THE FORMATION OF β-CARBOLINE ALKALOIDS MEDIATED BY SERINE HYDROXYMETHYLTRANSFERASE

Andrew G. M. PEARSON* and Anthony J. TURNER
Department of Biochemistry, University of Leeds, 9 Hyde Terrace, Leeds LS2 9LS, England

Received 2 November 1978

1. Introduction

5-Methyltetrahydrofolate (methyl-FH_4) has been reported to be the methyl donor in the enzymic N-methylation of biogenic amines in several tissues [1–5]. However a more rigorous analysis of the reaction has shown that N-methylation does not occur and that the transfer of one-carbon groups to biogenic amines from methyl-FH_4 results in the formation of alicyclic alkaloids [6–8]. These results have been interpreted in terms of an enzyme that cleaves methyl-FH_4 to produce FH_4 and formaldehyde, the latter subsequently reacting non-enzymically with the amines to produce alkaloids in a Pictet-Spengler condensation [9–11]. Our investigations into the mechanism of this reaction led us to the conclusion that the enzyme responsible for the transfer of one-carbon units from methyl-FH_4 to biogenic amines may be 5,10-methylene-FH_4 reductase (EC 1.1.1.68) [12]. A consequence of this mechanism is that methylene-FH_4, formed enzymically, is an intermediate in the transfer of the one-carbon unit (fig.1a).

If this hypothesis is correct, then other reactions generating methylene-FH_4 should be capable of mediating in the synthesis of alicyclic alkaloids from biogenic amines. One such enzyme is serine hydroxymethyltransferase (EC 2.1.2.1), which catalyses the reaction shown in fig.1b. Here we have tested the ability of serine hydroxymethyltransferase to transfer the β-carbon atom of serine to the biogenic amine tryptamine, with the resultant formation of the alkaloid 1,2,3,4-tetrahydro-β-carboline (fig.1).

Address correspondence to: A. J. T.

* Present address: Department of Biochemistry, University of the West Indies, Mona, Kingston 7, Jamaica

2. Materials and methods

Serine hydroxymethyltransferase was purified from ox liver by the method in [13], and shown to possess no detectable methylene-FH_4 reductase activity. Methylene-FH_4 reductase was purified from pig liver as in [12]. 1,2,3,4-Tetrahydro-β-carboline hydrochloride was synthesised by the method in [14]. The melting point and infrared spectra of the product were in agreement with data in [8,11]. Mass spectral analysis of the product showed a molecular ion at 172 amu, the base peak at 143 amu (loss of CH_2NH) and a fragment ion at 115 amu (indole nucleus) in agreement with [8]. All other chemicals and materials were obtained from standard commercial sources.

Serine hydroxymethyltransferase (0.05 units) was incubated in 0.5 ml total volume with the following components: 30 μmol potassium phosphate buffer (pH 7.4), 0.1 μmol pyridoxal 5′-phosphate, 0.1 μmol (0.5 μCi) L-[3-^{14}C]serine, 0.8 μmol FH_4, 4 μmol 2-mercaptoethanol and 10 μmol tryptamine hydrochloride. Blank incubations were performed both in the absence of FH_4, and with enzyme that had been boiled for 5 min. The conditions of the incubations were essentially those used in the routine assay of serine hydroxymethyltransferase activity [15].

Methylene-FH_4 reductase (0.04 units) was incubated in 0.6 ml total volume with the following components: 100 μmol potassium phosphate buffer

(pH 6.3), 0.33 μmol (0.5 μCi) 5-[^{14}C]methyl-FH_4, 1.2 nmol FAD, 1.0 μmol EDTA, 4.8 μmol ascorbate, 0.3 μmol menadione(2-methyl-1,4-naphthoquinone) and 10 μmol tryptamine hydrochloride. Blank incubations were performed with enzyme that had been boiled for 5 min. The conditions of the incubations were essentially those used in the routine assay of this enzyme [16].

All incubations were performed at 30°C for 30 min. The reactions were then stopped by the addition of 1.0 ml 0.6 M borate buffer, pH 10, and to each tube was added 10 μmol of either *N*ω-methyltryptamine or 1,2,3,4-tetrahydro-β-carboline hydrochloride. The products were extracted into toluene, evaporated to dryness and the residues taken up in acetone. Samples from each incubation were applied to a silica-gel thin-layer plate (Merck F254) which was developed in butan-1-ol/acetic acid/H_2O (60 : 30 : 10, by vol.), dried and sprayed with a solution of *p*-dimethylaminocinnamaldehyde to visualise the products. The respective product zones were scraped from the plate and their radioactivity determined.

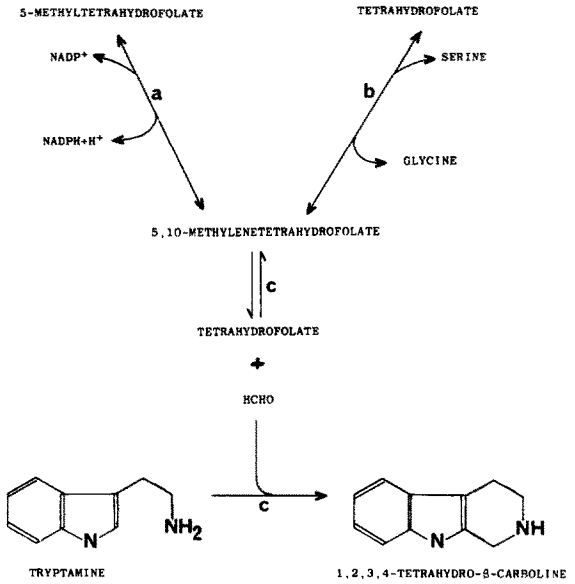

Fig.1. Proposed role of 5,10-methylenetetrahydrofolate in the transfer of one-carbon groups to biogenic amines. The enzymes involved are: (a) methylenetetrahydrofolate reductase (EC 1.1.1.68) and (b) serine hydroxymethyltransferase (EC 2.1.2.1). The condensation to form tetrahydro-β-carboline (reaction c) is presumed to be non-enzymic.

3. Results and discussion

Methylene-FH_4 reductase was shown to mediate in the formation of tetrahydro-β-carboline from tryptamine and methyl FH_4 (table 1), in agreement with the conclusions of other groups [6,8,10]. The probable mechanism of this one-carbon transfer process has been described in [12] and is summarized in fig.1. The results reported here (table 1) demonstrate that serine hydroxymethyltransferase can transfer the β-carbon atom of serine to tryptamine in

Table 1
Thin layer chromatography of the products of the incubation of serine hydroxymethyltransferase and methylene-FH_4 reductase with tryptamine

Enzyme incubation conditions	Radioactivity recovered from product zone (dpm)	
	THBC	NMT
Serine hydroxymethyltransferase	1500	360
Serine hydroxymethyltransferase (boiled)	200	20
Serine hydroxymethyltransferase (-FH_4)	130	–
Methylene-FH_4 reductase	600	20
Methylene-FH_4 reductase (boiled)	20	–

The experimental conditions are as described in the text

Abbreviations: THBC, Tetrahydro-β-carboline; NMT, *N*ω-methyltryptamine

a reaction dependent upon the presence of FH_4 which would further substantiate the proposed mechanism for β-carboline synthesis. A small amount of radioactivity was apparently isographic with the methyltryptamine as reported [10], but the major product was isographic with the β-carboline. No radioactivity was recovered from the product zones if the incubations were carried out in the absence of tryptamine. The difficulty encountered in separating the *N*-methyl amine from the cyclised amine led to the original confusion as to the nature of the product found [6–8]. Several different solvent systems were used in an attempt to increase the resolution on the chromatogram, and the one chosen afforded the best separation of those tested. I^+ is at present unclear why the proportion of radioactivity apparently isographic with the methyltryptamine is greater when serine hydroxymethyltransferase is used, although this may well be a reflection of the different incubation conditions required for the two enzymes. The major product in both cases was clearly the β-carboline.

Thus two enzymes, serine hydroxymethyltransferase and methylene-FH_4 reductase, each capable of generating methylene-FH_4, can mediate in the transfer of one-carbon units to biogenic amines in vitro producing alicyclic alkaloids. There has been considerable speculation in the literature concerning the possible formation of such compounds in vivo [17–20] since they are capable of inhibiting monoamine oxidase activity as well as the high-affinity uptake of amine neurotransmitters [21–25]. It is unlikely that methylene-FH_4 reductase mediates in the synthesis of β-carboline alkaloids in vivo, since this enzyme appears to function almost exclusively in the direction of methyl-FH_4 synthesis under physiological conditions [16]. However, serine hydroxymethyltransferase catalyses a readily reversible reaction and may be capable of participating in β-carboline synthesis in vivo in brain regions where indoleamine or catecholamine concentrations are high. In view of the pharmacological effects of these alkaloids, their possible formation in brain would merit further investigation.

Acknowledgements

We should like to thank John Hryszko for his excellent technical assistance. A.G.M.P. thanks the MRC for a studentship. This work was supported in part by a MRC Project Grant to A.J.T.

References

[1] Laduron, P. (1972) Nature New Biol. 238, 212–213.
[2] Banerjee, S. P. and Snyder, S. H. (1973) Science 182, 74–75.
[3] Hsu, L. L. and Mandell, A. J. (1973) Life Sci. 13, 847–858.
[4] Laduron, P., Gommeren, W. and Leysen, J. (1974) Biochem. Pharmacol. 23, 1599–1608.
[5] Waldmeier, P. C. and Maître, L. (1974) Experientia 30, 456–458.
[6] Mandel, L. R., Rosegay, A., Walker, R. W. and Vandenheuvel, W. J. A. (1974) Science 186, 741–743.
[7] Meller, E., Rosengarten, H., Friedhoff, A. J., Stebbins, R. A. and Silber, R. (1975) Science 187, 171–173.
[8] Wyatt, R. J., Erdelyi, E., DoAmaral, J. R., Elliot, G. R., Renson, J. and Barchas, J. D. (1975) Science 187, 853–855.
[9] Hsu, L. L. and Mandel, A. J. (1974) Life Sci. 14, 877–885.
[10] Rosengarten, H., Meller, E. and Friedhoff, A. J. (1975) Biochem. Pharmacol. 24, 1759–1762.
[11] Laduron, P. and Leysen, J. (1975) Biochem. Pharmacol. 24, 929–932.
[12] Pearson, A. G. M. and Turner, A. J. (1975) Nature 258, 173–174.
[13] Jones, C. W. and Priest, D. G. (1976) Arch. Biochem. Biophys. 174, 305–311.
[14] Vejdělek, Z. J., Trcka, V. and Protiva, M. (1961) J. Med. Pharmaceut. Chem. 3, 427–440.
[15] Taylor, R. T. and Weissbach, H. (1965) Analyt. Biochem. 13, 80–84.
[16] Kutzbach, C. and Stokstad, E. L. R. (1971) Biochim. Biophys. Acta 250, 459–477.
[17] Turner, A. J. (1977) Biochem. Pharmacol. 26, 1009–1014.
[18] Tipton, K. F., Houslay, M. D. and Turner, A. J. (1977) Essays Neurochem. Neuropharmacol. 1, 103–138.
[19] Sourkes, T. L. (1971) Nature 229, 413–414.
[20] Davis, V. E. (1973) Ann. NY Acad. Sci. 215, 111–115.
[21] Meller, E., Friedman, E., Schweitzer, J. W. and Friedhoff, A. J. (1977) J. Neurochem. 28, 995–1000.
[22] Buckholtz, N. S. and Boggan, W. D. (1976) Biochem. Pharmacol. 25, 2319–2321.
[23] Kellar, K. J., Elliot, G. R., Holman, R. B., Vernikos-Danellis, J. and Barchas, J. D. (1976) J. Pharmacol. Exp. Ther. 198, 619–625.
[24] Rommelspacher, H., Strauss, S. M. and Rehse, K. (1978) J. Neurochem. 30, 1573–1578.
[25] Alpers, H. S., McLaughlin, B. R., Nix, W. M. and Davis, V. E. (1975) Biochem. Pharmacol. 24, 1391–1396.