CLXXXIV. THE PURIFICATION OF URICASE

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In the course of an attempt to isolate the enzyme uricase, which oxidizes uric acid to allantoin, it has been found possible to obtain a preparation 550 times purer than the liver powder which is used as starting material [Davidson, 1938]. The purest preparation contains 0·15–0·20 % Fe, a trace of Cu, no Co or Mn and 14·4 % N. Small quantities are colourless, and even very large quantities have only a very pale brown colour. It is therefore not a haemin derivative. It is quite insoluble in water, almost insoluble in phosphate buffer *p*H 7·4 but soluble in alkaline solution, e.g. borate buffer *p*H 10. Truszkowski [1934] and Ro [1931] have already shown that uricase is soluble in alkaline solution.

In order to determine the enzymic activity a manometric test has been developed. The main chamber of the manometer vessel contains 1·0 ml. *M*/5 borate buffer *p*H 9 and 1·5 ml. enzyme preparation plus water. The potash tube contains 0·2 ml. 10 % KOH, and the side bulb 2·24 mg. uric acid as *M*/30 lithium urate. (0·56 g. uric acid is dissolved in 35 ml. boiling *N*/10 lithium hydroxide and the solution is made up to 100 ml. with water. This solution must be freshly prepared each day.) The gas space contains pure oxygen and the oxygen uptake is measured at 38° over a period of 30 min.

By the specific activity of the enzyme is meant the number of μl. O_2 which are taken up per min. in the first 30 min. by 1 mg. of dry enzyme preparation. The specific activity of the best preparation is 85–90 μl. per mg. per min. as compared with a value of 0·12–0·15 μl. per mg. per min. for the dry liver powder from which it is obtained.

The most convenient starting material has been found to be pig's liver, which has already been used by Kleinmann & Bork [1933] and by Keilin & Hartree [1936] for uricase studies. The pig's liver is finely minced and ground in a mortar with five parts of acetone. The solid material is centrifuged off and shaken with three parts of acetone. After centrifuging, the liver powder is dried at first rapidly in a current of air and then overnight in vacuum desiccators, and is then finely powdered and passed through a sieve. The resulting light brown powder has a specific activity of 0·12–0·15 μl. per mg. per min. and retains its activity for several months when stored in desiccators at room temperature. Samples showing a specific activity of less than 0·10 μl. per mg. per min. are discarded.

250 g. liver powder are stirred with 2·5 l. ice-cold *M*/10 phosphate buffer *p*H 7·4. After standing for 20 min. at 0° the mixture is centrifuged, and the extract, which contains much protein but only little uricase is discarded. The solid residue is then stirred with 5 l. *M*/10 borate buffer *p*H 10 at 38°. After 20 min. the mixture is rapidly cooled to 0° and centrifuged. The clear extract contains the bulk of the enzyme and has a specific activity of 0·5 μl. per mg. per min.

An equal volume of saturated ice-cold ammonium sulphate solution is added to this solution, and the resulting precipitate, which contains the enzyme, is centrifuged off at 0° and dissolved with shaking in 5 l. ice-cold distilled water.

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1/10 vol. saturated ice-cold ammonium sulphate is then added (no precipitate being formed), and the solution, which has a *p*H of 7·2–7·4, is heated to 55° for 5 min. with vigorous stirring, whereby a heavy flocculent precipitate of denatured protein is formed. The solution is rapidly cooled to 0° and the precipitate is centrifuged off and discarded. The enzyme solution, which now has a specific activity of 1·0–1·5 μl. per mg. per min., is treated with enough saturated ammonium sulphate solution at 0° to bring the degree of saturation to 0·5. The resulting precipitate, which contains the enzyme, is centrifuged off at 0° and dissolved in 1 l. of ice-cold distilled water. This solution is dialysed for 15 hr. in cellophane sacs against running distilled water at 0°. A brown precipitate which appears during the dialysis and which contains the enzyme is centrifuged off and washed with ice-cold water on the centrifuge. It is then rubbed in a cooled mortar with 400 ml. *M*/10 phosphate buffer *p*H 7·4 at 0° and centrifuged on the high speed centrifuge (15,000 r.p.m.). The extract, which contains much protein but little enzyme, is discarded. The residue is rubbed in a mortar with 250 ml. *M*/10 borate buffer *p*H 10 at room temperature and is centrifuged on the high speed centrifuge. The extraction with 250 ml. borate buffer is repeated, the extracts, which contain the bulk of the enzyme, are combined, and the residue, which consists of brown insoluble protein, is discarded.

The clear, pale yellow uricase solution which now has a specific activity of 10–25 μl. per mg. per min. is cooled to 0°, treated with an equal volume of saturated ice-cold ammonium sulphate solution, and the resulting precipitate, which contains the enzyme, is centrifuged off at 0° and dissolved in 60 ml. ice-cold water.

To the solution just enough (0·5–1·0 vol.) ice-cold 80 % acetone is added so that a flocculent precipitate appears after 20 min. The precipitate is centrifuged off and discarded. Acetone is removed from the supernatant fluid by vacuum distillation at 20° with two drops octyl alcohol and the resulting slightly turbid solution, after standing overnight at 0°, is centrifuged on the high speed centrifuge. A small precipitate of very high activity is thus obtained, and is washed three times on the centrifuge with ice-cold distilled water. It is then rubbed up with 5 ml. *M*/10 borate buffer *p*H 10 at room temperature, in which the enzyme dissolves, leaving a small inactive brownish residue which is centrifuged off at high speed and discarded.

The uricase solution is almost colourless. When it is dialysed against running distilled water at 0° the free enzyme is obtained. Specific activity 85–90 μl. per mg. per min. The yield of the purest preparation is about 5 mg. per 100 g. liver powder.

On standing the activity of the free enzyme gradually diminishes but in solution at *p*H 10 it is stable for several weeks. Some of the enzyme frequently precipitates out in an insoluble form. The enzyme cannot be dried without great loss of activity.

The velocity of the test reaction is dependent on the oxygen pressure and in a mixture of 2 % oxygen and 98 % argon it is only 7 % as great as 100 % oxygen. If the argon is replaced by carbon monoxide no inhibition by the carbon monoxide is found.

Very small quantities of cyanide, however, inhibit the reaction reversibly, as has been already pointed out by Keilin & Hartree [1936] and by Truszkowski [1930]. The enzyme therefore would appear to be a heavy metal compound, but it has nevertheless not yet been proved that the catalytic activity of the enzyme is due to the iron. Attempts to remove iron by dialysis with cyanide after the manner in which Kubowitz [1938] removed copper from phenoloxidase, and by

other methods, have not so far been successful. The colourless nature of the enzyme suggests that the iron is not present in the form of a haemin derivative. On the other hand the comparatively large amount of iron (of the same order as that found by Sumner & Dounce [1937] in crystalline catalase) and the consistency with which the value 0·15–0·20 % is found in repeated preparations, suggests that the iron is more than a mere impurity.

If it is assumed that the catalytic activity of the enzyme is due to the iron, then 1 mg. iron brings about the reaction of 57,000 μl. oxygen per min.

Summary

A method for the purification of the enzyme uricase is described.

The purest preparation has a specific activity, under stated conditions, of 85–90 μl. per mg. per min. and contains 0·15–0·20 % Fe.

Although uricase seems to be a heavy metal compound it has not yet been proved that the iron acts as the active group of the enzyme.

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