Purification of a N-Methyltransferase

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In order to study the mechanism of N-methylation a method has been worked out for the preparation of a pure N-methyltransferase. The enzyme selected for this purpose is histamine-N-methyltransferase which has previously been partially purified by Lindahl-Kiessling [1] and Brown *et al.*[2] The present communication describes the purification procedure and some inhibition experiments performed with the purified enzyme.

Determination of enzyme activity. 0.0164 μmole histamine-2-(ring)-^{14}C dihydrocloride, 0.163 μmole S-adenosylmethionine, 100 μl 0.5 M Tris buffer, pH 8.1, and 100 μl enzyme solution in a total volume of 220 μl were incubated in test tubes at 37°C for 30 min. The reaction was stopped by adding 100 μl of 10 N NaOH saturated with Na_2SO_4. The enzymatically formed methylhistamine was then extracted with 1000 μl of chloroform by shaking the sealed test tubes for 10 min. After centrifugation 200 μl of the chloroform phase were withdrawn and the radioactivity measured in a liquid scintillation counter.

Protein determination. Protein concentration of enzyme fractions were routinely estimated from the absorption of ultraviolet radiation at wave-lengths of 2800 Å and 2600 Å as described by Kalckar [3]. In some cases the results were checked with the procedure of Lowry *et al.*[4]

Enzyme preparation. About 200 g of frozen pig liver were cut into pieces and homogenized in 400 ml cold 0.1 M NaAc buffer, pH 5.6. After centrifugation at 40 000 *g* for 30 min, the supernatant was fractionated at -2°C with a mixture of ethanol and water (1:1). The precipitate formed between an alcohol concentration of 25—35% was collected by centrifugation and dissolved in 90 ml of 0.1 M NaAc buffer, pH 5.6, and dialyzed against the same buffer for 3 h. The material was poured into a 250 ml flask which was put into a water bath having a temperature of 41°C and kept there for 5 min. The enzyme solution was vigorously shaken and the temperature was not allowed to exceed 40°C. After heat denaturation the solution was immediately chilled in an ice bath and centrifugated at 10 000 *g* for 5 min. The supernatant was dialyzed over night against 2 l of 0.005 M sodium phosphate buffer, pH 7.5, containing EDTA at a concentration of 0.001 M. The precipitate formed during dialysis was removed by centrifugation at 10 000 *g* for 5 min. In the subsequent steps negative adsorption on a CM-cellulose column was used followed by positive adsorption on a DEAE-cellulose column. The columns had an inner diameter of 25 mm and were equipped with a glass filter disc at the bottom. The Whatman celluloses used were washed according to the directions of Peterson and Sober [5] and thoroughly equilibrated with 0.005 M phosphate buffer, pH 7.5. For the negative adsorption 5 g of CM-cellulose treated as above were allowed to settle by gravity and was then gently packed to give a height of 130 mm. The dialyzed material was applied to the column and allowed to soak in by gravity. When the protein solution had entered, the column was eluated with 0.005 M phosphate buffer, pH 7.5, until the optical density of the eluate at 2800 Å was less than 0.1. The eluate was then added to a DEAE-cellulose column packed as the CM-cellulose column above. When the solution had soaked in, 10 ml of 0.005 M phosphate buffer, pH 7.5, were used to rinse the top of the column followed by about 150 ml of 0.1 M phosphate buffer, pH 7.5. This step removes more than 95 % of total protein applied, leaving the enzyme on the column. The enzyme was eluted either directly with 0.15 M NaAc buffer, pH 4.6, or by using a gradient elution. In the latter case the mixing vessel initially contained 250 ml of 0.005 M NaAc buffer, pH 5.6, and the reservoir 500 ml of 0.15 M NaAc buffer, pH 4.6. When 5 ml fractions were collected, the protein as well as the enzyme activity appeared in one peak around fraction number 20. The eluate obtained in this way can be stored at + 4°C without adjusting pH or at -20°C after dialysis against phosphate buffer pH 7.5 without loosing activity. A typical result of the fractionation described above is summarized in Table 1. The increase of specific activity calculated from the first supernatant is about 260 times. Moreover, there is about a two-fold purification not accounted for here, from the homogenate to the first supernatant.

Ultracentrifugation, starch gel electrophoresis at pH 7.5 and 5.6 and the sym-

Table 1. Summary of purification procedure.

Step	Total protein mg	Total volume ml	Specific activity units/mg *	Total units	Recovery %
40 000 *g* supernatant	26 000	350	1.2	31 000	100
Ethanol precipitate	2 200	90	5.7	12 500	40
Heating at 40°C	1 900	90	5.3	10 100	33
Dialysis at pH 7.5	1 500	90	6.9	10 400	33
CM-cellulose column	1 000	150	11.3	11 300	36
DEAE-cellulose column	7	24	313	2 200	7

* 1 unit = that amount which will catalyse the formation of 1 mμmole methylhistamine under the conditions described in the text.

metrical curves of protein content and enzyme activity about the same fraction number during chromatography on DEAE-cellulose reveals an essential homogeneous enzyme preparation.

Inhibitors. In order to test the influence of some inhibitors the same procedure as for testing enzyme activity was used, the incubation time now being 60 min. The reaction mixture including the inhibitor was preincubated for 15 min before adding the substrate.

Of the common SH-inhibitors *p*-chloromercuribenzoate and phenylmercuriacetate at a concentration of 10^{-5} M and N-ethylmaleimide at a concentration of 10^{-4} M inhibited the reaction to 100 %. At a concentration of 10^{-3} M iodoacetamide and *o*-iodobenzoate showed an inhibition of 15 %. In all cases the inhibition was fully counteracted by adding the corresponding concentrations of reduced glutathione to the reaction mixture before preincubation.

The effect of some drugs on the enzyme reaction is summarized in Table 2.

Table 2.

Inhibitor	Inhibition % Inhibitor conc. M		
	10^{-5}	10^{-4}	10^{-3}
Bufotenine	6	21	
Chloropromazine	16	50	75
LSD	8	23	
Br−LSD	20	30	
Mescaline		0	21
Isomescaline		0	26
Serotonin	8	20	
Tryptamine	13	44	

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