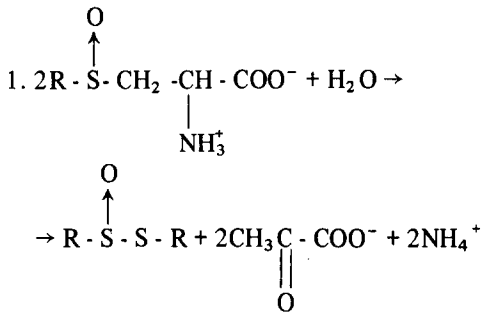
THE CLEAVAGE OF THE ETHER LINKAGE OF *O*-METHYL SERINE BY THE ALKYL CYSTEINE LYASE OF *ACACIA FARNESIANA*

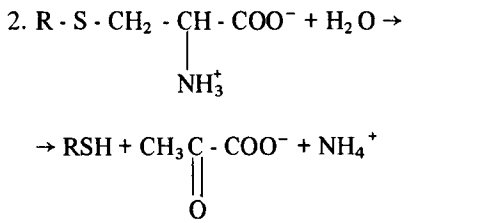
Mendel MAZELIS
Department of Food Science and Technology, University of California, Davis, CA 95616 USA

Received 7 April 1975

1. Introduction

A number of enzymes have been described which catalyse the cleavage of the C-S bond of S-alkyl cysteines. Some are very specific requiring that the sulphur atom be present in the oxidized form as the sulphoxide [1–5] and carrying out reaction 1 below, whereas the others will utilize either the thioether or sulphoxide form as a substrate [6–10] and catalyse reaction 2:





In the main these enzymes have been found in higher plants [1–8] but have also been reported in bacteria [9,10].

Recently we reported the purification and properties of the enzyme *S*-alkyl-L-cysteine (EC 4.4.1.6) from extracts of acetone powders of seedlings of *Acacia farnesiana* Wild. [8]. The enzyme protein was purified to homogeneity. It had a broad specificity with regard to the substituent replacing the thiol hydrogen and could utilize both the thioether and sulphoxide forms of the substrates. We have recently tested the oxygen and nitrogen analogues of *S*-methyl-L-cysteine as substrates for this enzyme. This paper presents the evidence for the cleavage of these analogues by the lyase which thus is one of the few examples known of the enzymatic cleavage of an ether linkage.

2. Materials and methods

S-Alkyl-L-cysteine lyase (EC 4.4.1.6) was purified from extracts of acetone powders of 5-day old seedlings of *A. farnesiana*. The germination of the seedlings, preparation of the acetone powder, and enzyme purification have been described previously [8]. The components of the reaction mixture and assay procedure were the same as previously reported [8] except for the substitution of *O*-methyl-DL-serine (OMS) or α-amino, β-methylaminopropionate, as the substrate where indicated. Pyruvate was determined by the direct method of Friedemann and Haugen [11] and NH_4^+ by direct Nesslerization of portions of the reaction mixture [8].

OMS was a product of Sigma Chemical Co. *S*-Methyl-L-cysteine was purchased from Cyclo Chemical Corp. A sample of α-amino, β-methylaminopropionate was generously provided by Professor E. A. Bell of

Kings College, University of London. Other chemicals were purchased from commercial sources.

3. Results and discussion

Under the assay conditions used the production of pyruvate from OMS was linear with time for the period of incubation, and also with the amount of enzyme added. The proof that pyruvate was a product was obtained by carrying out a large scale incubation and recovering the pyruvate produced as the crystalline 2,4 dinitrophenylhydrazone. The reaction mixture contained 2.8 mmol of OMS, 128 nmol pyridoxal phosphate, and 0.5 mg of enzyme, specific activity 33 U/mg in a vol of 5 ml. After incubation for 200 min at 30°C 5 ml of 10% (w/v) trichloroacetic acid was added. The resulting precipitate was removed by centrifugation and 100 ml of 0.5% (w/v) 2,4 dinitrophenylhydrazine in 2 M HCl was added to the supernatant solution, and the mixture stored at 10°C overnight. A precipitate was obtained which was collected by filtration and recrystallized from ethanol and water. The overall yield of recrystallized product was 145 mg. The ir spectrum and melting point was identical with an authentic sample of pyruvic-2,4-dinitrophenylhydrazone.

The close relationship between pyruvate and ammonia production during the cleavage of OMS is shown in table 1. The K_m of the enzyme for OMS was determined by the use of a computer program for a linear regression curve using a straight line form of the Michaelis-Menten equation. The correlation coefficient was greater than 0.99. The K_m calculated was 6.5 mM based on the L-OMS concentration and the V_{max} was 57 U/mg. Although the Dl-form of the substrate was utilized, it was assumed that the L-isomer was the substrate and that the D-form had no effect on the utilization of the L-isomer. In a similar manner the K_m and V_{max} values for L-djenkolate and *S*-methyl-L-cysteine was obtained. The results are presented in table 2. L-Djenkolate is one of the probable natural substrates in this plant species since it is a normal constituent of its non-protein N pool [12]. It is clear that L-djenkolate would be the preferred substrate compared to the others but substitution of the S atom by O has little influence on the rate of the reaction. The effect of substituting N for S was also examined by testing α-amino-β-methylamino propionate as the substrate. The relative activity of all analogue substrates relative to *S*-methyl-L-cysteine is given in table 3. Previous results [8] had shown that serine, *O*-phosphoryl serine and cysteine itself were not substrates. Apparently there is a requirement for an alkyl moiety on the β-substituent. The chemical similarity between

Table 1
Ratio of NH_3 to pyruvate production from DL-OMS

Time (min)	NH_3 / pyruvate
15	0.94
30	0.80
45	0.83
60	0.88

The reaction mixture was that used in the standard assay except for DL-OMS as the substrate. Final concentration of OMS was 10 mM and 8 μg of protein with a specific activity of 35 U/mg was utilized as the enzyme.

Table 2
Kinetic constants for various substrates

Substrate	K_m (mM)	V_{max} (U/mg)
L-djenkolate	0.3	76
S-methyl-L-cysteine	5.3	62
OMS	6.5	57

The results for OMS are based on the concentration of the L-isomer only. Standard reaction mixtures were used with each substrate. The reaction time was 3 min using 5 μg of enzyme with a specific activity of 40 U/mg.

Table 3
Relative activity of analogue substrates of alkyl cysteine lyase

Substrate	Relative activity
S-methyl-L-cysteine	100
OMS	67
DL-β-methylamino-α-aminopropionate	16

Standard reaction assay procedure was used in each case. The final substrate concentration (mM): *S*-methyl-L-cysteine (40); OMS (80); DL-β-methylamino-α-aminopropionate (80). In each instance 12 μg of enzyme with a specific activity of 33 U/mg was used.

O and S analogues as denoted by terms such as thioester or thioether appears to carry over to the action of this enzyme. The N analogue also is cleaved at a significant rate. The relative non-specificity of the enzyme in regard to this aspect of the substrate structure may be of some use in future for studies of the active site and mechanism of the reaction.

Few examples are known of the direct enzymatic cleavage of ether linkages. Two general types have been described. One proceeds via a hydroxylase mechanism involving molecular oxygen and NADPH [13] of a reduced pteridine [14]. Another known type is carbon-oxygen lyases which catalyse a β-elimination reaction to cleave the C-O bond [15–17]. The enzyme described in the present report belongs to the latter group. It would be interesting to know if the other enzymes in this group could also utilize the S-analogues of their usual substrates.

Acknowledgements

This work was partially supported by Contract 2-6633 from the National Aeronautics and Space Administration. I would like to thank Mr R. K. Creveling for his technical assistance in the preparation of the samples and ir spectroscopy.

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