TYROSINASE AND POLYPHENOLOXIDASE. THE ROLE OF METALLIC IONS IN MELANOGENESIS

by

DENIS KERTÉSZ

Research Department of the Istituto Regina Elena per lo Studio e la Cura dei Tumori, Roma (Italy)

The mechanism by which tyrosinase acts on monohydric phenols has been under discussion for a long time[1-5]. ONSLOW AND ROBINSON[6] were the first to propose that the monophenolase action is not due to the enzyme molecule itself but to the *o*-quinones produced by the enzymic oxidation of *o*-dihydric phenols. After the adverse criticism of PUGH[7] the same view was reaffirmed by KEILIN AND MANN[8] and by CALIFANO AND KERTÉSZ[9]. More recently WARBURG[10] states also that probably in the case of monohydric phenol oxidation *o*-diphenols are first formed by a slow reaction, and these then act catalytically by virtue of the change *o*-diphenol $\rightleftharpoons$ *o*-quinone. This is the same catalytic change which, in presence of traces of catechol, enables the enzyme to oxidize hydroquinone, ascorbic acid and reduced coenzymes I and II[10].

However, most authors, following NELSON AND DAWSON[5], believe that the oxidation of the monohydric phenols is a function of the enzyme molecule itself and that the enzyme is primed whilst oxidizing an dihydric phenol and so acquires the capacity to act on monohydric phenols. MALLETTE AND DAWSON[11], to explain the loss of monophenolase activity during the purification process, suppose that different centres of the same protein molecule are responsible for the monohydric phenol and dihydric phenol oxidation. The diminution of the activity on monohydric phenols (in their terminology the cresolase activity of tyrosinase) and the augmentation of the activity on *o*-dihydric phenols (in their terminology the catecholase activity of tyrosinase) would be the result of the greater fragility and loss of part of the molecule responsible for the cresolase activity, or of the unmasking of new active centres responsible for the catecholase activity during the purification process.

Evidence reported in this paper not only fails to support the above views, but shows that the cresolase or monophenolase activity of the enzyme belongs to free, and not protein-bound, metallic ions which hasten the spontaneous reaction[9] between *o*-quinones and monohydric phenols. In agreement between earlier theoretical[12] and experimental[13,14] work the oxidation of the monohydric phenols appears to be represented correctly by the following reactions

(I) *o*-dihydric phenol $\rightarrow$ *o*-quinone $\rightarrow$ ulterior products $\rightarrow$ melanin
(II) *o*-quinone + monohydric phenol + H_2O = 2 *o*-dihydric phenol

of which only the first of (I) is enzymatic.

References p. 179.

METHODS

Enzyme preparation. Different preparations of polyphenoloxidase apo-enzyme, from potato peelings extracted with HCN, were described earlier[15,16]. In this work a stable powder was used which is similar to one described recently[16], but of a more simple preparation. 1300 g of potato peelings are extracted with 700 ml of extraction liquid (0.05 molar KCN neutralized and buffered to pH 7). The extract is centrifuged for a few minutes to eliminate the starch and then brought up to 30% concentration in acetone and centrifuged again. The precipitate is discarded. The supernatant is brought up to 60% concentration in acetone and centrifuged. The precipitate is resuspended in 180 ml of extraction liquid. The trouble suspension obtained is brought up again to 60% concentration in acetone, filtered on a Buchner funnel and the precipitate is washed 5 times with acetone. It is dessicated first in an air current, later in a dessicator under vacuum. It can be pulverized after 24 hours; the powder is stable (at least 12 months at + 2° C) and it is not hygroscopic. During the whole procedure the extraction liquid is maintained at 0°, acetone at —20° C; all operations are made in a cold room at + 2° C (for further details see ref. 16). Immediately before use, the powder is suspended at a ratio of 50 mg/ml in Pyrex-redistilled water and the liquid is sharply centrifuged for 30 minutes to eliminate the abundant insoluble matters. The clear solution obtained is practically inactive without added copper on Dopa; its content in non-dialysable substances is 16 mg/ml and in copper 0.02%. 1 ml of the solution contains, if copper is added, 60 enzyme units (Fig. 1) as defined by LERNER *et al.*[17].

Activity determination. The activity of the enzyme was determined manometrically by measuring the oxygen uptake in the Warburg apparatus at 38° C. Substrates (in amounts shown in the legends of the figures) were added from the side arms after 15 minutes of temperature equilibration, at 0 time. The reaction mixtures still contained 0.25 ml of the enzyme solution, 1.25 ml of 0.1 molar phosphate or phosphate-citrate buffer at pH 6.8, the amount marked on the figures respectively of copper and of other metals and Pyrex-redistilled water to make the total volume up to 2.5 ml. The central well of the flasks contained 0.1 ml of NaOH 15%. All solutions were prepared with Pyrex-redistilled water; reagents were chosen and glass-ware washed metal-free. The importance of these precautions was emphasised by BARRON *et al.*[18], by WARBURG[10], and recently by the author[16].

Copper determination. Copper was determined with sodium diethyldithiocarbamate according to KEILIN AND MANN[8]. Colorimetric readings were made with a Coleman spectrophotometer, at 440 mμ.

RESULTS

Influence of metals on Dopa oxidation. Influence of copper is reported in Fig. 1. It is evident that the enzyme is already fully active in presence of 0.00005 millimols (0.08%) Cu and that presence or absence of excess copper is indifferent. The insignificantly lower activities of the enzyme measured with the highest amounts of copper are artefact and due to the fact that the enzyme is slightly and slowly inactivated in presence of a great excess of copper. This is shown by the two lower curves (A and B) of the Fig. 1, where the Warburg flasks containing 0.0001 millimols and 0.0016 millimols of Cu were reopened, the side arm cavities washed (with the aid of a pipette) 7 times with Pyrex-redistilled water and then filled with the same amount of Dopa solution as previously. The new substrate is added to the old test solution after 7 minutes of temperature equilibration. As the whole procedure did not take more than 15 minutes, the potato enzyme was held 120 minutes at 38° C in

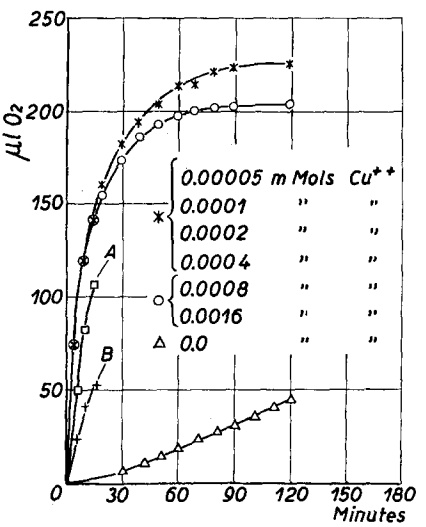

Fig. 1. The effect of varying amounts of copper on the enzymic oxidation of 1 mg Dopa. For curves *A* and *B* see text. All flasks contain the same amount of apo-enzyme corresponding (in presence of copper) to 15 enzyme units.

References p. 179.

contact with Dopa and Cu with almost continuous agitation before the second o time. The enzyme which acted in presence of 0.0001 millimol Cu is (calculating the greater dilution) only 12% inactivated; the enzyme which acted in presence of 0.0016 millimols of Cu is about 55% inactivated during this time.

As it was found by KUBOWITZ[19], copper is strictly specific and cannot be replaced by any other metal. Tests were made on: cobalt (as cobaltous chloride), vanadium (as divanadyl tetrachloride and vanadyl sulphate), nickel (as sulphate), zinc (as chloride), iron (as ferric chloride), chromium (as potassium chromic sulphate), magnesium (as sulphate) and manganese (as manganous sulphate). Cobalt in Vitamin B_{12} is also completely inactive, as is iron in Cytochrome C (experiments not reported).

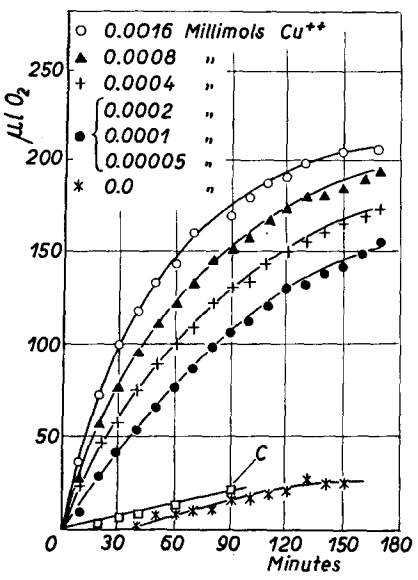

Fig. 2. The effect of varying amounts of copper on the enzymic oxidation of 0.90 mg tyrosine + 0.1 mg Dopa. Curve *C* represents maximum autoxidation, in presence of 0.0016 millimol copper and in absence of apo-enzyme. All other flasks contain the same amount of apo-enzyme as in Fig. 1.

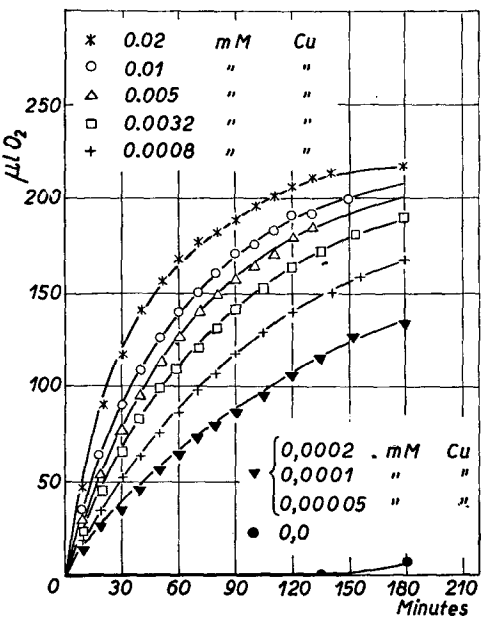

Fig. 3. The effect of varying amounts of copper on the enzymic oxidation of 0.90 mg tyrosine + 0.05 mg Dopa in 0.05 molar citrate-phosphate buffer. All flasks contain the same amount of apo-enzyme as in Fig. 1.

Influence of copper on tyrosine oxidation. Completely different is the action of copper on tyrosine oxidation. The enzyme has the same activity in presence of 0.00005, 0.0001 and 0.0002 millimols of copper, however (Fig. 2), further augmentation of this amount of copper determines further augmentation of the activity on tyrosine. Using citrate-phosphate buffer of MCILWAIN instead of SÖRENSEN's phosphates permits a greater variation of copper concentration and thus an enlargement of the previous findings (Fig. 3), but, as was found by MASON AND WRIGHT[10] for Dopa oxidation at pH 6.6, the enzyme is slightly less active in presence of citrate. The lower activity of the enzyme in this experiment is partly due also to the lower concentration of Dopa.

References p. 179.

Influence of other metals on tyrosine oxidation. The non-specificity of copper is shown in Fig. 4. Here, after having added 0.00005 millimols of copper, equimolecular amounts of cobalt, vanadium, nickel, zinc, iron, chromium, magnesium and manganese (as before) were added. Cobalt, vanadium and nickel accelerate significantly the activity, but copper is about 2.5 times as active as the other three. Presence or absence of zinc, iron, chromium, magnesium and manganese is indifferent. Cobalt in Vitamin B_{12} is inactive or at least is not more active than inorganic cobalt; Cytochrome C is slightly inhibitory (experiments not reported).

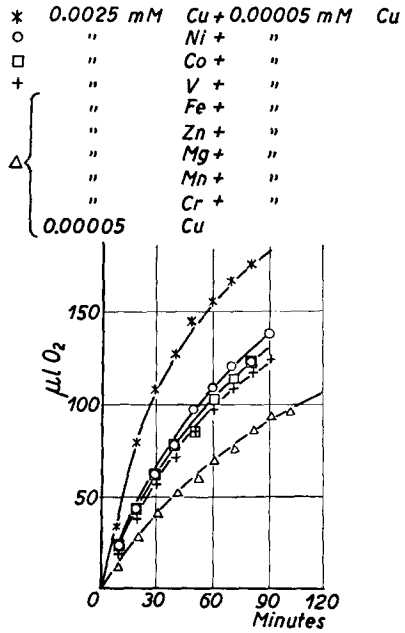

Fig. 4. The effect of equimolecular amounts of copper, nickel, cobalt, vanadium, iron, zinc, magnesium, manganese and chromium on the enzymic oxidation of 0.9 mg tyrosine + 0.05 mg Dopa. All flasks contain the same amount of apoenzyme as in Fig. 1.

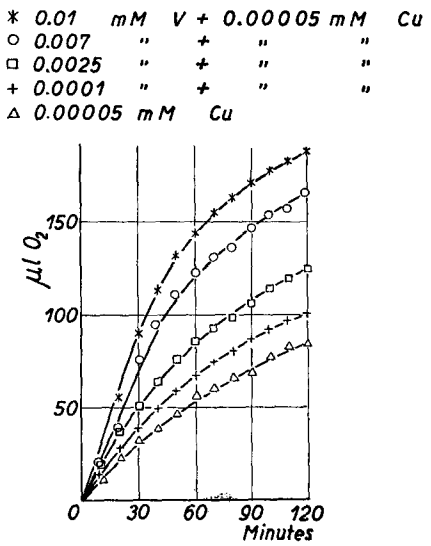

Fig. 5. The effect of varying amounts of vanadium on the enzymic oxidation of 0.9 mg tyrosine + 0.05 mg Dopa. All flasks contain the same amount of apoenzyme as in Fig. 1.

Fig. 5 shows that an augmentation of vanadium concentration determines a corresponding augmentation of activity on tyrosine.

Demonstration that copper accelerating tyrosine oxidation is free and non protein-bound. In the lowest of the curves reported in the Fig. 2 and 3 it was shown that the apo-enzyme has the same activity on tyrosine in presence of 0.00005 millimols, 0.0001 and 0.0002 millimols of copper and that the activity begins to increase only when this amount is surpassed. It is highly improbable that in all the following trials, especially with the highest concentration used, copper should remain always bound to protein. However, it can be easily demonstrated, by two different methods, that even the lowest amount of copper, which gives a measurable increase of the activity on tyrosine, is already in excess and is not protein-bound.

References p. 179.

One of the demonstrations is based on the fact, found by KUBOWITZ[19], that copper bound to polyphenoloxidase protein does not dialyse (if not against HCN). In Fig. 6 it is shown how dialysis against Pyrex-redistilled water influences the activity of the enzyme to which 0.0008 millimols copper were added. Dialysis does not modify the activity on Dopa, where only the protein-bound specific copper is acting, but reduces the activity on tyrosine at the same level as it would have without excess of copper.

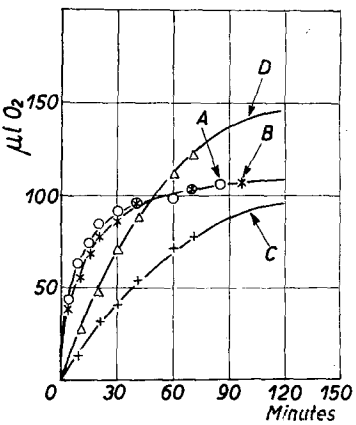

Fig. 6. The effect of dialysis on the enzymic oxidation of 0.5 mg Dopa (Curves *A* and *B*) and on the enzymic oxidation of 0.9 mg tyrosine + 0.05 mg Dopa (Curves *C* and *D*). After having added 0.0008 millimols of copper, *A* and *C* are dialysed (see text), *B* and *D* not. All flasks contain the same amount of apo-enzyme as in Fig. 1.

Another, yet more categoric proof can be presented by virtue of the complex interrelations existing between ascorbic acid, catechol, copper and the apo-enzyme. KUBOWITZ[10] and KEILIN AND MANN[8] have discovered that copper bound to catalytically active positions of the polyphenoloxidase protein becomes strictly specific for *o*-dihydric phenols and it loses its original capacity to oxidize directly ascorbic acid[18]; it can do so only in the presence of a catalytic amount of catechol, by virtue of the change *o*-dihydric phenol $\rightleftharpoons$ *o*-quinone. It was recently shown[16] that copper bound to proteins of the enzyme solution is incapable of the direct catalysis of ascorbic acid oxidation also when it occupies positions which are catalytically inactive, and that copper has a certain preference for the catalytically active positions of the apo-enzyme molecule. If copper is not in excess and it is all protein-bound, in presence of traces of catechol, an augmentation of the amount of apo-enzyme determines a proportional augmentation of the ascorbic acid oxidation, which follows exclusively the indirect mechanism of KUBOWITZ[19]. If, however, copper is in excess over the protein, then indirect and direct oxidation proceeds contemporaneously, and an augmentation of the amount of apo-enzyme not only does not determine an augmentation, but actually reduces the rate of ascorbic acid oxidation, because it reduces the quantity of free copper ions present[16]. In the experiment reported in Fig. 7, in presence of 0.0001 millimols of copper and of 0.0001 millimols of catechol a fourfold augmentation of the amount of apo-enzyme determines a proportional augmentation of the oxidation; on the contrary, in presence of 0.0004 millimols

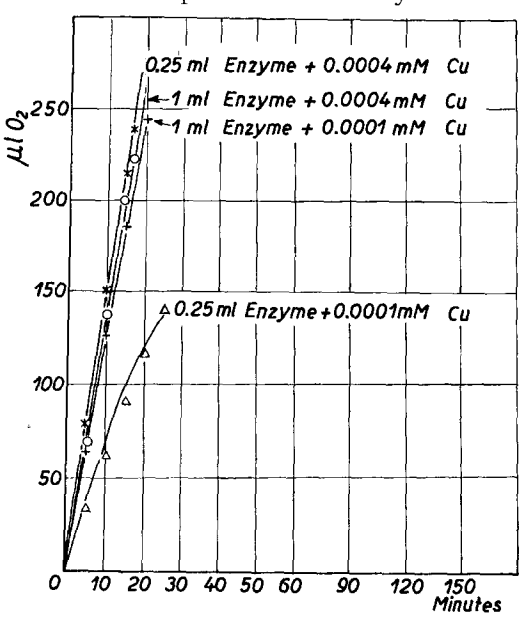

Fig. 7. The effect of varying apo-enzyme concentrations in presence of an excess (0.0004 millimols) and in presence of an amount not in excess (0.0001 millimols) of copper on the enzymic oxidation of 3.51 mg ascorbic acid + 0.01 mg catechol. 1 ml enzyme = 60 enzyme units.

References p. 179.

of copper and 0.0001 millimols of catechol, the originally high rate of ascorbic acid oxidation is in fact reduced by a fourfold augmentation of the apo-enzyme concentration.

DISCUSSION

ONSLOW AND ROBINSON[6] suggested that *o*-quinones react directly with monohydric phenols because they found that by repeated adsorptions and elutions they could reduce the activity of the potato-enzyme on tyrosine but not on catechol, and that the lost activity on tyrosine could be restored by adding traces of catechol. We arrived at the same conclusion[9] because we found that the enzymic oxidation of tyrosine, after the induction period, proceeds at a constant linear rate which is independent of the quantity of the *o*-dihydric phenol added to shorten the induction period, and that in the absence of enzyme *o*-benzoquinone can oxidise tyrosine to an *o*-dihydric phenol. The constant rate of the monohydric phenol oxidation was also found by BEHM AND NELSON[21]; and LERNER *et al.*[17] in the first of a series of valuable papers have not only rediscovered the same particularities of the monohydroxyphenol oxidation, but have also shown that the induction period is proportional to the negative logarithm of the amount of *o*-dihydroxyphenol added, as it was predicted theoretically[22].

In criticising the direct *o*-quinone theory, NELSON AND DAWSON[5] state: "Undoubtedly, the strongest argument against the view that products of catechol oxidation are alone responsible for the initial oxidation of monohydric phenols is the fact that the ratio of catecholase activity to monophenol action of the enzyme can be widely varied during the preparation of tyrosinase". As PUGH[7] rejected ONSLOW AND ROBINSON'S views principally because she could *not* vary the relative activity of the enzyme on catechol and on *p*-cresol, it is disconcerting to see the same argument used as the strongest one in an exactly contrary sense. Whatever is the value of this argument, the fact remains that the relative activity of the enzyme could be changed previously only in the sense of a loss of the monophenolase activity; a change more reasonable to explain on the basis of the *o*-quinone theory[8,10] than admitting a specific monophenolase activity of the enzyme itself, which leads one necessarily to attribute quite new and unprecedented properties to the tyrosinase molecule[11].

However, a real difficulty which indeed can not be explained by the simple *o*-quinone theory is represented by the different behaviour of enzymes of animal and vegetal origin. *Sepia*[9] and melanoma[17] enzymes oxidise Dopa very rapidly and tyrosine, if less rapidly, still at a considerable speed, whilst enzymes of vegetal origin[8,19] oxidise catechol and Dopa very rapidly, but their action on tyrosine is relatively moderate. So animal enzymes appear to function more as a tyrosinase and vegetal enzymes more as a catecholase or polyphenoloxidase; in the terminology of NELSON AND DAWSON[5] the first present a low catecholase/cresolase ratio, the second a high one. This different specificity of animal enzymes makes it necessary to postulate the presence of an additional factor or factors, which can accelerate the action of vegetal enzymes on monohydric phenols, especially on tyrosine.

It is shown in this paper that at least four metals, copper, cobalt, vanadium and nickel can accelerate the action of the potato enzyme on a monohydric phenol and can raise it to a higher level than it was in the original extract. Iron, zinc, magnesium, manganese and chromium were tried and found completely inactive, but an eventual

References p. 179.

activity of some other metal, owing to the little specificity of the reaction, cannot be excluded *a priori*.

The action of the four active metals is completely different in Dopa and in tyrosine oxidation. Copper is highly specific in Dopa, as it is in *o*-dihydric phenol oxidation in general[8,19] and cannot be replaced by any other metal as the prosthetic group of the enzyme; if the enzyme is once saturated by the relatively small amount of copper required, it is fully active and this activity cannot be augmented by an excess of the same or of any other metal. As the active copper is completely protein-bound and all other metal is inactive, dialysis against Pyrex-redistilled water does not have any influence on the activity on Dopa (Fig. 6).

Presence of copper in the small quantity sufficient to saturate the active positions of the enzyme molecule is also indispensable in tyrosine oxidation, but with this amount of copper alone the activity is only moderate. In agreement with the fact discovered by KUBOWITZ[19] that polyphenoloxidase protein can bind more copper than it uses as prosthetic group and that the surplus copper is fixed on catalytically inactive positions ("falsche Stellungen") of the molecule, the activity on tyrosine remains moderate until the inactive places also of the enzyme molecule and of the impurities (which of course have only inactive places) are occupied (Fig. 2 and 3). The activity begins to increase only when this amount of copper is once surpassed and where there are free and non-protein-bound copper ions in solution. As excess copper dialyses easily, activity on tyrosine is reduced to the minimum level by dialysis against Pyrex-redistilled water (Fig. 6); moreover, it catalyses directly ascorbic acid oxidation (Fig. 7), which can not be done by actively- or inactively-bound copper[16]. A definite relationship exists between copper concentration and rate of tyrosine oxidation; if the time required for the oxidation of 50% tyrosine is plotted against the logarithm of the amount of copper added, a linear curve is obtained which presents a sharp break at the value where there are no free ions in solution, that is where all copper is bound. Below this value, as stated before, the time of half oxidation remains constant (Fig. 8).

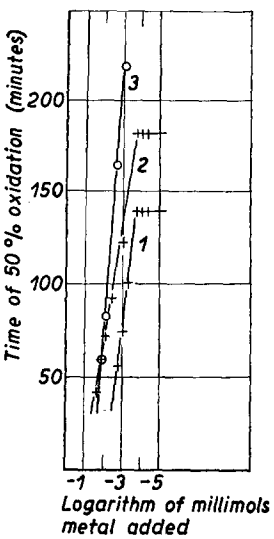

Fig. 8. Relationship between amount of metall added and enzymic tyrosine oxidation. Curve 1: Copper in phosphate buffer (from the experiment reported in Fig. 2). Curve 2: Copper in citrate-phosphate buffer (from the experiment reported in Fig. 3). Curve 3: Vanadium (from the experiment reported in Fig. 5).

The specificity of excess copper in tyrosine oxidation is limited to the fact that it is more active than cobalt, vanadium or nickel (Fig. 4). That the same quantitative relationship holds also for the other active metals as for copper is shown by curve 3 of Fig. 8, where half oxidation time is plotted against the logarithm of vanadium concentration.

The point and mechanism of intervention of metals results clearly from a comparison of their action on Dopa and on tyrosine. This comparison cannot be made on catechol and *p*-cresol; the difficulties of using catechol as substrate are well known[5,19,16] and *p*-cresol, besides being highly unphysiological, is too autoxidable to serve as a useful substrate to distinguish between monohydric phenol and *o*-dihydric phenol oxidation. Although the ulterior products of Dopa and of tyrosine oxidation (exactly as those of catechol and of *p*-cresol oxidation[23,24,25]) are unknown, it was shown by RAPER's fun-

References p. 179.

damental researches that the first product of the tyrosine oxidation is Dopa and the second is its *o*-quinone[1, 26, 27] and there can be no doubt that in the two over-all reactions:

(I) Tyrosine → Dopa → *o*-quinone → ulterior products → melanin
(II) Dopa → *o*-quinone → ulterior products → melanin

all the ulterior products, the nature and importance of which is yet under discussion[15, 28], are identical. As the over-all tyrosine oxidation (I) is much slower than over-all Dopa oxidation (II), the reaction tyrosine → Dopa is rate-determining in tyrosine oxidation and, the two reactions being in all other respects identical, all agents which accelerate tyrosine but not Dopa oxidation necessarily intervene in the reaction tyrosine → Dopa.

The so-called tyrosinase appears as a very complicated system, composed of: 1. an enzyme with copper as prosthetic group, specific for *o*-dihydric phenols; 2. an *o*-dihydric phenol; 3. certain free metallic ions. The complexity of this system is increased by the interdependence of its components inasmuch as the quantity of *o*-dihydric phenol (or respectively of *o*-quinone) present depends on the amount of the enzyme[12] and on both the quality and quantity of the metallic ions present. Yet more complications arise from the fact that only free metallic ions increase the originally low rate of tyrosine oxidation and in consequence all constituents which can bind metal ions, especially proteins inert on *o*-dihydric phenol oxidation, will influence the activity on monohydric phenols, as will the metallic ions always present in common laboratory distilled water. In this context it is useful to point out the decisive importance of the use of Pyrex-redistilled water: if the enzyme were prepared in common distilled water and dialysed afterwards against the same, its activity on tyrosine would not change and it would appear to belong to the protein molecule. The error introduced by the use of common distilled water is greater in the case of pure or of dilute enzyme solutions[16]; moreover it is constant in a given Laboratory and so escapes detection.

Although names are not of very great importance, the name "tyrosinase" is probably the most inappropriate among the numerous designations used for the enzyme studied in this paper. Admitting the direct action of this enzyme on monohydric phenols and calling it tyrosinase, we already possessed a most particular enzyme, which

1. if dilute, could act on other substances but not on its own substrate[29, 9];
2. had a much greater action on a number of other substances than on tyrosine[5, 8, 10];
3. could oxidise other substances immediately, but tyrosine only after an induction period[1–5];
4. could act at distance, without immediate contact with its substrate[13, 14];
5. ought to possess quite unique properties for an enzyme molecule[11].

The results reported in this paper are in agreement with the views that the oxidation of tyrosine is a non-enzymatic reaction between *o*-quinone and tyrosine and render the name tyrosinase still more inappropriate: this enzyme corresponds to the system polyphenoloxidase + *o*-dihydric phenol (or *o*-quinone) + metallic ions, which latter function probably as electron transmitters and hasten the non-enzymic reaction between tyrosine and *o*-quinone.

The author cannot see any justification for the conservation of the term "tyrosinase" except to keep the name under which the discovery and the fundamental researches of B. BERTRAND and of H. S. RAPER were made.

References p. 179.

SUMMARY

A study was made to explain the different behaviour of animal and of vegetal polyphenol-oxidases on monohydric- and dihydric-phenols.

1. It was found that the first phase of the tyrosine oxidation (the transformation of tyrosine in Dopa) is considerably accelerated by excess of copper, by cobalt, by vanadium and by nickel, whilst iron, zinc, chromium, magnesium and manganese are inactive.

2. Excess of copper, cobalt, vanadium and nickel have no action on the second, enzymic phase of tyrosine oxidation (the transformation of Dopa in its *o*-quinone) where only copper bound to polyphenoloxidase protein is active.

3. It was demonstrated, by two independent methods, that the acceleration of tyrosine oxidation is determined by free and non-protein-bound metal ions.

4. and that there is a quantitative relationship between metal concentration and velocity of tyrosine oxidation.

5. Tyrosinase is identified with a complex system composed of an enzyme specific for *o*-dihydric phenols, of an *o*-dihydric phenol (or *o*-quinone) and of free metallic ions. The role of the latter is to accelerate the spontaneous reaction between *o*-quinone and tyrosine.

6. The appropriateness of the name "tyrosinase" is discussed.

RÉSUMÉ

La présente étude a été entreprise afin d'essayer d'expliquer le comportement différent des polyphénoloxydases animales et végétales vis-à-vis des phénols mono- et divalents.

1. Nous avons trouvé que la première phase de l'oxydation de la tyrosine (transformation de la tyrosine en dopa) est accélérée considérablement par un excès de cuivre, par le cobalt, le vanadium et le nickel, tandis que le fer, le zinc, le chrome, le magnésium et le manganèse sont inactifs.

2. Un excès de cuivre, de cobalt, de vanadium ou de nickel n'exerce aucune action sur la seconde phase enzymatique de l'oxydation de la tyrosine (transformation de dopa en son *o*-quinone) où seul le cuivre lié à une protéine polyphenoloxydasique est actif.

3. Nous avons démontré par deux méthodes indépendantes que l'accélération de l'oxydation de la tyrosine est déterminée par des ions metalliques libres et non liés à une protéine et

4. qu'il existe une relation quantitative entre la concentration du métal et la vitesse de l'oxydation de la tyrosine.

5. La tyrosinase a été identifiée comme étant un système complexe composé d'un enzyme spécifique pour les phénols contenant 2-OH en position *o*-, d'un tel phénol divalent (ou d'une *o*-quinone) et d'ions métalliques libres. Le rôle de ces derniers consiste à accélérer la réaction spontanée entre l'*o*-quinone et la tyrosine.

6. La justesse du nom "tyrosinase" est discutée.

ZUSAMMENFASSUNG

Es wurde ein Versuch gemacht, um das verschiedene Verhalten von tierischen und pflanzlichen Polyphenol-Oxydasen gegen einwertige und zweiwertige Phenole zu erklären.

1. Es wurde festgestellt, dass die erste Phase der Oxydation von Tyrosin (die Verwandlung von Tyrosin in Dopa) durch einen Überschuss von Kupfer, durch Kobalt, durch Vanadium und durch Nickel sehr beschleunigt wird, während Eisen, Zink, Chrom, Magnesium und Mangan inaktiv sind.

2. Ein Überschuss von Kupfer, Kobalt, Vanadium und Nickel übt keine Wirkung auf die zweite, enzymatische Phase der Tyrosinoxydation (Verwandlung von Dopa in sein *o*-Chinon) aus; hier ist nur das an Polyphenol-Oxydase-Protein gebundenes Kupfer wirksam.

3. Es wurde mit Hilfe zweier unabhängiger Methoden bewiesen, dass die Beschleunigung der Tyrosin-Oxydation durch freie und nicht proteingebundene Metallionen bewirkt wird, und

4. dass zwischen der Metallkonzentration und der Geschwindigkeit der Tyrosin-Oxydation ein quantitatives Verhältnis besteht.

5. Tyrosinase wurde als ein komplexes System, bestehend aus einem für Phenole mit 2 OH-Gruppen in *o*-Stellung spezifischen Enzym, aus einem solchen zweiwertigen Phenol (oder *o*-Chinon) und aus freien Metallionen, identifiziert. Die Rolle der letzteren besteht darin, dass sie die spontane Reaktion zwischen *o*-Chinon und Tyrosin beschleunigen.

6. Die Angemessenheit des Namens "Tyrosinase" wird diskutiert.

References p. 179.

REFERENCES

1 H. S. RAPER, *Ergeb. Enzymforsch.*, 1 (1932) 270.
2 H. S. RAPER, in BAMANN-MYRBÄCK: *Methoden der Fermentforschung*, Leipzig (1941) 2476.
3 C. OPPENHEIMER, *Die Fermente u. i. Wirkungen*, Supplement, The Hague (1939) 1608.
4 W. FRANKE, in NORD-WEIDENHAGEN: *Handbuch der Enzymologie*, Leipzig (1940) 731.
5 J. M. NELSON AND C. R. DAWSON, *Advances in Enzymol.*, 4 (1944) 99.
6 M. W. ONSLOW AND M. E. ROBINSON, *Biochem. J.*, 22 (1928) 1327.
7 C. E. M. PUGH, *Biochem. J.*, 24 (1930) 1442.
8 D. KEILIN AND T. MANN, *Proc. Roy. Soc.*, B, 125 (1938) 187.
9 L. CALIFANO AND D. KERTESZ, *Nature*, 142 (1938) 1036; *Enzymol.*, 6 (1939) 233.
10 O. WARBURG, *Heavy Metal Prosthetic Groups*, Oxford (1949) 173.
11 M. F. MALLETTE AND C. R. DAWSON, *Arch. Biochem.*, 23 (1949) 29.
12 D. KERTÉSZ, *Enzymol.*, 12 (1948) 254; 13 (1949) 182.
13 D. KERTÉSZ, *Compt. rend. soc. biol.*, 143 (1949) 1469; *Nature*, 165 (1950) 523.
14 D. KERTÉSZ AND P. CASELLI, *Bull. soc. chim. biol.*, 32 (1950) 583.
15 D. KERTÉSZ, *Bull. soc. chim. biol.*, 32 (1950) 587.
16 D. KERTÉSZ, *Bull. soc. chim. biol.*, 33 (1951) 1400.
17 A. B. LERNER, T. B. FITZPATRICK, E. CALKINS, AND W. H. SUMMERSON, *J. Biol. Chem.*, 178 (1948) 185.
18 E. S. G. BARRON, R. H. DE MEIO, AND F. KLEMPERER, *J. Biol. Chem.*, 116 (1936) 626.
19 F. KUBOWITZ, *Biochem. Z.*, 298 (1938) 32.
20 H. S. MASON AND C. J. WRIGHT, *J. Biol. Chem.*, 180 (1949) 235.
21 R. C. BEHM AND J. M. NELSON, quoted as in press, ref. 5, page 138.
22 *loc. cit.* 12, page 260.
23 H. JACKSON, *Biochem. J.*, 33 (1939) 1452.
24 H. S. MASON, L. SCHWARTZ, AND D. C. PETERSON, *J. Am. Chem. Soc.*, 67 (1945) 1233.
25 E. A. H. ROBERTS AND D. J. WOOD, *Nature*, 165 (1950) 32.
26 H. S. RAPER, *Biochem. J.*, 20 (1926) 735.
27 C. E. M. PUGH AND H. S. RAPER, *Biochem. J.*, 21 (1927) 1370.
28 D. KERTÉSZ, *Nature*, 168 (1951) 697.
29 C. E. M. PUGH, *Biochem. J.*, 27 (1933) 478.

Received November 12th, 1951