of the very rare cases [12] of a d^3-system deviating from octahedral symmetry, and any information about its absorption spectrum will be highly desirable.

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Some Observations on Betaine-Homocysteine-Methyl-Transferases

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Experiments carried out in our laboratory suggest that enzymes capable of synthesizing methionine by means of a transfer of a methyl group from betaine (carboxymethyl-trimethyl-ammonium chloride) to homocysteine, are present in appreciable amounts only in the livers of vertebrate animals. Attempts to find such enzymes in microorganisms, plants or invertebrates have so far failed [1]. The apparent pH-optima of the betaine-homocysteine-methyltransferases of all the vertebrate livers tested occur between 7.0 and 7.8. These transferases are strongly inhibited by dimethylglycine, which besides methionine is a product of the enzymic reaction [1,2]. Choline can not replace betaine as the methyl donor with purified enzyme preparations nor does choline act as an inhibitor of the transferases.

In order to study the characteristics of this type of methyl group transfer in more detail, a procedure for the purification of the betaine-homocysteine-methyl-transferase of pig liver was worked out. It comprises the following steps:

1. One part of fresh pig liver is homogenized in three parts of water in a Waring blender. The pH of the homogenate is adjusted to 5.1 at 0°C and the thick "brei" obtained immediately centrifuged at 44 000 $\times$ g for 30 min.

2. The supernatant fluid is adjusted to a pH-value of 7.7 and poured into a series of test tubes which are then placed in a water bath at 80°C for 90 sec. An inactive precipitate is formed and is removed by centrifuging at 44 000 $\times$ g for 30 min.

3. The enzyme which is contained in the supernatant fluid from step 2, is adsorbed on calcium phosphate gel prepared according to Keilin and Hartree [3]. The enzyme is eluted from the gel by means of 0.15 M orthophosphate and 0.1 M pyrophosphate.

4. The eluates from step 3 are pooled and the pH adjusted to 7.2. Acetone is added to a final concentration of 50 %. The precipitate that forms is collected by centrifuging at 5 000 $\times$ g for 10 min, dissolved in water and dialysed against water at pH 8.5.

5. The pH of the dialysed enzyme preparation is adjusted to 7.50 and acetone is added to a concentration of 50 %. The precipitate is collected by centrifuging at 20 000 × *g* for 10 min and dissolved in 0.005 M phosphate buffer. The pH is adjusted to 6.5 and the solution centrifuged at 20 000 × *g* for 2 min. The supernatant fluid contains the enzyme. The enzyme preparation thus obtained is coloured and has a specific activity approximately 100 times that of the homogenate. The overall yield is of the order of 35 %.

Further purification has been attempted in many different ways. Preparative electrophoresis at pH 9.2 in a density gradient [4] separates the coloured material present in the step 5-preparation from the transferase. Experiments of this type suggest that 60—70 % of the total protein content of the step 5-preparation constitutes an enzyme which is electrophoretically homogeneous at alkaline pH-values. This enzyme can use dimethylpropiothetin as the methyl donor in place of betaine.

Colourless enzyme preparations with a high specific activity have also been obtained using ion-exchange chromatography on triethylaminoethyl cellulose according to Porath [5]. This purification method has also failed to separate betaine-homocysteine-methyl-transferase from material having "thetin homocysteine methylpherase" [6] activity.

Fractionation of the enzyme preparation from step 5 with ethanol has occasionally resulted in crystalline material with a specific activity of about 150 times that of the liver homogenate. Dimethylpropiothetin can replace betaine as the methyl donor both with the crystalline and all other active ethanol fractions obtained. The ratio between the amount of methionine formed with propiothetin and the amount formed with betaine is approximatively ten for these fractions.

If a solution of purified betaine-homocysteine-methyl-transferase is kept at 80°C its ability to transfer methyl groups from betaine is lost at the same rate as its ability to transfer methyl groups from dimethylpropiothetin. — Dimethylglycine, which interferes with the utilization of betaine as the methyl donor also inhibits the methyl transfer from porpiothetin.

A number of compounds were synthesized [7] and tested for their ability to replace betaine as the methyl donor. It was found that the methyl donor specificity of betaine-homocysteine-methyl-transferase is by no means absolute. Not only dimethylpropiothetin and dimethylacetothetin but also carboxymethyl-dimethylethyl-ammonium chloride, carboxymethyl-diethylmethyl-ammonium chloride, 1-carboxyethyl-trimethyl-ammonium chloride and the methyl ester of betaine can more or less efficiently replace betaine as the methyl donor at pH 7.8 in phosphate buffer. However, the enzyme does not seem to be able to transfer ethyl groups.

An interesting property of the purified enzyme is that it is stimulated by some commonly occurring metabolites and inhibited by others. This phenomenon will only be exemplified here with a few remarks on the stimulation by oxalate. An approximately 10 % increase in the activity of the enzyme, as measured by the amount of methionine formed per hour can be obtained with potassium oxalate concentrations of 0.1 mM. The stimulation increases with increasing oxalate concentrations up to about 10 mM. The activity of the stimulated enzyme is then approximately 50 % above that of the control. At still higher concentrations of oxalate the stimulation decreases. Stimulation of the enzyme activity by oxalate is more marked at saturation than at subsaturation levels of either of the substrates. The enzymic formation of methionine is increased by the addition of oxalate also when dimethylpropiothetin serves as the donor of the methyl group.

A full account of the work reported here will be published shortly.

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