Phytochemistry, Vol. 27, No. 6, pp. 1593–1599, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00

AFFINITY AND HYDROPHOBIC CHROMATOGRAPHY OF MUSHROOM TYROSINASE

JANIS INGEBRIGTSEN and WILLIAM H. FLURKEY

Department of Chemistry, Indiana State University, Terre Haute, IN 47809, U.S.A.

(*Revised received* 14 *September* 1987)

Key Word Index—*Agaricus bisporus*; *Agariaceae*; mushroom; tyrosinase; chromatography; affinity; immunoaffinity; hydrophobic.

Abstract—Mushroom tyrosinase was subjected to affinity and hydrophobic chromatography on 2-mercaptobenzimidazole, 2-mercaptobenzothiazole, methimazole, mimosine, and tropolone ligands coupled to solid supports. Mercaptobenzimidazole, mercaptobenzothiazole, and methimazole columns coupled to CNBr activated Sepharose and mimosine coupled to epoxy activated Sepharose were capable of retaining significant amounts of the enzyme under affinity chromatography conditions. Only mercaptobenzimidazole and mercaptobenzothiazole columns retained significant amounts of the enzyme under hydrophobic chromatography conditions. The enzyme was also adsorbed onto phenyl and phenylbutylamine columns under hydrophobic conditions. Increasing amounts of the enzyme were bound to several alkyl columns under hydrophobic conditions and the binding increased as the initial salt concentration increased. Mushroom tyrosinase was also adsorbed onto two antibody columns. One column retained the holoenzyme while the other column retained smaller molecular weight proteins related to the enzyme. All resins which bound the enzyme resulted in significant purification and removal of contaminating proteins compared to crude samples. These results suggest that mushroom tyrosinase can be purified and characterized using inhibitors, phenolics, alkyl groups, and antibodies coupled to solid supports.

INTRODUCTION

Affinity chromatography, and more recently hydrophobic adsorption chromatography, has proven to be a powerful technique in the isolation and purification of biological molecules. Even as early as 1953, Lerman [1] showed that phenolic and substituted benzoic acid inhibitors of mushroom tyrosinase (monophenol-monooxygenase, EC 1.10.3.1) coupled to cellulose could be used as effective affinity adsorbents. More recently, Gutterridge and Robb [2] coupled a competitive inhibitor of mushroom tyrosinase, 4-aminobenzoate, to Sepharose 4B and showed that this affinity adsorbent was useful for the purification of the enzyme. At approximately the same time, O'Neill *et al.*[3] coupled phenolic, catecholic, and benzoic groups to Sepharose 4B, aminophenyl bearing glass, and *p*-aminobenzyl cellulose to investigate which adsorbent showed specificity for the mushroom enzyme. Of these adsorbents, dopamine affinity columns were capable of retaining the enzyme from crude samples. Unfortunately these columns were found to undergo oxidation and could not be used repeatedly. A similar approach was used by Yonekura *et al.* [4] to purify the phenoloxidase from housefly larvae. These investigators used phenylalanine, *p*-aminobenzoate, a *p*-aminobenzoate derivative, and *N*-acetyl-L-phenylalanine as affinity resins. Of these only the *p*-aminobenzoate derivative appeared to bind polyphenoloxidase to any great extent. Menon and Haberman [5] used a different approach and coupled 3-iodotyrosine to Sepharose. This inhibitor coupled resin separated two or three isoenzyme forms and resulted in partial purification of the enzyme.

None of these studies investigated the possible use of hydrophobic adsorption or immunoaffinity chromatography, although Lerman [1] noticed that high salt increased nonspecific adsorption. This technique was used by Flurkey and Jen [6–9] to purifiy peach polyphenoloxidase. Later the method developed by Flurkey and Jen was adapted by Wisseman and Lee [10] and Wisseman and Montogomery [11] for the purification of grape and pear polyphenoloxidases. Few advances in immunoaffinity chromatography of mushroom tyrosinase or related enzymes have been reported except for that by Goldhirsh and Whitaker [12]. They used immunoaffinity columns to isolate mushroom tyrosinase.

Recently, Kahn and Andrawis [13] have investigated the effect of tropolone, methimazole, L-mimosine, 2-mercaptobenzimidazole, and 2-mercaptobenzothiazole on mushroom tyrosinase. Most of these inhibitors were characterized by low k_i's or low I_{50}'s. Some of them were reported to be copper chelators also. Although most of the affinity resins for mushroom tyrosinase have been *p*-aminobenzoic acid or *p*-aminobenzoic acid derivatives, the above inhibitors might be a more useful ligand. In view of the above, we decided to investigate if any of these inhibitors could be used as affinity adsorbents under low salt conditions (affinity) or high salt conditions (hydrophobic). Several alkyl and phenyl derivatives were also tested to determine if they could be used as affinity adsorbents for mushroom tyrosinase. Lastly, we prepared two different antibody (immunoaffinity) adsor-

Abbreviations. 2-Mercaptobenzimidazole, MBI; 2-mercaptobenzothiazole, MBT; methimazole, met; L-mimosine, mim; tropolone, tro; anti-mushroom tyrosinase A antibodies, anti-MTA; anti-mushroom tyrosinase B antibodies, anti-MTB.

bents to determine their usefulness in the purification of mushroom tyrosinase.

RESULTS

Two immunoaffinity resins were prepared by coupling anti-mushroom tyrosinase (anti-MTA) to CNBr activated Sepharose. Anti-MTA antibodies were produced against holoenzyme preparations. Anti-MTB antibodies were produced using a 26000 protein from purified mushroom tyrosinase preparations as the antigen [14]. When crude tyrosinase preparations were passed through anti-MTA columns major proteins of 47–48, 44–45, 24–26, 13–15, and a minor protein of 12–14 $\times 10^3$ were adsorbed and eluted from the columns at high pH (Fig. 1 lane 7). These proteins which cross-reacted with anti-tyrosinase antibodies will be referred to as *a,b,c,d,* and *e* respectively. Passage of a similar sample over anti-MTB columns showed that proteins *a,b,* and a small amount of *e* were not adsorbed while significant amounts of proteins *c, d,* and *e* were adsorbed. Since most of the enzyme activity was not adsorbed onto the anti-MTB column, the catalytic site must be present as *a* or *b* subunits. The M_rs of *a* and *b* correspond closely to the reported M_r for the large subunit of mushroom tyrosinase while that for *d* and *e* corresponds closely to that reported for the small subunit [15–17]. This suggests that protein *c* may be either a dimer of the small subunit, an inactive enzyme fragment, or a contaminant within the enzyme preparation. Both columns, especially anti-MTA, resulted in significant purification of the bound enzyme forms compared to the composition of the crude enzyme samples (compare lanes 7 and 11 to lane 3 and 8 in Fig.1) and demonstrate the usefulness of immunoaffinity chromatography applied to mushroom tyrosinase. These results also indicate that even though mushroom tyrosinase is the major component in commercial enzyme preparations a significant number of other proteins are present. The yields from the immunoaffinity columns were not as great as one would expect and suggest that much of the enzyme activity was lost or adsorbed onto the column (Table 1).

When mushroom tyrosinase was subjected to affinity chromatography under low salt conditions, the enzyme was adsorbed onto MBI, MBT, and met ligands coupled to CNBr activated Sepharose (Table 1). In general, the percent recovery was good but the percent enzyme bound was relatively low. Only mimosine coupled to epoxy activated Sepharose showed significant binding of tyrosinase. Yields and recoveries were generally lower for all ligands coupled to epoxy activated Sepharose. Mimosine coupled to Tresyl activated resins did not show as much binding as either CNBr or epoxy activated resins. Analysis of the bound protein fractions by SDS PAGE showed that the MBI, MBT, met, and mim columns retained proteins *a, b* and small amounts of *c* (Fig. 2a) Two additional proteins between 55 000 and 70 000 were also present in the bound fractions but we do not know if they are related to mushroom tyrosinasa. These bands did appear in all bound fractions in all chromatography runs.

Affinity chromatography of mushroom tyrosinase on phenolic substituted resins indicated that the enzyme was retained on phenylbutylamine agarose, but not on phenyl, phenylalanine, phenylboronic, or tyrosine substituted resins (Table 1). Although not readily apparent in

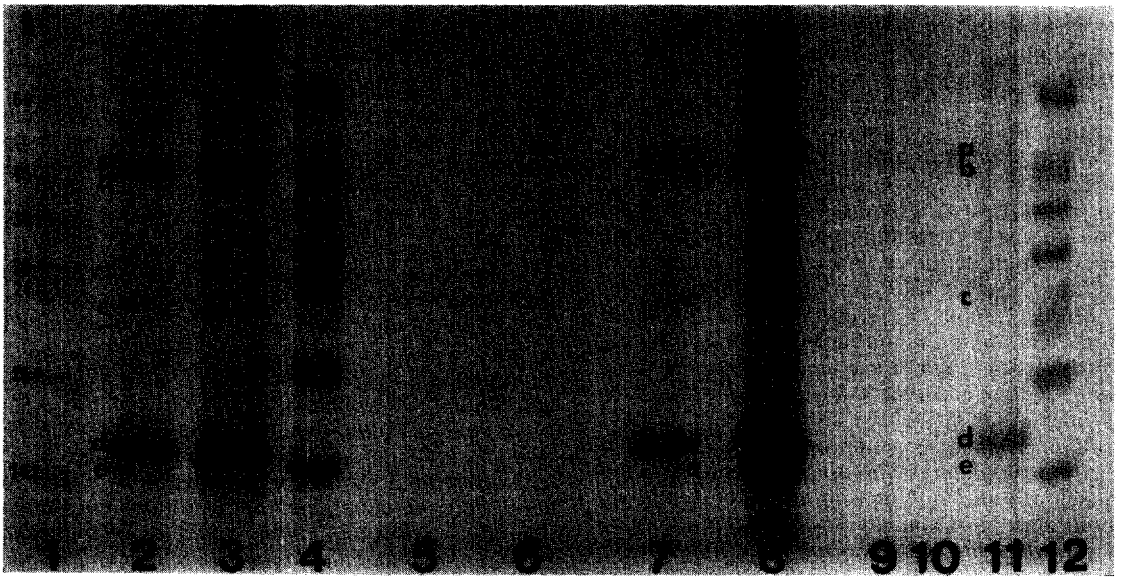

Fig. 1. SDS PAGE of immunoaffinity column fractions and crude mushroom tyrosinase. Lanes 1,4, and 12 contained M_r markers.Lanes 2,3, and 8 contained *ca* 15, 30, and 100 μg of resuspended mushroom tyrosinase solid respectively. Lanes 5 and 6 were unadsorbed fractions from anti-MTA columns. Lane 7 contained proteins from the bound fraction of anti-MTA columns. Lanes 9 and 10 contained proteins from unadsorbed fractions from anti-MTB columns while lane 11 contained proteins from the adsorbed fractions. Approximately one-sixth of the total fraction volume (adsorbed and unadsorbed) was precipitated with TCA and subjected to SDS PAGE for lanes 5–7 and 9–11. Letters (*a–e*) refer to major proteins associated with tyrosinase.

Table 1. Affinity chromatography of mushroom tyrosinase*

Type of affinity column	mg ligand/ g resin	% enzyme bound	% enzyme recovered
(A) CNBr activated resins			
MBI	12	38	93
MBT	12	25	35
met	0.9	44	95
mim	5.5	4	65
(B) Epoxy activated resins			
MBI	4.1	2	77
MBT	4.6	2	63
met	9.1	4	65
mim	3.7	49	49
tro	< 0.1	< 1	72
(C) Tresyl activated resins			
mim	0.6	13	67
(D) Phenolic substituted resins			
phenyl	—	< 1	68
phenylalanine	—	2	79
phenylboronic	—	< 1	78
phenylbutylamine	—	27	30
tyrosine	—	2	72
(E) Immunoaffinity resins			
anti-MTA	—	36	36
anti-MTB	—	8	80

* The % enzyme bound was determined from the ratio of adsorbed enzyme to the total amount of enzyme applied to each column. The % recovery was calculated from the ratio of unadsorbed and adsorbed enzyme to the total amount of enzyme applied.

Fig. 2a, very small amounts of proteins *a* and *b* were found in the bound fractions eluted from phenylbutylamine columns (Fig. 2). However, these may have been bound through electrostatic interactions rather than through affinity interactions since the amino group could be charged at pH 6.0.

Under conditions suitable for hydrophobic adsorption, most of the inhibitor affinity resins showed decreased enzyme recovery and lower amounts of enzyme bound (Table 2). Only MBI and MBT coupled to epoxy activated Sepharose appeared to bind appreciable amounts of the mushroom enzyme. Greater amounts were retained on epoxy activated resins compared to CNBr activated resins. However, some of this protein may be inactive enzyme since the recoveries were much lower. All adsorbed fractions contained proteins *a–e* in variable amounts (Fig. 2b). In contrast to SDS-PAGE patterns from affinity columns, the SDS-PAGE patterns of bound protein fractions from hydrophobic columns contained more of proteins *c–e*.

Mushroom tyrosinase was also adsorbed onto phenyl and phenylbutylamine Sepharose under hydrophobic conditions. Once again, the recoveries and percent enzyme bound were low. Analysis of the bound fractions by SDS PAGE showed that only two proteins were retained on phenyl columns (*a* and *b*) while four proteins (*a,b,d,e*) were recovered from phenylbutylamine columns (Fig. 2b). Phenylbutylamine columns also appeared to be somewhat selective in that more of protein *b* was retained in comparison to protein *a*. The reasons for this selectivity are not known at present.

In the above studies the inhibitors and antibodies were coupled directly to the resins without the use of a spacer arm, although epoxy activated Sepharose contains a long hydrocarbon arm. To determine what effect the spacer arm might have on retention and hydrophobicity, the enzyme was chromatographed on several resins containing increasing alkyl chain lengths. The enzyme was partially retained on blank agarose through hexyl columns run under conditions which promoted hydrophobic interactions (Fig. 3). As the alkyl chain length increased the percent enzyme bound also increased. A minimum chain length of C_7 to C_8 was needed for adsorption under these conditions. When the chromatography was carried out at higher salt concentrations, the enzyme was adsorbed onto shorter alkyl chain length columns. Surprisingly, at 50% ammonium sulphate, the enzyme was completely adsorbed onto all alkyl columns as well as blank agarose columns. On blank agarose columns this adsorption increased from 20 to 40 to 100% as the salt concentrations increased from 12.5 to 25 to 50% saturation. Therefore, the presence of an alkyl spacer arm may result in nonspecific adsorption of enzyme under high salt conditions.

DISCUSSION

Numerous reports have recently appeared in the literature using commercial sources of mushroom tyrosinase [12, 13, 18–28]. Of these, only a few reports have shown the purity of the starting material or have attempted to purify the partially purified commercial enzyme [12, 14,

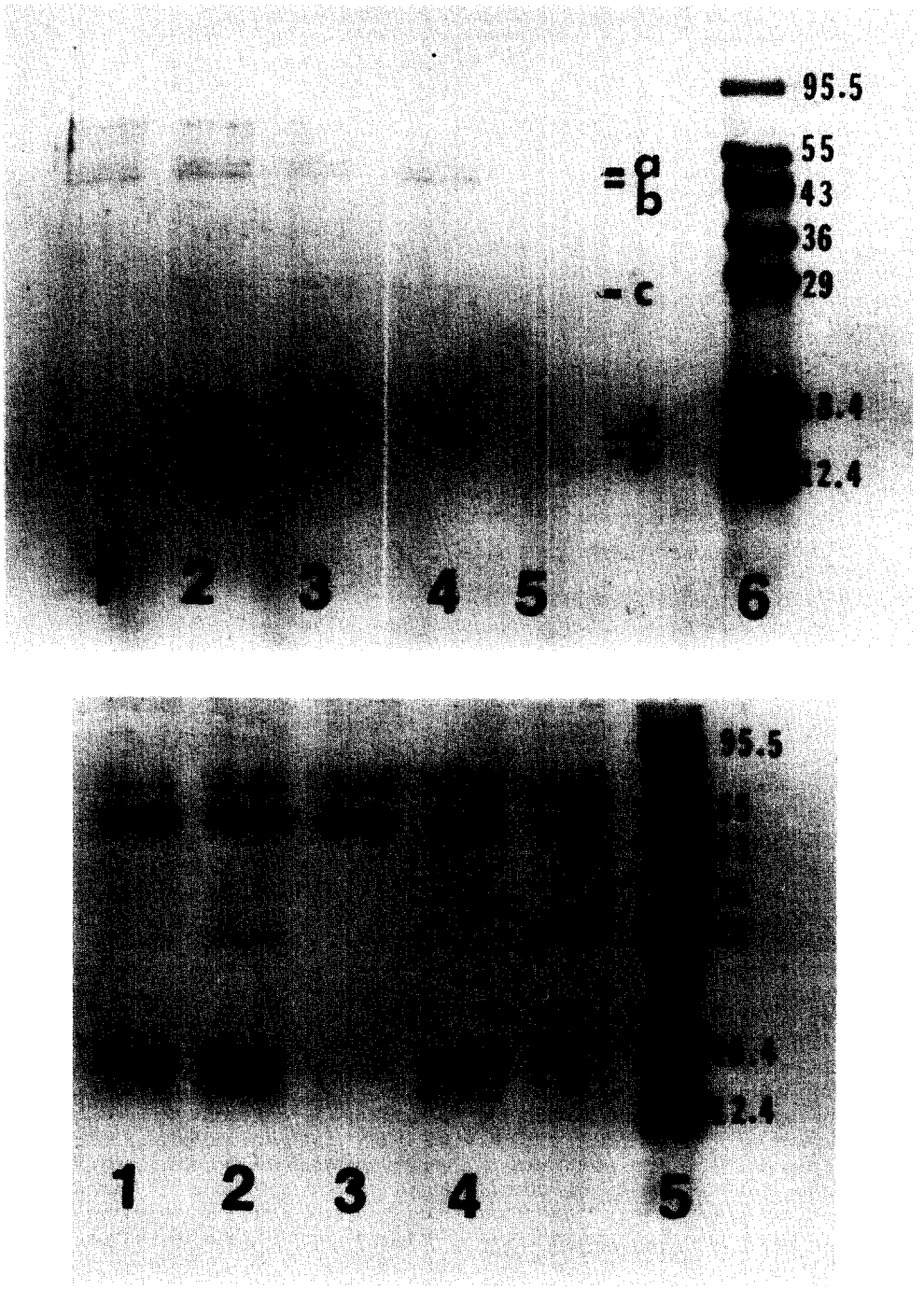

Fig. 2. SDS-PAGE of enzyme adsorbed to resins run under affinity or hydrophobic conditions. In A, samples from affinity columns were TCA precipitated and subjected to SDS PAGE. Lane 6 contained molecular weight markers. Lanes 1–5 contained sample from enzyme bound to MBI (1), MBT (2), met (3), and mim (4) coupled to CNBr activated resins and phenylbutylamine agarose (5). In B, samples were run under hydrophobic conditions and the bound fractions were TCA precipitated and subjected to SDS PAGE. Lane 5 contained M_r markers. Lanes 1–4 contained absorbed enzyme bound to MBI (1) and MBT (2) coupled to epoxy activated resins, phenyl (3) and phenylbutylamine (4) columns.

23, 26, 28]. Our results indicate that other proteins besides mushroom tyrosinase are present in commercial sources of the enzyme (Fig. 1). Thus, studies employing the commercial enzyme are somewhat suspect to interpretation since homogeneous enzyme was probably not used. Even though the commercial enzyme is not pure, it

Table 2. Hydrophobic chromatography of mushroom tyrosinase*

Type of affinity column	mg ligand/g resin	% enzyme bound	% enzyme recovered
(A) CNBr activated resins			
MBI	12	< 1	58
MBT	12	5	27
met	0.9	2	65
mim	5.5	4	56
(B) Epoxy activated resins			
MBI	4.1	37	44
MBT	4.6	10	11
met	9.1	4	54
mim	3.7	1	54
tro	< 0.1	< 1	64
(C) Tresyl activated			
mim	0.6	3	54
(D) Phenolic substituted resins			
phenyl		30	52
phenylalanine		< 1	58
phenylboronic		< 1	50
phenylbutylamine		19	44
tyrosine		1	38

*The % enzyme bound and the % recovery was determined as described in Table 1 and in the Experimental section.

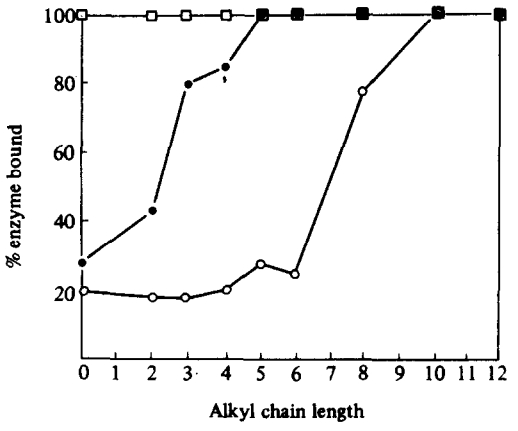

Fig. 3. Hydrophobic adsorbtion chromatography of mushroom tyrosinase on increasing alkyl chain length columns. The % enzyme bound was calculated from the ratio of bound enzyme to the amount of enzyme eluted in the high salt and low salt fraction. Columns were run at 12.5 (○), 25 (●), and 50 (□) % saturation respectively. Chromatography was carried as described in the Experimental section.

does represent a useful starting material to examine the application of affinity chromatography in the purification of mushroom tyrosinase.

The inhibitors, mim, met, MBI, and MBT, reported by Kahn and Andrawis [13] can be used as suitable ligands for affinity chromatography of mushroom tyrosinase. Under these conditions the enzyme was more likely to be adsorbed to MBI, MBT, and met coupled to CNBr activated resins than to epoxy activated resins. In contrast, the enzyme was more likely to be adsorbed to mim coupled to epoxy and Tresyl resins than to CNBr activated resins. This may be a result of different functional groups on the ligand coupling to each support. Resins containing tropolone did not bind the enzyme appreciably. Thus, the type of ligand and choice of solid support can affect the amount of enzyme adsorbed. Similar observations were reported by O'Neil *et al.* [3] and Yonekura *et al.* [4]. Recoveries and percent enzyme adsorbed to each resin were also similar to those reported earlier [1–5]. Unlike Gutteridge and Robb [2] and Yonekura *et al.* [4] we did not succeed in obtaining homogeneous enzyme when the adsorbed fractions were analyzed by SDS-PAGE. However, neither of the above two investigations subjected the preparations to SDS-PAGE, instead they used native PAGE as a criteria of purity. In any case, affinity chromatography of tyrosinase on inhibitor columns resulted in significant removal of contaminating proteins. Unfortunately, two proteins of higher M_r were consistently adsorbed onto all affinity columns. We do not know if they are related to mushroom tyrosinase, but this could be tested by Western blotting. We also observed that all mim coupled resins turned orange with time starting from the top of the column downward (data not shown). Presumably this occurs through some oxidation mechanism and may be related to a similar phenomenon reported by O'Neil *et al.* [3] for oxidation of dopamine bound ligands on Sepharose. Oxidation of bound mimosine would obviously limit the usefulness of this resin. However, we have noticed that the enzyme still binds to the coloured columns.

Under conditions suitable for hydrophobic chromatography more enzyme was bound to epoxy resins containing MBI and MBT than to CNBr activated resins containing the same ligands. Recoveries and percent enzyme bound were much lower than under affinity conditions, but the yield appeared to be greater when analysed by SDS-PAGE. The additional yield of protein

detected by SDS-PAGE may represent inactive enzyme accumulation. Hydrophobic chromatography on these resins also resulted in the adsorption of more of the 26000, 15000 and 13000 proteins. Tyrosinase was adsorbed onto phenyl Sepharose under high salt conditions much like that reported for peach [6–9], grape [10], and pear [11] polyphenolxidase. Unlike the above reports, yields and recoveries were much lower. The bound fraction from phenyl Sepharose columns did not contain the lower M_r proteins and suggests that chromatography on this resin may be useful in separation or purification of the large subunit of tyrosinase. Hydrophobic chromatography on phenylbutylamine columns was somewhat more selective in that one of the two higher M_r subunits was retained in greater proportion than the other. The enzyme was not adsorbed onto phenylalanine or tyrosine resins. These results confirm earlier reports by Flurkey and Jen [29]. We did notice, however, that tyrosine columns turned orange as the enzyme was passing through the column (data not shown). Because of the low recoveries from this resin, perhaps the enzyme was permanently adsorbed onto the column.

Hydrophobic chromatography on columns substituted with various alkyl chain lengths indicated that the enzyme could be adsorbed onto the spacer arm without an attached ligand. Under relatively mild hydrophobic conditions, the enzyme was adsorbed onto octyl, decyl, and docecyl columns. Increasing the salt concentration increased retention and binding to shorter alkyl chain lengths. These findings were similar to those reported by Flurkey and Jen [29] for peach polyphenoloxidase, who observed that the enzyme was slightly retained on hexyl and fully retained on octyl columns. Iborra *et al.* [30] also observed that greater amounts of dopa oxidase were adsorbed as the alkyl chain length increased. At very high salt concentrations the enzyme was adsorbed onto all alkyl columns as well as blank agarose columns. The reason for this observation is not known but may be related to protein crystallization on solid supports under high salt concentrations [31, 32]. Binding of the enzyme to spacer arms under high salt conditions may be a problem in gradient elution of the enzyme from ion exchange columns and may account for lower recoveries than expected.

Immunoaffinity chromatography of mushroom tyrosinase resulted in significant purification of the enzyme compared to crude samples. Our recoveries were somewhat better than those reported by Goldhirsh and Whitaker [12]. Likewise our SDS-PAGE patterns of bound enzyme were much different than the above investigators who estimated M_r of 105000, 53000, and 23000 for the mushroom enzyme purified by immunoaffinity chromatography. They also observed that immunoaffinity purified tyrosinase did not contain a 12000 component that was present in hydroxylapatite purified preparations. We did find a 13–15000 and 12–13000 component after immunoaffinity chromatography, suggesting that perhaps our antibody recognizes the L subunit while theirs did not. The tyrosinase purified by immunoaffinity chromatography in this report contained similar M_r proteins found in purified mushroom tyrosinase preparations purified by classical techniques [15–17].

The use of immunological techniques has significant application to mushroom tyrosinase. For example, Khan and Ali [26–28] used antibodies against the native enzyme to explore tyrosinase conformation and structure. They also showed that the Cu cofactor was not required for immunological cross reaction and the antigenic sites were distinguishable from the catalytic sites. Podila and Flurkey [14] have also shown that antibodies can be used to identify the translation products of mushroom tyrosinase. They suggested that the H and L may be translated from different genes since two distinct translation products were synthesized *in vitro*. It should also be possible to raise antibodies against the H and L subunits, isolate each individually, and examine what role L plays in relation to H subunits.

During the course of this investigation we encountered many of the same problems reported by others concerning affinity chromatography of mushroom tyrosinase. This was expected due to the nature of using inhibitors as the affinity ligand. Yields, recoveries, as well as the ability to reuse certain resins were the major problems encountered. Although the inhibitor ligands chosen in this study had low K_is, they can be used effectively in place of the more common *p*-amino benzoic acid derivatives as affinity resins to purify mushroom tyrosinase. Their usefulness depends upon the presence or absence of a spacer arm, choice of solid support and method of coupling, and the type of chromatography employed. The results from this study may aid in the rapid purification and characterization of the tyrosinase from a variety of sources.

EXPERIMENTAL

Materials. The following materials were purchased from Sigma Chemical Co.: L-mimosine; tropolone; L-dihydroxyphenylalanine (L-DOPA); mushroom tyrosinase (grade III, lot no. 84F–9640); cyanogen and epoxy activated Sepharose; agarose resins containing covalently bound phenyl, phenylalanine, phenylbutylamine, and tyrosine; alkyl columns containing covalently bound ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, decyl, and dodecyl groups; and phenylboronic acid coupled to agarose. Methimazole, 2-mercaptobenzimidazole, and 2-mercaptobenzothiazole were obtained from Aldrich Chemical Co. Tresyl chloride activated Sepharose was obtained from Pharmacia Fine Chemicals.

Preparation of affinity columns. Each inhibitor (50 mg) was dissolved in 10 ml of 0.1 M $NaHCO_3$ and 0.5 M NaCl at pH 8.3. Methimazole was gently heated, 1 ml of EtOH was added to MBI, and 1 ml of EtOH and 1 ml of Me_2CO was added to MBT in order to solubilize them. Coupling of the inhibitors, blocking of activated groups, and post coupling washing of the resins were carried out according to established procedures and the manufacturers instructions [33]. Each resin was suspended in 0.01 M Na-Pi buffer (pH 6.0 containing 0.01% NaN_3, wrapped in Al foil, and stored at 4° until used. The molar absorbtivity was determined for each ligand experimentally. This coefficient was used to calculate the amount of ligand not coupled. Substraction from the total ligand mass yielded the amount of bound ligand.

Affinity chromatography. Each resin, approximately 1 ml, was packed into a small column and equilibrated with 0.01 M Na-Pi buffer (pH 6.0). Mushroom tyrosinase (100–250 μg of solid powder) was dissolved in a small amount of equilibration buffer and applied to each column. The columns were washed with 10 ml of equilibration buffer and the fraction collected. Enzyme, adsorbed to the resin, was eluted with 0.5 M Na-Pi buffer (pH 6.0) in a 10 ml fraction. In some experiments, the columns were washed with 10 ml of 0.1 M acetate buffer (pH 4.0) containing 0.5 M NaCl followed by 10 ml of 25% ethyleneglycol to determine if enzyme was still adsorbed. All columns were run at 25°. The

amount of enzyme bound was determined quantitatively by enzyme assays and qualitatively by SDS-PAGE.

Hydrophobic chromatography. Each resin, approximately 1 ml, was packed into a small column and equilibrated with 0.01 M Na-Pi (pH 6.0) containing either 12.5, 25, or 50% $(NH_4)_2SO_4$. Mushroom tyrosinase (100–250 μg solid powder) was dissolved in the equilibration buffer and applied to each column. The columns were washed with 10 ml of equilibration buffer and the fraction collected. Adsorbed enzyme was eluted from the resin by washing the columns with 10 ml of 0.01 M Na-Pi (pH 6.0). Columns were washed with 25% ethylene glycol to remove tightly bound enzyme.

Immunoaffinity chromatography. Antibodies were prepared against the mushroom tyrosinase holoenzyme (anti-MTA) and against a 23–26 000 protein (anti-MTB) as described earlier [14]. The IgG fraction obtained from $(NH_4)_2SO_4$ pptn of each antisera was coupled to CNBr activated Sepharose [33]. Each immunoaffinity resin was packed into a small column and equilibrated with 50 ml of phosphate buffered saline. Mushroom tyrosinase (250 μg of solid powder) was dissolved in the equilibration buffer and recycled onto the column via a peristaltic pump for 2 hr at 25°. The columns were washed with phosphate buffered saline to remove nonadsorbed proteins.The enzyme was eluted from the affinity columns with 0.25 M Tris (pH 10.0).

Enzyme assays and electrophoresis. Mushroom tyrosinase was assayed in 2 ml of 0.05 M Na-Pi buffer (pH 6.0) containing 10 mM catechol. The increase in absorbance was monitored at 410 nm in an Hitachi 100–600 recording spectrophotometer. The initial velocity was determined from the linear portion of the curve. One unit of activity was defined as a change of 1.0 absorbance unit per min at 410 nm [9]. SDS-PAGE was performed on selected samples from resins containing adsorbed enzyme. Samples were pptd with trichloroacetic acid, washed with Me_2CO, and dissolved in two-fold concentrated Laemmli buffer before application onto SDS-PAGE [14]. Protein content of the commercial resuspended powder could not be determined with certainty using either the Lowry or Bradford protein assay, probably because of the brown pigments present. Therefore, the same amount of resuspended solid powder was applied to each column.

Acknowledgements— This work was supported in part from funds from the Indiana Academy of Sciences. We also thank P. Archer and K Divan for their secretarial assistance.

REFERENCES

1. Lerman, L. S. (1953) *Proc. Natl Acad. Sci.* **39**, 232.
2. Gutteridge, S. and Robb, D. A. (1973) *Biochem. Soc. Trans.***1**, 519.
3. O'Neill, S. P., Graves, D. J. and Ferguson, J. J. (1973) *J. Macromol. Sci.***A7**, 1159.
4. Yonekura, M., Shimoda, T. and Funatsu, M. (1981) *Agric. Biol. Chem.* **45**, 101.
5. Menon, I. A. and Haberman, H. F. (1975) *Acta Dermat.* **55**, 343.
6. Flurkey, W. H. and Jen, J. J. (1978) *J. Food Sci.* **43**, 1826.
7. Jen, J. J. and Flurkey, W. H. (1979) *Hortscience* **14**, 516.
8. Flurkey, W. H. and Jen, J. J. (1980) *Biochem. Physiol. Planzen* **175**, 637.
9. Flurkey, W. H. and Jen, J. J. (1980) *J. Food Biochem.* **4**, 29.
10. Wissemann, K. W. and Lee, C. Y. (1980) *J. Chromatogr.* **192**,232.
11. Wissemann, K. W. and Montgomery, M. W. (1985) *Plant Physiol.* **78**, 256.
12. Golan-Goldhirsh, A. and Whitaker, J. R. (1984) in *Nutritional and Toxicological Aspects of Food Safety* (Friedman, M., ed.) Vol. 177, pp.437–456. Plenum Press, New York.
13. Kahn, V. and Andrawis, A. (1985) *Phytochemistry* **24**, 905.
14. Podila, G. K. and Flurkey, W. H. (1986) *Biochem. Biophys. Res. Commun.* **141**, 697.
15. Strothkamp, K. G., Jolley, R. L. and Mason, H. S. (1976) *Biochem. Biophys. Res. Commun.* **70**, 519.
16. Gutteridge, S. and Mason, H. S. (1979) in *Biochemical and Clinical Aspects of Oxygen* (Caughey, W. S., ed.) pp. 589–602. Academic Press, New York.
17. Robb, D. A. (1979) *Biochem. Soc. Trans.* **7**, 131.
18. Kahn, V., Golan-Goldhirsh, A. and Whitaker, J. R. (1983) *Phytochemistry* **22**, 1875.
19. Golan-Goldhirsh, A. and Whitaker, J. R. (1984) *J. Agric. Food Chem.* **32**, 1003.
20. Andrawis, A. and Kahn, V. (1985) *Phytochemistry* **24**, 397.
21. Golan-Goldhirsh, A. and Whitaker, J. R. (1985) *J. Molec. Catalysis* **32**, 141.
22. Kahn, V. and Andrawis, A. (1986) *Phytochemistry* **25**, 333.
23. Boyer, R. F., Mascotti, D. P. and Schori, B. E. (1986) *Phytochemistry* **25**, 1281.
24. Andrawis, A. and Kahn, V. (1986) *Biochem. J.* **235**, 91.
25. Sugumaran, M. (1986) *Biochemistry* **25**, 4489.
26. Khan, I. A. and Ali, R. (1983) *J. Radiat. Res.* **24**, 154.
27. Khan, I. A. and Ali, R. (1985) *J. Radiat. Res.* **26**, 109.
28. Khan, I. A. and Ali, R. (1986) *J. Biochem.* **99**, 445.
29. Flurkey, W. H. and Jen, J. J. (1980) *J. Food Sci.* **45**, 1622.
30. Iborra, J. L., Cortes, E., Manjon, A., Ferragut, J. and LLorca, F. (1976) *J. Solid-Phase Biochem.* **1**, 91.
31. Gulewicz, K., Adamiak, D. and Sprinzl, M. (1985) *FEBS Letters* **189**, 179.
32. Porath, J. (1962) *Nature.* **196**, 47.
33. Wilchek, M., Miron, T. and Kohn, J. (1984) in *Methods in Enzymology* (Jakoby, W. B., ed.) Vol. **104**, pp. 3–55. Academic Press, Orlanda, Fl.