ADENOSINE DEAMINASE FROM CALF INTESTINAL MUCOSA

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In the course of studying the phosphoric acid esterases from calf intestinal mucosa (1, 2) the preparations were found to contain a potent deaminase for adenosine. The method of preparation is simple and the procedure indicates that this enzyme is quite stable. This deaminase is probably identical with that recently prepared by Brady (3) from the same material by a somewhat different procedure. In the present studies the action of the enzyme was followed manometrically; the utilization of this procedure for the estimation of adenosine is suggested.

EXPERIMENTAL

Preparation of Enzyme—The enzyme preparation described elsewhere (2) was used throughout these studies. The essential steps in the preparation are treatment of the mucosa with proteolytic enzymes, precipitation with 2 volumes of acetone with the addition of sodium acetate, treatment with Cγ alumina which removes impurities, and finally precipitation and drying with acetone.

Specificity of Deaminase—Ribonucleic acid, desoxyribonucleic acid (depolymerized (1)), and adenylic acid are deaminated at pH 7.6 but not at pH 5.9, whereas adenosine is deaminated at both pH values. Since the enzyme preparation contains phosphoesterases (2) which are inactive at the lower pH it was concluded that the first compounds are acted on only after dephosphorylation and that adenosine and desoxyriboadenosine were the specific substrates. The enzyme does not act on adenine.

Determination of Activity and Quantitative Application of Deaminase—The activity was determined by direct estimation of the NH₃ liberated and also manometrically from the absorption of CO₂ into the system made alkaline by the liberation of NH₃.

The direct procedure was used to demonstrate that NH₃ was formed from adenosine by the action of the enzyme and to determine the extent of the reaction. The enzyme was allowed to act on the substrate, the solution was then made alkaline with K₂CO₃, and the NH₃ was steam-distilled from a micro-Kjeldahl apparatus into the standard acid. Negligible blanks were obtained with the concentration of alkali provided by the K₂CO₃ with adenosine. In a typical experiment ¹ 90.7 per cent of the amino N was found as free NH₃.

¹ The amount of adenosine used was based on the N content of the sample.
Fig. 1. Quantitative liberation of NH₄ from adenosine. The experiments were performed at 37° with an atmosphere of 5 per cent CO₂-95 per cent N₂. The Warburg flasks contained 0.2 cc. of 0.5 M NaHCO₃, adenosine, and 0.16 mg. of enzyme (in the side arm); the total volume after mixing was 3.5 cc. The pH was 7.62. Adenosine, Mg.; Curve A, 1.39, Curve B, 0.93.

Fig. 2. Deaminase activity in relation to pH. The experiments were performed at 37° with 5.0 mg. of adenosine and with 0.16 mg. of enzyme (in the side arm); the total volume was 3.5 cc. NaHCO₃ and CO₂ were varied to give the pH values indicated; the points below pH 7.20 were obtained with an atmosphere of 100 per cent CO₂; those above were obtained with 5 per cent CO₂-95 per cent N₂.
The Warburg equipment was used for the manometric procedure. The absorption of CO$_2$ with excess substrate (5.0 mg. of adenosine per 3.5 cc.) was proportional to the amount of enzyme used (0.04 to 0.16 mg.). The deaminase is active with small amounts of substrate and so it can be used in quantitative experiments with adenosine. Experiments of this type are illustrated in Fig. 1. In this experiment 79 and 76 per cent, respectively, of the adenosine was accounted for by the c.mm. of gas absorbed.

![Graph](image)

**Fig. 3.** Effect of silver on deaminase. In these experiments the enzyme (0.16 mg.) and the silver were in the bottom of the flask and the adenosine (5.0 mg.) was in the side arm. A stock solution of 0.002 M silver nitrate was diluted 1:500 or 1:1000 just before use and amounts added to give the indicated moles per liter. The total volume in the flask was 3.5 cc.

At pH 7.62, 2 per cent of NH$_4$OH exists as NH$_3$ ($pK = 9.26$) which would not absorb CO$_2$. A correction must be made also for the acidic hydroxyl group resulting from the deamination ($pK$ of inosine = 8.75 (4)); at pH 7.62, 0.07 equivalent of CO$_2$ would be liberated by this group for each equivalent of adenosine deaminated. These corrections would increase the above figures to 88 and 85 per cent, values which are in fair agreement with the data obtained by direct measurement of the NH$_3$ formed.

**Optimum pH of Deaminase**—The optimum pH for the activity of the deaminase was determined as described under Fig. 2. There is a broad optimum at pH 7.0.
ADENOSINE DEAMINASE

Effect of Silver on Deaminase—The effect of silver on the deaminase was determined as described under Fig. 3, where the results are shown.

DISCUSSION

The deaminase is rather striking in its resistance to proteolytic enzymes and its stability when precipitated and dried with acetone. Schmidt (5) had observed that treatment with proteolytic enzymes\(^2\) was useful in isolating adenosine deaminase from muscle and had noted that \(C\gamma\) alumina would not absorb the deaminase. The enzyme described by Brady (3) from calf intestinal mucosa deaminated both adenosine and deoxyriboadenosine; the essential steps in its purification were the extraction of acetone-dried mucosa with water, precipitation of inactive proteins with salicylic acid, and precipitation and drying with acetone. 1 gm. of this material liberated 180 mg. of \(NH_3-N\) per minute at 18\(^\circ\), or, with a factor of 2 for each 10\(^\circ\) rise in temperature, 684 mg. per minute at 37\(^\circ\). The preparation used in the present studies caused the absorption of 61,200 c.mm. of \(CO_2\) per minute per gm., equivalent to 37.8 mg. of \(NH_3-N\) per minute. Further purification was attempted with the use of salicylic acid\(^3\) without success. The use of acid does not seem to be a promising purification procedure, since Brady (3) has found that exposure of the enzyme to pH 3.0 for 30 minutes will completely inactivate it.

The pH optimum of 7.0 obtained with the present preparation by the manometric method at 37\(^\circ\) is somewhat higher than 6.2 for the enzyme from rabbit liver (6) and 6.5 for the enzyme in laked rabbit blood (7).

The effect of silver on the deaminase is of interest because of the small amount required and it is useful because by the addition of silver the deaminase can be inhibited when it is desired to use the phosphoesterases in these preparations for preparing adenosine from nucleic acid (8, 9). The deaminase in its sensitivity to silver is comparable to urease. Sumner and MYRBACK (10) found that crystalline urease (2.5 mg. per liter) was 50 per cent inhibited by less than 0.3 \(\times 10^{-7}\) moles of silver per liter. The deaminase (45 mg. per liter) was 50 per cent inhibited by 4.0 \(\times 10^{-7}\) moles of silver per liter. In comparing the results it should be kept in mind that the deaminase is far from pure.

Adenosine can readily be determined manometrically with the deaminase.

\(^2\) Schmidt (5) chose to use preparations of papain rather than trypsin for this treatment because the latter contained adenosine deaminase. Manometric assays of Difco trypsin, 1:250 (1.0 mg. portions), which was used principally for preparing the enzyme used in the present studies, were negative for the deaminase.

\(^3\) The precipitate with this reagent is not obtained in consequence of its properties as a protein precipitant but because it gives the required pH for precipitation. Adjustment to the same pH with HCl will give a precipitate also.
The results obtained with a commercial sample of adenosine are somewhat low but this may be due to the presence of N-containing compounds other than adenosine in the sample. An experiment performed at pH 7.62, as was the experiment for Fig. 1, would also measure adenylic acid quantitatively. Adenosine triphosphate would be expected to be measured also (11). Adenosine can be estimated separately by performing the reaction at pH 5.9 at which the deaminase is still quite active but the phosphoesterase is inactive. The amount of enzyme used above completely deaminated 1.0 mg. of adenosine in 25 minutes at pH 5.9, but adenylic acid was not touched.

**SUMMARY**

An enzyme preparation from calf intestinal mucosa is described which deaminates adenosine and desoxyriboadenosine. The activity of the deaminase is conveniently measured manometrically; the use of the enzyme for estimating adenosine is suggested. The enzyme preparation contains phosphoesterases but these were inactive at pH 5.9, whereas the deaminase has considerable activity. The activity of the deaminase with adenosine as the substrate has a broad pH optimum at 7.0; the deaminase is very sensitive to silver, $4.0 \times 10^{-7}$ moles per liter causing 50 per cent inhibition.

**BIBLIOGRAPHY**

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