STUDIES ON THE SUBSTRATE SPECIFICITY OF THE PHOSPHATIDE METHYLATING SYSTEM OF MICROSOMES*

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Received October 23, 1961

Bremer and Greenberg (1961) observed that the *in vitro* addition of d,l-(N,N-dimethyl) distearoyl-α-cephalin enhanced the incorporation of C^{14}-methyl into choline by the microsome methylating system, while commercial dipalmitoyl-α-cephalin was inactive. Gibson, Wilson and Udenfriend (1961) similarly observed a lack of C^{14}-methyl incorporation with brain phosphatidylethanolamine or phosphatidyl serine. In this report evidence is presented to show that the same enzyme system will methylate monomethyl 1-(α)-distearoyl cephalin, but not 1-(α)-distearoyl cephalin.+

Experimental

The microsomal fraction of rat liver and radioactive S-adenosyl methionine-$C^{14}H_3$ were prepared as described previously (Bremer and Greenberg, 1961). Measurement of the radioactivity of the N-butanol extractable material was also performed as described in the above mentioned reference. The protein content of the microsomal suspension was determined by a biuret procedure (Layne, 1957).

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*Aided by research grants from the National Science Foundation (G12895) and the National Heart Institute (H 3074), National Institutes of Health (U.S. Public Health Service).

+ We are greatly indebted to Dr. Erich Baer of the University of Toronto for the generous gift of these phosphatides.

The incubations to test the substrate activity of the synthetic cephalins recorded in Table I were performed by incubating the amounts of rat liver microsomes shown in the table with S-adenosylmethionine (0.46-0.60 μmoles, 31,000 - 51,000 cts/min) Tris-HCl buffer (100 μmoles, pH 6.6) and the indicated amount of one of the cephalins in a total volume of 1 ml for 1 hour at 37°. In the incubations containing cholic acid, this was added to the phospholipid suspension in Tris buffer before addition of the microsomes or radiosubstrate. The mixture was then heated to boiling to produce an emulsion.

After incubation the mixture was extracted with N-butanol as described by Bremer and Greenberg. To demonstrate that the stimulation of incorporation really was the result of increased labelling of the phospholipid bases with methyl-C^{14} the butanol layer from incubation No. 1 was evaporated to dryness and the solid residue extracted with petroleum ether (B.P., 30-60°) and chloroform. The extract was then evaporated to dryness and 5 mg each of the appropriate phospholipid bases was added in dilute HCl. The mixture was hydrolyzed in 1.0 N HCl for 16 hours and chromatographed on Dowex 50 as described by Bremer and Greenberg.

A second experiment of the same nature at a lower cholic acid concentration was also run with results shown in Table II: microsomes (21.3 mg protein) were incubated with N-methylcephalin (3.08 mg) and S-adenosylmethionine (1.2 μmoles, 112,000 cts/min) in Tris-HCl buffer (200 μmoles, pH 8.6) with the addition of 2 mg cholic acid. As a control an identical incubation was run without N-methylcephalin. Other conditions were the same as in the experiments of Table I.

Results

Table I shows that the synthetic N-monomethylcephalin yielded a considerable increase in the radioactivity of the n-butanol extractable fraction. Hydrolysis and fractionation of the phospholipid bases of Experiment No. 1 in the manner described above, gave the following distribution of the $C^{14}H_3$ in the phosphatide bases: methylaminoethanol 0, dimethylaminoethanol 30, and choline 12.

Table I

Effect of Synthetic Cephalins on Incorporation of Methyl Groups

Experiment No.*	Methyl-cephalin mg	Cephalin mg	Cholic acid mg	Microsome protein mg	% Adenosylmethionine counts in n-butanol layer Total	per mg protein
1	1.4		5.0	15.6	54.0	3.46
2	0		5.0	15.6	4.84	0.31
3	1.0		2.0	26.0	46.2	1.78
4	0		2.0	26.0	12.0	0.46
5	0.9		0	24.0	15.4	0.64
6	0		0	24.0	16.3	0.68
7		1.5	1.0	22.6	14.5	0.64
8		0	1.0	22.6	13.3	0.59
9		2.48	1.0	10.6	9.8	0.92
10		0	1.0	10.6	10.4	0.98

* With a high ratio of cholic acid to protein in the incubation incorporation was inhibited, with a lower ratio there was poor stabilization of the added phospholipid suspension. Consequently the concentration of cholic acid was individually adjusted in each experiment. This makes the results comparable only in each experimental pair (No.1 with No. 2, No. 3 with No. 4, etc.).

The results given in Table II show a 11% conversion of the adenosylmethionine methyl in the butanol layer with the N-methylcephalin and 8.6 without.

Table II

Column Chromatographic Analysis of Butanol Extracts of N-Monomethylcephalin Incubations

	Net cts/min in butanol layer	% Conversion	Ratioactivity in Methyl aminoethanol % of original counts	Dimethyl aminoethanol	Choline in $C^{14}H_3$
With methylcephalin	13,200	11.0	0	1.8	11.2
Without methylcephalin	9,680	8.6	0	0	9.6

Column chromatography of the hydrolysate of the phospholipid bases indicated the occurrence of radioactive dimethylaminoethanol in the presence of the monomethylcephalin and only choline in its absence (Table II). This parallels the results obtained in the experiments described in Table I.

Discussion

These results and those of Bremer and Greenberg (1961) show that in order for the microsome methylating system of rat liver to methylate cephalin readily, the phospholipid must already be substituted in the amino group of the aminoethanol moiety. This implies that the introduction of the first methyl group by this system into the cephalin molecule is the rate limiting step in the synthesis of lecithin, as has already been suggested by Bremer and Greenberg (1961).

The lower amount of dimethylaminoethanol found in the second experiment could have resulted from the use of a lesser amount of cholic acid to solubilize the phospholipid. This may have caused less substrate to be available for the reaction.

It is interesting to note that the amount of radioactivity in the C^{14}-choline eluted from the columns after hydrolysis varied little among the experiments reported here (e.g. 12.0; 11.2; 9.6%). This could be taken to show that the increase in radioactivity in the phospholipids in the presence of added N-methylcelphalin was due to an increased amount of N-dimethylcephalin which would mean that none of the added N-methylcephalin was converted to lecithin. To resolve this problem the use of a P^{32}-labelled phosphatide is indicated.

References

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