Stereochemistry of the Glycine Reductase of *Clostridium sticklandii*

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Summary In the reductive deamination of glycine to acetic acid catalysed by glycine reductase of *Clostridium sticklandii* it is shown that both hydrogens are retained in the product and that the reaction proceeds with an inversion of configuration at the methylene carbon.

THERE is considerable interest in the elucidation of the mechanisms through which biological redox reactions are coupled to the formation of ATP from ADP and inorganic phosphate. In eucaryotic organisms most of the ATP is produced in the mitochondria and it is now a popular belief that the 'physico-chemical forces' participating in the phosphorylation reactions compulsorily utilise the structural features provided by a membrane organisation. By comparison, in certain bacteria although the *in vivo* ATP formation is catalysed by membrane associated enzymes, *in vitro*, the phosphorylation reactions can be achieved by completely solubilised preparations. One such phosphorylation reaction in *Clostridium sticklandii* is linked to the reduction of the amino acid glycine (equation 1) and occurs through the participation of a multicomponent system.

$$H_2N\text{–}CH_2\text{–}CO_2H + 2H + ADP + Pi \longrightarrow H\text{-}CH_2\text{–}CO_2H + NH_3 + ATP \quad (1)$$

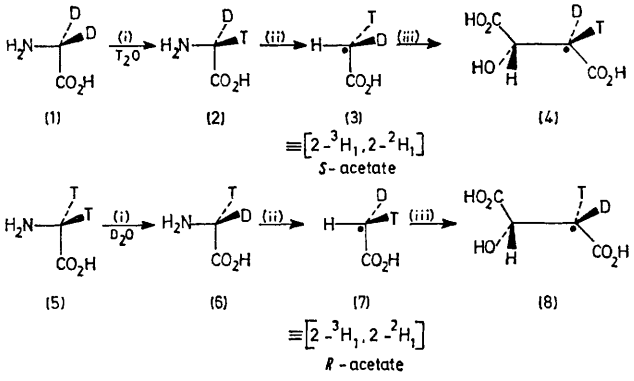
One of the components of the reaction is a low molecular weight selenium-containing protein called protein A which has been highly purified. The remaining components are less well characterised and have been collectively termed glycine reductase.[1] The physiological electron donor for the reaction (1) is ultimately NADH, but an electron transport chain mediates between NADH oxidation and glycine reduction. Dithiols, such as dithiothreitol, when used as artificial reducing agents in the solubilised preparations, bypass certain components of this electron transport chain.

TABLE

Source of acetate		$^3H/^{14}C$[a]	% Retention of ^3H
[2-$^3H_1$,2-$^2H_1$]-2*S*-Glycine	Starting malate	1·835	
	Equilibrated malate	0·546	29·7
[$^3H_2$]-Glycine	Starting malate	3·781	
	Equilibrated malate	1·893	50·1
[2-$^3H_1$,2-$^2H_1$]-2*R*-Glycine	Starting malate	1·848	
	Equilibrated malate	1·438	77·8

[a] Various samples of acetate, as indicated, were converted into malates. Aliquot portions of the malates were then treated with fumarase to equilibrate the C-3 *pro-R* hydrogen with protons of the medium. $^3H/^{14}C$ ratios refer to the recrystallised malate.

As an initial stage towards the understanding of these processes we have studied the mechanistic and stereochemical details of the chemical events which occur at C-2 of glycine during the reaction (1). Our first aim was to determine whether the two C-2 protons of glycine remain intact in the product. For this the enzyme was purified by a modification of the method of Turner and Stadtman[1] to give protein A with a specific activity of 28 μmol of glycine reduced/60 min/mg and 'glycine reductase' with a specific activity of 3 μmol glycine reduced/60 min/mg (*cf.* reported values[1] of up to 141 and 0·27 μmol glycine reduced/90 min/mg protein, for protein A and 'glycine reductase' fractions respectively). The protein A and 'glycine reductase' together, in the presence of ADP and dithiothreitol, catalysed glycine reduction to acetic acid. When [2-^{14}C, 2-$^3H_2$]-glycine with an initial $^3H/^{14}C$ ratio of 7·16 was incubated in the above preparation the product acetic acid, which was isolated as its *p*-bromophenacyl acetate derivative, had a $^3H/^{14}C$ ratio of 7·03, indicating a 99% retention of ^3H radioactivity and demonstrating that both C-2 protons of glycine are incorporated intact into the product acetic acid.†



SCHEME. (i) *via* serine transhydroxymethylase, (ii) *via* glycine reductase, and (iii) *via* malate synthetase.

In order to study the stereochemistry of the glycine reduction, enantiomeric, stereospecifically tritiated, deuteriated glycines were prepared using serine transhydroxy methylase purified from rabbit liver[2] which is known to catalyse the equilibration of the *pro-S* hydrogen of glycine with the medium.[3] [2-$^3H_1$, 2-$^2H_1$]-2*S*-glycine (**2**) was prepared by this enzymic exchange from [2-$^2H_2$]-glycine in ^3H_2O while [2-$^3H_1$, 2-$^2H_1$]-2*R*-glycine (**6**) was prepared from [2-$^3H_2$]-glycine in D_2O. [^{14}C]-Glycine was added to each specimen to give a $^3H/^{14}C$ ratio of about 1·8. These stereospecifically labelled samples of glycine were incubated with the partially purified glycine reductase preparation over a short period to restrict the contaminating labilisation of tritium† to <1% during conversion into chiral acetic acids.

For configurational analysis[4,5] acetic acid samples derived from stereospecifically labelled glycines as well as from randomly tritiated glycine (also containing ^{14}C-glycine) were converted into L-malate essentially as detailed by Rose.[6] Recrystallisation of the malate before and after fumarase equilibration gave the $^3H/^{14}C$ ratios shown in the Table. The malate derived from [2-$^3H_1$, 2-$^2H_1$]-2*S*-glycine (**2**) lost the majority of its C-3 *pro-R* tritium[7] while the malate derived from [2-$^3H_1$, 2-$^2H_1$]-2*R*-glycine (**6**) retained it. With these results and a knowledge of the stereochemistry of the serine transhydroxy methylase,[3] malate

† The glycine recovered from these incubations showed only a small loss of tritium. However, using enzyme preparations of lower purity, an extensive exchange of the C-2 hydrogen atoms of glycine with protons of the medium was apparent, presumably catalysed by contaminating enzymes.

synthetase,[4,5] and fumarase[7] reactions, it is concluded that the glycine reductase reaction proceeds with an inversion of configuration at the methylene carbon of glycine as illustrated in the Scheme.

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