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Human branched-chain L-amino acid aminotransferase: Activity and subcellular localization in cultured skin fibroblasts

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Summary. Assay conditions for measurement of human skin fibroblast branched-chain L-amino acid aminotransferase activity were established and applied to studies on subcellular distribution and kinetic properties of the enzyme. Digitonin fractionation of cultured cells revealed that the aminotransferase activity was mainly (at least about 95%) associated with mitochondrial citrate synthase activity. As tested with L-leucine, activity of the enzyme against amino group acceptors (forward reaction) was in the order 2-oxoglutarate ≥ branched-chain > straight-chain 2-oxo acids (C_3–C_8). With 4-methyl-2-oxopentanoate, activity against amino group donors (reverse reaction) was in the order L-glutamate ≥ branched-chain > straight-chain (C_2–C_6) and other L-amino acids. The data suggest that, in human fibroblasts, isoenzyme type I resides within the mitochondrial space. Possible implications for the metabolism of branched-chain compounds are discussed.

Keywords: Amino acids – Branched chain – Aminotransferase – Human

Introduction

Catabolism of branched-chain L-amino acids (BCAA) is initiated by reversible transamination (Harper et al., 1984). The reaction is catalysed by BCAA aminotransferase (AT; EC 2.6.1.42). Mammalian (iso)enzymes have been first isolated from hog heart (Ichihara and Koyama, 1966; Taylor and Jenkins, 1966) and later from rat liver (Aki et al., 1968) and heart (Wallin et al., 1988), hog brain (Aki et al., 1969), and pancreas of various species (Makino et al., 1984). So far organ distribution of enzyme activity, isoenzyme composition, and subcellular localization has been extensively studied in rat tissues (see Cappuccino et al., 1978; Harper et al., 1984; Ichihara, 1985, for refs.).

With respect to the human enzyme(s), information is far less abundant. Isoenzyme distribution in a variety of human tissues has been examined by Goto et al. (1977). Pancreatic enzymes have been studied by Makino et al.

(1984). Complementation studies with man-chinese hamster hybrids showed that expression of human BCAA-AT is controlled by two independent genes on chromosomes 12 and 19 (Jones and Moore, 1979; Naylor and Shows, 1980). Inherited deficiencies in enzyme activity have been reported (see Danner and Elsas, 1989, for refs.). However, data on enzymic properties are fragmentary (Goto et al., 1977; Makino et al., 1984) and virtually nothing is known on the subcellular compartmentation of activity in tissues (Schadewaldt et al., 1988a; Schadewaldt and Wendel, 1989).

In the present communication we report on the evaluation of assay conditions for measurement and on some properties of solubilized BCAA-AT from human skin fibroblasts as well as on the subcellular compartmentation of enzyme activity. Some preliminary results have been presented (Schadewaldt et al., 1988b).

Material and methods

Unless otherwise noted, all chemicals and reagents were obtained from Merck, Darmstadt, FRG, or Sigma, Munich, FRG. L-Alloisoleucine was purchased from Bachem, Heidelberg, FRG, 4-methyl-2-oxopentanoate (KIC), 3-methyl-2-oxobutanoate (KIV) and 2-oxopentanoate (sodium salts) were from Fluka, Buchs, Switzerland. Enzymes and coenzymes were from Boehringer, Mannheim, FRG. (S)-3-methyl-2-oxopentanoate (KMV) was prepared enzymatically from L-isoleucine according to Rüdiger et al. (1972). As checked by reversed phase high performance liquid chromatography of the quinoxalinol derivatives (Schadewaldt et al., 1989c) and by amino acid analysis, 2-oxo acids contained no detectable amounts of interfering compounds.

L-[1-^{14}C]Leucine (2.1 GBq/mmol), and L-[1-^{14}C]valine (2.2 GBq/mmol) were from DuPont NEN, Dreieich, FRG, or from Amersham Buchler, Brunswick, FRG. L-[1-^{14}C]Isoleucine (14.1 MBq/mmol) and L-[1-^{14}C]alloisoleucine (9.2 MBq/mmol) were synthesized as detailed elsewhere (Schadewaldt et al., 1989b). Prior to use, L-amino[^{14}C]acids were purified by ion exchange chromatography (Schadewaldt et al., 1990).

Human fibroblasts were raised from skin biopsies, multiplied in monolayer culture, and cell suspensions prepared as described (Schadewaldt et al., 1988a). For incubation experiments with intact cells, 0.4 ml of cell suspension (0.2–0.5 mg of cell protein) was inserted into the assay. For preparation of cell-free extracts, the suspension was freeze-thawed once with liquid N_2 and treated with Triton X-100 (final concn. 0.25%, v:v). Digitonin fractionation of cytosolic and mitochondrial enzymes as well as determination of total enzyme activities were essentially performed as given by Mackall et al. (1979).

Enzyme activities in cell-free extracts were measured at 25°C by standard spectrophotometric procedures: alanine aminotransferase (EC 2.6.1.12) (Bergmeyer and Bernt, 1974a), aspartate aminotransferase (EC 2.6.1.1) (Bergmeyer and Bernt, 1974b), citrate synthase (EC 4.1.3.7) (Bergmeyer et al., 1974a), lactate dehydrogenase (EC 1.1.1.27) (Bergmeyer and Bernt, 1974c), 3-phosphoglycerate kinase (EC 2.7.2.3) (Bergmeyer et al., 1974b).

BCAA-AT activity in the forward direction was estimated by measuring 2-oxo [^{14}C]acid production from the corresponding 1-^{14}C-labeled BCAA and in the backward direction by determining BCAA formation from the respective 2-oxo acid. Under standard assay conditions (30 min at 37°C in a shaking water bath) the medium (total volume 1 ml, for device see (Schadewaldt et al., 1989c)) comprised (final concentrations in parentheses) sodium pyrophosphate/HCl (80 mmol/l, pH 8.3), pyridoxalphosphate (0.05 mmol/l), and L-[1-^{14}C]leucine (1 mmol/l, 6–14 Bq/nmol) or KIC (1 mmol/l). 2-Oxoglutarate (10 mmol/l) and L-glutamate (100 mmol/l) were present as amino group acceptor and donor, respectively. Incubation was started by addition of cell-free extracts

(0.05–0.25 mg of cell protein) and terminated by injection of 0.5 ml sulfosalicylic acid (10%, w:v). Modifications are specified in the Results section. Similarly, intact fibroblasts (0.4 ml of suspension) were incubated in PBS (37°C, 90 min, final volume 1 ml) fortified with L-[1-^{14}C]leucine (1 mmol/l, 21 Bq/nmol) as described previously (Schadewaldt and Wendel, 1989). Duplicate samples were prepared throughout and appropriate blanks were run in parallel.

2-Oxo[1-^{14}C]acids accumulated during incubation with L-amino[^{14}C]acids were chemically decarboxylated by addition of H_2O_2. $^{14}CO_2$ was trapped and measured by liquid scintillation counting as given elsewhere in detail (Schadewaldt et al., 1989c). With intact cells, $^{14}CO_2$ originating from branched-chain 2-oxo acid dehydrogenase complex activity (BCOA-DH; not observed in incubations with cell-free extracts) was determined separately prior to H_2O_2 treatment.

Amino acids were measured by automatic amino acid analysis using ninhydrin detection and DL-norleucine as an internal standard (Benson et al., 1967; Schadewaldt et al., 1990). Protein concentration was estimated by the Lowry procedure (Lowry et al., 1951) with bovine serum albumin as a standard.

According to the Cleland notation (Henson and Cleland, 1964), the aminotransferase reaction proceeds via a Ping Pong Bi Bi mechanism. With substrates A and B and in the absence of products and inhibitory effects of substrate, the forward velocity is given by

$$v = (V_{max} * [B])/\{K_{mB} + [B] * (1 + K_{mA}/[A])\} \quad (1)$$

If the concentration of A is constant and B is the varied substrate, $(1 + K_{mA}/[A]) = F$ is constant and the reaction velocity is given by

$$v = \{(F^{-1} * V_{max}) * [B]\}/\{(F - 1 * K_{mB}) + [B]\} \quad (2)$$

or

$$v = V_{max,\,rel.} * [B]/(K_{mB,\,app.} + [B]) \quad (3)$$

The latter formula was used for non-linear regression analysis of kinetic data according to Wilkinson (1961). A computer program was used (Duggleby, 1981) which yielded values for concentrations of the varied substrates B with half maximal effect on the transamination rate ($K_{mB,\,app.}$), the belonging maximal velocity ($V_{max,\,rel.}$), and the respective asymptotic standard errors.

Other results are presented as means of two (deviation of individual values from mean $<$ or $<<$15%) or as means ± S.D. of at least 3 independent experiments with the number of experiments in parentheses.

Results

Determination of enzyme activity

We first established optimized conditions for measurement of human skin fibroblast BCAA-AT activity and examined possible interferences as discussed by Allison and Purich (1979).

A solubilized enzyme preparation was used. Among the various solubilization procedures tested, freeze-thawing and Triton X-100 treatment of intact cells appeared to be optimal with respect to yield and reproducibility (data not shown).

Under standard conditions at pH 8.5 (cf. Taylor and Jenkins, 1966; Ichihara and Koyama, 1966), no significant differences in L-leucine transamination were observed when incubations were carried out in: Tris/HCl, triethanolamine/HCl, sodium and potassium phosphate, or sodium pyrophosphate/HCl (final concentrations 80 mmol/l). Because Good buffers

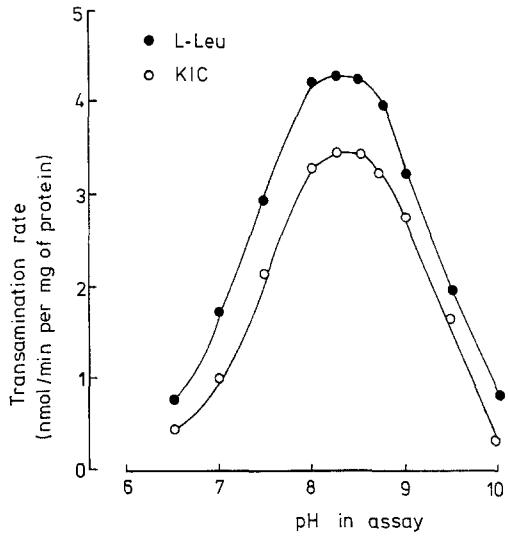

Fig. 1. pH dependence of human skin fibroblast branched-chain L-amino acid aminotransferase activity in the forward and backward direction. Cell free extracts were incubated in sodium pyrophosphate-phosphate buffer (80 mmol/l each) at the pH as indicated. Transamination was estimated from the production of KIC and L-leucine in assays (37 °C, 30 min) with L-[1-^{14}C]leucine (1 mmol/l)/2 oxoglutarate (10 mmol/l) and KIC (1 mmol/l)/L-glutamate (10 mmol/l), respectively (cf. Experimental section). Results are means (n = 2, deviation from mean <10%)

(Good and Izawa, 1972) interfered with the amino acid analysis and phosphate buffers exhibit a weak buffer capacity around pH 8.5, sodium pyrophosphate buffer was used for further analyses (transamination rate remained unaffected between 10 and 200 mmol/l). When the pH-dependence was examined, a rather broad pH-optimum around pH 8.3 was found for the forward as well as backward reaction (Fig. 1). Therefore, pH 8.3 was used in further assays.

Transamination acitivity was linearily correlated to the amount of cell protein (range: 0.02–04 mg/assay). Enzyme activity gradually declined during incubation. However, when incubations were terminated within 30 min, underestimation of the reaction rate was limited to <5%.

Transamination rates were optimal with pyridoxalphosphate concentrations between 0.02 and 0.05 mmol/l. At ≥0.1 and ≤0.02 mmol/l pyridoxalphosphate and on addition of mercaptoethanol (10 mmol/l) or dithiothreitol (5 mmol/l), transamination rates declined. Presence of EDTA (5 mmol/l) or preincubation of BCAA-AT (30 min at 0°C) with individual substrates (L-leu, L-glu, 2-oxoglutarate, KIC; 10 mmol/l) had no significant effect.

Stability of substrates in the absence of enzyme was assured in appropriate incubations with 1-^{14}C-labeled L-leucine or KIC (1 mmol/l) in the absence as well as in the presence of 2-oxoglutarate (10 mmol/l) and L-glutamate (100 mmol/l), respectively. Occurrence of unspecific side reactions in the presence of solubilized enzyme was also examined: Oxidative degradation could be excluded by $^{14}CO_2$ measurements after incubation with the above mentioned branched-chain compounds alone or in combination with the appropriate amino group acceptor and donor, respectively. In the absence of 2-oxoglutarate, no formation of 2-oxo[1-^{14}C]acid from labeled L-leucine occurred. In the absence of L-glutamate, incorporation of label from [1-^{14}C]KIC into amino acids was negligible (<0.05 nmol/min per mg of cell protein). These results suggested that other enzymes, e.g. alanine or aspartate

aminotransferase, should not interfere with the present activity assay to a significant extent.

Substrate consumption during incubation was generally rather low (< or <<5%). Nevertheless, special care was taken when a considerable difference occured between the concentration of the amino group donor and acceptor. It was secured that substrate consumption in the assays never exceeded 10%. Likewise, accumulation of products in the assays was limited to 40 nmol (≤0.04 mmol/l).

Effects of substrate concentration on the transamination rate in the forward and backward direction were studied with L-leucine/2-oxoglutarate and KIC/L-glutamate, respectively. Non-linear regression analysis of the kinetic data yielded concentrations with half maximal effects of 0.71 ± 0.01 mmol/l and 2.26 ± 0.12 mmol/l for L-leucine (in the presence of 2-oxoglutarate, 20 mmol/l) and 2-oxoglutarate (in the presence of L-leucine, 1 mmol/l), respectively (n = 14). Likewise, concentrations of KIC ([L-glutamate] constant, 50 mmol/l) and L-glutamate ([KIC] constant, 1 mmol/l) eliciting half maximal effects amounted to 0.32 ± 0.04 mmol/l and to 17.1 ± 1.0 mmol/l, respectively. Noteworthy, a more or less distinct inhibition of transamination acitivity was observed in the presence of higher concentrations of KIC (≥2 mmol/l) and 2-oxoglutarate (≥20 mmol/l), respectively.

Thus, use of saturating concentrations of L-amino [1-^{14}C]acids appeared to be somewhat impeded, because appropriate improvement of the sensitivity of the assay, i.e. increase of the sample/blank ratio, would have afforded rather large amounts of cell protein. With 2-oxo acid substrates, concentrations were limited by possible inhibitory effects (cf. Table 1). For comparative purposes, we therefore applied 1 mmol/l of branched-chain compounds in further experiments. Concentrations of 2-oxoglutarate and L-glutamate were adjusted to 10 mmol/l and 100 mmol/l, respectively.

Amino group acceptors and donors

The efficiency of various physiological and some artifical amino group acceptors was examined in assays with L-[1-^{14}C]BCAA (1 mmol/l). When compared on the basis of the initially applied concentration of 10 mmol/l, 2-oxoglutarate appeared to be the best 2-oxo acid substrate (Table 1).

Properties of a number of 2-oxo acid substrates were additionally characterized by measuring the concentration dependence in a concentration range where substrate inhibition could be largely excluded. An evaluation of the kinetic data by non-linear regression analysis is summarized in Table 2. Concentrations of 2-oxo acids with half maximal effect on the transamination of L-leucine were in the order branched-chain 2-oxo acids (S-KMV < KIC < KIV) < straight-chain 2-oxo acids (C6 < C5 < C8 < C4 << C3). An intermediate value was obtained for 2-oxoglutarate. Transamination activity with oxaloacetate was too low to be evaluated. Computed maximal transamination rates of L-leucine were highest in the presence of its related 2-oxo acid (KIC) followed by 2-oxoglutarate > branched-chain 2-oxo

Table 1. Activity of human skin fibroblast branched-chain L-amino acid amino-transferase

2-Oxo acid added (10 mmol/l)	Transamination rate of L-Leucine (nmol/min per mg of cell protein)	L-Val	L-Ile	L-Allo
2-Oxoglutarate	4.35 ± 0.16 (96%)*	2.18	5.15	1.80
Oxalacetate	0.013 ± 0.04			
Pyruvate	0.09 ± 0.01 (107%)			
2-Oxobutyrate	1.02 ± 0.01 (87%)	0.72	0.96	0.64
2-Oxovalerate	2.41 ± 0.05 (85%)	1.15	2.72	1.10
2-Oxocapronate	1.51 ± 0.14 (77%)	0.62	1.65	0.57
2-Oxooctanoate	0.27 ± 0.04 (112%)	0.12	0.33	0.13
2-Oxoisovalerate	2.45 ± 0.17 (64%)	1.23	3.19	1.15
2-Oxoisocaproate	3.32 ± 0.21 (55%)	1.15	4.79	1.14
(S)-3-Methyl-2-oxo-valerate	2.36 (51%)			
Phenylpyruvate	0.25			
4-Hydroxyphenyl-pyruvate	0.15			

Cell-free extracts were incubated under standard conditions in the presence of L-[1-^{14}C]BCAA (1 mmol/l, 8–16 Bq/nmol) and unlabeled 2-oxo acids as indicated. Branched-chain 2-oxo[1-^{14}C]acids were measured as transamination products. Results are means of two (deviation from mean <15%) or means ± S.D. ($n \geq 4$). *The data represent the fractional activity which was found experimentally (this table) as compared to a theoretically (uninhibited) transamination rate which can be calculated on the basis of the kinetic constants in Table 2.

acid (S-KMV > KIV) > straight-chain 2-oxo acids (C5 > C6 > C4 >> C3 ≥ C8).

Additionally, various L-amino acids were tested in the presence of KIC as amino group donors for the backward reaction. Statistical treatment of the data indicated that, quite similar to the findings with the corresponding 2-oxo acids, the efficacy was in the order branched-chain compounds ≥ L-glutamate > straight-chain compounds (Table 2).

Subecllular compartmentation

With L-[1-^{14}C]leucine (1 mmol/l), transamination activity in assays with intact cells amounted to 0.29 ± 0.06 (n = 10; 90 min incubation at 37 °C) as compared to a rate of 4.73 ± 0.85 nmol/min per mg of cell protein (n = 10; 5 different cell strains) in experiments with cell-free extracts. This indicated that only a minor part of the total transamination capacity was metabolically used in the intact cell.

Subcellular localization was investigated by measuring BCAA-AT activity (standard assay conditions) together with some marker enzymes in

Table 2. Characterization of various 2-oxo and L-amino acids as amino group acceptors and donors, respectively, for human skin fibroblast branched-chain L-amino acid aminotransferase reaction

	Estimated kinetic parameters of transamination		
	$K_{m, app}$ (mmol/l)	$V_{max, rel.}$ (nmol/min per mg of cell protein)	
Varied 2-oxo acid	L-[^{14}C]leucine present at 1 mmol/l		
2-Oxoglutarate[b]	1.52 ± 0.02	5.23 ± 0.03	
Pyruvate[c]	30.72 ± 2.29	0.32 ± 0.01	
2-Oxobutyrate[b]	3.62 ± 0.03	1.59 ± 0.01	
2-Oxovalerate[b]	0.64 ± 0.04	3.02 ± 0.08	
2-Oxocaproate[b]	0.41 ± 0.02	2.06 ± 0.04	
2-Oxooctanoate[b]	1.54 ± 0.13	0.28 ± 0.01	
2-Oxoisovalerate[a]	0.37 ± 0.02	4.00 ± 0.10	
2-Oxoisocaproate[a]	0.21 ± 0.01	6.16 ± 0.07	
(S)-3-Methyl-2-oxo-valerate[a]	0.09 ± 0.01	4.66 ± 0.18	
Varied L-amino acid	4-methyl-2-oxopentanoate present at 1 mmol/l		
Glutamate[c]	18.1 ± 1.5	10.8 ± 0.4	(3.9 ± 0.2)*
Aspartate[c]	10.0 ± 1.5	0.2 ± 0.1	(0.07)
DL-Aminoadipate[c]	n.d.	n.d.	(0.43)
Alanine[c]	86.8 ± 13.7	0.4 ± 0.1	(0.05)
2-Aminobutyrate[c]	35.8 ± 2.8	4.1 ± 0.2	(0.81)
Norvaline[d]	4.7 ± 0.3	5.2 ± 0.2	(3.0)
Norleucine[d]	1.7 ± 0.1	2.4 ± 0.1	(1.8)
[^{14}C]Valine[d]	2.8 ± 0.2	9.4 ± 0.3	(6.3 ± 0.9)
[^{14}C]Leucine[d]	0.7 ± 0.1	9.0 ± 0.1	(7.5 ± 1.6)
[^{14}C]Isoleucine[d]	0.5 ± 0.1	11.8 ± 0.1	(8.9 ± 2.5)
[^{14}C]Alloisoleucine[d]	1.6 ± 0.1	5.7 ± 0.1	(4.2 ± 0.4)
Glycine, Phenylalanine, Tyrosine, Methionine[c]		n.d.	(<0.1)

The data refer to experiments where 5 concentrations of the variable substrate were used (relation of concn. 20:15:10:5:1; ranges (mmol/l): [a]0.05–1.0, [b]0.5–10, [c]5–100, [d]1–20; 37 °C, 30 min). In experiments with L-[1-^{14}C]BCAA and constant [KIC], accumulated 2-oxo[1-^{14}C]acids and L-leucine were measured as transamination products, respectively. Concentrations with half maximal effect on the transamination rate ($K_{m, app.}$) and (extrapolated) maximal transamination velocity ($V_{max, rel.}$) were computed by non-linear regression analysis (see Experimental section). Results are means ± asymptotic standard errors (n = 10); n.d. not determinable. *Transamination rates as measured in the presence of 10 mmol/l of an individual L-amino acid are included for comparison in parentheses. Data are means (n = 2, deviation of individual values from mean < or << 15%) and means ± S.D. ($n \geqslant 3$).

Table 3. Compartmentation of selected enzymes in cultured human skin fibroblasts

Enzyme	Activity in fibroblasts		
	Total	Mitochondrial	Cytosolic
	(nmol/min per mg of cell protein)		
Lactate dehydrogenase	1480 ± 330	230 ± 80	1310 ± 340
Phosphoglycerate kinase	920 ± 180	120 ± 25	820 ± 170
Aspartate aminotransferase	69 ± 11	44 ± 8	24 ± 3
Alanine aminotransferase	4.2 ± 1.2	2.7 ± 0.7	1.2 ± 0.3
BCAA aminotransferase	4.3 ± 1.0	3.7 ± 0.9	0.3 ± 0.1
Citrate synthase	27 ± 5	27 ± 8	0.5 ± 0.4

Total activity was determined in cell-free extracts. In parallel, "cytosolic" and "mitochondrial" fractions were prepared by digitonin fractionation. In general, enzyme activities were assayed by common spectrophotometric procedures. BCAA-AT activity was estimated under standard assay conditions (see Experimental section). Results are means ± S.D. from experiments with different cell lines (n = 5).

"cytosolic" and "mitochondrial" fractions. The data in Table 3 show that no loss of enzyme activity occurred during digitonin fractionation. Separation of subcellular compartments appeared to be essentially complete as indicated by the low amounts of lactate dehydrogenase and phosphoglycerate kinase ($\leq$15%) versus citrate synthase (<2%) which were recovered in the mitochondrial and cytosolic fraction, respectively. About 95 percent of BCAA-AT activity was found to be associated with citrate synthase activity. Whether the remainder was actually resident in the cytosol or was rather attributable to incomplete separation of compartments remained obscure.

When the transamination activities against [1-^{14}C]-labeled L-leucine and L-valine were examined under standard assay conditions, essentially the same leucine/valine transamination ratios were found (mean 1.90 ± 0.14, n = 12) with the cytosolic, the mitochondrial as well as the total activity. This suggested that a common enzmye activity (presumably isoenzyme I) prevailed in the cells under investigation.

Discussion

The present results show that branched-chain L-amino acid aminotransferase activity (BCAA-AT) of cultured human skin fibroblasts can be reliably determined in cell free extracts without measurable interferences from side reactions. Presumably, aminotransferase type I prevails in human skin fibrobasts.

According to Goto et al. (1977), only isoenzyme I and III occur in human tissues. Isoenzyme I appears to be ubiquitous and most abundant in all tissues examined, except lung, ovary and brain. Furthermore, studies on BCAA-AT from various mammalian sources suggest that mitochondrial isoenzymes rather generally exhibit type I properties (Kadowaki and Knox, 1982; Montamat et al., 1978; Wallin et al., 1988).

Apparently, the enzyme under investigation exhibits quite similar properties (including pH optimum) as aminotransferases from other species and tissues (cf. Ichihara, 1985). Compiled data from the literature suggest that, when measured comparatively with 2-oxoglutarate as amino group acceptor, aminotransferase activities are higher with L-isoleucine (113 ± 19%, range 100–151%) and lower with L-valine (81 ± 23%, range 45–114%) than with L-leucine (≡ 100%) as a substrate (Aki et al., 1967, 1968, 1969; Goto et al., 1977; Ichihara and Koyama, 1966; Taylor and Jenkins, 1966). Exactly the same order was observed in the present study with the human skin fibroblast enzyme (Table 1). The data in Table 1 furthermore show that this order was retained with 2-oxo acids other than 2-oxoglutarate.

The considerable variability of apparent differences in substrate properties of the three BCAA (most prominent with L-val) mentioned above may at least in part be due to the fact that equal substrate concentrations in the assays led to different degrees of substrate saturation for the enzyme(s). Available data on half maximal effective concentrations are somewhat variable (L-leu and L-ile, about 0.4–4 mmol/l; L-val, about 1–10 mmol/l), however, the value for L-valine was consistently found to be about three times higher than for L-leucine or L-isoleucine (Aki et al., 1967, 1968; Goto et al., 1977; Kadowaki and Knox, 1982; Ichihara and Koyama, 1966; Montamat et al., 1978; cf. Table 5). Final clarification of differences in the substrate properties of BCAA would afford knowledge of absolute kinetic constants. However, appropriate data appear not to have been published (cf. Makino et al., 1984). In a recent study in our laboratory on the kinetic properties of rat heart mitochondria BCAA-AT, absolute Michaelis constants (± SEM) and relative V_{max} values for L-leucine, L-isoleucine, L-valine and L-alloisoleucine amounted to 0.49 ± 0.05, 0.53 ± 0.04, 2.88 ± 0.39, and 0.77 ± 0.04 mmol/l and to 100, 109, 107, and 58%, respectively (unpublished data). Whether this might be representative for (mitochondrial) aminotransferases from other sources as well, is now under investigation.

In a first comparison of the efficacy of various 2-oxo acids in the forward direction of transamination, 2-oxoglutarate appeared to be the best amino group acceptor (Table 1). A more detailed kinetic analysis of KIC production from L-leucine showed (Table 2) that minor effects of other 2-oxo acids were primarily referable to poor substrate properties (2-oxo acids $\leq C_4$, $\geq C_8$) or due to apparently inhibitory effects at higher substrate concentrations (C_5-, C_6-2-oxo acids). When the efficacy of various L-amino acids as amino group donors in the reverse reaction (formation of L-leu from KIC) was studied, short-chain ($\leq C_4$), long-chain and non-aliphatic L-amino acids (including L-methionine) proved to be poor substrates, as expected (Table 2). The natural BCAA were generally as effective as L-glutamate and apparently not inhibitory to human

Table 4. Compiled data on tissue activity and subcellular distribution of branched-chain L-amino acid aminotransferase in rat and man

Tissue	Branched-chain L-amino acid aminotransferase activity (umol/min per g wet weight)							
	Rat							Human
	Ref. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Heart	1.45	1.45	2.46 (58%)[a]	1.21	3.74 (56.%)	–	4.37 (103%)	0.52
Kidney	1.60	1.25	2.32 (66%)	1.05	1.68 (86%)	1.98 (74%)	3.49 (98%)	0.35
Brain	–	0.73	1.24 (49%)	0.78	1.15 (32%)	1.18 (23%)	1.55 (27%)	0.20
Muscle (mixed)	0.24	0.58	0.84 (72%)	0.47	1.07 (55%)	1.41 (54%)	1.10 (64%)	0.17
Liver	0.02	0.11	0.11 (80%)	0.10	0.10 (77%)	0.07 (57%)	0.05 (0%)	0.14
Lung	–	0.57	0.29 (77%)	–	0.51 (60%)	–	–	0.40
Spleen	–	0.63	0.36 (69%)	–	0.71 (79%)	–	–	0.33
Pancreas	–	–	–	–	2.92 (47%)	–	–	1.93
Ovary	–	–	0.84 (45%)	–	–	1.32 (30%)		0.29
Placenta	–	–	–	–	–	0.75 (25%)	–	0.25
Conditions								
L-Leucine	6.7	n.s.	107	60	6	6	15[b]	107
2-Oxoglut.	6.7	n.s.	6.7	20	3	3	1[c]	6.7
pH	7.4	7.4	7.4	6.8	8.6	8.6	7.8	7.4
Temp (°C)	37	38	37	30	37	37	37	37

Refs: (*1*) Ichihara and Koyama, 1966 (*2*); Krebs, 1972 (*3*); Ichihara et al., 1975 (*4*); Shinnik and Harper, 1976 (*5*); Cappuccino et al., 1978 (*6*); Kadowaki and Knox, 1982 (*7*); Hutson, 1988 (*8*); Goto et al., 1977

Selected data from references containing data on >3 different tissues are included (see refs. Liechty et al., 1987; Papet et al., 1988; Goodwin et al., 1987, and Montamat et al., 1978 for sheep and mouse tissue, repectively). [a]When reported, the portion of the mitochondrial (particulate) activity is given in parentheses. Assay conditions are included for comparison; n.s., not specified; substrate concentrations in mmol/l; [b]L-isoleucine and [c]3-methyl-2-oxopentanoate were used as substrates.

skin fibroblast aminotransferase. These results are complementary to previous reports on aminotransferase from other sources where the activity towards different L-amino acid substrates and 2-oxoglutarate as a common acceptor had been found similarly dependent on the structure of the side chain (Aki et al., 1967; Ichihara and Koyama, 1966; Ogawa et al., 1970; Taylor and Jenkins, 1966).

The present findings on substrate properties and apparent inhibitory effects of 2-oxo acids might have practical implications for the choice of substrates and concentrations, when assays for determination of BCAA-AT in cells and tissues are to be performed. In the past, quite different assay conditions have been applied (cf. e.g. Table 4) rendering comparison of data from different laboratories somewhat complicated. According to the details given above, e.g. L-leucine and 2-oxoglutarate at 5–10 and 10–20 mmol/l, respectively, and pH 8.3–8.5 might provide appropriate initial conditions for most purposes.

BCAA-AT activity in human skin fibroblasts (around 0.5 umol$*$min$^{-1}*$g^{-1} wet wt.) was comparable to the activities in other human tissues (see Table 4). As determined by the digitonin fractionation method, about 95% of the enzyme activity in fibroblasts was closely assocciated with citrate synthase activity indicating a predominant (if not total) mitochondrial localization (Table 3). To our knowledge, subcellular compartmentation in other human tissues has not been investigated. For comparison, results of compartmentation studies in a number of rat tissues are listed in Table 4. Additional sporadic reports on individual mammalian tissues have appeared (e.g. rat skeletal muscle (Odessey and Goldberg, 1979; Snell and Duff, 1985), heart (Hutson et al., 1988) and brain (Brosnan et al., 1985), mouse testis (Montamat et al., 1978)). Presumably due to the different methodologies used (for discussion see Hutson, 1988; Snell and Duff, 1985) the reported relations of mitochondrial to cytosolic BCAA-AT are at some variance, especially in tissues assumed to be primarily involved in overall BCAA metabolism (cf. Table 4). E.g., early differential centrifugation studies suggested that only a minor part of the enzyme activity in heart and skeletal muscle was intramitochondrial (Hutson et al., 1988; Ichihara and Koyama, 1966; Miller and Harper, 1984). More recent studies using differential extraction methods or extrapolation on the basis of soluble marker enzymes indicate, however, that at least in rat heart, kidney and red skeletal muscle tissue BCAA-AT activity is largely confined to the mitochondrial compartment (Hutson, 1988; Hutson et al., 1988; Snell and Duff, 1985), quite opposite to findings with liver and and brain (Table 4 and Brosnan et al., 1985). With respect to other cells and species, subcellular (iso)enzyme distribution clearly deserves further studies.

Intramitochondrial localization of BCAA-AT and rather close coupling between transamination and oxidative decarboxylation may provide an explanation for the observations with perfused rat kidney (Miller and Harper, 1984), and muscle (Schadewaldt et al., 1989a), and incubated human fibroblasts (Schadewaldt and Wendel, 1989) that branched-chain 2-oxo acids produced by intracellular transamination of the corresponding L-amino acids are more readily degraded by mitochondrial branched-chain 2-oxo acid complex than when added extraneously. Primarily mitochondrial transamination leads to compartmentation of (cytosolic) anabolic and (mitochondrial) catabolic pathways of (essential) BCAA and may thus prevent shortage of substrate for protein synthesis when availability is limited. Furthermore, direct connection with overall intramitochondrial metabolism via the 2-

oxoglutarate/L-glutamate couple might be advantageous for rapid adaption of BCAA catabolism to the energy demands of the cell (Hutson, 1988; Hutson et al., 1988). However, the actual physiological significance and regulation of mitochondrial co-localization of aminotransferase and 2-oxo acid dehydrogenase in some tissues and the apparent contrary in others is obscure at present and remains to be elucidated.

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