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APPLICATION OF E. COLI ASPARTATE TRANSAMINASE TO AMINO ACID SYNTHESIS

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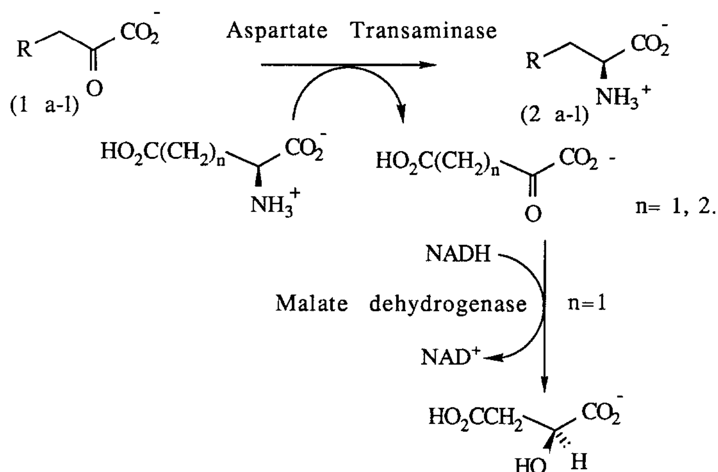
ABSTRACT: The kinetics and synthetic utility of the conversion of α-keto acids into L-α-amino acids using cloned E.coli Aspartate transaminase have been evaluated.

The rapid and enantioselective synthesis of α-amino acids has long attracted attention from synthetic chemists[1]. However whilst fermentation has been used for academic and industrial production of amino acids[2], no single in vitro enzymatic system is available for preparation of a wide range of L-α-amino acids. Herein is outlined a simple and efficient procedure for the preparation of a variety of aromatic and aliphatic L-α-amino-acids from readily available α-keto acids[3] using cloned E.coli Aspartate Transaminase.

E.coli Aspartate Transaminase has been cloned[4] and is available in large quantities. In order to evaluate the synthetic potential of this enzyme, its substrate specificity was investigated. Maximum velocity (V_{max}) and Michaelis constant (K_m) data[$] were determined using a NADH/Malate dehydrogenase coupled U.V. spectroscopic assay[6] (Scheme 1). V_{max}/K_m values, a measure of the catalytic efficiency of each substrate (1), are given in Table 1.

SCHEME 1



The enzyme kinetics indicated that keto acids of a wide range of structural types are satisfactory enzyme substrates and so preparative scale syntheses (1 mmol or greater) of both aromatic and aliphatic amino acids were carried out. 3-Arylpyruvic acid derivatives (1a-e) [close analogues of the native substrate phenylpyruvic acid (1a)] are readily converted into the corresponding L-α-amino acids (2a-e) but in addition the conversion of alkyl substituted α-keto acids (1f-l) could also be accomplished by increasing the enzyme concentration by

10-15 fold. The facility of the latter conversions is due to the availability of cloned enzymes in large quantities. The yields and optical purities (all greater than 90% ee) of amino acids 2 (a-e & h-l) synthesised enzymatically are given in Table 1. Since the large quantities of cloned E.coli Aspartate transaminase available allow efficient utilisation of α-keto-acids previously not thought to be substrates, this enzyme is an effective catalyst for the mild enantioselective preparation of a wide range of L-α-amino acids.

TABLE 1*

Substrate R (1)	K_m^S (mM)	V_{max} (μmolmin^{-1})	V_{max}/K_m (1min^{-1} x10^3)	Enzyme (kU1^{-1})	Time (h)	Yield %	$[\alpha]_D$
a) Ph	4.3	0.43	0.1	20	12	84	-34.2 (-33.7 to -35.2)
b) p-HOC_6H_4	2.0	0.78	0.39	30	4	80	-10.9 (-10.2)
c) o-MeC_6H_4	6.8	0.13	0.02	40	12	30	-16.1
d) 2-Napthyl	1.0	0.091	0.09				
e) 3-Indole	1.4	0.28	0.2	40	18	72	-30.1 (-31.5)
f) HO_2C	0.37	1.3	3.5				
g) HO_2CCH_2	7.5	0.05	0.007				
h) H	22	0.26	0.001	400	18	35	+13.4 (+14)
i) Me	20	4.5×10^{-3}	0.0007	400	18	37	+19.6 (+20.4)
j) $MeCH_2$	21	9.2×10^{-3}	0.0004	400	18	32	+23.0 (+24+/-2)
k) Me_2CH	19	6.9×10^{-3}	0.0036	200	18	40	+11.8 (+12.3)
l) $MeSCH_2$	13	0.1	0.008	100	12	58	+24.8 (+23.4)

*Optical rotations were obtained using conditions described in Catalogue Handbook of Fine Chemicals, Aldrich, 1986-7. Literature values are in brackets.
Either Aspartate (2F) or Glutamate (2g) can be used as the amino group donor.

Typical procedure:

Keto-acid (1) (1 mmol) and L-Glutamic acid (1.5 mmol) were dissolved in water (20 ml) at 40°C and the pH was adjusted to ca.8 using 1M sodium hydroxide and hydrochloric acid solutions. To this solution pyridoxal phosphate (5 mg/1) and Aspartate transminase (see Table 1) were added. After ca. 12h purification was accomplished by; (1) Addition of acetone (70% v/v) to precipitate the protein. (ii) Supernatant adjusted to pH ca 3.3, and eluted from alumina column with water (to remove excess Glutamic acid). (iii) Ion exchange chromatography on Dowex 50 WX 8-100, eluting with 1M aq. pyridine to produce pure L-α-amino acid (2).

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$ Aspartate transaminase obeys "Ping-Pong, Bi-Bi" kinetics[5]. Parameters were calculated by Lineweaver-Burke analysis:
Rate $V = V_{max}/\{1+K_m^A/[A]+ K_m^S/[S]\}$
where V_{max} is the maximal velocity at saturating levels of both substrates, [A] is the concentration of L-Aspartic acid, [S] is the concentration of keto acid (1) and K_m^A and K_m^S are Michaelis-Menten constants

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