The scope of amino donor specificity of glutamine transaminase and asparagine transaminase

Transamination of the α-amino group of glutamine (and some closely related analogs) and of asparagine is catalysed by two enzymes, glutamine transaminase and asparagine transaminase, discovered in rat liver by MEISTER *et al.*[1,2] and currently known to occur in many biological systems, including plants and bacteria. Both enzymes transfer the $-NH_2$ group to a large number of various α-keto acids. In contrast to their broad acceptor specificity, these transaminases are generally believed to have narrow specificity with respect to the $-NH_2$ donor[3].

The peculiar substrate specificity assumed for these so-called amino acid amide α-keto acid transaminases does not agree with some evidence –indicating reversibility of their action[4]. It is in conflict with the well-established, generally accepted "shuttle" mechanism of enzymic transamination, postulating two alternating binary half-reactions in which the transaminase acts as intermediary $-NH_2$ acceptor[5–7]. This mechanism carries the implication that a transaminase should catalyse reversible transamination reactions between the α-amino analogs of all α-keto acids that can accept $-NH_2$ groups from the pyridoxamine form of the enzyme, and any of these keto acids[7].

If the above mechanism is valid, then glutamine transaminase and asparagine transaminase should act, in accordance with the scope of their acceptor specificity[1–3], as fairly non-specific α-amino-α-ketomonocarboxylate transaminases.

This is probably in fact the case, as indicated by the following preliminary data obtained in experiments with extensively purified amino acid amide transaminases from rat liver.

The purification procedures developed by the authors[8], involving heat treatment, $(NH_4)_2SO_4$ fractionation, zone electrophoresis and adsorption on DEAE-cellulose, eliminate glutaminase, ketoamidase, most of the contaminating transaminases, and separate the glutamine and asparagine transaminases from each other. As compared with crude liver extract, the activity of the partially purified glutamine enzyme is raised 50- to 80-fold and that of the asparagine enzyme, 30- to 40-fold. The main, active component of each preparation (50–70 % of the total protein) is electrophoretically homogeneous; the maximal rates of transamination are fairly low as compared, for example, with alanine–glutamate transaminase.

With these enzyme preparations, the "adequate" amides and different α-amino acids were tested as $-NH_2$ donors for transamination with pyruvate, phenylpyruvate and α-ketoisocaproate.

The experimental results presented in Table I clearly show that both amide transaminases effectively catalyse $-NH_2$ transfer from a number of α-aminomonocarboxylic acids (at increased concentration levels) to pyruvate, phenylpyruvate and α-ketoisocaproate. With enzymes of animal origin, transaminations to the latter two keto acids from donors other than alanine, glutamate, glutamine or asparagine have not been demonstrated before.

The reaction rates were low with 14 mM α-aminomonocarboxylic acids, which indicates that their K_S values for the two transaminases studied are considerably higher than those of the "adequate" amino acid amides.

Abbreviations: DEAE-, diethylaminoethyl-; EDTA, ethylenediaminetetraacetate.

TABLE I

TRANSAMINATIONS CATALYSED BY "AMINO ACID AMIDE" TRANSAMINASES

Amino donors	*Glutamine transaminase* *Amino acceptors*			*Asparagine transaminase* *Amino acceptors*		
	pyruvate	*phenylpyruvate*	*α-ketoisocaproate*	*pyruvate*	*phenylpyruvate*	*α-ketoisocaproate*
	Amino group transfer (μmoles/mg enzyme protein)					
L-glutamine, 14 m*M*	—	5.4	—	—	0.15	—
L-glutamine, 67 m*M*	—	—	—	—	0.60	—
L-asparagine, 14 m*M*	—	0.18	—	1.90	1.70	—
L-asparagine, 67 m*M*	—	0.54	—	—	—	—
L-alanine, 67 m*M*	—	1.20	—	—	1.29	—
DL-aminobutyrate, 67 m*M*	3.75***	1.50	1.08	0.47	0.40	0.34
L-leucine, 67 m*M*	1.62	*	—	0.82	*	—
DL-norleucine, 67 m*M*	2.42	*	*	0.82	*	*
L-methionine, 67 m*M*	2.70	2.92	3.09	1.10	1.50	1.15
L-histidine, 67 m*M***	2.52	2.14	2.43	0.84	1.32	1.34
L-phenylalanine, 67 m*M*	2.22	—	*	0.73	—	*
L-glutamate, 67 m*M*	10.10***	0	0.88	4.10***	0	0.07

The enzyme preparations (0.3–0.5 mg glutamine transaminase, or 0.8–1.0 mg asparagine transaminase) were incubated for 2 h at 37° in 1 ml 0.05 *M* veronal buffer (pH 8.4) with the substrates indicated (28 m*M* pyruvate, 14 m*M* phenylpyruvate, 28 m*M* α-ketoisocaproate). Deproteinized filtrates of the incubated samples and blanks with omitted $-NH_2$ donor or acceptor were subjected to descending paper chromatography with appropriate solvent mixtures, followed by quantitative photometric estimation of the amino acids formed by transamination (alanine, phenylalanine or leucine) as the ninhydrin–copper complexes[8]. The $-NH_2$ transfer values presented are corrected by subtraction of the higher of the two incomplete blanks mentioned above.

* Chromatographic separation of amino acids inadequate; values omitted.

** Samples supplemented with 10^{-4} *M* EDTA to inhibit histidine deaminase.[2]

*** $-NH_2$ group transfer high, owing to the presence of contaminating L-alanine–glutamate transaminase.

It is worthy of note that the amide transaminases also manifest slow cross-reactions with increased concentrations of the "inadequate" amino acid amide (*cf.* ref. 9).

When asparagine transaminase was incubated with phenylpyruvate and non-saturating concentrations of (a) asparagine, (b) L-alanine, and (c) a mixture of both $-NH_2$ donors, phenylalanine formation was competitive rather than additive, *viz.*: a, 1.21; b, 1.50; and c, 1.66 μmoles phenylalanine. These data prove that the transamination of alanine was due to the asparagine transaminase and not to contamination of this enzyme preparation with phenylalanine–pyruvate transaminase.

It thus appears that the two liver enzymes discovered by MEISTER *et al.* in studies on the coupled transamination of glutamine and asparagine and hitherto considered as specific amino acid amide transaminases probably are, in fact, group-specific general monocarboxylate transaminases. The classification of these enzymes as glutamate and asparagine transaminases may be justified insofar as their affinity to the corresponding amino acid amides is, apparently, markedly higher than to other amino-monocarboxylate donors. However, the present evidence for a broad scope of donor as well as acceptor specificity of these transaminases calls for reinvestigation of a number of claims concerning the independent existence of enzymes specifically catalysing miscellaneous transamination reactions.

Further studies are under way on the substrate affinities and specifities of glutamine and asparagine transaminases.

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Received August 31st, 1960

Biochim. Biophys. Acta, 44 (1960) 187–189

Terminal incorporation of [^{14}C]AMP into s-RNA by bacterial enzymes

AMP, derived from ATP, is incorporated into terminal positions of soluble ribonucleic acid (s-RNA) by enzymes derived from rat liver. This attachment of AMP has been shown to occur predominantly in positions adjacent to cytidylic acid of s-RNA, thus establishing the terminal ribonucleotide sequence and the specificity of the reaction[1]. The enzyme fractions responsible for the incorporation of terminal ribonucleotides into s-RNA have been obtained primarily from extracts of rat liver[1–4].

Since an analogous type of s-RNA has been found to exist in *Escherichia coli*[5], the presence of enzymes associated with its synthesis has been sought in extracts of this microorganism. The availability of enzyme systems from two markedly different sources (animal and bacterial) responsible for the same reaction would permit a detailed comparative study and lead to a better understanding of these reactions. In the present communication the existence is demonstrated in *E. coli* of an enzyme system responsible for the terminal incorporation of [^{14}C]AMP from [^{14}C]ATP into s-RNA, in positions adjacent to cytidylic acid; this enzyme system has been partially purified.

Extracts of *E. coli*[6] were centrifuged for 1 h at 100,000 × *g* and the pH of the supernatant fraction was adjusted to 3.8. The resulting isoelectric precipitate (Table I, Fraction 1) retained more than 90 % of the ability of the crude extract to incorporate [^{14}C]AMP into RNA when [^{14}C]ATP was the precursor (results not shown). As shown in Table I (Fraction 1), after alkaline hydrolysis of the isolated radioactive RNA, 60 % of the incorporated [^{14}C]AMP was liberated as adenosine and 40 % as AMP. Further purification resulted in the isolation of a fraction (Table I, Fraction 2)

Abbreviations: AMP, ADP, ATP, adenosine mono-, di- and triphosphate; s-RNA, soluble ribonucleic acid.