XXXVIII. OXIDATIVE DEAMINATION BY A BASIDIOMYCETE ENZYME.

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THE oxidative deamination of amino acids by tyrosinase was first observed by Chodat and Schweizer [1913], who demonstrated qualitatively that an enzyme prepared from the potato, when in the presence of oxygen and at an alkaline reaction, oxidised certain amino acids with the production of carbon dioxide, ammonia, and an aldehyde containing one carbon atom less than the original amino acid. The above reaction is stated to proceed more readily and completely in the presence of certain phenols, Schweizer [1917] considering that this acceleration was caused by the combination of the phenol with the products of the reaction. Both *p*-cresol and catechol are mentioned in this connection, but in the experiments described by these authors *p*-cresol alone was used.

The fact that this phenol is readily oxidised by tyrosinase with the production of an orange-coloured substance was first observed by Chodat [Chodat and Staub, 1907], who subsequently found that if certain amino acids were added to *p*-cresol and tyrosinase, fluorescent cresol-blue pigments were formed; he did not, however, at first ascribe this phenomenon to the oxidation of the amino acids.

Chodat and Schweizer [1915] state that it is not known whether the oxidation of tyrosine proceeds in the same manner as that detailed for amino acids such as glycine and alanine; Schweizer [1917] describes, however, the detection of small quantities of ammonia with Nessler's reagent after tyrosine had been acted upon by the enzyme in the presence of *p*-cresol.

For the work described in this paper a tyrosinase was used from the basidiomycete *Lactarius vellereus*. A crude enzyme extract was prepared according to the method described by Onslow [1920] for the phanerogams. The plant tissue was rapidly and thoroughly pounded with 97 % alcohol and was repeatedly filtered to dryness on a Buchner funnel; the residue was then extracted with phosphate buffer of p_H 7.8 for about 10 minutes and filtered, the filtrate containing a crude solution of the enzymes present, including an exceedingly active tyrosinase. The extract gave a negative ninhydrin reaction, demonstrating its freedom from amino acids and polypeptides which might have participated in the reactions, described by Chodat and Staub, between

phenols and these substances in the presence of tyrosinase. Small amounts only of the enzyme were made each time; the extracts, however, were found to retain their strength for some days if kept in a refrigerator.

The apparatus used by us to investigate the oxidation of amino acids was a modified form of the Barcroft differential manometer, four instruments being shaken mechanically side by side in a water bath at 16°, this temperature being preferred to a higher one partly because of the great rapidity of the initial oxidations, and partly to facilitate speedy equilibration rather than on account of considerations of the optimum temperature for the tyrosinase reaction. The strengths of the solutions of amino acids and phenols were those used by Chodat and Schweizer [1913], the solvent being in every case phosphate buffer of p_H 7.8.

Fig. 1. Oxidation of glycine, leucine and alanine in the presence of *p*-cresol.
2 mg. *p*-cresol. 4.5 mg. glycine.
Equimolecular solutions of leucine and alanine

The enzyme extract alone was found to be entirely unable to oxidise glycine, alanine and leucine, though tyrosine showed a rapid oxygen uptake, accompanied by melanin formation. In the presence of *p*-cresol, however, the three former amino acids were readily oxidised (see Fig. 1). It will be seen that the

oxygen uptake of glycine is more rapid than that of leucine, which, in its turn is oxidised more readily than is alanine. With the enzyme preparation used, oxidation of glycine was complete in about 40 hours, the amount of oxygen used in excess of that absorbed by *p*-cresol alone being approximately that theoretically required for the equation

The oxidation of leucine or alanine was incomplete after 100 hours, when the shape of the curve for leucine indicates a diminution of the velocity of the reaction, possibly due to the inactivation of the enzyme. The form of these oxidation curves seems to indicate the existence of a coupled reaction, reminiscent of that obtained in the oxidation of unsaturated fats by the sulphhydryl grouping. It is noteworthy that the concentration of *p*-cresol does not appear to be a limiting factor of the velocity of the reaction; further work on the kinetics of the reaction is now in progress.

Fig. 2. Oxidation of glycine in the presence of (a) catechol, (b) resorcinol.

4.5 mg. glycine.

2 mg. catechol.

4.5 mg. glycine.

2 mg. resorcinol.

p-Cresol was then replaced by an equimolecular solution of catechol, and again oxidation of glycine was obtained. The initial oxygen uptake of catechol when catalysed by the *Lactarius* enzyme was extremely rapid, therefore a special form of bottle was used on the Barcroft manometer, a measured volume of the enzyme extract being introduced, from a side burette fitted into the bottle, after equilibrium had been attained; after addition of the enzyme the taps were immediately turned so as to connect the bottles and the manometer. The result of this experiment is shown in Fig. 2. It will be seen that

the oxidation of glycine in the presence of catechol is less rapid than in that of *p*-cresol, when an enzyme extract of approximately similar strength is used, oxidation being incomplete in 100 hours. For these experiments Merck's resublimed catechol was used; it may be noted that one molecule of catechol upon oxidation takes up one molecule of oxygen.

Using the same technique, one of us (M. E. R.) has shown that glycine and leucine are oxidised in the presence of *p*-cresol and a potato oxidase, the method of preparation of the enzyme being the same as that described for *Lactarius*. Glycine is also oxidised in the presence of catechol. The curves obtained are similar to those given for the oxidations in the case of *Lactarius*.

p-Cresol and catechol were then replaced by resorcinol. The reaction in the presence of this phenol is somewhat different, oxidation taking place only after a latent period which sometimes extended to 15 hours, the phenol itself without the amino acid in the presence of the enzyme taking up practically no oxygen during this period. Resorcinol, unlike catechol and *p*-cresol, is not autoxidised if allowed to stand exposed to the air for a considerable period, manifesting at the end of this time no peroxide formation on testing with guaiacum and horse-radish peroxidase.

The enzyme extract was found to be unable to oxidise glycine in the presence of carefully buffered solutions of *p*-hydroxybenzoic acid or aldehyde, nor were these substances themselves oxidised. Similarly the oxidation of protocatechuic acid and aldehyde was extremely slow in comparison with that of catechol, though there was evidence of oxidative deamination of glycine in the presence of the former.

Tyrosine itself is unable to replace *p*-cresol in the oxidation of amino acids. When glycine was added to a saturated solution of tyrosine in phosphate buffer of p_H 7·8, the oxygen uptake was no greater than that of a similar solution of tyrosine in the absence of glycine; on the other hand, no inhibition of the speed of oxygen uptake of tyrosine when in the presence of other amino acids was observed.

The decrease of amino nitrogen at the close of the reaction was investigated by means of the micro form of Van Slyke's apparatus. The solutions were removed from the bottles of the Barcroft manometer by means of a pipette, and the amino nitrogen content was compared with that of a control solution which had been prepared with boiled enzyme and which had been shaken concurrently in another Barcroft apparatus.

Results obtained were as follows:

Substance	O ₂ uptake of amino acid at 16° mm. ³	Amino N of control mg.	Amino N after deamination mg.	Loss of amino N mg.	Theoretical loss calc. from O ₂ uptake mg.
Glycine + <i>p</i> -cresol	640	0·844	0·054	0·790	0·800
Leucine + <i>p</i> -cresol	540	0·875	0·265	0·610	0·628
Glycine + catechol	380	0·842	0·346	0·496	0·509

A decrease of amino nitrogen, and the production of ammonia, was observed also in the case of glycine and resorcinol in the presence of the enzyme.

The results of estimations of the ammonia produced during the course of the reaction did not correspond exactly with the observed loss of amino nitrogen, giving slightly lower values. Estimations of the total nitrogen content, by Kjeldahl's method, before and after oxidation had taken place, showed however that no loss of nitrogen as ammonia from the alkaline fluid had occurred. It is possible that the aberrant nitrogen may have taken part in the production of the coloured complex.

That Chodat and Schweizer observed oxidation of amino acids by tyrosinase alone, though acceleration of the reaction was obtained in the presence of certain phenols, may possibly be explained by the supposition that the enzyme extract used by them, prepared from the potato, may have contained traces of an autoxidised *ortho*-dihydroxy compound [Onslow, 1920]. Schweizer states that he prepared tyrosinase according to the instructions given by Chodat in Abderhalden's *Handb. d. biochem. Arbeitsmethoden*. The preliminary stages of this method include mincing of the potatoes and maceration with water of the crude alcoholic precipitate containing the enzyme, during which processes a certain amount of oxidation is difficult to avoid. Autoxidised substances might possibly be adsorbed on the proteins present together with the enzyme, and be unremoved by subsequent reprecipitations.

The basidiomycete *Lactarius vellereus* does not appear to contain *o*-dihydroxy substances [Robinson, 1924]; the enzyme extracts used in our experiments, moreover, did not undergo spontaneous darkening or oxidation on being shaken in air for a considerable period.

SUMMARY.

A crude enzyme extract, containing tyrosinase, from the basidiomycete *Lactarius vellereus* is unable to oxidise the amino acids, glycine, leucine and alanine unless certain phenols be present. Oxidative deamination of glycine has been brought about in the presence of *p*-cresol, catechol and resorcinol. *p*-Cresol cannot be replaced in this reaction by *p*-hydroxybenzoic acid or aldehyde.

A possible explanation for the divergence of our results from those of Chodat and his co-workers is given in the text.

In conclusion we wish to express our thanks to Sir F. G. Hopkins, F.R.S., and to the Hon. Mrs Onslow for their continual interest during this work and for much valuable advice.

Note [by R. A. McCance]. Although neither *p*-cresol nor resorcinol decolorises methylene blue either alone or in the presence of tyrosinase, the system, glycine (or any amino acid) plus *p*-cresol, resorcinol, etc., plus tyro-

sinase has been found to decolorise methylene blue freely. Of the amino acids tried, glycine is the most active in this connection. Tyrosine can act in this connection in its capacity as an amino acid.

A study of this reaction has been made with a number of substances and the results and conclusions as to the nature of the chemical changes involved will be published shortly.

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