PN 10078

Phenylglyoxal aminotransferase activity in *Rhodopseudomonas spheroides*

During a study of the L-α-alanine:γ,δ-dioxovalerate aminotransferase (transaminase) of *Rhodopseudomonas spheroides*[1] it was observed that phenylglyoxal caused inhibition of enzyme activity, and appeared to act as a competing substrate. The α-aminoketone produced by the transamination of phenylglyoxal is ω-aminoacetophenone, a compound which has been implicated as an intermediate in the biosynthesis of ephedrine in *Ephedra distachya*[2]. Evidence has now been obtained suggesting that L-α-alanine:phenylglyoxal transaminase of *Rps. spheroides* is a distinct and specific enzyme. This enzyme has not previously been described.

Enzyme activity was assayed using the appropriate substrates, by a modification of the method described[3] for γ,δ-dioxovalerate transaminase whereby the alanine concentration used was 30 times that of phenylglyoxal. The α-aminoketone formed was estimated colorimetrically after condensation with acetylacetone, using Ehrlich's reagent[4]. Colour formation occurs in the cold, indicating a pyrrole unsubstituted at one of the α-positions, *i.e.* formed from ω-aminoacetophenone rather than α-amino-α-phenylacetaldehyde. The absorption spectrum of the coloured adduct, with λ_{max} at 560 mμ and a shoulder at 525 mμ ,is identical with that produced by authentic ω-aminoacetophenone. Paper chromatography of reaction mixtures yielded a ninhydrin-positive spot with the same R_F and colour characteristics as the authentic aminoketone. Pyruvate formation was detected and measured as the DNP-hydrazone, and approximately equalled ω-aminoacetophenone formation.

Phenylglyoxal transaminase was found to be purified by the procedure described[1] for dioxovalerate transaminase, and to approximately the same extent. Activity measurements were usually carried out at pH 7, as for the dioxovalerate system. The pH optimum, with α-alanine as the NH_2-group donor, was 6.5.

A study of NH_2-group donor specificity, with a 30–40% saturated $(NH_4)_2SO_4$ fraction[1] showed that γ-aminobutyrate transaminated at a rate 26% that observed with L-α-alanine, whereas the relative rates with δ-aminovalerate and ε-aminocaproate were 7 and 10%, respectively. These low rates of transamination with ω-amino acids contrast with the respective values of 76, 58 and 72% obtained with dioxovalerate as the NH_2-group acceptor. The presence of an equimolar amount of any of these ω-amino acids had virtually no inhibitory effect on transamination with α-alanine. No transamination between phenylglyoxal and β-alanine, β-amino-*n*-butyrate, α-amino-*iso*butyrate, β-amino-*iso*butyrate, serine, aspartate, or phenylalanine could be detected. α-Amino-*n*-butyrate gave 16% of the activity of α-alanine.

The following phenylglyoxal analogues caused the percentage inhibition shown when used at an equimolar concentration, *i.e.* 1.33 mM; diacetyl, 66; acetylacetone, 32; acetonylacetone, 31; phenylacetate, 19; β-phenylpropionate, 20; phenylpyruvate, 44; pyruvate, 45. None of the dioxo compounds appeared to inhibit by acting as competing substrates. Analogues related to ω-aminoacetophenone, which caused significant inhibition at a higher concentration only, *i.e.* 6.0 mM, were: β-phenylethylamine, 19; DL-β-hydroxy-β-phenylethylamine, 37; DL-β-phenylserine, 23; ephedrine, 38; *pseudo*-ephedrine, 34.

Enzyme inhibition occurred with a number of hydrazine and hydroxylamine derivatives which are potent inhibitors of dioxovalerate transamination, and which are also known to inhibit aromatic amino acid decarboxylases[5–7]. At a concentration

of 10 μM, the following compounds caused the percentage inhibition shown, 3-hydroxy-4-bromobenzyloxyamine, 84; 3-hydroxybenzyloxyamine, 82; *N*-(3-hydroxybenzyl)-*N*-methylhydrazine orthophosphate, 65. Aminooxyacetic acid[8] caused 89% inhibition. Under identical conditions these compounds inhibit dioxovalerate transaminase activity by 94, 34, 23, and 98%, respectively.

No inhibition could be detected using EDTA at concentrations less than 10 mM, and α,α'-dipyridyl caused only 24% inhibition at 1 mM.

During the course of this work it was noticed that phenylglyoxal transaminase activity was labile, and that, whereas storage of enzyme preparations had little effect on dioxovalerate activity, there was a marked decay in activity towards phenylglyoxal. This decay of activity was not affected by the presence of β-mercaptoethanol, but could be prevented by the addition of pyridoxal phosphate. It was found that $(NH_4)_2SO_4$ fractions containing phenylglyoxal transaminase could be resolved from coenzyme to the extent of 90% by thorough dialysis. A LINEWEAVER–BURK[9] plot, showing the effect of pyridoxal phosphate concentration on the reactivation of dialysed enzyme, gave a value of $3.4 \cdot 10^{-5}$ M for the enzyme–coenzyme dissociation constant. Pyridoxamine phosphate also restored the activity of dialysed enzyme, but was less effective than equimolar amounts of the aldehyde form. The enzyme–coenzyme dissociation constant in this case was $6.2 \cdot 10^{-4}$ M. Treatment of fully active enzyme fractions with an alkaline phosphatase preparation caused a rapid loss of transaminase activity. Alkaline phosphatases are known to hydrolyse pyridoxal phosphate[10,11], and the resolution of tryptophanase by this method has been reported[12].

Fractionation of particle-free extracts of *Rps. spheroides* showed that some separation of transaminase activities for dioxovalerate and phenylglyoxal could be achieved. $(NH_4)_2SO_4$ fractionation, of heated and protamine sulphate-treated extracts[1], has given the best results so far. The measurement of both activities with alanine as the NH_2-group donor showed that whereas phenylglyoxal was more rapidly transaminated than dioxovalerate in the 30–40% saturated fraction, dioxovalerate transamination was relatively more rapid in the 40–50% saturated fraction. The activity ratios, determined in the presence of an excess of pyridoxal phosphate, were 1.7 and 0.26, respectively. The presence of excess coenzyme caused a different degree of activation in each case. Specific activities for both enzymes were greater in the 30–40% saturated fraction, but no separation of the two enzyme activities in this fraction was achieved by column chromatography on DEAE-cellulose at pH 7.

Comparison of the two transaminase activities in non-fractionated particle-free extracts of different species of Rhodopseudomonas showed a wide variation. Whereas a strain of *Rps. spheroides* gave a ratio of 1.1 in favour of phenylglyoxal transaminase, the activity ratios for *Rps. capsulatus* and *Rps. palustris* were 2.9 and 0.7, respectively. These values were obtained in the presence of excess pyridoxal phosphate.

Attempts to determine the equilibrium constant for phenylglyoxal transamination by direct methods has not proved possible. As with dioxovalerate transaminase, excess substrate inhibition by the ketoaldehyde occurs unless the NH_2-group donor is in excess. Product inhibition by pyruvate also occurs, and evidence has been obtained that side-reactions become important if long reaction times are involved. With large amounts of enzyme, and 30-fold excess of alanine, 68% con-

version of phenylglyoxal to ω-aminoacetophenone has been observed after incubation at the optimum pH for approx. 3 h at 37°. By varying the amount of enzyme used, and measuring the conversion of substrate which occurs during incubation for times of this order, a linear relation was obtained between the reciprocals of percentage conversion and enzyme concentration. Extrapolation of these results suggested that 75% conversion would occur at an infinitely high enzyme concentration. Values of 56 and 27% conversion were obtained for relative alanine concentrations of 10- and 3.33-fold, respectively. Chromatographic evidence has been obtained for the formation of alanine during incubation of pyruvate with ω-aminoacetophenone. Alanine formation was dependent on the presence of enzyme preparation, and on the concentration of aminoketone. These results suggest that at equimolar alanine and phenylglyoxal concentrations, the equilibrium position would be unfavourable for ω-aminoacetophenone formation.

An examination of the effect of substrate concentrations on phenylglyoxal transaminase activity, gave values of 12.0 and 0.6 mM for the apparent Michaelis constants of alanine and phenylglyoxal, respectively. These results are similar to those obtained for the alanine-dioxovalerate system[1].

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A coupling factor in photophosphorylation

Several reports have recently appeared which described the isolation from mitochondria of factors which were necessary for phosphorylation but not for electron transport in oxidative phosphorylation[1–6]. The experiments reported herewith will demonstrate the isolation of a similar factor from chloroplasts. It is required for photophosphorylation but not for photoreduction.