LXXXII. THE NATURE OF TYROSINASE.

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The view that tyrosinase is a mixture of a number of components has been held by several investigators. Haehn [1920] reported that potato tyrosinase lost its activity on dialysis or ultrafiltration, but regained it on addition of the ultrafiltrate or boiled juice. He concluded that the activator was inorganic in nature. Raper and Wormall [1923], while partially confirming Haehn's finding, noticed that the boiled juice of new but not of old potatoes had an accelerating effect. They also adduced evidence to show that the activator in potato juice is organic in nature.

In an earlier paper [Narayanamurti and Ramaswami, 1929] it was shown that on ultrafiltration of *Dolichos* tyrosinase the residual liquid on the ultrafilter was active and that the addition of the ultrafiltrates to the residual liquid did not cause any acceleration. The following additional results have so far been obtained.

Experimental.

In this investigation the enzyme prepared by three different methods, *viz.* (*a*) ordinary dialysis, (*b*) ordinary dialysis followed by ultrafiltration, and (*c*) ordinary dialysis followed by precipitation with alcohol, has been studied.

Preparation of dialysed extracts. About 200 g. of the finely ground meal of the ungerminated seed of *Dolichos lab lab* were extracted with 600 cc. of distilled water in glass-stoppered bottles at room temperature for 3 days in presence of toluene. After filtering through paper pulp the extract (containing a large quantity of protein) was dialysed in large parchment filters against flowing distilled water for 1 week, when all the protein was precipitated. The solution was then centrifuged to remove suspended matter, the resulting liquid being clear.

Purification by repeated precipitation with alcohol. Dialysed extracts were precipitated with 95 % alcohol, the flocculent precipitate being allowed to settle in an ice-chest and separated by decantation and filtration. The residue on the filter was ground up with distilled water and filtered, alcohol again being added to the filtrate. The whole of the first precipitate does not dissolve and the undissolved residue possesses some activity. The precipitated enzyme is very small in quantity and is dried *in vacuo*.

Ultrafiltration. The ultrafiltration apparatus used was an improved form of that described by Brukner [1926]. About 200 cc. of the dialysed extract were filtered through parchment paper at a pressure of 50 kg. The solid residue was removed, ground up with distilled water and filtered through ordinary filter-paper. This filtrate was again subjected to ultrafiltration, the whole process being repeated a third time, the ultrafiltrates being collected each time. The residue finally obtained was dried in a vacuum desiccator.

Effect of addition of ultrafiltrate on the activity of the enzyme. The effect of addition of the ultrafiltrate on the activity of the enzyme was investigated at 30° and the results are given in Table I, the activity being expressed in cc. thiosulphate.

Table I.

80 cc. of 0·06 % tyrosine solution and 20 cc. of acetate buffer at p_H 6·5 and 10 cc. of toluene were taken in each case.

No.	Enzyme solution	Water or ultrafiltrate	Time (hrs.) 2	4	5
I	20 cc. dialysed extract	10 cc. water	4·5	5·2	6·3
II	20 cc. of a solution of ultrafiltered enzyme	,,	2·0	3·4	4·0
III	,,	10 cc. ultrafiltrate 1	1·6	1·9	3·3
IV	,,	10 cc. ultrafiltrate 2	1·4	2·9	3·5
V	,,	10 cc. ultrafiltrate 3	1·3	3·1	3·7
VI	,,	10 cc. boiled juice	1·8	3·4	4·1

It is clear that the enzyme has not lost its activity on ultrafiltration and that the addition of boiled juice or the ultrafiltrate does not cause any acceleration. On the other hand, slight inhibition is caused, this being greatest with ultrafiltrate 1 and least with 3. The solid content of enzyme solution I (dialysed) was 4 times that of the ultrafiltered enzyme solution but its activity was only 1·5 times as great. Results of experiments done at the same solid content are given in Table II.

Table II.

Time in hours	Activity in cc. thiosulphate: Ultrafiltered enzyme	Dialysed extract
1	1·0	0·6
2	1·6	—
4·5	3·3	1·5
7·0	4·5	2·0

The results clearly indicate that the ultrafiltered enzyme is more than twice as active as the dialysed preparation. Conductivity measurements showed that the conductivity of the ultrafiltered enzyme was one-twelfth that of the dialysed preparation. It is therefore evident that ultrafiltration is a good method of purification and that the enzyme is not separated into two components as claimed by Haehn.

Comparison of precipitated enzyme with the dialysed extract. The results are given in Table III.

Table III.

	Activity in cc. thiosulphate	
Time in hours	Precipitated enzyme	Dialysed extract
0·5	4·0	5·2
1·0	8·1	6·4
2·0	10·6	9·5

It is evident that the precipitated enzyme is slightly more active than the dialysed extract.

Electro-osmosis. It has been shown previously [Narayanamurti and Norris, 1928] that cholam malt diastase could be separated into two components by electro-osmosis. Similar experiments were tried with tyrosinase, but it was found that the addition of anode and cathode cell liquids to the middle cell liquid caused only a slight diminution of activity, thus again showing that tyrosinase cannot be separated into two components.

Summary.

Dolichos tyrosinase purified by ultrafiltration is more active than the dialysed preparation. Addition of ultrafiltrate causes no acceleration; on the other hand, slight inhibition is observed.

On repeated precipitation tyrosinase does not lose its activity.

Addition of cathode cell or anode cell liquid to the middle cell liquid of tyrosinase subjected to electro-osmosis in a five-celled apparatus does not cause any increase in activity. Slight inhibition is caused.

All these results clearly indicate that tyrosinase cannot be separated into two components. Any activator present must be in the colloidal condition or bound to a colloidal carrier.

References.

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