THE pH STABILITY OF PROTYROSINASE AND TYROSINASE*

BY THOMAS HUNTER ALLEN, ARTHUR B. OTIS, AND JOSEPH HALL BODINE

(From the Zoological Laboratory, The State University of Iowa, Iowa City)

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Protyrosinase is changed into tyrosinase by a variety of reagents and treatments (1), and as such its activity may then be measured. Since variation of the pH is generally known to affect the properties of soluble proteins, it seemed of interest to determine this effect upon protyrosinase.

Methods

Grasshopper egg protyrosinase was extracted according to a described procedure (2). A number of 1.0 ml. portions of this extract were mixed in test tubes with 5.0 ml. portions of sodium hydroxide or hydrochloric acid dissolved in 0.9 per cent sodium chloride. The concentrations of the acid solutions ranged from 1.1 to $4.6 \times 10^{-4}$M, whereas those of the base solutions ranged from 2.7 to $43.6 \times 10^{-4}$M. As controls, other 1.0 ml. portions of protyrosinase were mixed with 5.0 ml. portions of 0.9 per cent sodium chloride. The pH values of these different mixtures were recorded with a glass electrode (Leeds and Northrup, No. 7661 Universal pH potentiometer).

In order to determine total as well as free hydrogen and hydroxyl ion, 5.0 ml. portions of the various acid and base solutions were mixed with 1.0 ml. portions of the final solution of 0.9 per cent sodium chloride against which the protyrosinase had been dialyzed. This was necessary, because the dialysate solution in itself was found to have a slight binding capacity.

All the tubes containing treated protyrosinase were stored at 25°C. Those tubes to which base had been added were stored in a closed chamber in the presence of 10 per cent potassium hydroxide. At intervals, 2.5 ml. of 0.2 M phosphate buffer of pH 6.7 were added to each of these tubes, whereupon the pH changed to 6.7 ± 0.1.

In some experiments the protyrosinase extract was heat treated in order to obtain a tyrosinase (1). To do this a tube containing a sample of protyrosinase was heated for 5 minutes in water kept at 70°C. 1.0 ml. portions of this tyrosinase were treated according to the procedure described above for protyrosinase.

The contents of the tubes were next analyzed for protyrosinase, tyrosinase, and inactive decomposition products. These analyses were performed with a Warburg apparatus by measuring the velocity of oxidation of tyramine hydrochloride. One 1.7 ml. portion from a tube was mixed in a manometer flask with 1.0 ml. of 0.9 per cent sodium chloride. Another similar portion was mixed in another flask with 0.7 ml. of the sodium chloride solution and 0.3 ml. of $5.1 \times 10^{-4}$M sodium dodecyl sulfate. (This amount of sodium dodecyl sulfate was enough (2) to change all protyrosinase

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into tyrosinase.) 0.3 ml. of 0.4 per cent tyramine hydrochloride was placed in the side bulb of each flask. After equilibration in a water bath at 24.9°C. the contents of the flasks were mixed. The reciprocal of the time for the uptake of the initial 100 μl. of oxygen was found. This specific reaction velocity for that analysis of the control in the presence of sodium dodecyl sulfate was given the value of unity. Differently treated portions of the same extract were compared on the basis of the relation which their specific reaction velocities bore to that of the control.

RESULTS

The set of curves in the upper part of Fig. 1 describes the attainment of equilibrium among protyrosinase, tyrosinase, and inactive products at various pH values from 4.40 to 10.60. The upper curve in each member of this representative set pertains to the sum of the relative amounts of protyrosinase and tyrosinase which exist during exposure to any one pH. Each of the lower curves is determined by the relative amount of tyrosinase which is produced by the pH treatment. When either both curves or the upper curve decline toward the abscissa, as at pH 10.60 or 4.40, it seems that inactive products are being formed in the one case from tyrosinase and protyrosinase and in the other from protyrosinase alone. After some 3,000 minutes, equilibrium values seem to be attained. Curves A and D (Fig. 1), constructed from a total of 24 such equilibrium values, may be called pH stability diagrams of protyrosinase. The left hand limb of curve A portrays the shift of protyrosinase into inactive products, whereas the right hand limb from pH 9.30 to pH 10.34 records the inactivation of both protyrosinase and tyrosinase. There seems to be a mixture of tyrosinase and protyrosinase at the latter hydrogen ion concentrations, since the ascending and then descending parts of curve D record the shift of protyrosinase into tyrosinase and thence into inactive products. The activation of protyrosinase by treatment with high hydroxyl ion concentrations seems to be irreversible. A sample of protyrosinase which had been exposed to pH 10.08 for 1460 minutes showed no reversion of tyrosinase to protyrosinase for so long as some 3,000 minutes at 25°C. after lowering the pH to 6.72 by adding phosphate buffer.

Since a tyrosinase results from exposure of protyrosinase to high hydroxyl ion concentrations, it seems necessary to see whether this "new" tyrosinase resembles that produced by other methods. Its thermolability and sensitivity to cyanide and diethyldithiocarbamate were therefore tested. This tyrosinase was destroyed by heating at 90°C. for 5 minutes. It was completely poisoned by $1 \times 10^{-4}$ cyanide or diethyldithiocarbamate. These characteristics proved to be identical with those of tyrosinase produced by the action of sodium dodecyl sulfate.

The pH stability diagram of heat produced tyrosinase is indicated by curves B and C which were constructed according to the procedure described in the legend of Fig. 1. Because of some heat inactivation, curve B, throughout most
of its course, is at a lower level than curve A. Although the heat treatment produced 0.6 tyrosinase (curve C), it also resulted in the destruction of 0.2 of

the mixture of protyrosinase and tyrosinase (curve B). The right hand limbs of curves B and C apparently coincide with those of curves A and D. This coincidence is probably due to the fact that in these cases only tyrosinase was

![Diagram of Fig. 1: The effect of exposing protyrosinase to various hydrogen ion concentrations at 25°C. The ordinates are the ratio of the specific reaction velocity to that of the control tube. The abscissae for the upper set of curves are given in thousands of minutes. The abscissa for the lower curves is given in pH units. Each pair of the upper curves was constructed from data of the analyses performed on protyrosinase which had been exposed to the indicated pH. The upper member of each pair shows the activity obtained in the presence of sodium dodecyl sulfate, whereas the lower member shows the activity in the absence of dodecyl sulfate. Equilibrium values from the upper members were used in constructing curve A. Similar values from the lower members were used for curve D. The foregoing procedure was also used in constructing curves B and C for heat-produced tyrosinase.](https://jgp.rupress.org/content/153/2/396/F1)
destroyed. Protosynase (curve D) at high hydroxyl ion concentrations apparently changes into tyrosinase which in turn changes into inactive products. Since the left hand limbs of curves B and C do not coincide with that of curve A, it seems necessary to conclude that tyrosinase is more stable than protosynase through the lower ranges of pH.

DISCUSSION

In a former study (3) it was found that certain polar-nonpolar anions would activate protosynase, whereas similar cations had no such influence. This difference between effects of oppositely charged ions also seems to apply for the hydrogen and hydroxyl ions. Activation does not seem to occur through ranges of increasing $C_H$, but it does occur beyond a $C_{OH}$ of $2.0 \times 10^{-5}$M. It, therefore, seems that a positive charge is borne by the part of protosynase which is primarily affected in the process of activation. Although activation may not necessarily proceed in the same manner, it should be pointed out that the concentration of either the most efficient alkyl sulfate or the hydroxyl ion required for half activation is of the same order of magnitude. This total concentration for the tetradecyl sulfate ion (2) is $5.0 \times 10^{-5}$M of which the relative amounts bound and free are unknown. For the hydroxide the total concentration is $6.4 \times 10^{-5}$M and the OH concentration is $3.84 \times 10^{-6}$. Other parts of protosynase than those involved with activation seem to bind hydroxyl ions. Thus at pH 9.04, where no activation occurred, the total concentration of hydroxyl ions was $2.3 \times 10^{-5}$ of which 0.5 was bound and 0.5 was free.

So far in this discussion the similarity in the effects produced by alkyl sulfates and hydroxyl ions has been described. It should be noted, however, that the hydroxyl ion also causes an inactivation of tyrosinase. Such a destruction has not been found for even the greatest concentration of alkyl sulfate which its solubility in 0.9 per cent sodium chloride permits one to use (2). Perhaps this effect can be explained by assuming that hydroxyl ions first split protosynase into tyrosinase which next is changed from a native into a denatured product. Under these conditions the latter effect does not seem to be produced by the alkyl sulfates.

Protosynase is a complex entity which consists at the least of protein and a potentially active prosthetic group containing copper (4). Therefore, it is interesting to compare the pH stability of protosynase with that of other copper proteins, notably the hemocyanins. Ultracentrifugal analyses show that a greater number of lower molecular weight components appear as a solution of hemocyanin is brought to critical extremes of pH (5). Such a dissociation is reversible, whereas the activation of protosynase seems to be irreversible. If the activation does involve the reversible formation of smaller molecules, the tyrosinase thus produced may be able to reassociate into larger
particles without necessarily reverting to protyrosinase. In this connection, it seems interesting to note that when hemocyanin is split into smaller particles by exposure to pH 8.5 and is then returned to pH 6.8, these small particles combine to give particles having sedimentation constants identical with those of the original hemocyanin but possessing different electrophoretic properties (6).

**SUMMARY**

1. pH stability diagrams for protyrosinase and for tyrosinase were constructed.

2. Above pH 7.30 protyrosinase is unstable. Between pH 7.30 and pH 9.30 there is a partial destruction. Beyond pH 9.30 it changes irreversibly into tyrosinase which in turn is destroyed beyond pH 10.12.

3. Through the lower ranges of pH protyrosinase is less stable than tyrosinase. The former is destroyed below pH 4.80, while the latter is unaffected until the pH drops below 4.10.

4. The tyrosinase produced at high pH values resembles that produced by other methods.

**BIBLIOGRAPHY**