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Enzymic acetylation of the stereoisomers of α- and β-methyl choline*

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A NUMBER of workers have investigated the *N*-alkyl group substrate specificity of choline acetyltransferase (acetyl-CoA:choline *O*-acetyltransferase; EC 2.3.1.6) (ChAc),[1–4] and in a recent study we reported on the enzymic acetylation of a group of choline analogues where the methyl groups on the quaternary nitrogen atom were replaced successively by ethyl groups.[5] The results suggested that replacement of methyl by ethyl groups resulted in a lower affinity of the substrate to the active site of the enzyme and that the rate of acetylation was proportional to the binding force of the quaternary nitrogen to a negative charge at the active site of the enzyme.

In the present work we have attempted to elucidate further the mode of interaction of choline and some choline analogues with ChAc. Choline analogues were used with methyl groups substituted in the methylene chain. These compounds possess an asymmetric carbon atom and the stereoisomers of both α- and β-methyl choline were employed.

Partially purified ChAc was prepared from bovine caudate nucleus as described previously.[5] ChAc activity was determined using ^{14}C acetyl-CoA as substrate by a modification of the method of McCaman and Hunt[6] and the incubation conditions were as described by Hemsworth and Smith.[5]

The choline analogues D-(+)-α-methyl choline, L-(−)-α-methyl choline, D-(−)-β-methyl choline and L-(+)-β-methyl choline were prepared by Prof. A. H. Beckett and Dr. J. W. Clitherow, Chelsea School of Pharmacy, London University, England. The absolute configurations of these compounds have been established by Beckett *et al.*[7] (+)-α-Methylcholine was shown to be related to D-(−)-alanine hydrochloride and (+)-β- methylcholine to L-(+)-lactic acid. The stereospecificity of these compounds can be confirmed by the use of Cahn's "sequence rule"[8] with the aid of CPK and Prentice-Hall framework molecular models.

Concentrations of substrate from 10^{-5} to 2×10^{-2} M were employed. Time studies demonstrated that the rates of acetylation of choline and the choline analogues were linear for at least 15 min at the concentrations and under the incubation conditions employed. In this study, 15-min incubations were used to obtain the presented data. L-β-Methyl choline was not a substrate for ChAc. The other compounds were acetylated at a lower rate than choline.

Choline showed substrate inhibition as reported previously,[4, 5] but up to 2×10^{-2} M none of the other substrates exhibited substrate inhibition. It has been shown previously that ChAc is a two substrate enzyme and each substrate affects the affinity of the other for the enzyme.[9] At a constant concentration of acetyl-CoA, apparent Michaelis–Menten constants were determined for each substrate from the graphs (Fig. 1) according to the method of Lineweaver and Burk,[10] and the apparent V_{max} for each substrate was also determined (Table 1). Choline has a much lower apparent K_m than the other choline analogues, indicating that choline has a greater affinity for ChAc.

Models of choline and the choline analogues are shown in Fig. 2. If the choline molecule is assumed to adopt the conformation shown when bound to the catalytic site of ChAc, then the relative rates of acetylation of the other substrates could be explained in terms of interference by the added methyl groups with binding of the substrate molecule to the active site of the enzyme or with transfer of the acetyl group to the substrate hydroxyl group. D-α-Methylcholine was a better substrate for ChAc than

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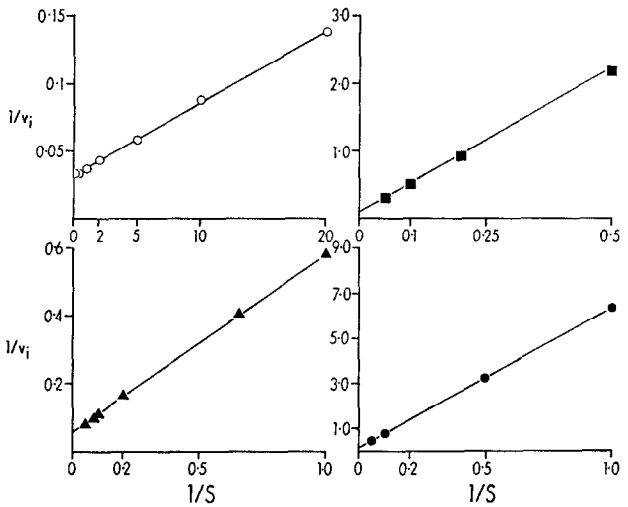

FIG. 1. Derivation of apparent Michaelis–Menten constants by the method of Lineweaver and Burk.[10] The incubation conditions were as described in the text. S, concentration of substrate (mM); v_i, initial velocity of reaction (μmoles of acetylated product/ml of enzyme/hr). ○, choline; ▲, D-α-methylcholine; ■, L-α-methylcholine; ●, D-β-methylcholine.

TABLE 1. APPARENT MICHAELIS–MENTEN CONSTANTS, K_m (M) AND VALUES OF V_{max} (μmoles ACETYLATED PRODUCT FORMED/ml ENZYME/hr) FOR CHOLINE AND THE CHOLINE ANALOGUES WHERE THE CONCENTRATION OF ACETYL-CoA IN THE INCUBATION SYSTEM WAS $4{\cdot}5 \times 10^{-5}$ M

Substrate	Apparent K_m	Apparent V_{max}
Choline	$1{\cdot}7 \times 10^{-4}$	31·0
D-α-Methylcholine	$8{\cdot}9 \times 10^{-3}$	17·1
L-α-Methylcholine	$3{\cdot}9 \times 10^{-2}$	9·88
D-β-Methylcholine	$4{\cdot}6 \times 10^{-2}$	7·62

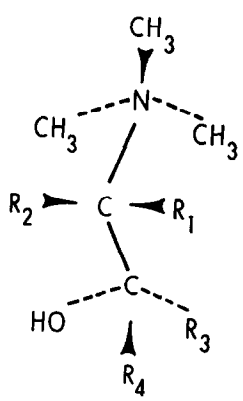

FIG. 2. Proposed conformation of choline and α- and β-methylcholines when bound to the active site of ChAc. R_1, R_2, R_3 and R_4=H=choline; R_1=CH_3, R_2, R_3 and R_4=H=D-α-methylcholine; R_2=CH_3, R_1, R_3 and R_4=H=L-α-methylcholine; R_3=CH_3, R_1, R_2 and R_4=H = L-β-methylcholine; R_4=CH_3, R_1, R_2 and R_3=H=D-β-methylcholine.

L-α-methylcholine, and Fig. 2 shows that in D-α-methylcholine the substituted methyl group is further from the OH group than in the L-isomer. With L-α-methylcholine the substituted methyl group may affect, by steric hindrance, the interaction between the acetyl group provided by acetyl-CoA and the hydroxyl group on the substrate molecule. The steric hindrance effect of the methyl group in the β-substituted methylcholines is probably greater than in the α-substituted compounds as reflected in the lower acetylation rates, and in the case of L-β-methylcholine no acetylation is observed.

It is of interest that in the case of hydrolysis of the acetyl-β-methylcholines by bovine erythrocyte acetylcholinesterase the D-isomer is not hydrolyzed.[11] The rate of the hydrolysis of L-acetyl-β-methylcholine is much lower than that of the α-isomers, suggesting a similar effect of steric hindrance by the substituted methyl group.

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Antagonism of the anti-tumour effects of asparaginase by methotrexate

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A RECENT publication has reported that the inhibitory effects of methotrexate against the L5178Y mouse leukaemia can be completely antagonised by pretreatment with asparaginase, which is also tumour inhibitory in this system.[1]

In experiments on the mouse R_1 lymphoma we have shown that combinations of asparaginase and methotrexate given simultaneously, are significantly less effective than if asparaginase is given prior to methotrexate treatment, or vice versa.

Materials and methods

The R_1 lymphoma was induced by irradiation of a CBA mouse and has, since induction, been maintained in the same strain, by routine transplantation of ascites cells. A sub-line of the tumour, showing partial resistance to asparaginase, was used in these experiments. The optimum tumour