Tetrahedron Letters No.11, pp. 875-877, 1969. Pergamon Press. Printed in Great Britain.

THE ABSOLUTE CONFIGURATION OF STEREOSPECIFICALLY TRITIATED GLYCINES

By M. Akhtar and P.M. Jordan

Department of Physiology & Biochemistry, University of Southampton, SO9 5NH, England

(Received in UK 16 January 1969; accepted for publication 4 February 1969)

The enzyme serine hydroxymethylase[1] catalyses the interconversion of glycine and serine as shown in equation (i), the cofactors pyridoxal phosphate and tetrahydrofolate being required for the reaction[2].

$$\text{glycine} + \text{formaldehyde} \rightleftharpoons \text{L-serine} \qquad \text{(i)}$$

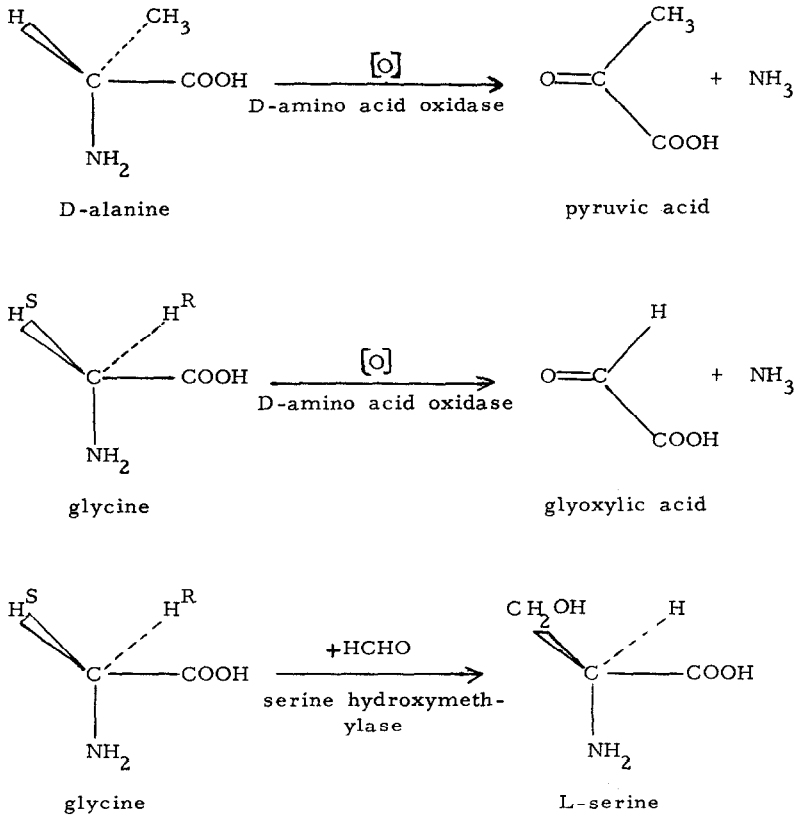
In a recent communication[3] it was reported that the incubation of glycine with serine hydroxymethylase in the absence of formaldehyde resulted in the rapid stereospecific exchange of one of the two α-hydrogen atoms of glycine. Using this method [2RS-$^3H_2$] glycine gave rise to a monotritiated glycine which we called "A-tritioglycine". The use of non-radioactive glycine in the presence of tritiated water yielded glycine with the opposite orientation of tritium which we called "B-tritioglycine"[*1]. We now report on the absolute configuration of these compounds.

It has been shown that purified preparations of the enzyme D-amino acid oxidase, which normally convert D-alanine into pyruvic acid, may also accept glycine as a substrate in the latter case the product being glyoxylic acid[4]. In the conversion of D-alanine to pyruvate the α-hydrogen atom of the former is removed, and since the α-hydrogen atom of

---

*1 Reincubation of these samples with serine hydroxymethylase under the same conditions resulted in the loss of only a small amount of tritium activity from "A-tritioglycine" whereas "B-tritioglycine" lost the majority of its tritium. These incubations showed that at least 85% of the tritium activity was stereospecifically located in each case.

L-alanine is enzymically inert we have assumed that the hydrogen atom of glycine with the 'S' configuration will be removed in the formation of glyoxylate by the enzyme. This can be shown more clearly by the scheme below.



When stereospecifically tritiated glycine ($^{3}H/^{14}C$ 1.71), obtained by the equilibration of [2RS-$^{3}H_2$] glycine with serine hydroxymethylase, was oxidised with D-amino acid oxidase, the derived glyoxylate had a $^{3}H/^{14}C$ of 1.41. The 83% retention of tritium activity in the experiment shows that the labelled hydrogen of glycine had the 'R' configuration. This tritiated stereoisomer of glycine is therefore [2R-^{3}H] glycine. In another experiment glycine ($^{3}H/^{14}C$ 2.60), obtained from the incubation of non-radioactive glycine with serine hydroxymethylase in the presence of tritiated water, when incubated with D-amino acid oxidase

gave glyoxylate with a $^{3}H/^{14}C$ of 0.42. The loss of 84% of the tritium activity in the experiment shows that the precursor glycine contained tritium associated with the 'S' hydrogen atom. This tritiated stereoisomer of glycine is therefore [2S-^{3}H] glycine*2. As expected the oxidation of randomly labelled [2RS-$^{3}H_2$] glycine ($^{3}H/^{14}C$ 2.91) with D-amino acid oxidase gave glyoxylate with a $^{3}H/^{14}C$ of 1.43, containing half of the original tritium activity.

When [2R-^{3}H] glycine was incubated with serine hydroxymethylase in the presence of formaldehyde, the biosynthetic serine was found to contain all the original tritium activity. The conversion of [2S-^{3}H] glycine into serine however, resulted in a total loss of tritium label. Serine synthesised from randomly tritiated [2RS-$^{3}H_2$] glycine, contained half the tritium activity of the precursor glycine. Since the 'R' hydrogen atom of glycine has the same absolute configuration as the α-hydrogen atom of L-serine (see scheme opposite), we therefore conclude that the overall conversion of glycine into serine occurs with retention of configuration.

Our method is not only convenient for the synthesis of stereospecifically tritiated glycines, but can also be applied to the synthesis of the corresponding deutero-compounds.

REFERENCES

(1) Huennekens, F.M. & Osborn, M.J. Advance. Enzymol. 21, 369, 1959.

(2) Blakley, R.L. Biochem. J. 65, 331, 1957.

(3) Akhtar, M. & Jordan, P.M. Chem. Comm. 1691, 1968.

(4) Neims, A.H. & Hellerman, L. J. Biol. Chem. 237, PC 76, 1962.

---

*2 [2R-^{3}H] glycine is therefore equivalent to "A-tritioglycine", and [2S-^{3}H] glycine to "B-tritioglycine".