THE STEREOCHEMISTRY OF THE INCORPORATION OF FORMATE-^{3}H INTO L-SERINE IN RAT LIVER

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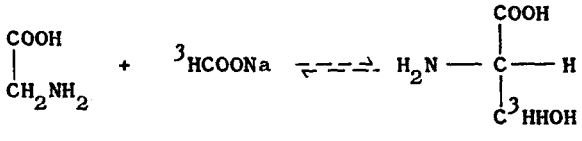
SUMMARY : In rat liver, the incorporation of formate-^{3}H into L-serine is partially stereospecific. The implications of this result are discussed.

We have studied the stereochemistry of formate-^{3}H incorporation into L-serine. This result derives from a study of the chirality



of C-3 in L-serine, which is a consequence of the stereospecificity of preceding enzymatic steps.

The formate condenses with tetrahydrofolate to give 10-formyl H_4 folate, then 5,10-CH=H_4 folate. Blakley (1964) showed that the reduction (a), catalyzed by 5,10-methylenetetrahydrofolic dehydrogenase (E.C. 1.5. 1.5) from bakers'yeast, is stereospecific

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Abreviations used : E enzyme ; H_4 folate, 5,6,7,8-tetrahydrofolate ; 5,10-CH = H_4 folate , 5,10 methenyltetrahydrofolate ; 5,10-CH_2-H_4 folate, 5,10 methylenetetrahydrofolate ; P-Pal, pyridoxal phosphate. Y-ADH, yeast alcohol dehydrogenase ; LDH, lactic dehydrogenase.

(a) $5,10\text{-CH} = H_4$ folate + NADPH $\underset{E}{\rightleftharpoons}$ $5,10\text{-CH}_2\text{-}H_4$ folate + NADP+

for side A of the coenzyme. It should be noted that if the reduction (a) is stereospecific, the two hydrogens of the methylene bridge, which are configurationally non-equivalent, are of different origin ($\underline{\underline{H}}COO^-$; NADP$\underline{\underline{H}}$). However, methylenetetrahydrofolate racemizes so easily (Equation b) (Kisliuk 1957) that it is not possible to study the stereochemistry of (a) in respect to the

(b) $5,10\text{-CH}_2\text{-}H_4$ folate + $H_2O \rightleftharpoons H_4$ folate + HCHO non enzymatic

$5,10\text{-CH}_2\text{-}H_4$ folate. But, we can use this methylenetetrahydrofolate for transhydroxymethylation (Equation c) and see if the stereochemistry of the methylene group appears at C-3 of L-serine. This supposes that (c) is also stereospecific and that its rate is at least as great as that of the non enzymatic decomposition of methylenetetrahydrofolate in the equilibrium (b). If this is not the

(c) $5,10\text{-CH}_2\text{-}H_4$ folate + P-Pal-glycine + $2H_2O$ $\underset{E}{\rightleftharpoons}$

L-serine + H_4 folate + P-Pal

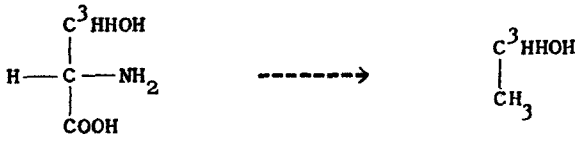
case, the hydroxymethyl of the L-serine formed will be racemic. It may be seen that if the enzymatic reactions (a) and (c) are completely stereospecific then the labelled hydrogen coming from the formate has either the R or S configuration in the hydroxymethyl of L-serine.

METHODS AND RESULTS

L-serine-3-^{3}H was prepared from glycine and formate-^{3}H by incubation of rat liver slices (Kruhøffer 1951 ; Blakley 1954).

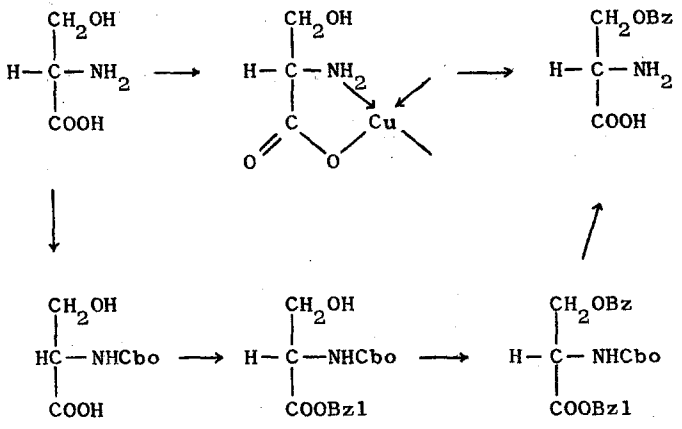
To determine the configuration of tritium in the hydroxymethyl of the so prepared L-serine, the serine was degraded into ethanol following the reaction shown in the table.

We have confirmed that this degradation occurs without appreciable racemization (less than 10 %)

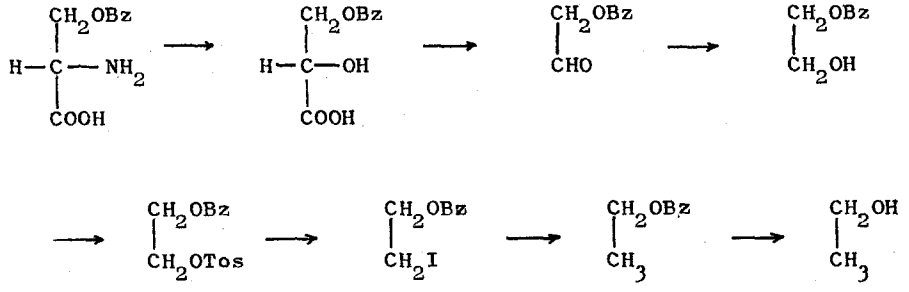


DEGRADATION OF SERINE

a) Preparation of Obenzoyl serine :

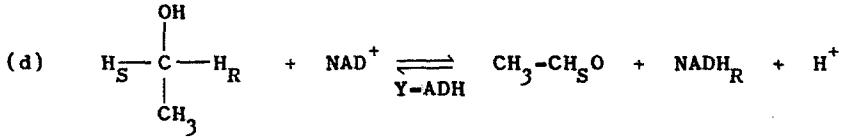


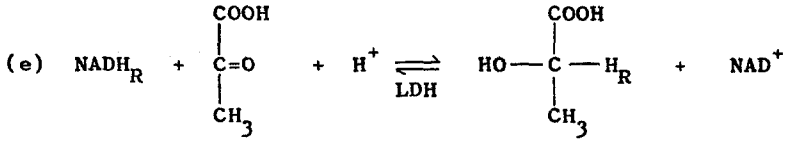
b) Degradation of Obenzoyl serine :



Bz = Benzoyl ; Cbo = Carbobenzoxy ; Bzl = Benzyl ; Tos = p-Toluene-sulfonyl.

To determine the configuration of the ethanol-1-^{3}H thus obtained we used the method described by Arigoni et al. (1966) ;





We isolated acetaldehyde as its dimedone derivative and the lactic acid as its p-phenylphenacyl ester.

Isotope effects in the determination of the configuration of ethanol 1-^{3}H :

In order to estimate the optical purity of the ethanol-1-^{3}H obtained by degradation we first compared our results with those found in the case of ethanol-1-^{3}H racemic and (S) ethanol-1-^{3}H. Under our conditions we found :

Ethanol-1-^{3}H racemic	acetaldehyde	: 45 %
	lactic acid	: 30 %
(S)-ethano-1-^{3}H	acetaldehyde	: 90 %
	lactic acid	: 0 %

(% of specific radioactivities with respect to the starting ethanol)

The difference between the percentages found and the theoretical percentage (50 %, 100 % respectively) are due in the case of acetaldehyde to a secondary kinetic isotope effect (unpublished results)* and in the case of lactic acid to the superposition of two primary kinetic isotope effects (Thomson 1964 ; Palm 1966).

Optical purity of the ethanol from L-serine 3-^{3}H :

Results : corrected for isotope effects (acetaldehyde : 1,11 ; lactic acid 1,65)

Experiment	1	2	3
acetaldehyde	68 %	71 %	72 %
lactic acid	-	18 %	28 %
optical purity of the ethanol	0,36	0,42	0,44

(% of specific radioactivities with respect to the starting ethanol)

CONCLUSION : The hydrogen from the formate enters the hydroxymethyl group of L-serine in preponderantly the S configuration.

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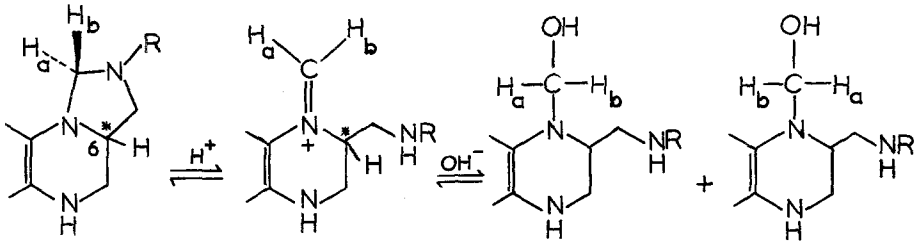
* At long reaction time, when the equilibrium is established, the activity of the acetaldehyde reaches 50 %. Therefore, there is no measurable isotope effect on the equilibrium (d). We have also shown that under our conditions there is no isotope effect on the formation of the dimedone derivative.

Optical purity at C-3 in L-serine is at least 36 to 44 %.

DISCUSSION :

Dehydrogenases involving NAD^+ or $NADP^+$ are known to be stereospecific with respect to the substrats(alcohol dehydrogenases...). We believe that the enzymatic reactions (a) and (c) are both stereospecific and that the partial racemization could be due to competition between the transhydroxymethylation (c) and the non enzymatic equilibrium (b) *.

However, one cannot exclude the possibility of an assymetric induction at one stage in the reaction pathway due to the assymetric center at C-6, of the tetrahydrofolate.



1 2 3

The non-enzymatic attack of OH^- on the immonium ion 2 could take place preferentially on one side of the molecule. If this were the case then the hydroxymethyltetrahydrofolate 3 would be the active form of the coenzyme which is fixed on the enzyme during the transhydroxymethylation. However, previous work seems to show that the active [redacted] form of the coenzyme is 1.

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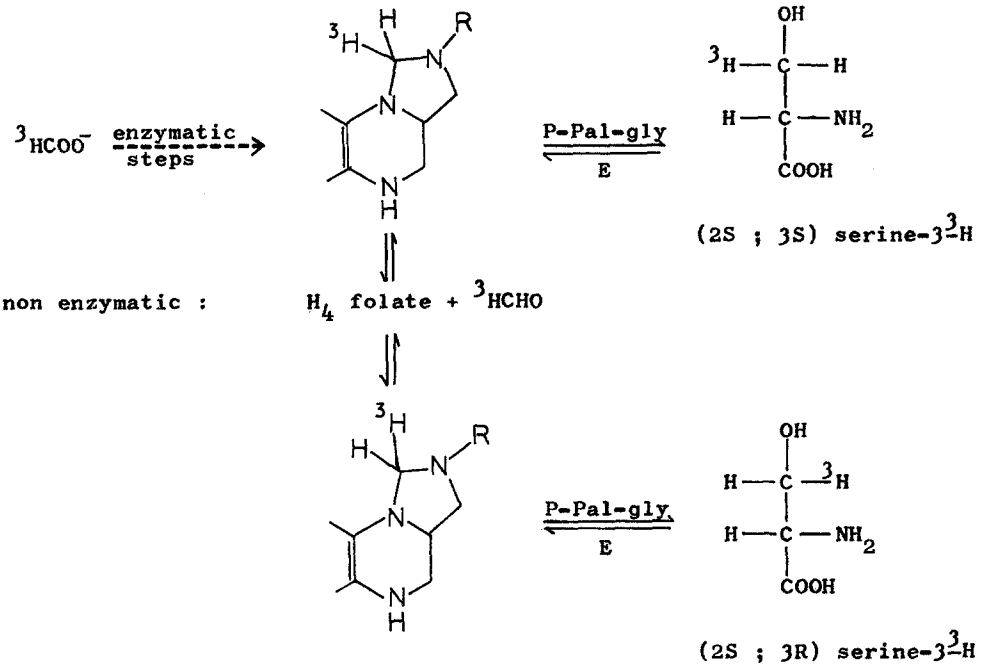
* or to the existence of two enzymatic systems of opposite stereochemistry.

We suggest the following scheme :



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