THE ACTION OF ADENOSINE DEAMINASE ON S-ADENOSYLHOMOCYSTEINE AND RELATED COMPOUNDS

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The action of "non-specific" adenosine deaminase (EC 3.5.4.4) from Aspergillus oryzae (Mitchell and McElroy, 1946; Kaplan, 1955; Sharpless and Wolfenden, 1967) on biological adenosine sulfur compounds and related substances has been examined. The enzyme was found to deaminate the thioethers S-adenosylhomocysteine, 5'-methylthioadenosine, 5'-ethylthioadenosine and their sulfoxides to the corresponding inosine derivatives. In contrast to this, the related adenosine sulfonium compounds proved resistant toward the enzyme; tested were S-adenosylmethionine, S-adenosylethionine, 5'-dimethylsulfoniumadenosine, and S-adenosyl(L-2-hydroxy-4-methylmercapto)butyric acid. The difference between the thioethers and the sulfonium compounds is of interest in relation to the mode of action and specificity of the enzyme, and it is important for preparative and analytical purposes. Spectrophotometric assay of enzymatic processes in which the sulfonium compounds act as donors and the thioethers listed above are formed, becomes possible by subsequent action of the deaminase. The latter converts the adenosine thioethers into the corresponding inosine compounds with a shift of the ultraviolet absorption maximum from 260 mμ to 249 mμ. The deamination is rapid and quantitative

and thus reveals the extent or progress of the initial enzyme reaction such as transmethylation. Adenosine deaminase from intestine (Kalckar, 1947) did not deaminate S-adenosylhomocysteine, 5'-methylthioadenosine, and S-adenosylmethionine.

All adenosine sulfur compounds were obtained by procedures developed in this Laboratory (Schlenk and Tillotson, 1954; Duerre, 1962; Schlenk et al., 1965; Shapiro and Ehninger, 1966). A generous supply of S-adenosylhomocysteine has been made available to us also by the Biochemical Research Center of Boehringer Mannheim, Tutzing, Germany. The deaminase was prepared from Sanzyme (Sankyo), an Aspergillus oryzae preparation obtained from Calbiochem, Los Angeles, California. Fractionation with acetone and alcohol (Kaplan, 1955; Minato et al., 1966; Sharpless and Wolfenden, 1967) gave more than 200-fold purification, and the quantities employed for the tests (5 to 25 µg) showed almost negligible absorbance in the critical ultraviolet range. S-Adenosylmethionine: homocysteine methyltransferase was purified by alcohol fractionation (Shapiro et al., 1964).

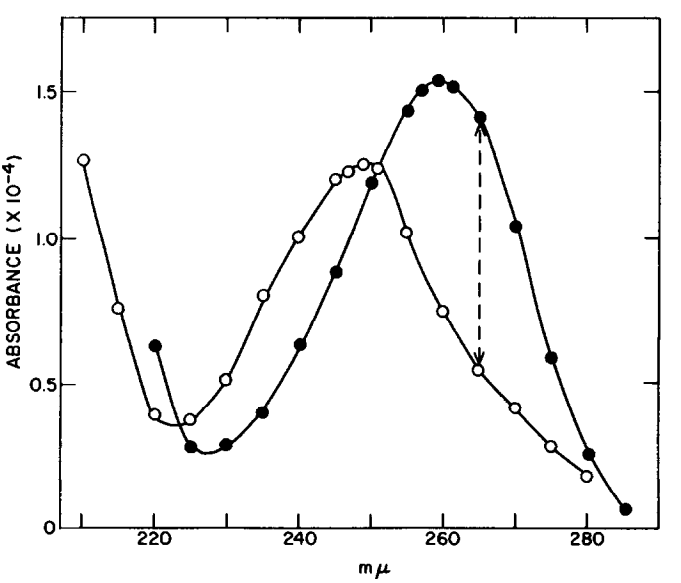

Fig. 1. Absorption spectrum of S-adenosylhomocysteine (●) and S-inosylhomocysteine (0).

The shift in the absorption spectrum caused by conversion of S-adenosylhomocysteine into S-inosylhomocysteine is shown in Fig. 1.

The absorption spectra of 5'-methylthioadenosine, 5'-ethylthioadenosine, and their hypoxanthine derivatives are identical with those shown in Fig. 1 in the range from 240 mμ to 280 mμ. The spectrophotometry of the deaminations described here is carried out most conveniently at 265 mμ, as recommended for adenosine by Kalckar (1947). For a light path of 1.0 cm, the decline of absorbance at 265 mμ by 0.1 unit (Fig. 1) indicates the deamination of 11.6 mμmoles of adenosine compound per ml.

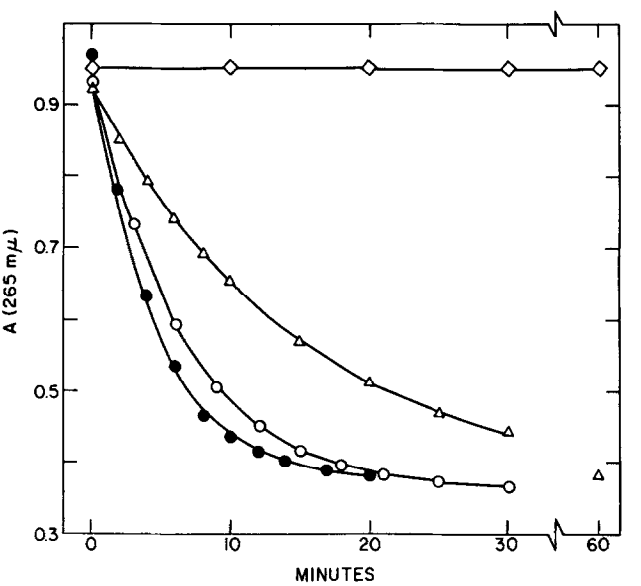

Fig. 2. Spectrophotometric measurement of the deamination of adenosine and related sulfur containing compounds by the "non-specific" deaminase from Aspergillus oryzae. The concentration of the substrates was 0.067 mM in 0.1 M potassium phosphate buffer, pH 6.8. Purified enzyme (4.4 μg/ml) was added, and the progress of the deamination was measured by the decline of absorbance at 265 mμ. The temperature was 25°. S-Adenosylmethionine, ◇; S-adenosylhomocysteine, Δ; adenosine, 0; 5'-methylthioadenosine, ●.

Fig. 2 shows an example of the deamination. S-Adenosylmethionine was inert, whereas 5'-methylthioadenosine reacted faster than adenosine, and S-adenosylhomocysteine was deaminated more slowly. S-Adenosylethionine and 5'-ethylthioadenosine (not shown in Fig. 2) behaved like the corresponding methyl compounds. 5'-Dimethylsulfoniumadenosine (Parks and Schlenk,

1958) and S-adenosyl-(L-2-hydroxy-4-methylmercapto)butyric acid (Schlenk et al., unpublished) were not deaminated. The DL-racemates of S-adenosylhomocysteine sulfoxide (Duerre, 1962) and of 5'-methylthioadenosine sulfoxide were obtained by oxidation of the thioethers with H_2O_2. The fact that their deamination was quantitative proves that the steric configuration of the sulfoxide group is of no consequence for the action of the enzyme.

The deaminase was used for preparative isolation of 5'-methylthioinosine and S-inosylhomocysteine. The latter compound has not yet been described and was examined, therefore, in some detail. It responded to the ninhydrin reagent which showed the amino group of the homocysteine part to be unimpaired. The response to the platinum iodide spray (Toennies and Kolb, 1951) indicated an intact thioether group. Hydrolysis in 0.1 N HCl at 100° for 2 hours led to hypoxanthine and S-ribosylhomocysteine; the latter was identical in all respects with the product obtained by acid hydrolysis of S-adenosylhomocysteine (Duerre, 1962).

Table 1. Spectrophotometric assay of S-adenosylhomocysteine and 5'-methylthioadenosine by deaminase in presence of an excess of S-adenosylmethionine (66.7 mμmoles/ml). The reactions were carried out in UV absorption cells in 0.1 M potassium phosphate buffer, pH 6.8, at 25°. After mixing the compounds and measuring at 265 mμ, 17.5 μg of purified deaminase was added to each cell. The reaction was completed after 5 minutes with 5'-methylthioadenosine, and after 10 minutes with S-adenosylhomocysteine.

S-Adenosylhomocysteine		5'-Methylthioadenosine	
added	recovered	added	recovered
(mμmoles/ml)			
33.3	33.0	33.3	33.5
16.7	16.2	16.7	17.4
6.7	6.0	6.7	6.6

The presence of an excess of S-adenosylmethionine causes little inhibition of the deamination of the thioethers (Table I). The deamination of the adenosine thioethers can be carried out also in perchloric acid extracts after neutralization with $KHCO_3$ and removal of $KClO_4$. The

presence of 0.1 N ClO_4^- caused little delay of the deamination whereas 0.3 N ClO_4^- was noticeably inhibitory.

The use of the deaminase for the assay of transmethylations in which S-adenosylhomocysteine is formed appears to be particularly promising. The quantity of S-adenosylhomocysteine corresponds to the methyl transfer and can be determined spectrophotometrically by deamination. In practice, samples may be withdrawn from the transmethylation experiment for subsequent deamination, or the deaminase may be added to the transmethylation system for immediate action. In the latter case, the process can be studied in a spectrophotometer cell, provided that the deamination is rapid compared with the transmethylation. This is exemplified in Fig. 3 with the reaction sequence,

L-Homocysteine + S-adenosylmethionine →

→ L-methionine + S-adenosylhomocysteine (a);

S-Adenosylhomocysteine → S-inosylhomocysteine (b).

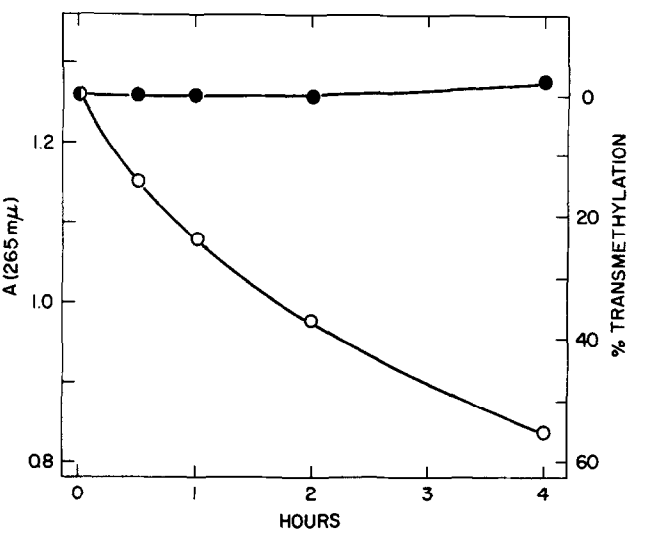

Fig. 3. Spectrophotometric assay of S-adenosylmethionine:homocysteine methyl transferase (Shapiro et al., 1964). In the complete system (O), the concentrations were (in μmoles/ml): potassium phosphate, pH 6.8 (85); Zn^{++} (0.017); mercaptoethanol (1.0); L-homocysteine (1.3); S-adenosylmethionine (0.089); purified methyl transferase (0.2 mg/ml); purified deaminase (12 μg/ml). In the control experiment (●) homocysteine was omitted. The incubation was carried out at 37°.

The decline in absorbance during the experiment indicates the formation of S-inosylhomocysteine according to Reaction (b). For each 0.1 unit decrease in absorbance at 265 mμ, 11.6 mμmoles of S-adenosylhomocysteine, formed in the transmethylation process (Reaction a), have been deaminated.

Analogous techniques appear possible in enzymatic reactions leading from S-adenosylmethionine to 5'-methylthioadenosine, such as the formation of homoserine (Shapiro and Mather, 1958) or spermidine (Tabor et al., 1958).

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REFERENCES

Duerre,J.A., Arch.Biochem.Biophys. 96, 70 (1962).
Kalckar,H.M., J.Biol.Chem. 167, 445 (1947).
Kaplan,N.O., in S.P. Colowick and N.O.Kaplan: Methods in Enzymology, II, 475 (1955).
Minato,S., T.Tagawa, M.Miyaki, B.Shimizu, and K.Nakanishi, J.Biochem. 59, 265 (1966).
Mitchell,H.K., and W.D.McElroy, Arch.Biochem.Biophys. 10, 351 (1946).
Parks,L.W., and F.Schlenk, J.Biol.Chem. 230, 295 (1958).
Schlenk,F., and J.A.Tillotson, J.Biol.Chem. 206,687 (1954).
Schlenk,F., C.R.Zydek, D.J.Ehninger, and J.L.Dainko, Enzymologia 29, 283 (1965).
Shapiro,S.K., and D.J.Ehninger, Analytical Biochem. 15, 323 (1966).
Shapiro,S.K., and A.N.Mather, J.Biol.Chem. 233, 631 (1958).
Shapiro,S.K., D.A.Yphantis, and A.Almenas, J.Biol.Chem. 239, 1551 (1964).
Sharpless,T.K., and Wolfenden, in S.P.Colowick and N.O.Kaplan: Methods in Enzymology XII A, 126 (1967).
Tabor,H., S.M.Rosenthal, and C.W.Tabor, J.Biol.Chem. 233, 907 (1958).
Toennies,G., and J.J.Kolb, Anal.Chem. 23, 823 (1951).