Participation of phenylacetyl-adenylate in the enzymic synthesis of phenylacetylglutamine

In a previous communication from this laboratory[1], the enzymic synthesis of phenylacetylglutamine from phenylacetyl CoA* and L-glutamine catalyzed by human tissues was described. Homogenates of human liver and kidney, enzyme preparations purified from the supernatant fraction of these homogenates, and particulate fractions isolated from these tissues catalyzed this reaction; comparable preparations from rat and beef liver were inactive. These results are consistent with studies *in vivo* in which orally administered phenylacetate gave rise to urinary phenylacetylglutamine in man and the chimpanzee, while in other mammals only phenylacetylglycine was formed[2,3]. Particulate preparations from human liver also catalyzed phenylacetylglutamine formation in the presence of phenylacetate, ATP and CoA-SH; with such preparations, synthetic phenylacetyl CoA served in place of ATP, CoA-SH and phenylacetate.

The present report describes the participation of phenylacetyl-AMP in phenylacetylglutamine synthesis. Synthetic phenylacetyl-AMP (prepared by reaction between phenylacetic anhydride and adenylic acid) has been found to replace phenylacetate and ATP in phenylacetylglutamine synthesis by the particulate preparation (Table I). Phenylacetyl-AMP is a more effective precursor of phenylacetylglutamine in the presence of CoA-SH than is a mixture of phenylacetate and ATP; approximately 10–20 times higher concentrations of phenylacetate were required to produce amounts of phenylacetylglutamine equivalent to those formed from phenylacetyl-AMP under the same conditions. However, phenylacetyl-AMP is active only in the presence of CoA-SH. The evidence suggests the following reaction sequence:

(1) phenylacetate + ATP $\rightleftarrows$ phenylacetyl-AMP + pyrophosphate
(2) phenylacetyl-AMP + CoA-SH $\rightleftarrows$ phenylacetyl CoA + AMP
(3) phenylacetyl CoA + L-glutamine $\rightleftarrows$ phenylacetylglutamine + CoA-SH

In addition to the evidence for reaction (3) reported previously[1], the formation of phenylacetylglutamine from phenylacetyl CoA and L-glutamine is associated with stoichiometric formation

TABLE I

SYNTHESIS OF ACYL-AMINO ACIDS FROM ACYL-AMP DERIVATIVES

	Acyl-amino acid formed (mμmoles)
Phenylacetyl-AMP	
Complete system§	35.0
Complete system; CoA-SH omitted	4.3
Complete system; acylating enzyme omitted	31.0
Complete system; mitochondria omitted	7.6
Complete system; phenylacetyl-AMP omitted	0
Benzoyl-AMP	
Complete system§	25.0
Complete system; CoA-SH omitted	1.0
Complete system; mitochondria omitted	2.1
Complete system; benzoyl-AMP omitted	0

§ The complete system contained the following components per ml of reaction mixture: CoA-SH, 0.18 μmole; G-SH, 7 μmoles; $MgCl_2$, 1.1 μmoles; sodium phosphate at pH 8.2, 70 μmoles; ^{14}C-L-glutamine, randomly labeled, 0.6 μmole (approximately $1 \cdot 10^6$ c.p.m.); 3 mg of acylating enzyme purified from the cellular supernatant of human kidney[1]; 6 mg of an 0.02 *M* potassium phosphate (pH 7.5) extract of an acetone powder of liver mitochondria[4]; and 0.66 μmole phenylacetyl-AMP. 0.5 μmole benzoyl-AMP and 0.42 μmole glycine-1-^{14}C (approximately $1.2 \cdot 10^6$ c.p.m.) were used in the hippurate experiments. Incubations were carried out for 2 h at 37.5°. The radioactive acyl-amino acids formed were determined as described previously[1]. Similar values were obtained when G-SH was omitted.

* The following abbreviations are used: adenosine-5'-monophosphate, AMP; coenzyme A, CoA or CoA-SH; adenosine triphosphate, ATP; glutathione, G-SH; counts per minute, c.p.m.

of free sulfhydryl groups. Reaction (2) is consistent with the data reported here and with the observed disappearance of free sulfhydryl groups, estimated as described by MAHLER *et al.*[4], when phenylacetyl-AMP and CoA-SH were incubated with the enzyme preparation (Table II).

Particulate preparations of human liver and kidney also catalyze hippuric acid formation from benzoate, ATP, CoA-SH and glycine; benzoylglutamine is not formed when glycine is replaced by glutamine. The mechanism of hippuric acid synthesis appears to be similar to that of phenylacetylglutamine formation; thus benzoate, ATP and CoA-SH may be replaced by benzoyl CoA[5], and benzoyl-AMP replaces benzoate and ATP in the presence but not in the absence of CoA-SH (Table I). Disappearance of free sulfhydryl groups was observed to take place in the course of the reaction between benzoyl-AMP and CoA-SH (Table II). The present results strongly suggest the participation of acyl-adenylic acid derivatives as intermediates in the formation of phenylacetylglutamine and of hippuric acid. As in the activation of acetate[6] and of fatty acids[7], acyl-AMP formation appears to precede transfer of the acyl group to CoA-SH.

TABLE II

DISAPPEARANCE OF FREE SULFHYDRYL GROUPS IN THE PRESENCE OF ACYL-AMP AND CoA-SH (REACTION (2))

	-SH (mμmoles)
Complete system* (+ phenylacetyl-AMP)	60.0
Complete system (+ benzoyl-AMP)	49.3
Complete system; acyl-AMP omitted	2.75

*The complete system contained the following components per ml of reaction mixture: CoA-SH, 0.18 μmole; $MgCl_2$, 1.1 μmoles; sodium phosphate at pH 8.2, 70 μmoles; 6 mg of mitochondrial extract, and either 0.66 μmole phenylacetyl-AMP or 0.5 μmole benzoyl-AMP. Incubations were carried out for 2 h at 37.5°. Analytical reagents used for the determination of free -SH groups were added directly at the end of the incubation period.

Although human liver mitochondria were able to catalyze the overall formation of phenylacetylglutamine or hippurate from the corresponding free acid or acyl-AMP derivative, preparations of rat and beef liver mitochondria did not catalyze phenylacetylglutamine formation unless the acylating enzyme preparation purified from the supernatant fraction of human tissue was added. The available evidence therefore suggests that, although activation of benzoate and phenylacetate may be catalyzed by the tissues of several species, there is a marked species specificity involved in the reaction between the acyl CoA derivative and amino acid (reaction (3)). The enzyme that catalyzes reaction (3) is readily separable from the system that catalyzes reaction (1) and (2).

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