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Betaine-Homocysteine-Methyl-Transferases

III. The Methyl Donor Specificity of the Transferase Isolated from Pig Liver

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Studies have been made on the specificity of the methyl donor requirement of a betaine-homocysteine-methyl-transferase purified from pig liver. It was found that a number of betaine derivatives and sulfonium compounds could replace betaine as methyl donor. No evidence was obtained for ethyl group transfer.

Many of the ammonium compounds tested as methyl donors interfered with the utilization of betaine, and betaine itself decreased the rate of methyl transfer from certain sulfonium compounds.

The data presented are tentatively interpreted and discussed.

In a previous communication [1] methods were reported for the preparation of a purified betaine-homocysteine-methyl-transferase from pig liver. It is the purpose of this article to describe some studies on the specificity of the methyl donor requirement of this enzyme preparation. The problem has already been discussed briefly in two earlier papers from this laboratory [2,3], and has been investigated also by other research groups [4,5].

The ability of the substances tested as methyl donors to compete with betaine (carboxymethyl-trimethyl-ammonium chloride) for the active donor site on the transferase was also studied in the investigation reported here.

EXPERIMENTAL

The enzyme preparation used in the experiments described below, was obtained from pig liver by the method given in Ref.[1] (alternative 1). As judged from electrophoretic and ultracentrifuge studies the transferase contributed approximatively 90 % of the total protein of the preparation. The protein content of the incubation mixtures was determined using the method suggested by Kalckar [6]. Enzyme activity was measured as the amount of methionine formed per hour during the initial phase of the reaction when the amount of dimethylglycine was small. The incubations were carried out aerobically at 37°C.

Methionine was determined microbiologically according to Steele *et al.*[7] The nitroprusside method [8] was found unsuitable for this purpose as many of the compounds tested

interfered with the colour formation. Ethionine, however, was measured chemically by determining the optical density at 520 mμ of the nitroprusside reaction product.

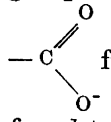
Of the compounds tested as methyl donors betaine, choline, dimethylglycine, acetyl choline, dimethyl-β-propiothetin, trimethylamine hydrochloride, methyl methionine sulfonium chloride, ergothioneine · HCl, and S-adenosyl methionine iodide were commercial products purchased from California Corporation for Biochemical Research, Cal., USA; L. Light & Co., England; Fluka AG, Switzerland, and Nutritionals Biochemicals Corporation, Ohio, USA. Betaine aldehyde; DL-carnitine, O-acetyl-DL-carnitine; muscarine iodide; N-trimethyl taurine; dimethyl acetothetin and trimethylsulfonium chloride were kindly supplied by Drs. D. R. Strength, S:t Louis University School of Medicine, USA; G. S. Fraenkel, University of Illinois, USA; F. Hoffman-La Roche & Co, Switzerland; D. Ackermann, University of Würzburg, Germany; F. Challenger, University of Leeds, England and G. A. Maw, A. Guinness, Son & Co, Ltd., Dublin, Eire.

The methyl and ethyl esters of betaine were synthesized from the methyl and ethyl esters of bromoacetic acid and trimethylamine [9]. The betaine amide was prepared from chloroacetamide and trimethylamine [9]. The nitrile corresponding to betaine was obtained by reacting dimethyl-amino-acetonitrile prepared according to von Braun [10] with methyl iodide. The betaine-like compounds that are formed when one, two or all three of the N-methyl groups are replaced by ethyl groups were prepared *via* dimethyl- or diethyl-amino-acetonitrile [10] and ethyl bromide or methyl iodide [11]. The nitriles were converted to the corresponding betaines according to Klages and Margolinsky [12]. The α-methyl betaine was synthesized by N-methylation of α-alanine with methyl iodide [11]. Propiobetaine was obtained from trimethylamine and propionic acid lactone [13] and butyrobetaine from α-amino buturic acid and methyl iodide [14]. All the compounds synthesized in the laboratory were recrystallized to constant melting points.

RESULTS AND DISCUSSION

It is obvious from the results presented in Tables 1 and 2 that the betaine-homocysteine-methyl-transferase of pig liver can use a variety of compounds as methyl group donors. However, neither choline nor betaine aldehyde can replace betaine in this function (Table 1). The methyl and ethyl esters of betaine, on the other hand, are excellent donors of methyl groups, being equally potent in this respect as betaine itself. This was found to be the case over a wide range of concentrations. It is of interest to notice in this connection the complete lack of donor activity of the betaine amide.

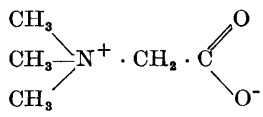
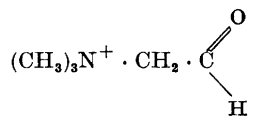
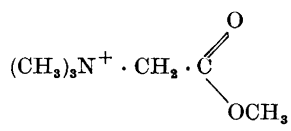
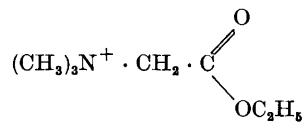
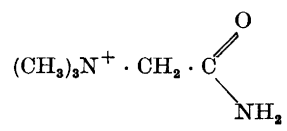
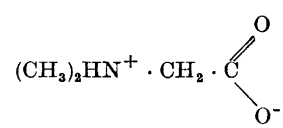
The "betaine nitrile" seems to possess some ability to donate methyl groups with the enzyme preparation used. This would suggest that the



function, present in all the other compounds in Table 1 that were found to act as methyl donors, is not essential for donor activity.

If one of the three N-methyl groups of betaine is replaced by an ethyl groups, a compound is formed that has a methyl donor activity that is only 30 % of that of betaine. Replacement of two of the N-methyl groups by ethyl group lowers the donor activity to 6 %. Ethyl groups cannot apparently be handled by the enzyme, since no ethionine could be detected in incubation mixtures containing betaine derivatives in which one, two or all three of the N-methyl groups had been replaced by ethyl groups. Attempts to detect ethionine were made either by the nitroprusside method or by paper chromatography. It is of course possible that the amount of ethionine formed was too small to be detected by these methods.

Table 1. The ability of some nitrogenous compounds that are structurally related to betaine to serve as donors of methyl groups using a preparation of betaine-homocysteine-methyl-transferase. The enzyme activity is also given for incubation mixtures containing both the compound and betaine at the concentration ratio of 2:1. All incubation mixtures contained 0.025 M DL-homocysteine and approximatively 0.2 mg protein per ml. Anions are omitted in the formulae.

Name	Formula	Relative activity	
		0.05 M of the compound alone %	0.1 M of the compound + 0.05 M of betaine %
Betaine (carboxymethyl-trimethyl-ammonium chloride)		100	100
Choline	$(CH_3)_3N^+ \cdot CH_2 \cdot CH_2 - OH$	0	100
Betaine aldehyde		0	80
Betaine methyl ester		100	130
Betaine ethyl ester		100	108
Betaine amide		0	93 *
2-Acetonitrile-trimethyl-ammonium chloride	$(CH_3)_3N^+ \cdot CH_2 \cdot C \equiv N$	12	55
Dimethylglycine		0	0

* Tested at 0.05 M together with 0.05 M betaine.

Table 1 (cont.)

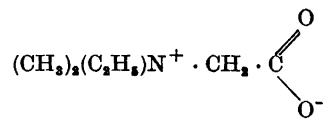
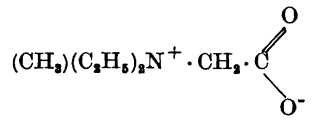
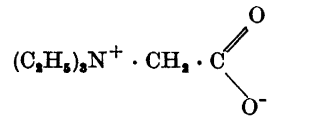
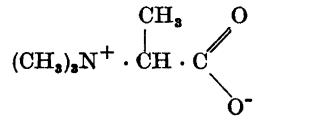
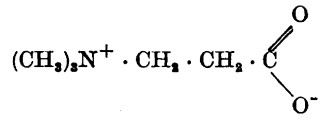
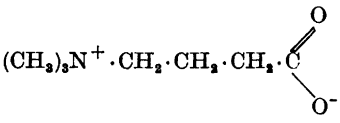
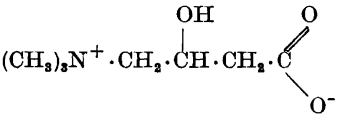
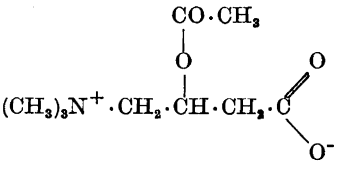
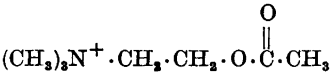
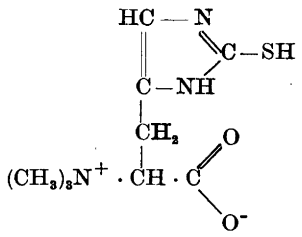
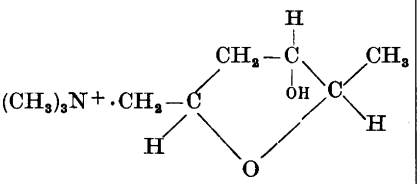
Carboxymethyl-dimethyl-ethyl-ammonium chloride		30	72
Carboxymethyl-diethyl-methyl-ammonium chloride		6	100
Carboxymethyl-triethyl-ammonium chloride		No ethionine	93
1-Carboxyethyl-trimethyl--ammonium chloride (α-methylbetaine)		13	85
2-Carboxyethyl-trimethyl-ammonium chloride (propiobetaine)		0	95
3-Carboxypropyl-trimethyl-ammonium chloride (butyrobetaine)		0	—
DL-Carnitine		0	90
O-Acetyl-DL-carnitine		0	77
Acetyl choline		0	100

Table 1 (cont.)

L-Ergothioneine hydrochloride		0	0 *
Muscarine iodide [18]		0	69
N-Trimethyl taurine	$(CH_3)_3N^+ \cdot CH_2 \cdot CH_2 \cdot SO_3^-$	0	115
Trimethyl-ammonium chloride	$(CH_3)_3N$	0	100

* When tested at a concentration of 0.01 M together with 0.05 M betaine the relative activity was 77 %.

In connection with a discussion on N-substituted betaines it is relevant to draw attention to the fact that dimethylglycine, which at the pH tested can be considered as a betaine molecule in which one of the N-methyl groups has been replaced by a hydrogen atom [15], cannot act as a donor of methyl groups although its affinity to the active center on the enzyme appears to be considerable.

With the purified betaine-homocysteine-methyl-transferase used in the present study, α-methyl betaine showed 13 % donor activity. The decrease in activity caused by the introduction of the α-methyl group in betaine is probably to a large extent due to steric hindrance. With a crude methyl-transferase from *Anodonta cygnea* the α-methyl betaine was found to have 45 % of the donor activity of betaine [3]. The reason for this discrepancy between the two enzyme preparations is not known. It is possible that the betaine-homocysteine-methyl-transferase of *Anodonta cygnea* has a different steric configuration around its active donor site than the betaine-homocysteine-methyl-transferase of pig liver. It is also conceivable that *Anodonta* liver contains more than one transferase. The difference between the results with the two enzyme preparations may of course be caused by the different degree of saturation at which the substrates were tested with the two transferases.

The latter explanation seems less plausible, however, as it has not been possible to increase appreciably the relative activity of α-methyl betaine for the transferase of pig liver by increasing its concentration in the incubation mixture several times above that used in Table 1.

Ergothioneine which has a larger substituent than the methyl radical in the α-position, can apparently not donate its methyl groups under the conditions of the test. It is of interest to notice, however, that ergothioneine is a very potent methyl-transferase inhibitor.

The length of the carbon chain of the ammonium compounds tested is obviously of vital importance for determining the ability to serve as a donor of methyl groups. Propiobetaine, butyrobetaine, carnitine and O-acetylcarnitine were all found to be completely inactive (Table 1). This is in contrast to the results obtained with the sulfonium compounds (Table 2). Not only dimethyl-acetothetin but also dimethyl-β-propiothetin and methyl methionine sulfonium chloride are excellent methyl group donors. Also the difference in behaviour of trimethyl ammonium chloride and trimethyl sulfonium chloride should be noted; the latter but not the former is able to act as methyl group donor.

Only a qualitative comparison can be made between the results reported here and those of other investigators. There is as far as this comparison is

Table 2. The ability of some sulfonium compounds to serve as donors of methyl groups using a preparation of betaine-homocysteine-methyl-transferase. The enzyme activity is also given for incubation mixtures containing both the test compound and betaine at the concentration ratios shown in the table. All incubation mixtures contained 0.025 M DL-homocysteine and 0.2 mg protein per ml.

Name	Relative activity		
	0.05 M of the compound alone	*x* M of the test compound and *y* M of betaine	
	%	%	*x* : *y*
Betaine	100	100	—
Dimethyl acetothetin	1 300	1 000 840	0.05 : 0.10 0.05 : 0.20
Dimethyl-β-propiothetin	850	200 170	0.05 : 0.10 0.05 : 0.20
Methyl methionine sulfonium chloride	1 200	350	0.05 : 0.20
S-Adenosyl-methionine iodide *	22	—	—
Trimethyl sulfonium chloride	500	125	0.05 : 0.10

* Tested at a concentration of 0.005 M.

possible, a general agreement between the methyl donor specificity of the horse liver thetin homocysteine methylpherase of Durell *et al.*[4], the rat liver thetin homocysteine transmethylase of Maw [5], the betaine-homocysteine-methyl-transferase from *Anodonta cygnea* [3] and the methyl transferase purified from pig liver. However, S-adenosyl-methionine was reported to be unable to donate its methyl group using the enzyme isolated from horse liver by Durell *et al.*[4] whereas this compound was found to possess donor activity with our pig liver transferase. Sufficient material has not been available to investigate further the reason for this difference in results. It should be mentioned that the S-adenosyl-methionine used by Durell *et al.*[4] was prepared by them, whereas the compound used in our studies was of commercial origin (California Corporation for Biochemical Research, Cal., USA). Shapiro [16,17] found that S-adenosyl-methionine prepared from yeast cells could donate its methyl group to homocysteine in cell free extracts from both rat liver and from some microorganisms.

Many betaine-like compounds that are not themselves methyl donors have the ability to inhibit the transfer of a methyl group from betaine to homocysteine (Table 1). This is for instance the case with dimethylglycine, betaine aldehyde, betaine amide, "triethyl betaine", propiobetaine, DL-carnitine, O-acetyl-DL-carnitine, ergothioneine, and muscarine [18]. The inhibition is most likely caused by competition between the inactive ammonium compounds and betaine for the active donor site on the transferase. Such a competition is probably also the reason for the decrease in transmethylase activity caused by some of the less potent methyl donors listed in Table 1, *e.g.* betaine nitrile, "mono- and diethylbetaine" and α-methyl betaine. The phenomenon is apparent also from the data in Table 2, where it is shown that betaine, which is less potent as a methyl donor than dimethyl-acetothetin, dimethyl-β-propiothetin, methyl methionine sulfonium chloride or trimethyl sulfonium chloride can markedly interfere with the methyl transfer from the latter compounds. In a previous communication from this laboratory [2] it was reported that all attempts have failed to separate the transferase that uses betaine as the methyl donor from a transferase that uses thetins. It was also observed at that time, that the conditions that altered the rate of transmethylation from betaine to homocysteine also altered in a similar manner the transfer of methyl groups from dimethyl-β-propiothetin to homocysteine [2]. All data accumulated so far therefore indicate that betaine and the thetins are bound to the same active center on the enzyme. However, further studies are necessary to prove conclusively that this is the case.

It appears likely from the observations presented in Table 1 that methyl donors of the ammonium type have to be bound at two (or more) points to the enzyme surface before they can perform their function.

It should be emphasized that the investigation described in this paper must be considered as being of an exploratory nature only. For a correct interpretation of the observations, the studies should be extended over a greater number of compounds. It is also necessary that the tests be performed under a variety of conditions to make possible kinetic evaluation of the data.

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