group on the enzyme is involved, inactivation could proceed as shown in Scheme 1. An addition reaction analogous to that proposed in reaction (3) has been clearly demonstrated in a model system (Morisaki & Bloch, 1972).


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**Glycidaldehyde, an Inhibitor Directed towards the C1-Unit-Binding Site of Serine Transhydroxymethylase**

H. A. EL-OBEID and M. AKHTAR

*Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 5NH, U.K.*

Serine transhydroxymethylase (L-serine-tetrahydrofolate 5,10-hydroxymethyltransferase, EC 2.1.2.1) catalyses the reversible reaction:

$$\text{Glycine} + \text{methylene-tetrahydrofolate} \rightleftharpoons \text{L-serine} + \text{tetrahydrofolate}$$  \hspace{1cm} (1)

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Scheme 1. *Postulated sequence for the reaction catalysed by serine transhydroxymethylase*

Abbreviation: H$_4$PteGlu, tetrahydrofolate.
Recent mechanistic studies in this laboratory (Jordan & Akhtar, 1970) have suggested that the overall conversion occurs through the sequence shown in Scheme 1. This involves in the initial step the formation of glycine–pyridoxal phosphate–enzyme complex (II), which in the next stages undergoes deprotonation to give the carbanion intermediate (V). The latter species (V) then reacts with formaldehyde, released at the active site from methylene-tetrahydrofolate, to give the Schiff base of L-serine–pyridoxal phosphate–enzyme (VI), which finally decomposes to furnish the products.

![Graph](image)

Fig. 1. Time-course of the inactivation of serine transhydroxymethylase activity by glycidaldehyde in the absence and in the presence of the amino acid substrates

Curve A, no addition; curve B, preincubated with 10mM-glycidaldehyde; curve C, preincubated with 10mM-glycidaldehyde and 25mM-glycine; curve D, preincubated with 10mM-glycidaldehyde and 25mM-L-serine. The inactivation experiments were performed and the residual enzymic activity was assayed by using the methods described previously (Akhtar & El-Obeid, 1972).
The proposal that the transfer of the $C_1$ unit in the serine transhydroxymethylase reaction occurs via the participation of an enzyme-bound formaldehyde led to the deduction that the enzyme must contain a binding site for the carbonyl group and suggested that an aldehyde containing a suitable alkylating group may selectively modify an active-site residue. This communication describes experiments carried out with glycidaldehyde:

\[ \begin{array}{c}
\text{C} \\text{H} \\
\text{H} \\
\text{O} \\
\end{array} \]

Cytoplasmic serine transhydroxymethylase was prepared from rabbit liver by the method described previously (Akhtar & El-Obeid, 1972). The effect of glycidaldehyde on the enzyme was studied as follows. The enzyme (1–2 units) was treated with glycidaldehyde at 37°C, and samples were removed at various time-intervals and assayed for the enzyme activity towards the synthesis of L-serine. The inactivation reaction was time-dependent and followed saturation kinetics. Under the same conditions glycidol had no significant effect on the enzyme activity. The time-course of inactivation was also studied in the presence of the two amino acid substrates. Fig. 1 shows that L-serine protected the enzyme against inactivation by glycidaldehyde. In the presence of glycine, however, the rate of inactivation by the inhibitor was significantly enhanced.

These experiments allow two main conclusions to be drawn. First, the group on the enzyme that is modified by glycidaldehyde is protected by the $-\text{CH}_2\text{OH}$ moiety of L-serine. The enzymic group participating in the inactivation process therefore must be involved in the manipulation or the binding of the $-\text{CH}_2\text{OH}$ moiety of L-serine and hence by implication of the $C_1$ unit in the forward reaction (eqn. 1). Secondly, the activity of the group under discussion is enhanced by the binding of glycine to the enzyme. The latter observation, when taken in conjunction with previous studies (Schirch & Jenkins, 1964; Jordan & Akhtar, 1970) showing that the binding of tetrahydrofolate to the enzyme activates the group participating in the deprotonation reaction (Scheme I, IV–V), highlights that the binding of each substrate to the enzyme promotes a conformational change resulting in enhanced activity of the groups participating in the subsequent stage of the reaction.

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Kinetics and Binding of Difluoro-oxaloacetate to Aspartate Aminotransferase

PATRICIA A. BRILEY, ROBERT EISENTHAL and ROGER HARRISON

Biochemistry Group, School of Biological Sciences, University of Bath, Bath BA2 7AY, U.K.

The kinetics and binding of difluoro-oxaloacetate with pig heart cytoplasmic aspartate aminotransferase (EC 2.6.1.1) have been investigated in order to determine the suitability of the fluoro compound as a substrate analogue in $^{19}$F n.m.r. studies (Martinez-Carrion et al., 1973). Difluoro-oxaloacetate is a potent inhibitor of the transamination reaction of $\alpha$-oxoglutarate and aspartate (Kun et al., 1963). The inhibition was competitive with $\alpha$-oxoglutarate as the varied substrate (0.07–0.33 mM) at saturating concentration of aspartate ($K_i$, 5 $\mu$M). In a separate series of experiments the effect on the rate of various concentrations of the inhibitor (0–40 $\mu$M) at several concentrations of $\alpha$-oxoglutarate and constant aspartate concentration was measured. Plots of $1/V$ against $[I]$ were linear, confirming the strict competitive nature of the inhibition with respect to $\alpha$-oxoglutarate.