Non-enzymic transamination between glycine and glyoxylate

Non-enzymic transamination reactions of the type

$$R'CH(NH_2)CO_2H + RCOCO_2H \rightleftharpoons R'COCO_2H + RCH(NH_2)CO_2H$$

under physiological conditions have been reported by NAKADA AND WEINHOUSE[1] for limited cases where R = H. The equilibria of the reactions were completely in favour of glycine formation. No evidence was obtained for non-enzymic transamination in the case where R and R' are alkyl derivatives.

TABLE I

NON-ENZYMIC TRANSAMINATION BETWEEN [2-^{14}C]GLYCINE AND GLYOXYLATE

Additions	*Glyoxylate activity (counts/min/μmole)*				
	0 h	*3 h*	*5 h*	*28 h*	*98 h*
—	1,510	1,580	1,720	3,470	13,500
Cu^{++}	1,510	15,900	22,100	58,300	101,000
EDTA	1,510	1,490	1,570	1,870	2,220

[2-^{14}C]Glycine (0.005 *M*) (1.25 μC/)mole) and sodium glyoxylate (0.005 *M*) were incubated in Sørensen's 0.067 *M* phosphate buffer, pH 7, at 37°. Additions of $CuSO_4$ (0.5 m*M*) and ethylenediaminetetraacetate (EDTA) (5 m*M*) were made as indicated. Glyoxylate was isolated as the 2,4-dinitrophenylhydrazone and further purified by paper chromatography in *n*-butanol–0.3 % NH_3 (R_F *trans* isomer, 0.24; *cis* isomer, 0.36). Glyoxylate 2,4-dinitrophenylhydrazone (*trans* isomer) was counted at infinite thinness and estimated spectrophotometrically at 367 mμ in 0.5 % $NaHCO_3$.

The results presented in Tables I and II demonstrate a facile non-enzymic transamination between [^{14}C]glycine and glyoxylate at pH 7 and 37°. The reaction is apparently dependent on co-ordination-complex formation as indicated by the Cu^{++} catalysis and the inhibition by ethylenediaminetetraacetic acid. The reaction is also catalysed by OH^- and abolished at pH's lower than 6. The effect of the added Cu^{++} is reversed in the presence of pyridoxal phosphate (Table II). A comparison of the glycine–glyoxylate reaction with the homologous alanine–pyruvate system is shown in Table II.

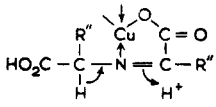
TABLE II

COMPARISON OF THE NON-ENZYMIC TRANSAMINATION BETWEEN (a) [1-^{14}C]GLYCINE AND GLYOXYLATE AND (b) [1-^{14}C]ALANINE AND PYRUVATE

System	*Additions*	*Keto acids counts at 24 h as % of total counts*
Glycine–glyoxylate	—	11.3
	Cu^{++}	30.6
	Cu^{++} + $PyrPO_4$	9.7
Alanine–pyruvate	—	< 1
	Cu^{++}	< 1
	Cu^{++} + $PyrPO_4$	77.7

[1-^{14}C]Amino acid (0.01 *M*, 0.8 μC/μmole) and keto acid (0.05 *M*) were incubated in Sørensen's 0.067 *M* phosphate buffer, pH 7, at 37°. Additions of $CuSO_4$ (1 m*M*) and pyridoxal phosphate ($PyrPO_4$) (10 m*M*) were made as indicated. Keto acid 2,4-dinitrophenylhydrazones were isolated, purified and counted as described in Table I.

The mechanism of the glycine–glyoxylate non-enzymic transamination may be envisaged as involving the co-ordination complex of a Schiff base, (I, R″ = H), which undergoes prototropic rearrangement:



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The facile transamination noted in the glycine–glyoxylate system as compared with the alanine–pyruvate system (Table II) would appear to be related to the absence of an α-alkyl substituent which through electron–release effects would inhibit prototropic rearrangement.

The effect of pyridoxal phosphate on the catalysis of the glycine–glyoxylate system by added Cu^{++} would appear from spectrophotometric evidence to be due to the formation of a pyridoxylideneglycine–copper complex of the type proposed by METZLER *et al.*[2] In view of the lack of significant participation of glycine in the pyridoxal-catalysed reactions described by METZLER AND SNELL[3], the formation of such a pyridoxylideneglycine–copper complex would effectively remove Cu^{++} from solution and so inhibit the transamination. The pronounced effect (Table II) of the α-alkyl substituent on the amino acid–pyridoxal phosphate–Cu^{++} reaction is in a direction opposed to its electron–release effect on the prototropic pyridoxylidene–glycine–azomethine structure. The effect of the α-alkyl substituent may here be envisaged as an influence on the free-energy change of the reaction exerted through hyperconjugative and inductive interaction with the carbonyl group of the keto acid product of the reaction. This formulation of the α-alkyl–substituent effect satisfactorily explains the observations of Table II and of METZLER AND SNELL[3], who have reported that the effect of an α-substituent in shifting the equilibrium of their amino acid–pyridoxal–Cu^{++} reaction towards keto acid and pyridoxamine formation is in the order

$$CH_{3}-,\ CH_3CH_2CH_2-,\ CH_3CH(CH_3)CH_2- > CH_3CH_2CH(CH_3) \gg CH_3CH(CH_3) \gg H$$

NAKADA AND WEINHOUSE[4] have reported on the basis of experiments employing an isotope-trapping technique that glyoxylate is an intermediate in the production of [^{14}C]formate from [^{14}C]glycine by rat-liver homogenates. It should be appreciated that the results of such experiments must be regarded as somewhat equivocal in view of the facile non-enzymic transamination reaction between glycine and glyoxylate described in this communication.

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