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Nonenzymatic Transamination of Phenylglycine with 2-Oxoglutaric Acid Mediated by a Micellar Pyridoxal Model

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Phenylglycine undergoes transamination with 2-oxoglutaric acid in the presence of N-dodecylpyridoxal chloride (DPL) and hexadecyltrimethylammonium chloride (CTACl) under mild conditions. This is the first example of nonenzymatic overall transamination realized in the absence of metal ions.

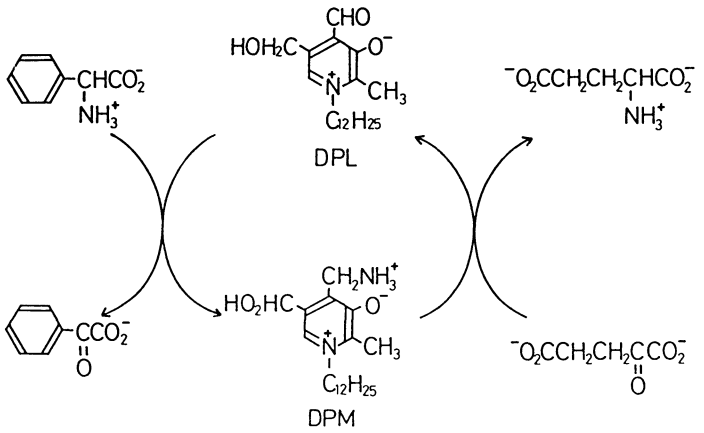
Transamination of amino acids is one of the reactions that are catalyzed by pyridoxal 5'-phoshate (PLP).[1] In one typical example of transaminase reaction aspartate and glutamate are interconverted by a sequence of reactions shown below, where PMP stands for pyridoxamine 5'-phosphate:

Aspartate + PLP ⇌ Oxalacetate + PMP (1a)

2-Oxoglutarate + PMP ⇌ Glutamate + PLP (1b)

Aspartate + 2-Oxoglutarate ⇌ Oxalacetate + Glutamate (1)

Transamination can be effected nonenzymatically with pyridoxal or its analogues usually in the presence of metal ions.[2-4] Most of the work reported to date dealt with either one of the partial reactions (Eqs. 1a or 1b) separately,[5,6] and few efforts have been devoted to the study of overall transamination in nonenzymatic systems. Murakami and collaborators recently developed a lipophilic pyridoxal model which can bring about overall transamination of amino acids and keto acids, but the catalytic activity of their model was still dependent on copper (II) ion.[7] In the meantime, we reported on a potent pyridoxal model (DPL, for struc-



Scheme 1.

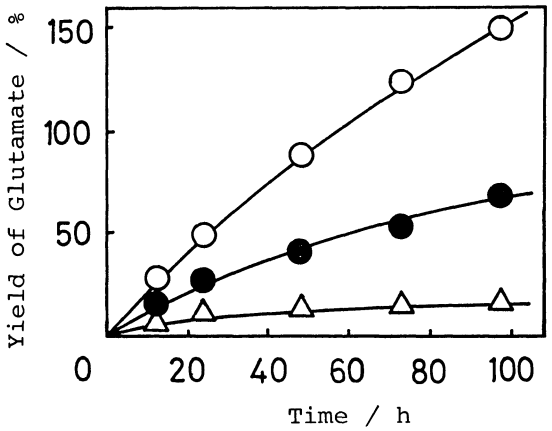


Fig. 1. Time course for the formation of glutamic acid by DPL-mediated transamination of 2-oxoglutaric acid with phenylglycine at pH 7.8 and 30.0 °C; 0.20 mM DPL, 2.0 mM phenylglycine, 3 (△), 6(●) or 12 (○) mM 2-oxoglutaric acid, 1.0 mM CTACl, 0.10 mM EDTA.

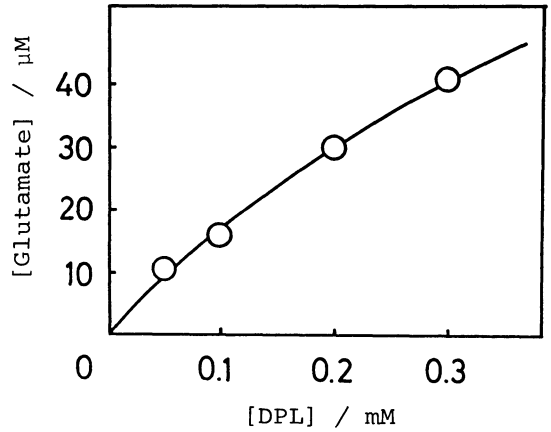


Fig. 2. Dependence on DPL concentration of the rate of transamination of 2-oxoglutarate with phenylglycine at pH 7.8 and 30.0 °C; 2.0 mM phenylglycine, 12 mM 2-oxoglutarate, 1.0 mM CTACl, 0.10 mM EDTA. Shown are the amounts of glutamate formed in 6 h of reaction.

ture see Scheme 1) which is capable of promoting half-transamination of ordinary amino acids under near-physiological conditions.[8-10] We would like to demonstrate that the same model is able to catalyze overall transamination of amino acids and keto acids in the absence of metal ions.

Half-transamination of DPL was carried out as reported previously with the following modifications.[9] Phenylglycine was replaced for phenylalanine and the concentration of hexadecyltrimethylammonium chloride (CTACl) was reduced to 1 mM. Occurrence of transamination was readily discerned by a decrease in the absorption at 395 nm of the Schiff base of DPL and phenylglycine. A reaction solution for overall transamination contained 0.20 mM DPL, 2.0 mM phenylglycine, 12 mM 2-oxoglutarate, 1.0 mM CTACl and 0.10 mM EDTA in 30 mM potassium phosphate buffer (pH 7.8). This mixture (1.0 ml) was incubated at 30.0 °C and aliquots (50 μl) were withdrawn at intervals. Following lyophilization the sample was incubated overnight in the dark with 50 μl of 10 mM dansyl chloride and 50 μl of 0.20 M sodium hydrogencarbonate at room temperature.[11] The resulting dansylated amino acids were analyzed by reversed phase HPLC.[12]

Formation of glutamate in the above reaction mixture was readily established by HPLC as well as by TLC on silica gel. No glutamate was generated upon omission of DPL from the reaction mixture, ruling out the possibility of direct transamination of phenylglycine with 2-oxoglutarate. The time course for this overall transamination at three 2-oxoglutarate concentrations is presented in Fig. 1. Note that the yield of glutamate in 96 h at the highest keto acid concentration employed amounted to 148% relative to the DPL concentration. This indicates that the reaction is truly catalytic and that DPL is turning over (Scheme 1).

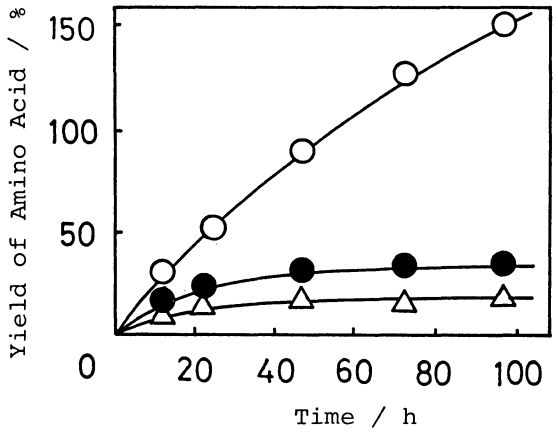


Fig. 3. Time course of DPL-mediated transamination of 2-oxoglutarate (○), pyruvate (△) or glyoxylate (●) with phenylglycine at pH 7.8 and 30.0 °C; 0.20 mM DPL, 2.0 mM phenylglycine, 12 mM keto acid, 1.0 mM CTACl, 0.10 mM EDTA.

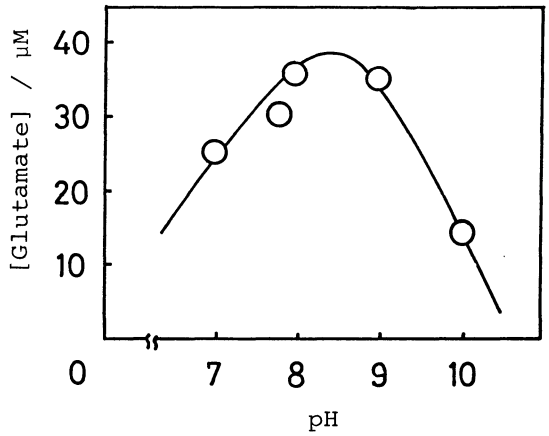


Fig. 4. pH-Dependence of the rate of DPL-mediated transamination of 2-oxoglutarate with phenylglycine at 30.0 °C; 0.20 mM DPL, 2.0 mM phenylglycine, 12 mM 2-oxoglutarate, 1.0 mM CTACl, 0.10 mM EDTA. Shown are the amounts of glutamate formed in 6 h of reaction.

Figure 2 illustrates a dependence of the rate of transamination on DPL concentration. The rate increases as the DPL concentration is increased, but the rate tends to level off at the higher DPL concentrations. This could suggest formation of a complex between DPM and keto acid at a step preceding the rate-determining tautomerization of the ketimine.[13)]

Of the four keto acids tested as amino-acceptor substrate (2-oxoglutarate, oxalacetate, pyruvate and glyoxylate), 2-oxoglutarate was the most reactive (Fig. 3). Of interest is the finding that oxalacetate, a close homolog of 2-oxoglutarate, did not react under the present conditions. The reason for this peculiarity is not known.

The pH dependence of the overall transamination of phenylglycine with 2-oxoglutarate has also been studied. As shown in Fig. 4, the rate is maximal at pH 8-9. This skewed bell-shaped rate profile appears to reflect the concentration of the active Schiff base of DPM and 2-oxoglutarate.[14)]

Finally, the rate of the partial and overall transamination is compared. As reported previously, the first half-transamination (Eq. 1a) of DPL with amino acid proceeds at a rate of ca. 1×10^{-4} s^{-1} at 30 °C.[9)] On the other hand, the rate of glutamate formation is ca. 1×10^{-6} s^{-1} under the similar conditions. This indicates that the second partial reaction (Eq. 1b) limits the overall transamination in the present system. Presumably, unfavorable Schiff base formation between DPM and keto acid may be responsible for this small rate of the second half-transamination. As described previously, formation of an aldimine of DPL is favorable only for those amino acids carrying a hydrophobic side-chain.[8)] The same must be true for the ketimine formation; since none of the keto acids employed bear such a

hydrophobic substituent the steady-state concentration of the ketimine of DPM and keto acid may be low.

In summary, it was shown that overall transamination of amino acids is effected by DPL and CTACl under mild conditions without relying on metal ions.

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12) HPLC (high performance liquid chromatography) was run on TSK ODS-120T (4.6 × 250 mm) with 50 mM ammonium acetate (pH 4.5) containing the following percentage of acetonitrile as eluant: dansylglutamic acid, 25%, retention time 24 min (flow rate 0.4 ml/min); dansylglycine, 28%, 24 min; dansylalanine, 31%, 22 min. Dansylamino acids were quantitated by integrating peak areas on a Shimadzu Chromatopac C-R1A. All experiments were carried out at least twice, and the data were reproducible within ± 3 % of the mean.
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