CHARACTERIZATION OF AN ENZYME YIELDING FORMALDEHYDE FROM-5-METHYLTETRAHYDROFOLIC ACID

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1. Introduction

In 1972, 5-methyltetrahydrofolic acid was reported to be the methyl donor in the enzymatic N-methylation of dopamine into epinine in the brain [1]. Afterwards, the same reaction was described for various biogenic amines in several tissues [2–8]. Although the enzyme has been extensively characterized [4,5] two main problems are requiring further elucidation. Firstly, when using indolealkylamines as substrate, we were unable to identify the corresponding N_{ω}-, N_1- or O-methylated compounds. Secondly, abnormally high blank values were often observed especially when the incubation was run without substrate, the latter being added to the mixture after fixation [9]. One could infer from this that the reaction was more than just a methyltransfer.

Recent investigations allowed us to identify the reaction mechanism by which the labelled methyl of 5-methyltetrahydrofolic acid is incorporated in the biogenic amines [9]. The purpose of the present paper is to describe a formaldehyde forming-enzyme, which should catalyze the initial step of the reaction, and to compare its properties with those of the previously described 5-methyltetrahydrofolate-N-methyltransferase (5 MT-NMT).

2. Materials and methods

Enzyme from pig brain and rat kidney was prepared by ammonium sulphate precipitation (30–60%) as previously described [5]. Further purification was achieved using a DEAE A-50 Sephadex column (1.5 × 25 cm). To this purpose an ammonium sulphate

enzyme fraction was first dialyzed against a buffered solution (0.05 M triethanolamine pH 6.9, 10^{-3} M EDTA and 0.1% NaN_3) and loaded on the column. Elution was performed by initiating a linear gradient of 0.5 M buffered NaCl in a mixing chamber containing 150 ml of the same buffer.

The formaldehyde formation was measured in incubation mixtures containing 0.25 μCi 5-[^{14}C-methyl]tetrahydrofolic acid (spec. act. 61 mCi/mmole, Radiochemical Centre, Amersham), 50 μmole sodium phosphate buffer pH 6.4 and an enzyme aliquot in a total volume of 0.5 ml. After incubation at 37°C, the reaction was stopped by chilling in ice and adding 0.1 ml of 100 mM mercaptoethanol. Then the mixture was diluted with 1.5 ml of 0.2 M ammonium acetate and passed through a DEAE A-25 Sephadex column (1 × 10 cm), previously equilibrated with a solution containing 0.2 M ammonium acetate and 10 mM mercaptoethanol. By washing the column with an additional 10 ml of the same solution, [^{14}C]formaldehyde was collected in the effluent and separated as such from 5-methyltetrahydrofolate and its derivatives which all remained bound to the resin. An aliquot of the effluent was immediately counted for the radioactivity in a liquid scintillation spectrometer. The [^{14}C]formaldehyde was further identified by the dimedon precipitation method [11] slightly modified as follows: the column effluent (10 ml) was brought up to pH 4.5 with 1 M acetic acid, 10 ml of a 0.4% aqueous dimedon solution and then 0.100 ml of a 4% formaldehyde solution were added. In this way a 20% molar excess of dimedon over formaldehyde was maintained. The formaldemethone was allowed to settle overnight at 4°C and then the precipitate was collected by sucking through a small millipore filter (0.45 μ).

Table I

Recovery of standard [^{14}C]formaldehyde after DEAE A-25 Sephadex column and dimedon precipitation in different conditions

Formalde-hyde amount	Effluent composition	Radioactivity in CPM $\times 10^{-3}$ (% of input)					
		Column effluent		Dimedon precipitate		Dimedon filtrate	
0.5 μCi	0.2 M NH$_4$ acetate	622	(84)	590.8	(80)	84.8	(11)
0.1 μCi		123.6	(85)	110.3	(76)	20	(14)
0.02 μCi		22.2	(83)	18.9	(71)	4.4	(16)
0.5 μCi	0.2 M NH$_4$ acetate	650	(88)	418.6	(57)	261	(35)
0.1 μCi	+ 10 mM mercaptoethanol	136	(94)	83	(57)	52	(36)
0.02 μCi		25.6	(95)	16	(59)	10	(37)
0.5 μCi	0.2 M NH$_4$ acetate	656	(89)	447	(60)	244	(33)
0.1 μCi	+ 10 mM mercaptoethanol	123	(85)	85	(59)	59	(41)
0.02 μCi	+ enzyme aliquot (2.6 mg prot.)	26	(96)	16	(59)	11	(40)

After washing with 5 ml of the 0.4% dimedon solution the filter was transferred to a counting vial and radioactivity was measured. Blanks were obtained from incubation mixtures at zero time. Control experiments using several amounts of standard [^{14}C]formaldehyde (spec. act. 4.4 mCi/mmole, Radiochemical Centre, Amersham) were run in various conditions as indicated in table 1.

5 MT-NMT, histamine-N-methyltransferase and catechol-O-methyltransferase were assayed according to methods already described [5,11,12].

3. Results and discussion

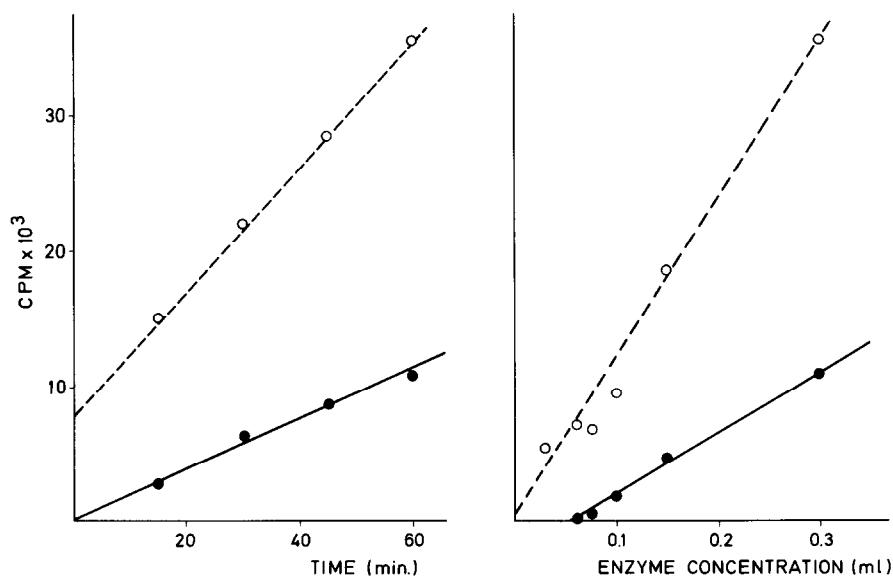Table 1 shows the efficacy of the dimedon precipi-

Fig. 1. Labelled formaldehyde formed in mixtures incubated with pig brain as a function of time and enzyme concentration. Radioactivity was measured in the DEAE column effluent (o– – –o) and dimedon precipitate (●———●).

tation using standard [^{14}C]formaldehyde under various experimental conditions. With mercaptoethanol only 60% of labelled formaldehyde was precipitated. Since 5-methyltetrahydrofolate interacts with dimedon, a prior formaldehyde isolation on column was needed and with this technique mercaptoethanol addition was necessary to prevent the degradation of folate derivatives. The results as presented in table I do not reveal any significant interaction between formaldehyde and proteins. This was also confirmed by acid precipitation.

In order to assess the enzymatic formation of formaldehyde, 5-[^{14}C-methyl] tetrahydrofolate was incubated with pig brain or rat kidney enzyme and radioactivity was measured in the column effluent and after dimedon precipitation. As shown in fig. 1, the formaldehyde production followed a linear course along with time (up to 60 min) and also in direct proportion to the enzyme concentration. The discordance in radioactivity between the column effluent and the dimedon precipitate may partially by explained by the 60% recovery due to mercaptoethanol, however a secondary reaction with 5-methyltetrahydrofolate cannot be ruled out. As shown in fig. 2, optimum activity was reached between pH 6 and 6.5, which is quite similar to the pH curve previously described for 5 MT-NMT [4,5]. Similarly, fig. 3 shows an identical thermal

inactivation curve in formaldehyde production as in 5 MT-NMT activity, when 4-methoxy-3-hydroxy-
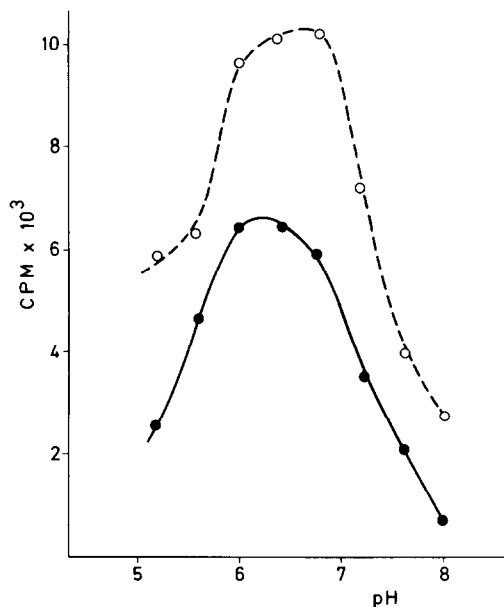

Fig. 2. Influence of pH on the labelled formaldehyde formation with a rat kidney enzyme. Radioactivity was measured in the DEAE column effluent (o————o) and dimedon precipitate (●————●).
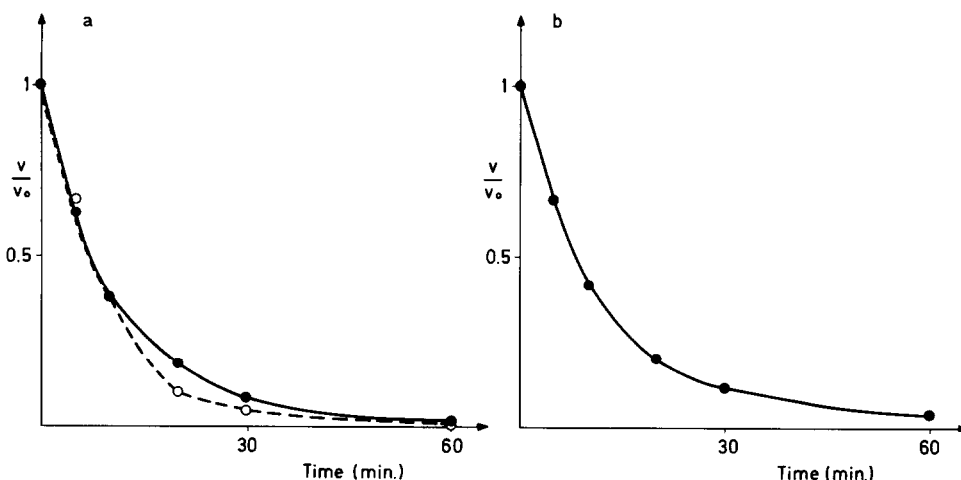

Fig. 3. Thermal inactivation of a rat kidney enzyme preincubated at 45°C as a function of time. a) labelled formaldehyde was measured in the DEAE column effluent (o————o) and dimedon precipitate (●————●) after incubation of 5-[^{14}C methyl] tetrahydrofolate at 37°C with preincubated enzyme. b) 5 MT-NMT activity was measured from the same preincubated enzyme fractions using 4-methoxy-3-hydroxyphenylethylamine as substrate.
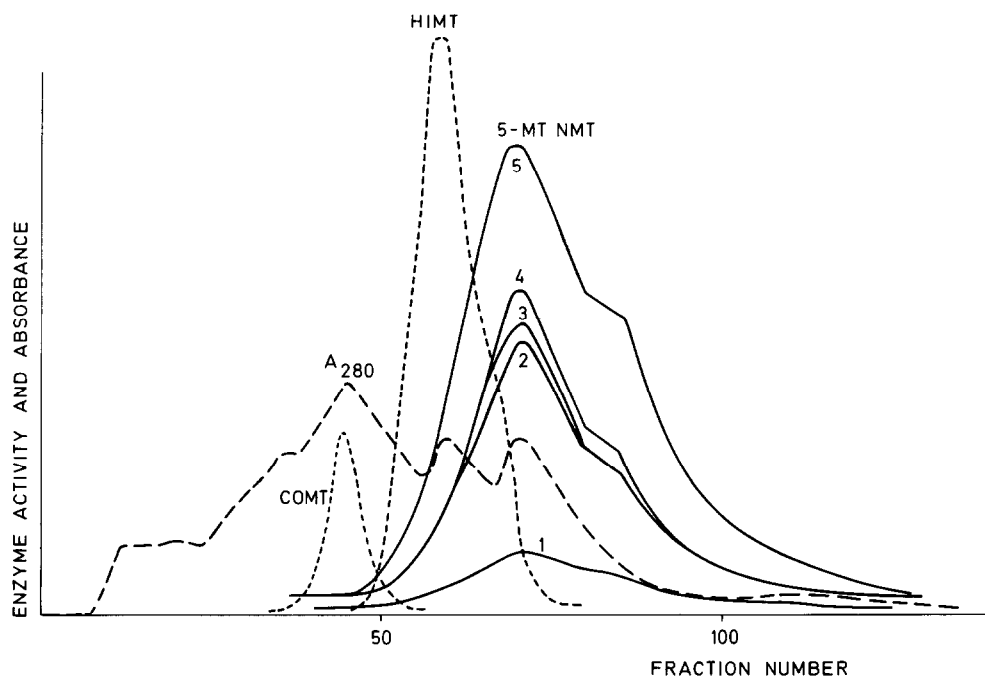
Fig. 4. Elution pattern of an ammonium sulphate enzyme preparation from rat kidney through a DEAE A-50 Sephadex column (cf. Materials and methods). Absorbance at 280 nm (- -). Histamine N-methyltransferase (HINMT) and catechol-O-methyltransferase (COMT) (- - - -), 5 MT-NMT activity (———) was measured: 1) without amine substrate, 2) with tryptamine, 3) 5-hydroxytryptamine, 4) N_1-methyltryptamine and 5) 4-methoxy-3-hydroxyphenylethylamine as substrate.

phenylethylamine was used as substrate. Finally as shown in fig. 4, several enzyme activities were measured in fractions obtained after purification of rat kidney enzyme on DEAE A-50 Sephadex column. Enzymes requiring S-adenosyl-methionine, like histamine N-methyltransferase and catechol-O-methyltransferase, presented a different pattern, not only as compared to each other, but also to 5 MT-NMT. For this latter an identical elution pattern was obtained using either tryptamine, 5-hydroxytryptamine, N_1-methyltryptamine or 4-methoxy-3-hydroxyphenylethylamine as substrate. Moreover by incubating the fractions without substrate a similar distribution pattern of radioactivity was obtained, presumably due to labelled formaldehyde extracted in the isoamyl alcohol toluene (2:3) mixture. Control experiments have shown that only 15% of formaldehyde can be extracted in this way. The foregoing results provide evidence that formaldehyde may be formed enzymatically from 5-methyltetrahydrofolate in various tissues. Since the enzyme displays the same

physical properties as 5 MT-NMT, it might be suggested that the same protein is involved. Rather as a direct methyltransfer to the nitrogen, the enzyme should in a first step catalize the formaldehyde production, which then further reacts with biogenic amines. This was confirmed by mass-spectrometrical identification of tetrahydroisoquinoline and β-carboline in 5 MT-NMT incubations using catecholamines and indolealkylamines respectively [13]. The same products were found in reaction mixtures just containing formaldehyde and amine substrates in buffer thus involving a Pictet–Spengler condensation. Consequently, this reaction occurs spontaneously and represents the second step in alkaloid formations, in contrast with the first step of the reaction where formaldehyde is formed enzymatically. The present results bring evidence of this enzymatic process; whether the aldehyde formation may occur in vivo remains to be further elucidated. Nevertheless, it is very likely, since alkaloids, such as tetrahydroisoquinoline and tetrahydropapaveroline

derivatives were detected in the urine of parkinsonian patients treated with L-Dopa [14]. If formaldehyde is indeed produced in vivo through this pathway, it is tempting to postulate that, because of its high affinity for biogenic amines and probably also for certain macromolecules, this formaldehyde production could play some role in the ageing process.

Acknowledgements

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