ON THE CATION SENSIBILITY OF THE VITAMIN B_{12}-DEPENDENT METHIONINE SYNTHETASE (5-METHYLTETRAHYDROFOLATE-HOMOCYSTEINE-METHYLTRANSFERASE FROM *ESCHERICHIA COLI*

Harold RÜDIGER

Institut für Biochemie der Universität Köln, 5000 Köln-1 An der Bottmühle 2 W. Germany

Received 28 June 1973

1. Introduction

The activity of many enzymes is known to be modified by various alkali and alkaline earth cations (see [1–5]). Though these ions only are able to exert relatively weak coordinative forces, the complex formation apparently enables them to keep enzyme proteins in specific biologically active conformations. Some B_{12}-dependent enzymes have turned out to belong to this group of cation-sensitive enzymes [6–12]. Toraya et al. [6–8] have shown that the active form of the deoxyadenosyl cobalamin-dependent propanediol dehydratase contains potassium ions which are supposed to facilitate the binding between enzyme and B_{12} coenzyme thus enhancing the enzymatic activity.

These results made it appear worthwhile to investigate the influence of alkali and alkaline earth ions on the activity of methionine synthetase, the enzyme catalyzing the final step in methionine biosynthesis:

5-methyltetrahydrofolate + homocysteine ⇌ tetrahydrofolate + methionine

and representing one of the few vitamin B_{12}-dependent enzymes which do not exclusively occur in microorganisms but also in higher organisms [13].

2. Experimental

Metal salts were employed as chlorides, 5-methyltetrahydrofolate and FMN as tetramethylammonium salts. Homocysteine was prepared by tetramethylammonium hydroxide hydrolysis of the corresponding thiolactone and neutralization by hydrochloric acid. Methionine synthetase was purified from vitamin B_{12}-grown *Escherichia coli* B cells as described [14] and transferred to 0.01 M tetramethylammonium-*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate (HEPES) buffer pH 7.2 by gel filtration over a column of Sephadex G-25. In each assay, about 2.8 μg of the enzyme preparation (7.2 μmoles/hr/mg protein) were allowed to react with substrates and cofactors as described [15] for 1 hr at 31°C under an atmosphere of argon in the presence of salts as indicated, except that the mixture was buffered by 0.04 M tetramethylammonium–HEPES buffer pH 7.2 and tetramethylammonium ions were substituted for sodium ions throughout. The amount of tetrahydrofolate formed was determined by conversion to the methionyl derivative and by measuring either the increase in absorption at 366 nm [16] or the spectrum in the wavelength range 300–400 nm.

3. Results and discussion

Most of the monovalent cations studied activate methionine synthetase (table 1), the optimal ion concentration in all cases for activation is about 67 mM. The 'natural' alkali ions and the unsubstituted ammonium ion differ from the 'unnatural' substituted ammonium ions ($TrisH^+$, $(CH_3)_4N^+$) in that they display a more pronounced activating effect. Thus, the activation is not solely due to ionic strength, though ion specificities are low.

Table 1
Influence of monovalent cations at optimal concentration (67 mM) on the activity of methionine synthetase.

Cation added as chloride	Relative activity
No cation (control)	1.00
Li^+	1.34
Na^+	1.52
K^+	1.45
NH_4^+	1.47
$TrisH^+$	1.21
$(CH_3)_4N^+$	0.99

Since the values are taken from different experiments, relative activities are given.

Of the monovalent cations investigated, only the tetramethylammonium ion is indifferent to methionine synthetase. This ion therefore was used as counterion. Enzyme activities in absence of tetramethylammonium ions could not be determined since methionine synthetase displays a rather narrow pH optimum and therefore must be buffered sufficiently to avoid uncontrolled variations in activity.

In contrast to the alkali ions, the alkaline earth ions differ significantly from each other (fig. 1). Mag-

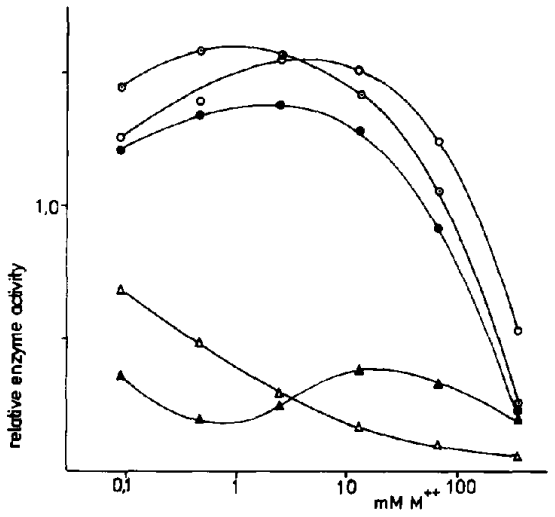

Fig. 1. Influence of bivalent cations on methionine synthetase activity: (o—o—o) magnesium; (•—•—•) calcium; (○—○—○) manganese; (△—△—△) strontium; (▲—▲—▲) barium ions.

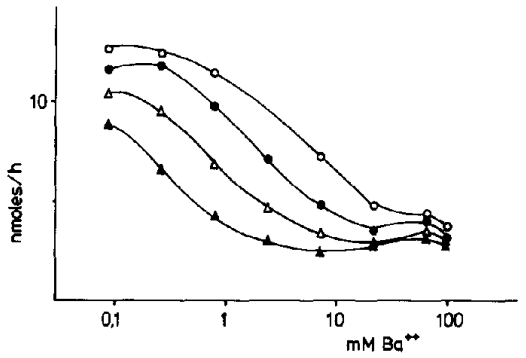

Fig. 2. Interaction of methionine synthetase, barium and sodium ions: (○—○—○) 200; (•—•—•) 66.7; (△—△—△) 22.2; (▲—▲—▲) 0.0 mM sodium ions.

nesium and to a lesser extent calcium activate the enzyme in a way similar to the alkali ions though the maximum in activity is reached at lower ionic strength as in the case of the alkali ions. Manganese closely resembles magnesium as it does in many enzymic reactions, but activates slightly better than magnesium at low concentrations. Strontium decreases the activity within the whole concentration range studied (0.09–200 mM, only a part is shown in fig. 1), beryllium ions inhibit the enzyme even more strongly (50% inhibition at 0.3 mM). The inhibition by these two ions depends on their concentration continually and unequivocally. In contrast, barium ions display a particularly complicated inhibition pattern. At low barium concentrations, the enzyme is inhibited slightly stronger than by strontium. At high barium concentrations, however, the inhibition is superimposed by an activation with a maximum at 22 mM barium.

When sodium and potassium or sodium and magnesium ions are present simultaneously, the enzyme is not activated more than by the single ions. An activating ion as e.g. sodium can, however, protect the enzyme from the influence of an inhibiting ion as e.g. barium, as may be seen in fig. 2. The complex and presumably composed inhibition pattern of barium appears to change to a more normal one at high sodium concentrations.

In different experiments, the inhibition by barium ions yielded curves of similar shape but of different absolute values. It therefore seemed reasonable to study the influence of the most labile cofactor of the

Table 2
Interaction of methionine synthetase, barium ions and S-adenosylmethionine.

S- adenosylmethionine	Barium chloride (2.5 mM)	Enzyme activity (nmoles/h)
10^{-5} M	–	7.7
10^{-5} M	+	1.5
10^{-4} M	–	8.2
10^{-4} M	+	3.2
10^{-3} M	–	10.6
10^{-3} M	+	9.7

Table 3
Interaction of methionine synthetase, sodium ions and S-adenosylmethionine.

S-adenosylmethionine	Sodium chloride (200 mM)	Enzyme activity (nmoles/hr)
4.57×10^{-8} M	–	0.1
4.57×10^{-8} M	+	0.8
1.37×10^{-7} M	–	2.3
1.37×10^{-7} M	+	4.6
4.11×10^{-7} M	–	4.6
4.11×10^{-7} M	+	7.6

enzyme, namely S-adenosylmethionine, as related to the inhibition by barium. The result of this experiment is shown in table 2. At moderate barium concentrations, S-adenosylmethionine is able to overcome the inhibiting effect of barium.

Barium ions appear rather to inhibit than to denaturate the enzyme: An enzyme sample which had been incubated at an inhibiting concentration of barium chloride (22 mM) at 0°C for 20 hr, was nearly fully active after having been separated from low molecular weight substances by gel filtration. Only insignificantly low amounts of cobalt of the same order of magnitude as found in a blank could be detected in the low molecular weight fractions by atomic absorption analysis indicating that the inhibition by barium is not due to resolution of the B_{12} cofactor from the apoenzyme, though a loosening of the bonds between B_{12} and protein cannot be excluded.

Table 3 shows the interaction of the two activating species sodium ions and S-adenosylmethionine. Both cations may substitute for each other, though a basic level of S-adenosylmethionine is necessary to achieve an activation by sodium ions, while at high S-adenosylmethionine concentrations the relative activation by sodium ions diminishes.

The modification of methionine synthetase activity by mono- and bivalent cations has hitherto escaped attention. Normally, alkali ions containing buffers and saturating concentrations of S-adenosylmethionine are used which will suppress inhibition by low levels of bivalent cations. The molecular mechanism by which activating and inhibiting ions operate cannot be deduced from the experiments described above. It may, however, be supposed that, similarly to the activation of dioldehydratase [6–8], the cations act on the enzyme protein conformation thus altering the affinity for B_{12} or other cofactors and substrates.

The activation by alkali ions may be physiologically important because it helps to save the high energy compound S-adenosylmethionine. If, however, this activating cofactor is employed in high concentrations, as is usually done in enzymatic tests, a part of its activating effect, in addition to the known methylation of the enzyme-bound vitamin B_{12} [13], may be attributed to its cationic character.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft.

References

[1] Schweet, R. and Heintz, R. (1966) Ann. Rev. Biochem. 35, 723.
[2] Webster, L.T. (1966) J. Biol. Chem. 241, 5504. Webster, L.T. (1966) J. Biol. Chem. 242, 1232.
[3] Schoner, W. (1971) Angew. Chemie 83, 947.
[4] Taylor, E.W. (1972) Ann. Rev. Biochem. 41, 577.
[5] Morrison, J.F. and Heyde, E. (1972) Ann. Rev. Biochem. 41, 29.
[6] Toraya, T., Sugimoto, Y., Tamao, Y., Shimizu, S. and Fukui, S. (1970) Biochem. Biophys. Res. Commun. 41, 1314.
[7] Toraya, T., Sugimoto, Y., Tamao, Y., Shimizu, S. and Fukui, S. (1971) Biochemistry 10, 3475.
[8] Toraya, T., Kondo, M., Isemura, Y. and Fukui, S. (1972) Biochemistry 11, 2599.

[9] Stadtman, T.C. and Renz, P. (1968) Arch. Biochem. Biophys. 125, 226.
[10] Morley, C.G.D. and Stadtman, T.C. (1970) Biochemistry 9, 4890.
[11] Morley, C.G.D. and Stadtman, T.C. (1972) Biochemistry 11, 600.
[12] Schneider, Z. and Johnson, B.C. (1971) Arch. Biochem. Biophys. 144, 491.
[13] Rüdiger, H. and Jaenicke, L. (1973) Mol. Cell. Biochem. 1, in press.
[14] Rüdiger, H. and Jaenicke, (1970) European J. Biochem. 16, 92.
[15] Rüdiger, H. and Jaenicke, L. (1969) European J. Biochem. 10, 557.
[16] Rüdiger, H. and Jaenicke, L. (1968) FEBS Letters 1, 293.