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Betaine-Homocysteine-Methyl-Transferases

I. Distribution in Nature

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Various types of living material have been homogenized and incubated with betaine and homocysteine in order to test their ability to form methionine by means of methyl group transfer. Enzymes that catalyze this reaction are called betaine-homocysteine-methyl-transferases and were found in the livers of all the vertebrates investigated, ranging from *Homo sapiens* to *Petromyzon fluviatilis*. These enzymes were not found in appreciable amounts in any other vertebrate organ except in the kidney of the guinea pig. The only non-vertebrate in which a betaine-homocysteine-methyl-transferase has been detected so far is the pond mussel *Anodonta cygnea* [4]. Methyl transferases were not found in plants and microorganisms.

The transferases were partially purified from some materials. Dimethyl-β-propiothetin but not choline could replace betaine as methyl group donor in all cases tested. Dimethylglycine but not choline markedly inhibited the transfer reaction. The methyl-transferase of human liver was much more stable to heat than that of pike liver. The apparent pH-optima of the transferases studied varied from about 7.0 for pike to about 8.5 for pond mussel. Only L-homocysteine seemed to be methylated.

In the present paper some observations are reported on the distribution in Nature of enzymes that catalyze the transfer of a methyl group form betaine (carboxymethyl-trimethyl-ammonium chloride) to homocysteine. These enzymes will be called betaine-homocysteine-methyl-transferases. A comparison of some of the properties of transferases of different origin will also be made. Methionine and dimethylglycine are produced in the enzymic reaction [1,2] and enzyme activity has been measured as the amount of methionine formed per hour.

Some of the results of this investigation have already been briefly mentioned elsewhere.[2–4] The existence of a betaine-homocysteine-methyl-transferase in *Anodonta cygnea*, for instance, has recently been reported from this laboratory [4] and will therefore not be dealt with in detail here.

EXPERIMENTAL

The material to be tested for betaine-homocysteine-methyl-transferase activity was homogenized in a Potter-Elvehjem-homogenizer (or crushed in a mortar and homogenized) in ice-cold 0.05 M phosphate buffer having a pH-value of 7.8. In some cases other pH-values were tried (see Table 1). Betaine hydrochloride (California Corporation for Biochemical Research, Cal., USA, or L. Light & Co., Ltd., England) and DL-homocysteine (Nutritional Biochemicals Corporation, Ohio, USA) were added to the homogenate and the mixture was incubated aerobically for 2—4 h at 37°C. Another mixture consisting of homogenate, DL-homocysteine and buffer was incubated under the same conditions and served as the blank.

Methionine was determined chemically by the method suggested by McCarthy and Sullivan [5] and modified by Borsook and Dubnoff [6] and others [7]. The red colour obtained upon addition of the hydrochloric-phosphoric acid mixture was developed overnight at 4°C [7]. The solution was then filtered and the light absorption at 5 100 Å was measured in a Beckman Model DU spectrophotometer. Also a microbiological method using *Leuconostoc mesenteroides* P-60 according to the procedure given by Steele *et al.*[8] was used for the estimation of methionine. Standard methionine solutions were prepared from L-methionine (Nutritional Biochemicals Corporation, Ohio, USA).

RESULTS

Distribution studies. Table 1 summarizes the results of the attempts to find betaine-homocysteine-methyl-transferase activity in various types of living material. Only when significant amounts of methionine could be detected by both the chemical and the microbiological methods of analysis was the presence in the incubation mixture of a methionine producing system considered as certain. A pig liver homogenate was always run at the same time as the unknown sample to ensure that the test system functioned. The data presented here de not permit a direct comparison of the concentrations of the transferases in the various materials, as the specific activity of the different transferases are not known. It is also probable [3] that the homogenates used contained inhibitors or activators. A very great variation in activity was furthermore encountered between different samples of the same test material, *e.g.* between different human livers.

With some organs the test was repeated many times. For instance, about forty human livers from patients suffering from various diseases were studied *. The data presented in Table 1 are the highest and the lowest values observed and give an indication of the variation in transferase activity that was encountered in these patients. In none of the cases could transferase activity be detected in the blood.

It can be seen in Table 1 that betaine-homocysteine-methyl-transferases were found in the livers of all vertebrate animals investigated ranging from *Homo sapiens* to *Petromyzon fluviatilis*. These enzymes could not be detected in any other organ of these animals except in the kidney of the guinea pig. Using dimethyl-β-propiothetin as the methyl donor, methyl group transfer was found to take place also in rat kidney homogenates. The pancreas, spleen, small intestine, lung, heart, striated muscle, testicles, cerebellum and cerebrum seemed to lack betaine-homocysteine-methyl-transferase activity.

* These samples were kindly supplied by Dr. W. Graf, St. Erik's Hospital, Stockholm.

Table 1. Distribution in Nature of betaine-homocysteine-methyl-transferases.

Name	Latin name	Material tested	pH of incubation mixture	Conc. in incubation mixture Bet.	Conc. in incubation mixture DL-Hcyst.	Transferase activity Chemical determ.	Transferase activity Microbiol. determ.
				mM	mM	μg L-methionine per g and h.	
Human	*Homo sapiens*	liver	7.8	110	24.6	125[a]	130
		»	7.8	110	24.6	690[b]	—
		blood	7.8	110	24.6	0	0
Monkey	*Macaca rhesus*	liver	7.8	5.5	12.3	510	450
		pancreas	7.8	5.5	12.3	0	40
		spleen	7.8	5.5	12.3	0	30
		small intestine	7.8	5.5	12.3	0	0
		cerebellum	7.8	5.5	12.3	0	0
		cerebrum	7.8	5.5	12.3	0	0
		liver	7.8	110	24.6	490	560
		pancreas	7.8	110	24.6	0	0
		spleen	7.8	110	24.6	0	0
		small intestine	7.8	110	24.6	0	0
		lung	7.8	110	24.6	0	0
		cerebellum	7.8	110	24.6	0	0
		cerebrum	7.8	110	24.6	0	0
Hedgehog	*Ecinaceus europaeus*	liver	7.8	110	12.3	2 600	2 650
Dog[c]	*Canis familiaris*	liver	7.8	5.5	12.3	250	250
		»	7.8	5.5	12.3	210	260
		kidney	7.8	5.5	12.3	0	0
		»	5.0	5.5	12.3	0	0
		pancreas	7.8	5.5	12.3	0	0
		»	5.0	5.5	12.3	0	0
		spleen	7.8	5.5	12.3	0	0
		»	5.0	5.5	12.3	0	0
		small intestine	7.8	5.5	12.3	0	0
		» »	5.0	5.5	12.3	0	0
Horse	*Equus caballus*	liver	7.8	110	24.6	1 200	1 450
		»	7.8	110	24.6	1 300	1 360
Pig	*Sus domesticus*	»	7.8	110	24.6	900	1 000
Calf	*Bos taurus juv.*	»	7.8	110	24.6	350	500
		»	7.8	110	24.6	400	400
Sheep	*Ovis aries*	»	7.8	110	24.6	170	180
Guinea pig	*Cavia cobaya*	liver	7.8	110	24.6	2 050	2 200
		kidney	7.8	110	24.6	1 000	1 100
		heart	7.8	110	24.6	—	0

[a] Lowest value observed. [b] Highest value observed. [c] (Alsatian) Police dog 7 years old.

Name	Latin name	Material tested	pH of incubation mixture	Conc. in incubation mixture Bet.	Conc. in incubation mixture DL-Hcyst.	Transferase activity Chemical determ.	Transferase activity Microbiol. determ.
				mM	mM	µg L-methionine per g and h.	
Rat	*Rattus norvegicus* (albino var.)	liver	7.8	110	24.6	750	850
		kidney	7.8	110	24.6	0	0[d]
		testicle	7.8	110	24.6	0	0
Rabbit	*Oryctologus cuniculus*	liver	7.8	110	24.6	800	850
Chicken	*Gallus domesticus* juv.	liver	7.8	5.5	12.3	125	—
		kidney	7.8	5.5	12.3	0	—
		pancreas	7.8	5.5	12.3	0	—
		liver	7.8	5.5	12.3	330	400
		kidney	7.8	5.5	12.3	0	10
		pancreas	7.8	5.5	12.3	20	0
		small intestine	7.8	5.5	12.3	0	40
		liver	7.8	110	24.6	780	810
Pigeon	*Columbia livia*	liver	7.8	110	24.6	530	650
Turkey	*Meleagris gallopavo*	liver	7.8	110	24.6	1 260	1 470
Grass snake	*Coronella austriaca*	liver	7.8	5.5	12.3	190	320
Frog	*Xenopus loevis*	liver	7.8	110	12.3	135	160
Pike	*Esox lucius*	liver	7.8	5.5	12.3	185	210
		roe	7.8	5.5	12.3	0	20
		muscle	7.8	5.5	12.3	20	35
		liver	7.8	5.5	12.3	185	310
		muscle	7.8	5.5	12.3	0	25
		liver	7.8	22	24.6	610	625
		muscle	7.8	22	24.6	0	0
Perch	*Perca fluviatilis*	liver	7.8	5.5	12.3	95	100
		soft roe	7.8	5.5	12.3	10	0
		muscle	7.8	5.5	12.3	0	0
		liver	7.8	5.5	12.3	170	250
		»	7.8	110	24.6	250	500
		»	7.8	110	24.6	540	620
Cod	*Cadus callarias*	liver	7.8	110	24.6	375	430
Burbot	*Lota lota*	liver	7.45	225	27.0	200	—
Lamprey	*Petromyzon fluviatilis*	liver	7.8	5.5	12.3	260	275
		rest	7.8	5.5	12.3	0	0

[d] Methionine was formed in kidney homogenates when dimethyl-β-propiothetin was used as methyl donor.

Name	Latin name	Material tested	pH of incubation mixture	Conc. in incubation mixture DL-Bet.	Conc. in incubation mixture DL-Hcyst.	Transferase activity Chemical determ.	Transferase activity Microbiol. determ.
				mM	mM	μg L-methionine per g and h.	
Ant	*Formica rufia*	whole	7.8	5.5	12.3	0	—
		»	4.5	5.5	12.3	—	20
		»	7.8	5.5	12.3	0	0
Bee	*Apis mellifica*	whole	7.8	5.5	12.3	0	0
		»	5.0	5.5	12.3	0	0
Banana fly	*Drosophila melanogaster*	whole	7.8	5.5	12.3	0	0
Spider	not identified	whole	7.8	5.5	12.3	0	0
Lobster	*Homarus gammarus*	intestine	7.8	22	12.3	0	65[e]
		»	6.0	22	12.3	0	40
		»	3.0	22	12.3	0	0
		liver	7.8	110	24.6	0	0
		»	6.0	110	24.6	0	0
		»	7.8	110	24.6	0	0[f]
Edible snail	*Helix pomatia*	liver	7.8	110	12.3	0	0
Cuttle-fish	*Eledone cyrrosa*	liver	7.8	110	24.6	0	0[e]
		»	6.0	110	24.6	60	0
		»	3.0	110	24.6	0	0
Sea mussel	*Mytilus edulis*	all except the shell	7.8	5.5	12.3	0	0
Mussel	*Cardium edule*	» »	7.8	5.5	12.3	0	0
Pond mussel	*Anodonta cygnea*	liver	7.8	110	12.3		g
Protozoa	*Tetrahymena pyriformis*	whole	7.8	110	24.6	0	0
Sugar beet	*Beta vulgaris* L. ssp. *esculenta* var. *altissima*	root	7.8	5.5	12.3	0	0
		»	2.7	5.5	12.3	0	0
		»	4.3	5.5	12.3	0	0
		»	6.4	5.5	12.3	0	0
		»	9.05	5.5	12.3	0	0

[e] Frozen to death.

[f] Liver excised while the animal was alive and immediately homogenized in ice-cold buffer and a portion of the homogenate pipetted into a solution of betaine and homocysteine.

[g] For results see Ericson, L.-E. *Nature* **185** (1960) 465.

Name	Latin name	Material tested	pH of incubation mixture	Conc. in incubation mixture Bet.	Conc. in incubation mixture DL-Hcyst.	Transferase activity Chemical determ.	Transferase activity Microbiol. determ.
				mM	mM	μg L-methionine per g and h.	
Oat	*Avena sativa*	seeds	7.8	5.5	12.3	0	0
Wheat	*Triticum sativum*	»	7.8	5.5	12.3	0	0
Mushroom	not identified	whole	7.8	5.5	12.3	25	7
»	*Pleurotus ostreatus* var. *pulmonarius*	cap with gills	7.8	5.5	12.3	0	0
Pea	*Pisum sativum*	pods + peas	7.8	5.5	12.3	0	15
Leek	*Allium porrum*	bulb	7.8	5.5	12.3	0	0
Birch	*Betula verrucosa*	leaves	7.8	5.5	12.3	0	—
		»	7.8	5.5	12.3	0	0
Elder	*Sambucus racemosa*	leaves	7.8	5.5	12.3	—	0
		»	7.8	5.5	12.3	0	0
Mountain-ash	*Sorbus aucuparia*	leaves	7.8	5.5	12.3	0	0
Potatoe	*Solanum tuberosum*	tuber + stem + leaves	7.8	5.5	12.3	0	0
Tomato	*Solanum esculentum* var. *ribesiforma*	stem + leaves	7.8	5.5	12.3	0	0
Tobacco	*Nicotiana rustica*	leaves	7.8	5.5	12.3	0	0
	Nicotiana silvestris	leaves	7.8	5.5	12.3	0	0[h]
		»	4.0	5.5	12.3	0	0
		»	9.0	5.5	12.3	0	0
Microorganisms	*Escherichia coli* 113—3	whole cells	7.8	110	12.3	0	0[i]
	Lactobacillus leichmannii	» »	7.8	110	12.3	0	0[i]
	Rhodotorula gracilis	» »	7.8	110	12.3	0	0[i]
	Saccharomyces carlbergensis	» »	7.8	110	12.3	0	0[i]
	Penicillium N_zA_{19}	» »	7.8	110	12.3	0	0[i]
	Streptomyces griseus	» »	7.8	110	12.3	0	0[i]

[h] Immediately after harvesting the leaves were put into ice-cold buffer to which betaine and homocysteine had been added.

[i] The cells were crushed in a mortar with sand and buffer at + 2°C.

With the method used betaine-homocysteine-methyl-transferase activity could not be detected in any non-vertebrate except in *Anodonta cygnea* [4]. Nor was it found in the plants or the microorganisms tested.

The chemical method of methionine determination gave in most cases slightly lower values than the microbiological method. The data indicate that only L-metionine was formed in the enzymic reaction and consequently that only L-homocysteine was methylated.

Some properties of the methyl-transferases from various sources. It has been shown by Sloane *et al.*[9] and from this laboratory [2-4,10] that dimethylglycine is a potent inhibitor of betaine-homocysteine-methyl-transferase. It is also well established that choline can not function as a donor of methyl groups with enzyme preparations lacking choline oxidase activity,[1,3,6,10-12] nor does choline interfere with the utilization of betaine.[2-4,10] It was considered of interest to determine whether the methyl-transferases of some of the organs investigated here fulfilled these criteria. Partially purified enzyme preparations were therefore made from human, monkey, chicken and pike livers and incubated with combinations of betaine, choline, dimethylglycine and homocysteine. The results, which are summarized in Table 2, indicate that dimethylglycine caused an almost complete inhibition of the methyl transfer when present in the incubation mixture at the same molar concentration as betaine. Choline had in no case an inhibitory effect nor did it act as a methyl donor. In other experiments, dimethyl-β-propiothetin was found to be an excellent methyl donor with these purified enzyme systems and with some liver homogenates from other animals.

Table 2. The influence of choline and dimethylglycine on the amount of methionine formed by betaine-homocysteine-methyl-transferases of different origin.

Enzyme purified from	Betaine 0.1 M	Choline 0.1 M	Dimethyl-glycine 0.1 M	DL-Homo-cysteine 0.015 M	Relative transferase activity %
Human liver	+	−	−	+	100
	−	+	−	+	0
	+	+	−	+	100
	+	−	+	+	0
Monkey liver	+	−	−	+	100
	−	+	−	+	0
	+	+	−	+	103
	+	−	+	+	5
Chicken liver	+	−	−	+	100
	−	+	−	+	0
	+	+	−	+	115
	+	−	+	+	7
Pike liver	+	−	−	+	100
	−	+	−	+	0
	+	+	−	+	102
	+	−	+	+	0

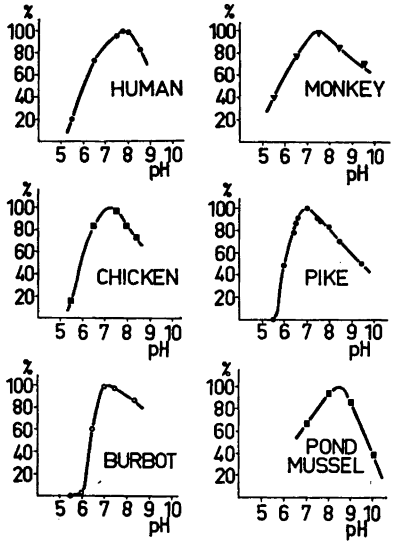

Fig. 1. Apparent activity-pH curves for betaine-homocysteine-methyl-transferases of different origin.

The apparent pH-optima of the transferases of human, monkey, chicken, pike and burbot livers were also determined. Fig. 1 shows the activity-pH curves for these enzymes and also, for the sake of comparison, the corresponding curve for the transferase of *Anodonta*-liver. The lowest apparent pH-optimum that was observed occurred at about 7 (pike) and the highest at 8.5 (pond mussel).

In connection with the purification of some of the transferases mentioned above it was noticed that the transferases of fish livers were much more *heat labile* than those of human, pig and rat livers. Crude and purified betaine-homocysteine-methyl-transferase of pig liver, for instance, could be kept at 80°C for several minutes without appreciable loss of activity (*cf.* Ref.[10]), whereas pike liver transferase suffered complete loss of activity from such treatment. This difference in heat stability is exemplified in Fig. 2. The curves shown in this figure were obtained with solutions of transferases purified from human liver and from pike liver containing approximately 0.5 % of protein and having a pH of 7.5. The solutions were heated at 60°C and the enzyme activity of samples taken at different time intervals was determined. The results show that the pike liver enzyme is much more sensitive to heat than the enzyme from human liver.

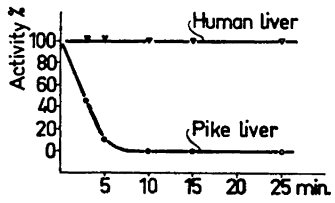

Fig. 2. A comparison of the temperature stability of betaine-homocysteine-methyl-transferase purified from human and from pike liver. The enzyme preparations were adjusted to pH 7.5 and kept at 60°C for various periods of time.

It might also be mentioned that the betaine-homocysteine-methyl-transferases of different origin behaved quite differently during the purification procedure. The transferase from human liver could be purified according to the first three steps used in the fractionation of the pig liver enzyme [10]. This was not possible with the transferase of pike liver. It was, as just mentioned, destroyed on heating at 80°C (step 2 in the fractionation of pig liver transferase [10]) and was not adsorbed on calcium phosphate gel under conditions which would retain the pig liver enzyme. The pike liver transferase was therefore purified by means of pH-adjustment and precipitation with ethanol.

DISCUSSION

The results of this investigation make it probable that betaine-homocysteine-methyl-transferases occur in the livers of all vertebrates. In other organs (*e.g.* kidneys) of vertebrates these transferases are encountered only occasionally.

A betaine-homocysteine-methyl-transferase has been found also in a member of the *Mollusca* [4]. This shows that formation of methionine by means of methyl group transfer does take place also in lower animals.

That betaine-homocysteine-methyl-transferases could not be observed in plants or in microorganisms must not be taken as conclusive evidence that they do not exist in such materials. It may only show that the method used was unsuitable for their detection. However, to the author's knowledge, no evidence for the transfer in plants or microorganism of an intact methyl group from betaine or from a thetin to homocysteine has yet been provided by any other research group. Dubnoff [13,14] found that in the presence of homocysteine, dimethyl-β-propiothetin but not betaine could replace the vitamin B_{12}-requirement of a mutant of *E. coli* but states [14] that "this reaction is not the simple methyl transfer demonstrated in animal tissues". Schlenk and De Palma [15] could not demonstrate any methyl group transfer from betaine, choline, dimethyl-acetothetin or dimethyl-β-propiothetin to homocysteine in *Torulopsis utilis*. Dimethyl-acetothetin and dimethyl-β-propiothetin are inactive as methyl donors also in *Aerobacter aerogenes*, *Saccharomyces cerevisiae* and some strains of *E. coli* [16]. Other examples of the inability of choline, betaine and the thetins to serve as donors of methyl groups in microorganisms have been cited.[17] — Challenger and his collaborators have isolated dimethyl-β-propiothetin from red [18] and green [19] algae but no evidence is as yet available indicating that this compound can donate its methyl groups to homocysteine in these or other plants. Dimethyl-acetothetin, although a potent methyl group donor in animal systems, has not been found to occur naturally.

On the other hand, methyl group transfers *from* methionine derivatives * to various acceptors take place in both microorganisms and plants as well as in animals. This has been amply demonstrated for microorganisms by Schlenk and De Palma [20], Shapiro [16,21,22], Alexander and Schwenk [23], Parks [24], Melville *et al.*[25], Birch *et al.*[26] and Remy [27], and for plants by Byerrum *et al.*[28–30],

* Dimethyl-β-propiothetin is here not considered as a derivative of methionine although it could be looked upon as a breakdown product of this amino acid.

Kirkwood *et al.*[31,32] and Mudd [33,34]. It is of interest to recall that in some of these reactions methionine is produced by methyl group transfer to homocysteine. Examples of such reactions are

S-methylmethionine + homocysteine → 2 methionine

and

S-adenosylmethionine + homocysteine → S-adenosyl homocysteine + + methionine

which have been studied in particular by Shapiro [16,21,22] and by Schlenk and De Palma [20]. S-Methylmethionine stimulated methionine formation also with the horse liver "thetin-homocysteine methylpherase" of Durell *et al.*[12], with the "thetin-homocysteine transmethylase" purified from rat liver by Maw [35] and with our betaine-homocysteine-methyl-transferase from pig liver [10]. For the moment it therefore seems that S-methylmethionine and betaine are interchangeable as methyl donors to homocystein in animal but not in microbial transferase reactions.

After this investigation had been completed and the results prepared for publication, a paper by Maw appeared [36] which dealt with the distribution of thetin-homocysteine transmethylase. The data presented here are in general agreement with the conclusions presented by Maw.

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