THE ALKALINE HYDROLYSIS OF S-ADENOSYLMETHIONINE IN TRITIATED WATER[1]

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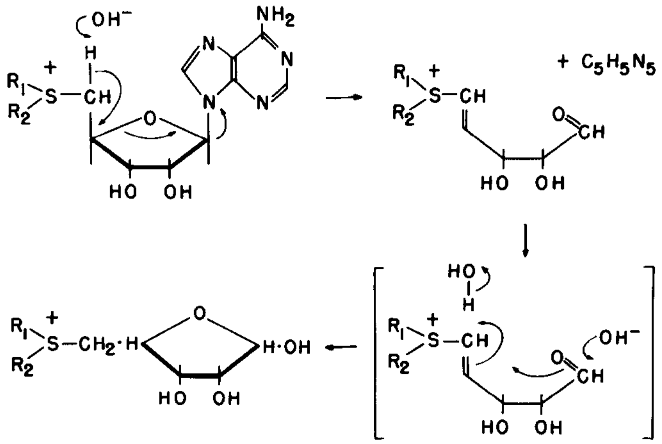
Alkaline hydrolysis of S-adenosylmethionine under mild conditions yields adenine and a pentose-containing sulfonium compound which tentatively has been termed S-ribosylmethionine (Parks and Schlenk, 1958). It was demonstrated that the unusual lability of the glycosidic bond is caused by the presence of the sulfonium group in the molecule, but details of the mechanism of hydrolysis remained obscure.

In a recent note, Frank et al. (1961) have suggested an ethylenic intermediate with the double bond at carbon atoms 4 and 5 of the ribose. After loss of the adenine, furanoid ring closure at carbon 4 can occur in either direction, yielding derivatives of D-ribose and L-lyxose:



R_1 : $-CH_3$

R_2 : $-CH_2CH_2CH(NH_2)COOH$

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Experiments with non-biological model compounds support this reaction sequence. Extensive racemization at carbon atom 4 in the carbohydrate part was ascertained by degradation and polarimetry (Frank et al., 1961).

It appeared desirable to test the proposed mechanism using the biological methyl donor S-adenosylmethionine. In particular, the transitory formation of an ethylenic intermediate appeared amenable to verification by carrying out the alkaline hydrolysis in tritiated water. The isotope should be incorporated at carbon atom 5, whether the intermediate forms a D-ribose or L-lyxose sulfonium compound.

S-Adenosylmethionine, prepared from yeast as the sulfate (Schlenk et al., 1959), was dissolved in tritiated water, neutralized, and adjusted with $Ba(OH)_2$ solution to 0.1 N. All operations were carried out at ice-bath temperature. The concentration of the sulfonium compound was 36.5 µM/ml, the radioactivity of the tritiated water was 2.93×10^9 cpm/ml. After 4 hours the solution was neutralized with 1 N H_2SO_4 and the precipitate of $BaSO_4$ removed. Samples of the solution (0.1 to 0.3 µM) were analyzed by paper chromatography (Parks and Schlenk, 1958). Scanning of the papers for radioactive spots showed considerable activity in the orcinol- and ninhydrin-positive material ("S-ribosylmethionine") formed by splitting of the glycosidic bond of S-adenosylmethionine. The radioactivity persisted after repeated chromatography. No activity was found in the adenine, nor in the methionine obtained by further alkaline hydrolysis at 100° C. for 5 minutes. It is obvious, therefore, that the activity is located in the pentose. To eliminate the possibility of a nonspecific tritiation of the carbohydrate part, adenosine, 5'-methylthioadenosine, ribose, and lyxose were exposed under identical conditions to tritiated water and 0.1 N $Ba(OH)_2$. No radioactivity was found in these compounds after chromatographing spots containing 0.3 µM and scanning the papers.

For quantitative measurement of the radioactivity in the pentose sulfonium compound, 2.0 ml of the hydrolyzate was analyzed by chromatography with Dowex 50 (H^+) resin, 100 to 200 mesh, in a column 8.5 x 0.5 cm, using 1.0 N HCl for elution (Expt. A). In a second experiment (B) the material was first purified by large-scale paper chromatography of 1.0 ml of hydrolyzate and elution of the bands containing the sulfonium compound; the combined eluates were applied to the column. On elution with 1.0 N HCl, the pentose sulfonium compound appeared ahead of adenine. Orcinol tests (Mejbaum, 1939) were carried out to determine its concentration, and liquid scintillation counting was used for assay of the radioactivity. Table I shows the data obtained with the appropriate 50-ml fractions of the eluate.

Table I

Radioactivity in eluate fractions of S-pentosylmethionine

Experiment and fraction	Pentose, μM/ml	Radioactivity, cpm/ml	Specific activity, cpm/μM
A 6	0.183	5476	29,900
7	0.276	7760	28,100
8	0.141	4440	31,500
B 7	0.043	1156	26,900
8	0.11	3005	27,400
9	0.131	3474	26,600

In the orcinol tests the time of heating was extended to 40 minutes, and the acidity of the reference standards was adjusted to match that of the acid eluates. Lyxose and ribose gave virtually identical results. In the determination of the radioactivity, the inhibition caused by the acidity of the eluates was less than 10%; a correction was made by using acidified reference standards. As expected, the specific activity of the compound was about 50% of that of the water in the incubation mixture (53,000 cpm/μM); only one hydrogen or tritium atom is bound per molecule of pentose.

The present data on the alkaline hydrolysis of S-adenosylmethionine are in agreement with the mechanism suggested by Frank *et al*. (1961). Transitory desaturation of the ribose and re-hydration after, or concomitant with, the splitting of the glycosidic bond is indicated by the uptake of tritium into the carbohydrate part. Because of the racemization resulting from this process the product should be termed S-pentosylmethionine rather than S-ribosylmethionine.

References

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