OXIDATION BY TYROSINASE OF COMPOUNDS CONTAINING TYROSYL GROUPS AS STUDIED BY ABSORPTION SPECTROSCOPY*

by

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The successive steps involved in the oxidation by tyrosinase of tyrosine to melanin have been studied extensively in the past by RAPER[22, 23], NELSON AND DAWSON[19], and MASON[15, 16] who generally agree that the reaction proceeds through the formation of dopa and hallochrome with the eventual formation of the polymeric pigment, melanin. Certain derivatives of tyrosine are also suitable substrates for tyrosinase[13, 14], and SIZER[30–33] has indicated that certain tyrosyl groups of susceptible proteins can be oxidized by this enzyme. The nature of the action of tyrosinase on protein materials is not clear, however; although the great importance of tyrosyl groups in determining the biological activity of proteins[21] makes this problem of considerable interest. The present investigation is an attempt to elucidate the mechanisms and details of this tyrosinase reaction by utilizing as models of the proteins certain natural mixtures of polypeptides as well as tyrosine derivatives in which amino or carboxyl groups, or both, are protected by chemical combination. This problem is well suited to study by the technique of kinetic ultraviolet spectroscopy at both room temperature[3, 8, 9, 11, 15, 30] and at very low temperatures[4, 12, 25–29].

METHODS

The kinetic spectrophotometric method as developed by MASON[15–17] was used for following tyrosinase action except that NaCN (neutralized) was added to stop the reaction in the digest samples to be studied spectroscopically. The addition of cyanide did not prevent autoxidation from occurring in the samples, but in the early stages of the reaction any autoxidation is relatively slow[23]. Samples were studied both at room temperature with the Beckman Spectrophotometer (Model DU) and also at the temperature of liquid nitrogen (77° K.) with the Hilger spectrograph (Model E-1), because of indications that low temperature spectroscopy might yield information on changes in fine structure of broad absorption bands[1, 4, 10, 12, 26–29]. Absorption curves at 77° K. were obtained from the Hilger plates by using the microphotometer plate-matching method of SINSHEIMER *et al.*[26]. In these low temperature studies the experimental samples were mixed with 1.5 parts of glycerol (Merck) and frozen in liquid nitrogen. Just before examination, the solutions were thawed briefly in order to fill the absorption cells (quartz, 1 mm path length) and the cells immersed in liquid nitrogen for exami-

* Supported in part by a grant from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council.

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References p. 599/600.

nation[10]. Since the low temperature technique furnished reliable relative rather than absolute optical densities[26], and since direct comparison of absorption curves determined with different concentrations of absorbing materials is desired, results are presented as plots of log optical density against wavelength for the room temperature data and of normalized density (*i.e.* per cent of density at an absorption peak) against wavelength for the low temperature data. Since data obtained by the two techniques at 293° and 77° K. are in good qualitative agreement (see Figs. 1 and 2) only one group of curves is presented. This group is usually the 77° K. results because of the wealth of detail which they furnish.

For all studies, 5–30 ml of *M*/15 or *M*/200 phosphate buffer (either p_H 6 or 7), containing that amount of substrate which would absorb at a suitable initial density at 275 mμ, were incubated at 25° C with a small amount of mushroom tyrosinase (0.2–0.5 ml Treemond tyrosinase: a typical stock solution contains 3,700 Miller Dawson units and 2.2 mg dry weight per ml), chosen to give a suitable rate of reaction. Detailed protocols for the various oxidation experiments are given with the figure headings. Ether was added as a preservative in those reactions lasting more than two hours; it was evaporated from the digest sample before spectroscopic examination.

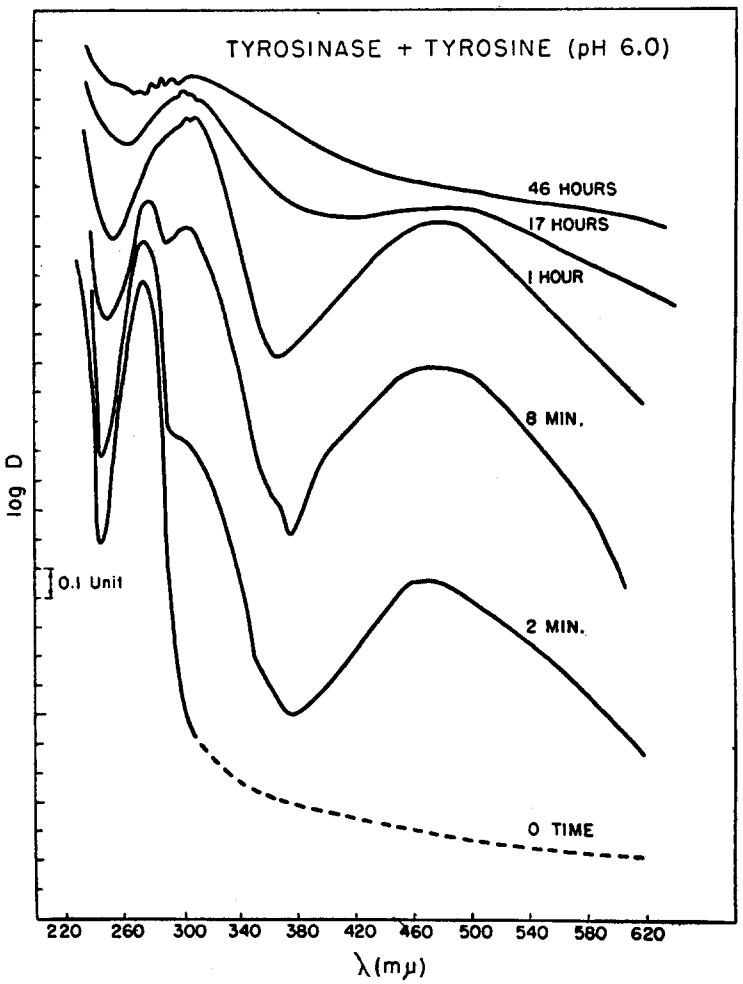

Fig. 1. Action of tyrosinase on tyrosine

50 ml of p_H 6.0 *M*/200 phosphate buffer containing 0.1 mg/ml tyrosine plus 1 ml tyrosinase (diluted 1:10) were incubated at room temperature. 3 ml aliquots were removed at times indicated, cyanide added, and absorption spectra measured at 293° K.

References p. 599/600.

RESULTS

Tyrosine

The 293° and 77° K. spectra of the tyrosinase-tyrosine system as functions of reaction time are presented in Figs. 1 and 2. The development of intermediary peaks at 305 and 475 mμ during the reaction is doubtless due to the formation of hallochrome[15]. The curves also demonstrate a decrease in ratio of maximum to minimum absorption and a general increase in optical density with time as the continuous high absorption of melanin pigments is approached[8, 9, 15, 30]. Although the formation of dopa as an intermediate has been established by chemical tests[23], there is no spectroscopic evidence for dopa in the 293° K. studies. The increased resolution of the 77° K. technique, however, does permit the identification of dopa formed from tyrosine in the reaction mixtures. The secondary tyrosine peak at 282 mμ continually increases with time (Fig. 2) until it finally absorbs more strongly than the primary tyrosine peak at 276 mμ. This increase in absorption at 282 mμ is probably due to dopa which has an absorption peak at 282 mμ[16]. Direct comparison of the original microphotometer traces of the dopa and tyrosinase-tyrosine spectra makes this conclusion more certain.

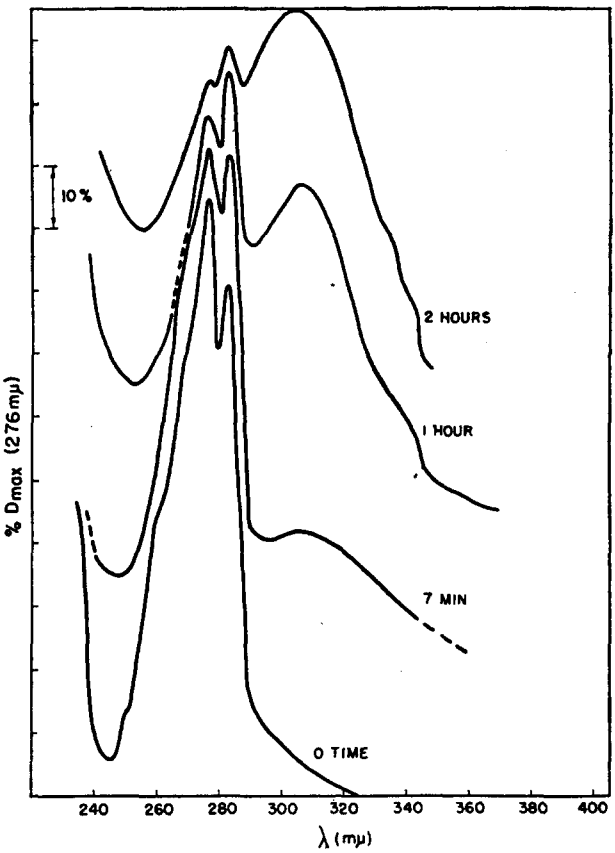

Fig. 2. Action of tyrosinase on tyrosine
10 ml of pH 5.95 *M*/15 phosphate buffer containing 2 mg/ml of tyrosine plus 0.66 ml tyrosinase were incubated at room temperature with constant stirring. 1 ml aliquots were removed at the times indicated, cyanide added, and the aliquots shaken with 1.5 ml of glycerol. The glycerol solutions were immediately immersed in liquid nitrogen and kept immersed until briefly thawed in order to fill the absorption cells. Absorption spectra were determined at 77° K.

The addition of the simple non-polar amino acid norleucine to the system had no effect on the absorption spectrum changes, while cystine delayed the formation of coloured products for several hours. The reaction was also retarded by 60% glycerol.

Serum Albumin

Negative spectroscopic results were obtained for the action of tyrosinase on crystallized bovine serum albumin (Armour) confirming the report that albumin is refractory to tyrosinase[30].

References p. 599/600.

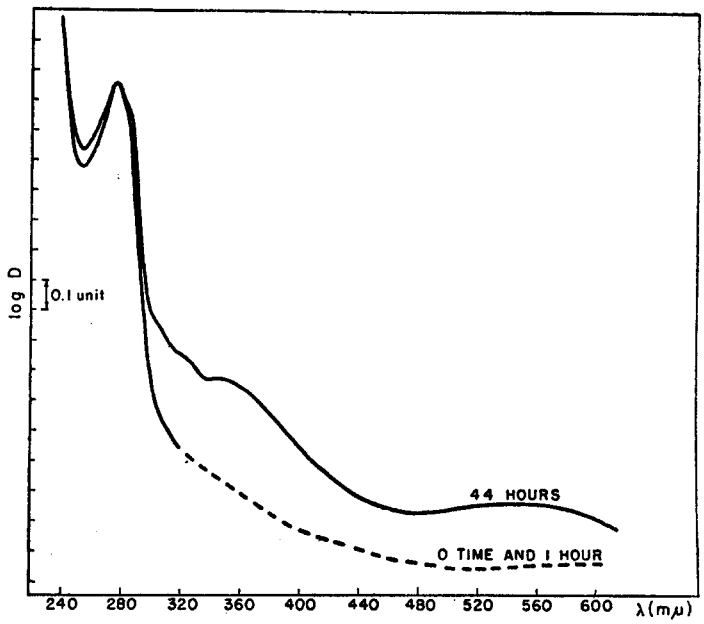

Fig. 3. Action of tyrosinase on native insulin
50 mg of pure zinc-free insulin were dissolved in dilute HAc and adjusted to a clear solution at p_H 7.5 with NH_4OH, 0.1 ml of tyrosinase was added and the mixture was incubated at room temperature under ether with frequent stirring. 3 ml aliquots were removed at the times indicated, cyanide added, and the absorption spectra determined at 293° K.

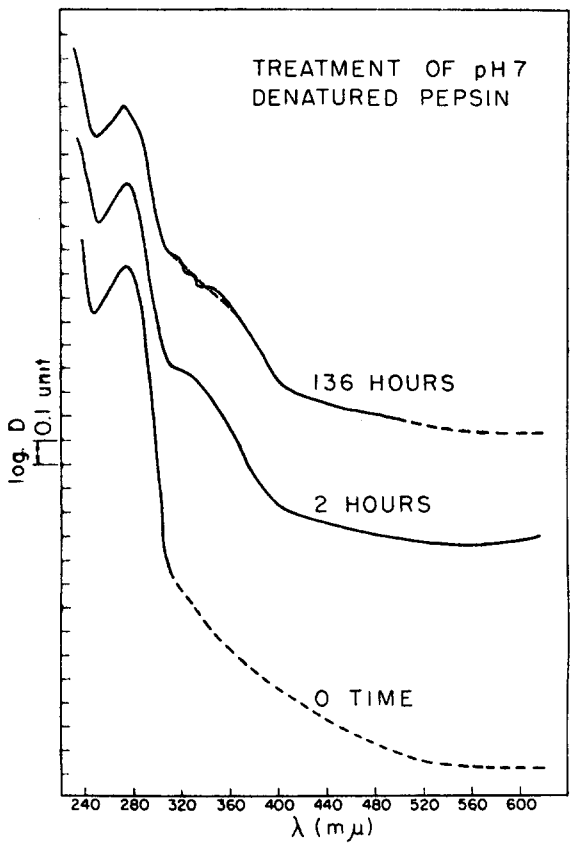

Fig. 4. Action of tyrosinase on p_H 7 denatured pepsin
35 mg of crystalline pepsin were dissolved in 2 ml of p_H 7.0 $M/15$ phosphate buffer and incubated at room temperature under ether overnight. The ether was removed by aspirating and the solution was made up to 10 ml with additional buffer. 0.02 ml of tyrosinase was added and the digest incubated at room temperature. 3 ml aliquots were removed at the times indicated, cyanide was added, and absorption spectra determined at 293° K.

Insulin

Crystallized insulin (Armour) is relatively very resistant to tyrosinase and the small changes in absorption spectrum (Fig. 3) are scarcely more than could be accounted for by the autoxidation of the tyrosinase alone.

Pepsin

The action of tyrosinase on crystallized pepsin (Armour) at p_H 7.0 is very marked when studied spectroscopically (Fig. 4). In particular, the rise in the minimum, the development of a shoulder at 325 mμ, the absence of the hallochrome peak and the failure of melanin precipitates to form are all characteristic of the action of tyrosinase on tyrosine in combined form, *e.g.* in proteins, as opposed to free tyrosine. It is suggested that the enzyme acts on denatured rather than native pepsin. This would seem to be the case because pepsin is rapidly inactivated at p_H 7^{20} and a fresh solution of pepsin at p_H 7 is more slowly oxidized by tyrosinase than one which has stood for some time*

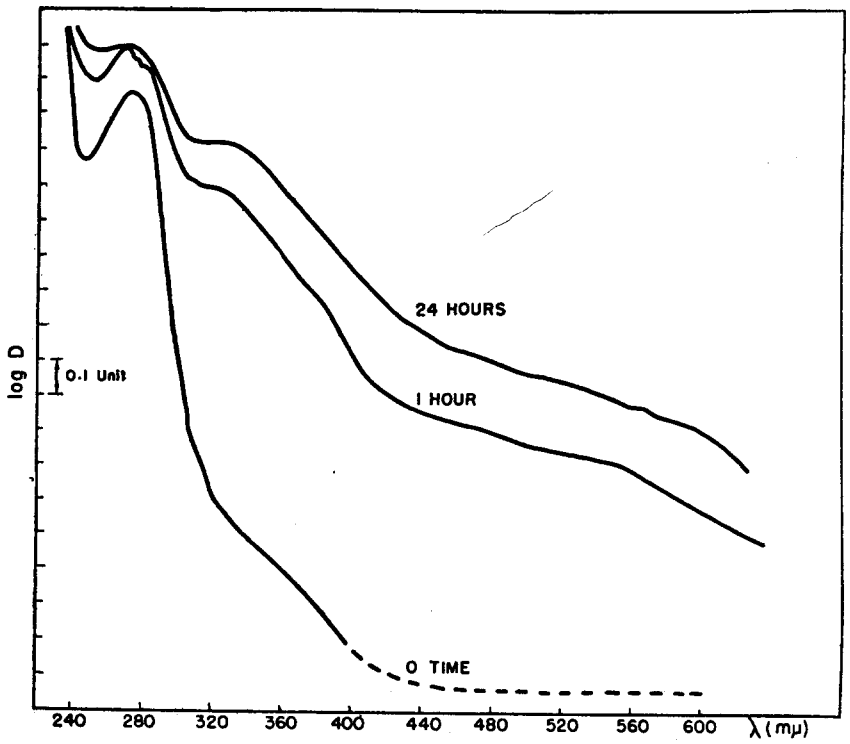

Fig. 5. Action of tyrosinase on pepsin subjected to peptic hydrolysis
35 mg of crystalline pepsin were dissolved in 2 ml of p_H 7.0 *M*/15 phosphate buffer and incubated overnight at room temperature under ether. The ether was removed by aspirating and the pepsin solution adjusted to p_H 1.0 with 10% HCl. 7 mg of crystalline pepsin were added and the digest was incubated at room temperature for 72 h. The solution was then made up to 25 ml with 7.0 phosphate buffer, immersed in boiling water for 5 minutes, and centrifuged. The supernatant was remade to 25 ml with additional p_H 7.0 buffer and incubated at room temperature with 0.1 ml tyrosinase. 3 ml aliquots were removed at the times indicated, cyanide was added, and absorption spectra determined at 293° K.

* Preliminary studies at p_H 5.6 indicate that tyrosinase can slowly oxidize native pepsin and thrombin.

References p. 599/600.

Protein-Partial Hydrolysates

The relative resistance of most proteins to attack by tyrosinase may be related to the steric unavailability of tyrosyl groups at the surface of the molecule or to phenolic hydrogen bonding to the side chains of other amino acids[5, 18, 24, 33], or both. Since SIZER[80] had already demonstrated that proteins refractory to tyrosinase could be rendered susceptible by preliminary treatment with proteases, this method was adopted in the present study. Partial hydrolysis was brought about using either pepsin or chymotrypsin at its p_H optimum.

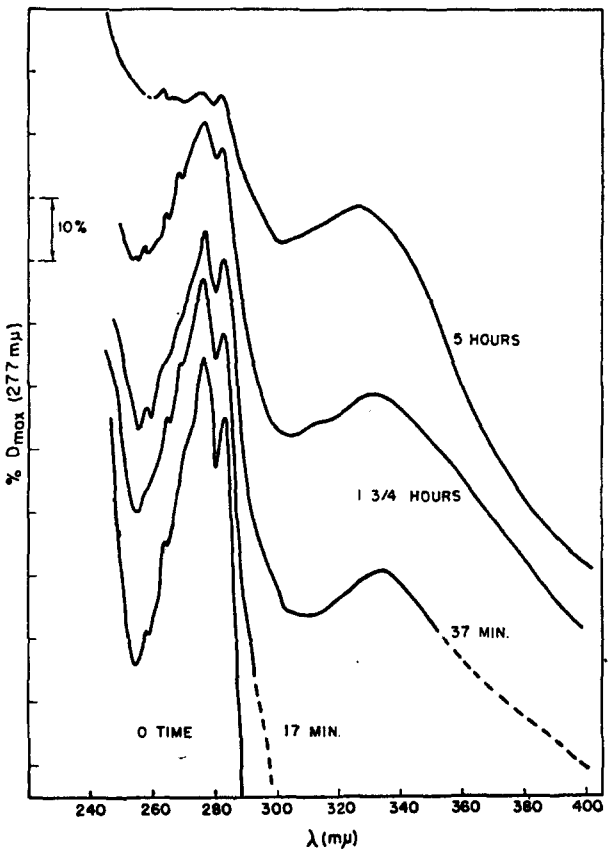

Fig. 6. Action of tyrosinase on insulin subjected to peptic hydrolysis

200 mg of crystalline insulin were dispersed in 5 ml of p_H 5.95 *M*/15 phosphate buffer and acidified with glacial acetic acid to solution at p_H 3.3. The solution was then adjusted to p_H 1.7 with concentrated HCl and 50 mg of crystalline pepsin were added. The digest was incubated at 37° C for 24 hours, immersed in boiling water for ten minutes, centrifuged, and the supernatant clear yellow solution adjusted to p_H 6.0 with NH_4OH. The solution was then incubated with 1.0 ml of undiluted tyrosinase stock at room temperature. 0·5 ml aliquots were removed at the times indicated, cyanide was added, and the aliquots shaken with 0.75 ml of glycerol. Absorption spectra of the glycerol solutions were measured at 77° K.

In the case of denatured pepsin, partially hydrolyzed by pepsin (Fig. 5), the absorption curves closely resemble those with pepsin at p_H 7 (Fig. 4); the results with a chymotryptic hydrolysate of pepsin are also quite similar except in the 325 mμ region. The hydrolysate is oxidized by tyrosinase more rapidly than the unhydrolyzed protein and the rise in the minimum absorption is more pronounced. Since interest centers around the action of tyrosinase on the partially hydrolyzed protein rather than on any tyrosine or small tyrosine peptides, the action of tyrosinase was also studied after the removal by dialysis of small molecules of molecular weight less than 12,000. (Cenco cellophane tubing was used in the dialysis). Results with the dialyzed protein hydrolysate were very similar to those for the undialyzed, except that the total increase in optical density is not as great in the dialyzed preparation.

Effects of tyrosinase on insulin partially hydrolyzed by either pepsin or chymotrypsin (with or without subsequent dialysis) are marked as compared with the native insulin. This difference is illustrated by the low temperature study presented in Fig. 6. Although results with insulin partially hydrolyzed by

References p. 599/600.

both enzymes are similar, the absorption band at 325 mμ is more pronounced in the oxidized peptic hydrolysate than in the chymotryptic one. This band is clearly demonstrated at low temperature in Fig. 6. A similar difference was noted between the digests of denatured pepsin hydrolyzed by the two different proteases. It seemed possible that these differences might be related to the formation of hydrolysate containing polypeptide residues, possessing pre-dominantly free tyrosyl amino groups by peptic, but predominantly free tyrosyl carboxyl groups by chymotryptic, hydrolysis of the protein[2]. Evidence on this point is presented in the next section.

Tyrosinase gives rise to a yellow-brown colour with protein hydrolysates and susceptible proteins; no melanin is formed. The complete lack of selective absorption in the visible region and specifically the absence of the 475 mμ hallochrome peak from the spectra of proteins or protein partial hydrolysates after treatment with tyrosinase indicates that no more than a trace of free tyrosine could have been present in the original substrates.

Tyrosine Derivatives

In view of the characteristic differences between the action of tyrosinase on tyrosine on the one hand, and the action of the enzyme on proteins and partial hydrolysates on the other, it seemed advisable to test certain synthetic derivatives of tyrosine as tyrosinase substrates. It was hoped that such studies might offer an explanation for the 325 mμ shoulder in the absorption spectra and the failure to form hallochrome and melanin from the proteins and their partial hydrolysates.

p-Methoxytyrosine. This substrate* in which the phenolic hydroxyl group is replaced by a methoxyl group is completely refractory to tyrosinase, confirming the results of LERNER *et al.*[13, 14] with mammalian tyrosinase. On the basis of this result, it seems plausible that hydrogen bonding of tyrosyl groups in proteins[33] might in part explain the resistance of

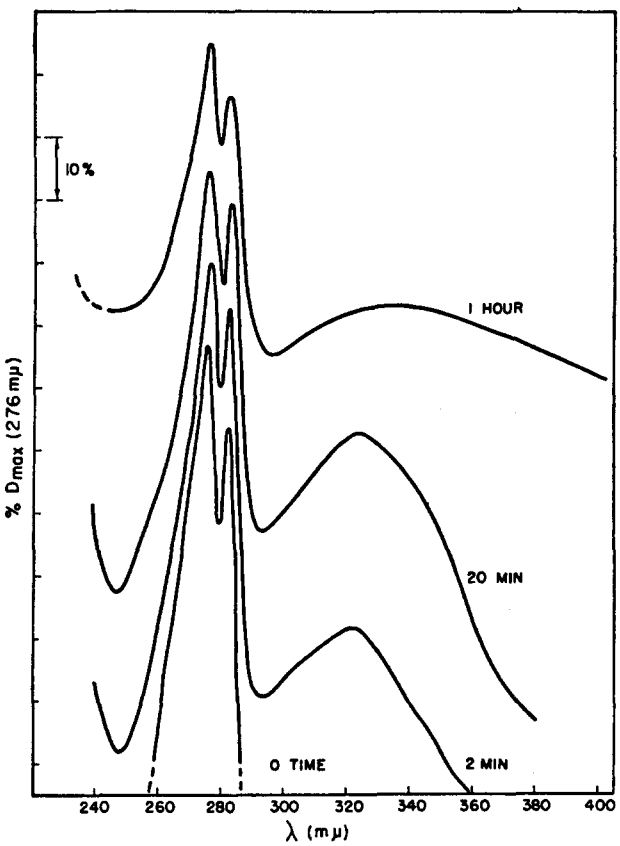

Fig. 7. Action of tyrosinase on tyrosine ethyl ester 5 ml of pH 6.0 *M*/15 phosphate buffer containing 2 mg/ml of tyrosine ethyl ester were incubated with 0.5 ml of tyrosinase at room temperature with constant stirring. 1 ml aliquots were removed at the times indicated, cyanide added, and the aliquots shaken with 1.5 ml portions of glycerol. The absorption spectra of the glycerol solutions were measured at 77° K.

* Kindly furnished by Drs. R. L. SINSHEIMER and J. F. SCOTT, Biology Dept., M.I.T.

References p. 599/600.

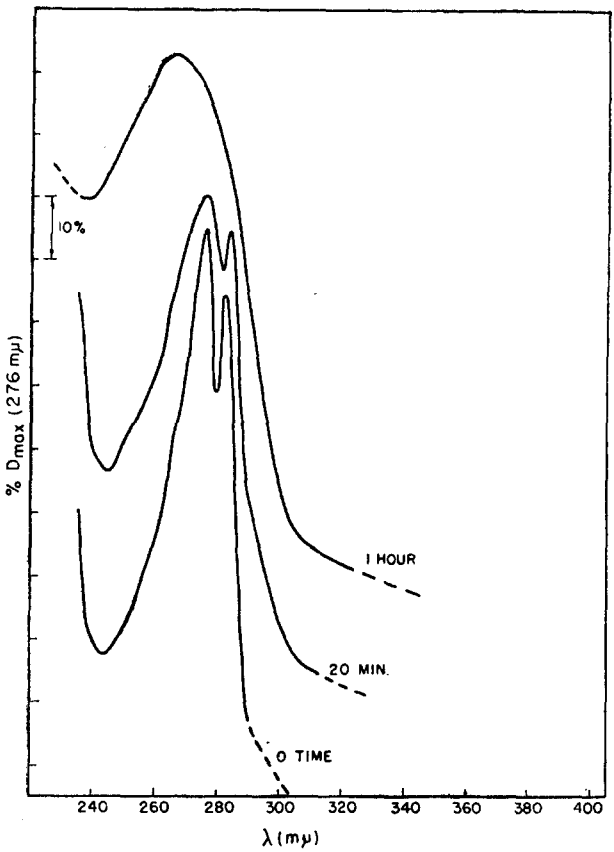

Fig. 8. Action of tyrosinase on N-formyl tyrosine
5 ml of pH 6.0 *M*/15 phosphate buffer containing 2 mg/ml of N-formyl tyrosine were incubated at room temperature with constant stirring with 0.5 ml of tyrosinase. 1 ml aliquots were removed at the times indicated, shaken with 1.5 ml portions of glycerol, and immediately immersed in liquid nitrogen. Absorption spectra were determined at 77° K.

most tyrosyl groups of proteins to tyrosinase as discussed above.

Tyrosine Ethyl Ester. The very rapid oxidation of tyrosine ethyl ester by tyrosinase is shown in Fig. 7. This action is characterized by the formation of esterified dopa, as indicated by a drop in the ratio of primary to secondary tyrosine peak. No hallochrome analog which absorbs like hallochrome is formed, and no melanin, although a greenish pigment does precipitate from the solution. The band at 325 mμ is of special interest since it corresponds to the 325 mμ shoulder of the tyrosinase-treated proteins and partial hydrolysates, and is thus evidently characteristic of the oxidation of tyrosyl residues with chemically combined carboxyl groups. As discussed above, the much greater prominence of the 325 mμ shoulder in the spectra of oxidized peptic, as compared with chymotryptic, hydrolysates is consistent with the theory[2] that pepsin liberates from proteins fewer tyrosyl carboxyl groups than does chymotrypsin.

*N-Formyl Tyrosine**. Tyrosine oxidizes N-formyl tyrosine much more slowly than tyrosine ethyl ester, although marked changes in absorption are also brought about (Fig. 8). The most striking effect of tyrosinase action, in addition to the formation of the dopa analog, is a marked increase in absorption in the region of the tyrosine minimum and a decrease in the ratio of maximum to minimum absorption also characteristic of the action of tyrosinase on susceptible proteins and their partial hydrolysates. Room temperature kinetic spectroscopic studies of the oxidation of N-formyl tyrosine do indicate the production of some selective absorption at 325 mμ, but it is much less marked than with the ester. No melanin is produced from N-formyl tyrosine. Instead, tyrosinase action ceases with the formation of a reddish-brown colour in the oxidized solution with a broad absorption band maximum at about 267 mμ. Thus differences in

* Recently a sample of N-glycyl-*l*-tyrosine (Delta Chemical Works) has been treated with tyrosinase. Preliminary spectroscopic studies on this system indicates, as would be expected, that tyrosinase action on this peptide closely parallels the action of tyrosinase on N-formyl tyrosine.

References p. 599/600.

the absorption at 325 mμ in the oxidation products of a peptic and chymotryptic hydrolysate find a plausible explanation in terms of results with these two synthetic derivatives.

N-Formyl Tyrosine Ethyl Ester. The course of the tyrosinase oxidation of N-formyl tyrosine ethyl ester (see Fig. 9) resembles in a general way that of the unesterified N-formyl tyrosine. Rates of oxidation are similar, as is the marked decrease in ratio of maximum to minimum, and also the final development of a single broad peak at about 271 mμ. As with both of the other tyrosine derivatives, the formation of a dopa analog intermediate is indicated by a decrease in the ratio of primary to secondary tyrosine peaks. In addition, however, a definite absorption band develops at 325 mμ, as occurs with the ethyl ester; and a brown turbid solution develops suggesting some polymerization, although no true melanin is formed. Thus, the oxidation of this compound, in which both tyrosyl amino and carboxyl groups are hindered, appears similar in most respects to the oxidation of the parent compounds in which only one or the other of these groups is combined. The spectroscopic studies of tyrosinase oxidation of N-formyl tyrosine ethyl ester further show striking similarities to comparable studies on certain proteins and their partial hydrolysates where susceptible tyrosyl residues probably do not have free carboxyl or amino groups.

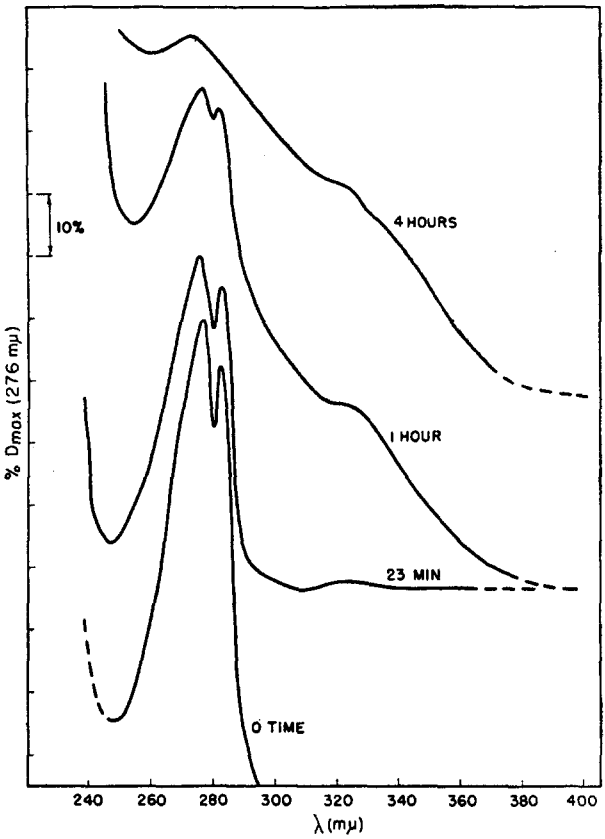

Fig. 9. Action of tyrosinase on N-formyl tyrosine ethyl ester 5 ml of pH 6.0 $M/15$ phosphate buffer containing 2 mg/ml of N-formyl tyrosine ethyl ester were incubated with 0.5 ml of tyrosinase with constant stirring. 1 ml aliquots were removed at the times indicated, cyanide added, and the aliquots shaken with 1.5 ml portions of glycerol. Absorption spectra were determined at 77° K.

Although the identification by chemical and spectroscopic means of the oxidation intermediates is required as the next step, these studies with tyrosine derivatives throw considerable light on the course of the oxidation of proteins and their partial hydrolysates. They also offer an explanation of certain differences observed between the action of tyrosinase on tyrosine and on proteins and their partial hydrolysates.

References p. 599/600.

SYNTHESIS OF TYROSINE DERIVATIVES

l-Tyrosine Ethyl Ester

Preparation of this compound was based on the method of FISHER[6]. M.p. from ethyl acetate was 101–102° uncorrected (reported 108–109°). Recrystallized 3 times from ethyl acetate and once from ethanol-ether and dried over P_2O_5 in vacuum at 80° without further change in m.p. Analysis: $C_{10}H_{15}NO_3$

Calc:	C — 63.20	H — 7.18	N — 6.70
Fnd:	C — 62.74	H — 7.42	N — 6.72

N-formyl-l-tyrosine

Preparation of this compound was based on the method of FISCHER[7] m.p. after drying: 170–172° uncorrected (reported 170–172°). M.W.: calc: 209; found: 207. Analysis: $C_{10}H_{11}NO_4$.

Calc:	C — 57.39	H — 5.26	N — 6.70
Fnd:	C — 57.03	H — 5.57	N — 6.79

N-formyl-l-tyrosine Ethyl Ester

Preparation of this compound was based on the method of FISCHER[7] for N-formyl-*l*-tyrosine. Tyrosine ethyl ester previously prepared was used as a starting material. M.p. from chloroform petroleum ether was 105–106°. After recrystallization from both chloroform-petroleum ether and ethyl ether-petroleum ether, the m.p. was unchanged. Analysis: $C_{12}H_{15}NO_4$.

Calc:	C — 60.75	H — 6.33	N — 5.91
Fnd:	C — 60.49	H — 6.59	N — 5.95

SUMMARY

Kinetic absorption spectroscopy at both room temperature and at 77° K. has been applied to the study of the action of tyrosinase on tyrosine and on compounds containing tyrosyl groups. Evidence is obtained that the oxidation of all susceptible substrates proceeds through the 3, 4-dihydroxyphenyl-alanine group (dopa) as an intermediate. Only in the case of tyrosine is there formed a typical hallochrome intermediate and, as the end product, a black, insoluble melanin pigment. The enzymatic oxidation of tyrosine is retarded by cystine and glycerol, but not by norleucine.

Serum albumin is found to be completely refractory to tyrosinase and insulin is only slightly oxidized, while denatured pepsin is strongly affected. Partial hydrolysates of pepsin and insulin are profoundly oxidized by tyrosinase. The oxidation by tyrosinase of proteins and protein partial hydrolysates is characterized by an over-all increase in optical density, by a pronounced decrease in the ratio of maximum to minimum absorption and by the development of a shoulder in the 325 mμ region. Differences, in the oxidation of tyrosine and of proteins by tyrosinase are ascribed to the fact that some tyrosyl groups in proteins are refractory because they de not have free phenolic hydroxyl groups. Other differences are related to the fact that in proteins both the tyrosyl amino and carboxyl groups are usually combined in peptide bonds. Studies of the action of tyrosinase on certain synthetic derivatives of tyrosine are consistent with this interpretation. These derivatives included *p*-methoxytyrosine, which is refractory to tyrosinase, and tyrosine ethyl ester, N-formyl tyrosine, N-formyl tyrosine ethyl ester and glycyl-*l*-tyrosine which were readily oxidized. In the case of derivatives in which carboxyl or amino, or both, groups were combined, the action of tyrosinase was parallel in most respects to its action on proteins and protein partial hydrolysates.

RÉSUMÉ

Les spectres de l'absorption cinétique à température de chambre comme à température de 77° K sont été appliqués aux études de l'action de la tyrosinase sur la tyrosine et sur des composés

References p. 599/600.

lesquelles contiennent des groups de tyrosyl. Il a été prouvé que l'oxydation de tous les substrats susceptibles procède à travers le groupe de la 3, 4-dihydroxyl-phényl-alanine (dopa) comme intermédiaire. Seulement dans le cas de la tyrosine un intermédiaire typique de hallochrome est formé, et comme produit final un pigment noir, insoluble de mélanine. L'oxydation enzymatique de la tyrosine est retardée par la cystine et le glycerol, mais pas par la norleucine.

L'albumine du sérum a été trouvé complètement réfractaire à la tyrosinase et l'insuline est seulement légèrement oxidée, pendant que la pepsine dénaturée est fortement attaquée. Les hydrolyses partiales de la pepsine et de l'insuline viennent profondement oxydés par la tyrosinase. L'oxydation des protéines et des résidus de l'hydrolyse partiale des protéines est caracterisée par l'augmentation générale de la densité optique, par un abaissement du rapport de l'absorption maximale et minimale, et par le développement d'une épaule dans la région de 325 mμ. Les différences de l'oxydation par la tyrosinase de la tyrosine et des autres protéines sont dûs au fait que quelques groupes de tyrosyl dans les protéines sont réfractaires parce qu'elles ne contiennent pas des groupes de phénol-hydroxyl libres. D'autres différences ont rapport avec le fait que dans les protéines les groupes tyrosyl, aminiques et carboxyliques sont usuellement combinées en liaison peptidiques. Des études de l'action de la tyrosinase sur certain dérivés synthétiques de la tyrosine s'accordent avec cette interprétation. Ces dérivés includent la *p*-méthyltyrosine, laquelle est réfractaire à la tyrosinase, l'ester éthylique de la tyrosine, la N-formyltyrosine, l'ester éthylique de N-formyltyrosine, et la glycyl-*l*-tyrosine, lesquelles sont oxydées promptement. Dans le cas des dérivés dans lesquels les groupes carboxyliques, aminiques ou touts les deux sont combinés, l'action de la tyrosinase est parallèle dans la plupart des regards à son action sur les protéines ou sur les hydrolyses partiales des protéines.

ZUSAMMENFASSUNG

Die Einwirkung von Tyrosinase auf Tyrosine und auf Tyrosylgruppen enthaltende Verbindungen wurde mittels kinetischer Absorptionsspektroskopie bei Zimmertemperatur sowie bei 77° K untersucht. Es wird gezeigt dass die Oxydation aller oxydierbaren Substrata durch die 3, 4-Dihydroxyl-phenylalanin-gruppe (Dopa) als Zwischenstufe vorgeht. Nur im Falle von Tyrosin ergibt sich ein typisches hallochromes Zwischenglied, und als Endprodukt ein schwarzes unlösbares Pigment, Melanin. Die enzymatische Oxydation des Tyrosins wird durch Cystin und Glycerin verzögert, aber nicht durch Norleucin.

Serum-albumin wurde gegen Tyrosinase als vollkommen refraktär gefunden und Insulin wird nur in geringem Masse oxydiert, wogegen denaturiertes Pepsin sehr stark angegriffen wird. Produkte der teilweisen hydrolytischen Spaltung von Pepsin und Insulin werden durch Tyrosinase weitgehend oxydiert. Die Oxydation der Proteine und der hydrolytischen Spaltprodukte der Proteinstoffe von Tyrosinase ist charakterisert durch eine allgemeine Erhöhung der optischen Dichte, durch eine ausgesprochene Verringerung des Verhältnisses der maximalen zur minimalen Absorption und durch die Entwicklung einer Erhöhung im Bereich um 325 mμ. Unterschiede in der Oxydation des Tyrosins und der Proteine durch Tyrosinase werden der Tatsache zugeschrieben dass einige Tyrosylgruppen in den Proteinen refraktär sind, da sie keine freie phenolische Hydroxyl-gruppen besitzen.

Andere Unterschiede werden der Tatsache zugeschrieben dass in Proteinen die Tyrosylamino-, sowie die Carboxyl-gruppen gewöhnlich in peptische Verbindungen verbunden sind. Studien der Einwirkung von Tyrosinase auf bestimmte synthetische Derivate des Tyrosins sind mit dieser Interpretation im Einklang. Diese Derivate schliessen auch *p*-Methoxy-Tyrosin ein das gegen Tyrosinase refraktär ist, so wie Tyrosin-ethyl Ester, N-formyl-Tyrosin, N-formyl-tyrosin-ethylester und das Glycyl-*l*-Tyrosin, die mit Leichtigkeit oxydiert wurden. Im Falle von Derivaten bei welchen die Carboxyl- oder Amino- oder beide Gruppen verbunden waren, war die Wirkung der Tyrosinase in den meisten Fällen parallel zu ihrer Wirkung auf Proteine und die Spaltungsprodukte der teilweisen Hydrolyse derselben.

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Received October 13th, 1950