cinnaminal was generated/mol of enzyme min$^{-1}$, in reasonable agreement with the value of 0.037 min$^{-1}$ determined for hydrogen peroxide production from TCP by lysyl oxidase (see Table I). It was found that neither cinnamaldehyde nor cinnaminal inhibited lysyl oxidase activity against n-hexylamine or elastin, making it unlikely that the aldehyde product of TCP oxidation caused the inhibition seen with TCP.

**Effect of Tranylcypromine on Reduction of Enzyme-Substrate Complexes**—The copper-dependent amine oxidases appear to involve initial formation of a Schiff base with the carbonyl cofactor at the active site, as illustrated by the

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* NMR and mass spectroscopic data describing intermediates and products of the reaction between TCP and 9,10-phenanthrenequinone are available by communicating directly with Dr. Herbert M. Kagan.
tritium in the reduced complexes of lysyl oxidase with amine substrates or inhibitors does not rule out the initial formation of a Schiff base between TCP or hexylamine with this enzyme and is consistent with evidence that this lysyl oxidase contains an o-quinone cofactor (4, 18). However, the apparent lack of tritium incorporation with TCP beyond that retained in the enzyme reduced in the absence of an amine does argue against the presence of a Schiff base formed between the cinnamaldehyde product of TCP oxidation and a free amino group of the enzyme.

**Spectral Properties of TCP-treated Lysyl Oxidase**—Lysyl oxidase was incubated with 5 mM TCP as described, dialyzed over a 24-h period against 16 mM potassium phosphate, 6 M urea, pH 7.8, and then against 16 mM potassium phosphate, pH 7.8. As shown (Fig. 2), the spectrum of the enzyme which had been incubated with tranylcypromine was elevated over that of the enzyme sample which had been incubated in the absence of the inhibitor and dialyzed as described. The spectrum of the modified enzyme displayed absorbance maxima at 258 and 262 nm and a shoulder at 268 nm. The absorbance of the TCP-treated enzyme was decreased below that of the control between 277 and 300 nm. These spectral changes indicated that a modification of the enzyme occurred, which was not reversed by dialysis against a reversibly denaturing concentration of urea, suggesting that TCP was either very tightly or covalently bound. However, subsequent treatment of the TCP-modified, dialyzed enzyme with 2% sodium dodecyl sulfate at 100°C for 2 min followed by dialysis against 6 M urea, 16 mM potassium phosphate, pH 7.8, and then against this phosphate buffer, alone, yielded an enzyme whose spectrum no longer displayed the increased absorbance in the UV region (Fig. 2). This result argues against a covalent adduct as the source of the spectral effects. To further probe enzyme-TCP interactions, the active site of the enzyme was irreversibly modified by reaction with 1 mM 1,2-diaminoclohexane at 37°C for 45 min. Free reagent was removed by exhaustive dialysis against 6 M urea, 16 mM potassium phosphate, pH 7.8, and then against this buffer in the absence of urea. Oxidative processing of this inhibitor appeared to result in the formation of a stable pyrazine ring between the 1,2-diamine, subsequent treatment with tranylcypromine resulted in very similar changes in the enzyme spectrum with peaks generated at 258 and 262 nm and with evidence for a shoulder in the region of 268 nm (Fig. 2). The spectrum of the enzyme modified with DACH alone and then dialyzed was nearly fully recovered (94% of control), as expected. However, preincubating the enzyme with 5 mM TCP followed by incubation with 1 mM DACH or preincubating with DACH followed by incubation with TCP resulted in the irreversible loss of 93% of enzyme activity in each case. The activity of each modified enzyme was compared to a control incubated and dialyzed under identical conditions in the absence of either inhibitor. After blocking of the cofactor with the diamine, subsequent treatment with tranylcypromine resulted in very similar changes in the enzyme spectrum with peaks generated at 258 and 262 nm and with evidence for a shoulder in the region of 268 nm (Fig. 2). The spectrum of the enzyme treated with TCP alone, or that of the enzyme treated with TCP and then with DACH, was only slightly different from that of the enzyme treated with DACH followed by TCP. Since the covalent modification of the active site carbonyl cofactor by DACH had relatively little effect on the spectral change induced by TCP, these agents apparently interact at different sites on this enzyme. It remains possible that subtle conformational effects accompanying the modification by DACH may underlie the slight difference in the spectral change induced by TCP. In any event, these results indicate that the TCP did not protect against the irreversible loss of activity caused by the DACH, whereas irreversible modification with the diamine did not prevent the spectral change resulting from incubation with TCP.

**DISCUSSION**

A variety of irreversible inhibitors of lysyl oxidase have been described that are competitive with alkylamine or elastin substrates of this enzyme (4, 5, 26, 27). In contrast, TCP appears to be the first inhibitor of lysyl oxidase to be identified as noncompetitive and as a reversible inhibitor of catalytic function. In spite of the noncompetitive nature of the inhibition, TCP exhibited substrate activity. The substrate potential, although limited, was shown by the enzyme-dependent release of H_2O_2 in enzyme-TCP incubation mixtures, by the detection of the cinnamaldehyde product of TCP processing by lysyl oxidase, and by a corresponding degree of inactivation of the enzyme upon the chemical reduction of the TCP-lysyl oxidase complex, the latter consistent with interaction of TCP with the carbonyl cofactor.

A possible mechanism for the oxidation of TCP to cinnamaldehyde by lysyl oxidase is presented in Fig. 3. This scheme is consistent with the order of substrate binding and product release known to be followed by this enzyme during amine oxidation (25, 27). The scheme also accounts for the present observation that apparently stoichiometric quantities of cin-
Tranylcypromine Inhibition of Lysyl Oxidase

namaldehyde and hydrogen peroxide were released as reaction products of the oxidation of TCP by lysyl oxidase. In Fig. 3, oxidation of TCP is presumed to initiate by the formation of a Schiff base adduct (Fig. 3, I) with a carbonyl of the o-carbonyl cofactor (V). It is suggested that the strained cyclopropyl ring may then disrupt so that an electron pair linking C1 and C2 of TCP moves to initiate reduction of the cofactor with compensating electron migration from a C5-H bond forming the α,β-double bond, thus yielding intermediate II. The subsequent hydrolysis of II yields cinnamaldehyde and the reduced eneamino-hydroxyl form of the cofactor, III. The aminophenol can be oxidized by molecular oxygen to IV from which ammonia is removable by hydrolysis, regenerating active enzyme, V.

Although TCP noncompetitively inhibits lysyl oxidase, the evidence for oxidative processing of the TCP to cinnamaldehyde indicates that a catalytically productive interaction also occurs. Quantitation of cinnamaldehyde production and peroxide release indicated that productive catalysis amounted to ≤0.03 mol/mol of enzyme, consistent with the conclusion that processing of TCP as a substrate itself becomes limited by an independent mode of binding at a noncompetitive site or sites. It is of interest that TCP has been previously shown to irreversibly inactivate mitochondrial monoamine oxidase and that oxidative processing of this inhibitor by this flavin-dependent enzyme also results in cinnamaldehyde formation (23). The enzyme inactivation was attributed to adduct formation between the cinnamaldehyde product and an attacking nucleophile of the mitochondrial enzyme, suggested to be a thiol group (9). It has been noted that the inactivation could result from the addition of an enzyme nucleophile at the α,β-ununsaturated moiety of cinnamaldehyde (28) and that, under certain conditions, this adduct may be reversibly disrupted (23, 28). As noted, the present results do argue against the possibility that the inhibition is due to a cinnamaldehyde-enzyme Schiff base complex. However, other possibilities, such as reversible adduct formation between a processed form of TCP and a thiol group of the enzyme require additional investigation.

Although the present results do not permit a definitive description to be made of the mechanism of the noncompetitive inhibition, the presence of TCP protected against the reductive trapping of n-hexylamine to the enzyme, and this can be taken to indicate that the bound inhibitor blocks the access of productive amine substrates to the active site. This could result from an unfavorable conformational change induced by this noncompetitive interaction, which masks the active site against all substrates, including TCP. It is also possible that the noncompetitive mode of interaction may result in the retention of TCP as a partially processed species at the active site. Although presently not available, isotopically labeled forms of TCP, as well as additional information about the topology of lysyl oxidase, should facilitate discrimination between these and other alternatives. It should be noted that a dual mode of binding of tranylcypromine has also been observed in plasma amine oxidase with similar Ig, kinetic behavior, and cinnamaldehyde release.3 It is notable that TCP and cyclopropylamine exhibited completely different modes of inhibition and that hydrogen peroxide release was not detectable in incubations of lysyl oxidase and cyclopropylamine, indicating that the enzyme did not catalyze the oxidative turnover of cyclopropylamine as a substrate. Thus, the benzene moiety of TCP appears to account for this difference in reactivity. As noted, a benzene moiety two carbons distant from the amino function in the phenethylamine substrate of lysyl oxidase enhanced substrate potential relative to a linear alkylamine substrate that lacked a benzene substituent (Table I), indicating that the aromatic ring can alter the interaction of ligands at the active site. This conclusion has been further supported by a more extensive study of the relationship between substrate potential and aromatic substituents of alkylamines (29). Prior studies have indicated that cyclopropylamine or an oxidatively processed derivative thereof can covalently modify other enzymes, including methylene dehydrogenase (21) and plasma amine oxidase (6, 7), and, thus, the inactivation of lysyl oxidase by cyclopropylamine presumably reflects the potential of the strained cyclopropyl ring to undergo such attack. Since this does not occur with TCP, it seems reasonable to suggest that the cyclopropylamine ring of TCP may be in a different orientation to the residues at the active site than that of cyclopropylamine because of the influence of the aromatic substituent. Additional studies are required to assess such possibilities.

In addition to interactions that lead to cinnamaldehyde formation, the present data provided evidence that at least one other mode of interaction with TCP occurred. Thus, there appears to be a tight interaction at an enzyme site or sites distinct from the active site, as indicated by the spectral change, which is not reversed by dialysis in 8 M urea but which is reversed by treatment with SDS. Moreover, blocking of the carbonyl cofactor by its prior, irreversible reaction with 2,2-diamino-1,4-benzenedicarboxylic acid forming a pyrazine adduct does not prevent the subsequent generation of this spectral change by TCP, thus arguing against catalytically productive TCP-enzyme complexes as the origin of this chromophoric interaction. This mode of binding also appeared not to stem from Schiff base formation between the cinnamaldehyde product of TCP oxidation and a free amino group of the enzyme in view of the apparent lack of tritium incorporation upon reduction of the enzyme-TCP complex. Moreover, the restoration of catalytic function by dialysis of the TCP-enzyme complex under conditions in which the spectral effect persisted indicates that this interaction is not related to the reversible, noncompetitive inhibition observed.

Undesirable side effects accompanying the use of TCP as an antidepressant are well documented and include addiction (30), hypertension (21), and untoward psychological symptoms (32). Although documentation appears to be lacking that tranylcypromine induces connective tissue defects, the present report raises the possibility that chronic administration and/or localized tissue concentration of this agent could inhibit the insolubilization of collagen and elastin fibers during active periods of synthesis as in wound healing and during development by virtue of its inhibition of lysyl oxidase.

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REFERENCES

Tranylcypromine Inhibition of Lysyl Oxidase


