Aminotransferase Activities of Branched Chain Amino Acids in *Escherichia coli*

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Leucine aminotransferase (EC 2.6.1.6) catalyzes the reversible transamination of 2-oxoglutarate and branched chain amino acids. Purified enzyme preparations isolated from chicken liver [1] and from pig heart [2] are like the transaminase B purified by Rudman and Meister [3] from *E. coli* specific for leucine, isoleucine, and valine. Some evidence has been presented that the number of aminotransferases catalyzing transamination reactions of leucine, isoleucine, and valine is more than one.[4]

Previous results suggest that a single enzyme catalyzes the transamination of branched chain amino acids in *E. coli*.[3,5] In this study aminotransferase activities for leucine, isoleucine, and valine were assayed in certain *E. coli* strains in which the specific activities of leucine aminotransferase varied considerably. Also a partial purification of this enzyme was carried out.

Growing E. coli. The following *E. coli* strains were used: U5-41, a wild valine-resistant strain, isolated as presented earlier,[5] K-12, a valine-sensitive strain from WHO International E. coli Centre, Copenhagen, Denmark, M-4862, a valine-requiring mutant originally developed by Dr. H. E. Umbarger and kindly provided by Dr. M. J. Pine (Roswell Park Memorial Institute, Buffalo, N.Y.), and K-12, a valine-resistant strain developed by cultivating the valine-sensitive K-12 in the presence of 1 mM L-valine in the minimal medium (MM) in sequential cultures until it grew well in the medium. The M-4862 strain has abnormal reductoisomerase activity and is resistant to valine.

Cultures were grown on a glucose-yeast extract-citrate-tryptone agar as described earlier.[5] Transfers were made to an inoculation medium (IM) containing 1 % Bacto-yeast extract (Difco), 1 % Bacto-tryptone, and 0.5 % dipotassium monophosphate that had been autoclaved at 120° for 7 min. Usually overnight incubation at 37° without shaking was carried out to produce cells for the inoculum. The cells were washed twice with cold 0.9 % sodium chloride and were transferred to a minimal mineral salt medium containing 0.1 % ammonium chloride, 0.7 % disodium hydrogen phosphate, 0.3 % potassium dihydrogen phosphate, 0.5 % sodium chloride, 0.01 % magnesium sulfate heptahydrate and 0.2 % D(+)-glucose. The mixture was shaken in a rotatory shaker (model A from E. Buehler, Tübingen, Germany) at 200 rpm at 37° until the cells were harvested.

Preparation of extracts. The cells were harvested in the late exponential phase where the activity of leucine aminotransferase is a maximum,[5] washed twice with saline and centrifuged at 5000 *g* for 10 min at 2–4°. The cell pellet was stored at −35° before use. The cells were broken by freezing and thawing in the presence of lysozyme as described by Ron, Kohler and Davis.[6] The buffer was a 10 mM Tris-HCl buffer, pH 8.0, 10 mM in 2-mercaptoethanol and 10 μM in pyridoxal-5′-phosphate (Fluka AG, Buchs, Switzerland). The slimy lysozyme extract was treated in a sonic oscillator (model DF-101, 250 W, 10 kc, Raytheon Company, Waltham, Mass.) for 3 min. This extract was centrifuged at 100 000 *g* for 1 h in a M-50 MSE ultracentrifuge (Measuring & Scientific Equipment Ltd., London, England). The supernatant (S-100) was the crude cell-free extract used in the experiments and as starting material for enzyme purification.

Heat treatment. The S-100 extract was incubated with 1 mM 2-oxoglutarate at 65° for 5 min. S-100 usually contained 3–10 mg protein per ml. The precipitated material was removed by centrifugation at 100 000 *g* for 30 min.

Ammonium sulfate fractionation. Ammonium sulfate was added to the solution remaining after the heat treatment up to 40 % saturation after it had been cooled in ice and the mixture was incubated at 0° for 15 min before centrifugation as above. The supernatant was then adjusted to the 70 % saturation level with ammonium sulfate and the precipitate was centrifuged as above after it had been stored in ice for several hours. The pellet was dissolved in a small volume of preparative disc-electrophoresis buffer 10 mM in 2-mercaptoethanol and 10 μM in pyridoxal-5′-phosphate.

Preparative disc electrophoresis. A Shandon preparative acrylamide electrophoresis apparatus (Shandon Scientific Co., Ltd., London, England) was used. The 10–12 cm long acrylamide gel was used without a spacer gel employing the technique of Ornstein and Davis as described by Chang, Srb and Stewart.[7] 2 ml fractions were collected at 2–4°. The eluting buffer was a 0.2 M Tris-acetate buffer, pH 8.0,

Table 1. Partial purification of leucine aminotransferase from *Escherichia coli.*

Enzyme preparation	Volume ml	Protein mg	Activity units/ml	Specific activity units/mg	Purif. factor	Recovery %	Relative activity Leu:Ile:Val
Crude extract, S-100	25.2	8.3	3.4	0.4	1	100	1.0:0.9:0.7
After heat treatment	22.4	1.3	5.0	3.7	9	131	1.0:0.9:0.7
After ammonium sulfate precipitation	1.0	9.6	110.9	7.5	18	129	1.0:0.8:0.7
Disc electrophoresis	8.0	3.1	8.1	25.8	63	59	1.0:0.8:0.6

and all the fractions possessing leucine aminotransferase activity were collected.

Enzyme assays. Colorimetric aminotransferase assays were carried out with 2-oxoglutarate as amino group acceptor by a method described elsewhere.[8] When the amination activities of branched chain keto acids with various amino acids were studied, a paper chromatographic method was employed.[5] The activity was expressed as μmoles of product formed in 1 min at 30°.

The protein contents of the enzyme preparations were determined by the method of Lowry, Rosebrough, Farr and Randall [9] using serum albumin as standard.

Results and discussion. The following specific activities were obtained for different *E. coli* strains when leucine, isoleucine, and valine, in this order, were amino group donors to 2-oxoglutarate: 0.45, 0.43, and 0.29 units/mg protein for the U5-41 strain, 0.27, 0.22, and 0.12 units/mg protein for the valine-sensitive K-12 strain, 0.47, 0.40, and 0.21 units/mg protein for the valine-resistant K-12 strain, and 1.11, 1.08, and 0.78 units/mg protein for the M-4862 strain. These specific activity values show that the relative activity with respect to leucine, isoleucine, and valine is practically the same regardless of the bacterial strain, indicating the presence of a single aminotransferase for these amino acids.

Results of attempts at partial purification of the leucine aminotransferase are presented in Table 1. A very simple purification procedure yielded a yellow product which was eluted as a single activity and protein peak from the preparative acrylamide gel just after the bromophenol blue indicator. Two other major protein peaks were eluted later. A 63-fold purification was attained and the product transaminated leucine, isoleucine, and valine, but not aromatic amino acids. The relative activities with respect to leucine, isoleucine, and valine remained practically the same during the purification. The same observation was made when S-100 was fractionated on hydroxylapatite and Sephadex G-100. In these fractionations isoleucine-2-oxoglutarate and valine-2-oxoglutarate transamination catalysts gave single peaks but leucine-2-oxoglutarate aminotransferase gave two peaks and the position of the second peak was the same as that of the phenylalanine-2-oxoglutarate transamination catalyst peak. It was about one tenth the size of the main peak. The main peak was identical in shape and position to the two other peaks indicating activity for isoleucine and valine. This result is in accordance with the results for transaminases in *E. coli* [3] and *V. cholerae.*[10]

The ability of several amino acids to donate amino groups to various branched chain keto acids in the presence of various *E. coli* extracts is shown in Table 2. The specific activity of the glutamate-2-oxoisocaproate transamination catalyst, taken to be 100 %, was 0.29 units/mg protein. The corresponding activity of leucine-2-oxoglutarate transaminase in Expt. B in Table 2 was 0.45 units/mg protein. Table 2 shows that several amino acids, including leucine, isoleucine, valine, methionine, and aromatic amino acids, donate an amino group to branched chain keto acids. A similar broad spectrum in the transamination of branched chain keto acids has been reported for other biological materials.[11,12]

Table 2. Amination of branched chain keto acids with various amino acids by *Escherichia coli* extracts.

	Cell strain	Amino donor	Specific activity: per cent			
			Amino acceptor			
			KIC	KMV	KIV	KGA
Experiment A	U5-41	glutamate	*100*	95	71	
	»	glutamine	31	35	32	
	»	valine	155	151		
	»	leucine			138	
	»	isoleucine			138	
	»	methionine	19	22	17	
	»	phenylalanine	19	22	19	
	»	tyrosine	16	17	14	
	»	tryptophan	22	12	17	
Experiment B	U5-41	glutamate	*100*	99	58	
	K-12, s	»	82	32	20	
	M-4862	»	184	130	101	
	U5-41	leucine			156	*100*
	K-12, s	»			110	60
	M-4862	»			216	247
	U5-41	isoleucine			161	96
	K-12, s	»			102	49
	M-4862	»			242	240
	U5-41	valine				65
	K-12, s	»				27
	M-4862	»				173

Abbreviations: KIC=2-oxoisocaproate, KMV=2-oxo-3-methylvalerate, KIV=2-oxoisovalerate, and KGA=2-oxoglutarate.

The data in Table 2 show that the changes mentioned in the first paragraph of this section are reflected also here and provide evidence that a single aminotransferase catalyzes branched chain amino acid-2-oxoglutarate transamination reaction. Other tested amino acids, including alanine, had a weak or no ability to donate amino groups. The ability of aromatic amino acids to donate an amino group is interesting because the partially purified preparation of leucine aminotransferase from *E. coli* did not effect the amination of 2-oxoglutarate.

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