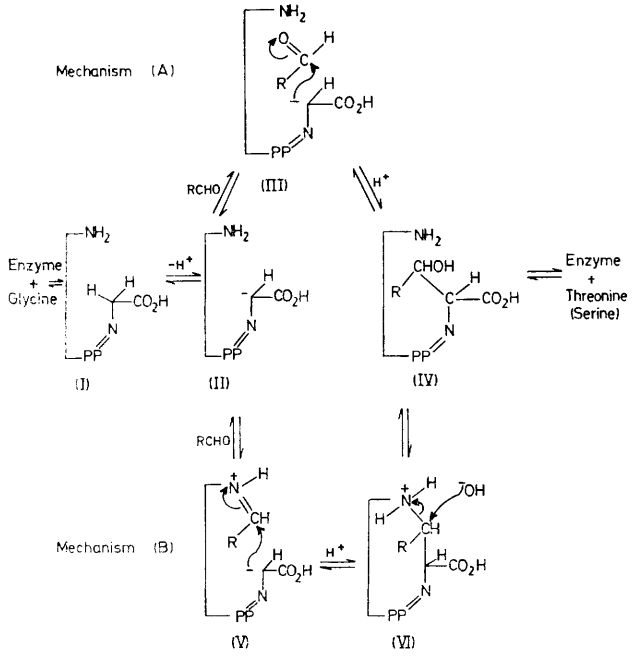
Mechanism of C-2–C-3 Bond Formation and Cleavage in Serine Transhydroxymethylase Reactions

By PETER M. JORDAN, HUMEIDA A. EL-OBEID, DAVID L. CORINA, and MUHAMMAD AKHTAR*
(*Department of Physiology and Biochemistry, University of Southampton, Southampton* SO9 3TU)

Summary It is shown that in the serine transhydroxymethylase-catalysed aldol cleavage of threonine containing ^{18}O at the hydroxy-group the label is quantitatively transferred to acetaldehyde.

SERINE transhydroxymethylase (5,10-methylenetetrahydrofolate–glycine hydroxymethyltransferase, EC 2.1.2.1) catalyses the reversible reaction in equation (1) and also possesses an additional activity responsible for the cleavage of allothreonine and/or threonine [equation (2)] which is independent of tetrahydrofolate[1,2] (threonine aldolase activity). The enzyme requires pyridoxal phosphate for full catalytic activity.



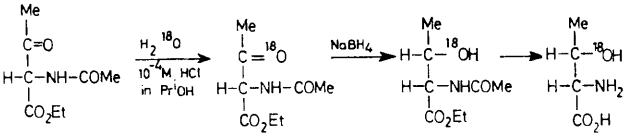
SCHEME 1. Structure (I) represents glycine bound to the enzyme–pyridoxal phosphate complex through a Schiff base linkage.

This communication describes a study carried out on the threonine aldolase activity of the enzyme.

Glycine + methylenetetrahydrofolate ⇌
L-serine + tetrahydrofolate (1)

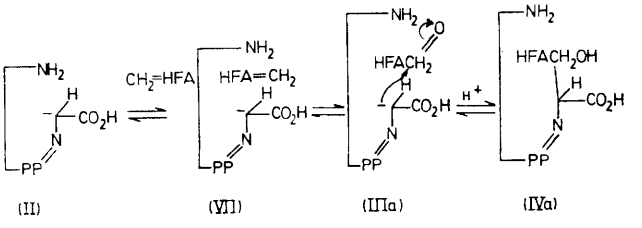
Glycine + acetaldehyde ⇌ allothreonine (or threonine) (2)

Recent mechanistic and stereochemical work[1–4] on the enzyme has established that for the reaction in the forward direction the first event is the formation of the enzyme–pyridoxal-glycine complex (I) which undergoes a deprotonation to give the carbanion species (II). Several mechanisms are possible[2,3] through which this carbanion species may participate in the subsequent condensation process.



SCHEME 2.

Two of these possibilities, mechanisms (A) and (B), are outlined in Scheme 1. Mechanism (A) involves the direct condensation of the carbanion (II) with the aldehyde[2,3] unit through the sequence (II) ⇌ (III) ⇌ (IV), after which the free amino-acid product is released by conventional reactions. Mechanism (B) involves the prior activation of



CH_2=HFA= Methylenetetrahydrofolic acid

SCHEME 3.

the aldehyde by Schiff base formation[1,5] with a $-NH_2$ group at the enzyme active site and condensation through the sequence (II) ⇌ (V) ⇌ (VI) ⇌ (IV) → product.

These two alternatives may be experimentally distinguished by using threonine labelled with ^{18}O in the hydroxy-group, since only in mechanism (A) will the label be retained in the product aldehyde. Using the synthetic sequence shown[6] in Scheme 2 and starting with water containing 42% ^{18}O atom excess, ^{18}O labelled threonine was obtained. Mass spectral analysis of its tri-trimethylsilyl derivative showed that the OH group of the amino-acid contained 38·5% ^{18}O atom excess.

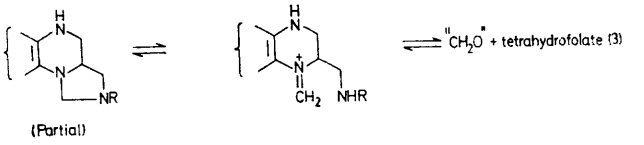
TABLE

Time/min		EtOH/μmol	% ^{18}O atom excess in EtOH
0	Complete system	0	—
12	Complete system	3·7	37%
12	Alcohol dehydrogenase omitted	0	—
12	Serine transhydroxy-methylase omitted	0	—

The reaction of threonine with serine transhydroxymethylase results in the formation of acetaldehyde [reverse of equation (2)]. In a model experiment using authentic [^{18}O]acetaldehyde it was shown that under the conditions of incubation and isolation, the ^{18}O in the acetaldehyde was removed by a hydration–dehydration mechanism. We therefore designed a method by which the acetaldehyde produced by the enzyme was stabilised immediately by reduction with alcohol dehydrogenase in the presence of NADH. When purified serine hydroxymethylase was coupled to alcohol dehydrogenase in this way, it was possible to demonstrate the formation of ethanol (as measured by g.l.c.) with a concomitant decrease in the NADH absorption at 340 nm.

[^{18}O]Threonine (38·5% ^{18}O excess) was now incubated with serine transhydroxymethylase and alcohol dehydrogenase together with NADH at pH 7·1 and the reaction was followed to completion in a spectrophotometer cell (12 min). The ^{18}O content of the ethanol produced during the incubation was determined by separation of the ethanol from the incubation mixture by g.l.c. using a column of 10% Carbowax 1540 on Teflon, followed by mass spectral analysis using an MS30 spectrometer.

These results show that during the conversion of threonine into acetaldehyde the ^{18}O label is almost quantitatively retained. It follows therefore that the mechanism of the enzyme reaction does not involve a covalent linkage between acetaldehyde and the enzyme and thus lends support to a direct condensation between the glycine carbanion and the carbonyl group of the acetaldehyde by mechanism (A). The same mechanistic principle when extended to the tetrahydrofolate linked, glycine ⇌ serine interconversion [equation (1)] requires that in this case also the crucial bond forming event may involve the reaction of the carbanion directly with 'formaldehyde' released at the active site by the sequence in Scheme 3, as was suggested previously.[2,3] Since it is known[7] that the enzymic reaction is stereospecific with respect to the two C-3 hydrogen atoms of serine, the transfer of the C_1 unit to and from tetrahydrofolate must involve a tightly bound form of 'formaldehyde,' the formaldehyde being generated from methylenetetrahydrofolate by reaction (3).



(*Received*, 10*th November* 1975; *Com.* 1251.)

[1] L. Schirch and T. Gross, *J. Biol. Chem.*, **1968**, **243**, 5651.
[2] P. M. Jordan and M. Akhtar, *Biochem. J.*, 1970, **116**, 277.
[3] M. Akhtar, H. A. El-Obeid, and P. M. Jordan, *Biochem. J.*, 1975, **145**, 159.
[4] L. Schirch and W. T. Jenkins, *J. Biol. Chem.*, 1964, **239**, 3801.
[5] M. S. Chen and L. Schirch, *J. Biol. Chem.*, **1973**, **248**, 3631, 7979.
[6] The starting ketone used below was prepared by the method of K. Pfister, C. A. Robinson, A. C. Shabica, and M. Tishler, *J. Amer. Chem. Soc.*, 1949, **71**, 1101.
[7] J. Beillmann and F. Schuber, *Biochem. Biophys. Res. Comm.*, 1967, **27**, 517.