Effect of 1-Hydroxymethyl- and 1-Methyl-1-aminoalkanes

on Enzymatic Leucine-2-oxoglutarate Transamination

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Studies were made on the effect of some 1-hydroxymethyl- and 1-methyl-1-aminoalkanes on the enzymatic leucine-2-oxoglutarate transamination reaction catalyzed by *Escherichia coli* cell extract, and on the transamination of the analogs and their effect on the growth of *E. coli*. All the analogs inhibited the enzymatic leucine-2-oxoglutarate transamination reaction and this inhibition was competitive with leucine. The analogs were not able to donate an amino group to 2-oxoglutarate when *E. coli* cell extract was used as enzyme source and they acted as growth inhibitors when *E. coli* cells were cultured in a simple glucose-mineral salt medium. It is concluded that the carboxylate group of leucine is essential for the enzymatic leucine-2-oxoglutarate transamination reaction.

In the transamination reaction between an amino acid and 2-oxoglutarate, the 2-amino group and α-carbon hydrogen play the key role in the formation of a Schiff base with amino acid and pyridoxal-5′-phosphate and in the formation of the end product, a keto acid. The role of the carboxyl group of the amino acid in the reaction is poorly understood. There is some experimental evidence to suggest that the carboxylate anion is involved in the reaction and it is supposed that this anion becomes attached to the apoenzyme part, effecting the breakage of a bond specific to the reaction.[1–3]

We selected some 1-hydroxymethyl- and 1-methylalkylamines and tested their effect on the enzymatic leucine-2-oxoglutarate transamination reaction and their ability to donate an amino group to 2-oxoglutarate catalyzed by partially purified *E. coli* leucine aminotransferase (EC 2.6.1.6).

MATERIALS AND METHODS

Organism. A wild-type *Escherichia coli* U5-41 was used in the growth inhibition studies and as source for leucine aminotransferase. The compositions of the transfer, inoculation and test media have been reported earlier.[4]

Assay methods. Chromatographic and isotopic methods were used in the leucine aminotransferase activity determinations. The isotopic method, in which the reaction

mixture contained 5 mM L-leucine, 25 mM 2-oxoglutarate and 10 μM pyridoxal-5'-phosphate in 50 mM Tris-HCl buffer, pH 8.0, has been published elsewhere.[5] Analogs were added to the reaction mixture to give 10 or 30 mM concentrations. In this assay 0.05 − 0.1 μCi of uniformly labeled L-leucine-U-C-14 (specific activity 344 mCi/mmol) was added to the reaction mixture to the volume of 1 ml. In some experiments lower leucine concentrations were used to obtain higher counts and these experiments are mentioned later. The radiocarbon was counted in a scintillation solution containing 3.92 g of PPO (2,5-diphenyloxazole) and 0.08 g of bis-MSB (p-bis(o-methylstyryl)benzene) in 1 l of toluene. The liquid scintillation counter was Decem NTL[314] from Wallac Co., Turku, Finland.

The chromatographic method was a modification of a previously published method in which glutamate formed from 2-oxoglutarate was estimated after it had been separated by paper chromatography and sprayed with ninhydrin. The modification consisted of using lower substrate concentrations, as in the isotopic assay.

Enzyme preparation. Leucine aminotransferase (EC 2.6.1.6) was partially purified from a wild-type *E. coli* U5-41 by a method presented earlier.[4]

Analogs and their preparation. The following 2-amino compounds were purchased as follows: 2-aminoethanol, DL-2-aminobutyric acid from British Drug Houses Ltd, Poole, England, 2-aminopropanol, 2-aminobutanol, 2-aminopropane, 2-aminobutane, 2-amino-4-methylhexane from Koch-Light Laboratories Ltd, Colnbrook, England, 2-aminoethane, 2-amino-3-methylpentane, 2-amino-6-methylheptane from Fluka AG, Buchs, Switzerland, 2-aminopentane and 2-aminohexane from K & K Laboratories Inc., Hollywood, California. Leucinol, isoleucinol, valinol, norleucinol, and norvalinol were prepared from the corresponding L-amino acids by the method of Vogl and Pöhm.[6] An ascending paper chromatographic method, with butanol-acetic acid-water 4 : 1 : 1 (v/v/v) as solvent, was used to test that the preparations were free from amino acids, and the absence of a carboxylic group and the presence of the amino group were demonstrated by infrared analysis. All other reagents were chromatographically pure reagent grade compounds.

NMR spectra. The differences between chemical shifts were determined on a 60 MHz Perkin-Elmer R 10 NMR spectroscope at 33.5°.

Table 1. Effect of 1-hydroxymethyl- and 1-methyl-1-alkylamines on the enzymatic leucine-2-oxoglutarate transamination catalyzed by partially purified leucine aminotransferase from *E. coli*. Initial velocities were followed in the reaction mixture containing 5 μmol L-leucine, 3.4 × 10⁻⁴ μmol uniformly labelled L-leucine (0.1 μCi), 25 μmol 2-oxoglutarate, 0.01 μmol pyridoxal-5-phosphate in 50 mM Tris-HCl buffer, pH 8.0, plus 10 mM or 30 mM analog. The total reaction volume was 1.0 ml and the activities are compared to the activity without analog which is taken as 100.

Analog	Concentration	
	10 mM	30 mM
None	100	100
2-Aminoethanol	91	70
2-Aminopropanol	85	77
2-Aminobutanol	96	71
Norvalinol	80	—
Norleucinol	91	84
Valinol	60	—
Leucinol	90	70
Isoleucinol	89	72
2-Aminoethane	76	33
2-Aminopropane	84	30
2-Aminobutane	62	25
2-Aminopentane	57	40
2-Aminohexane	67	25
2-Amino-3-methylpentane	59	36
2-Amino-4-methylhexane	74	37
2-Amino-6-methylheptane	78	26
L-Isoleucine	51	19

RESULTS

Effect of analogs on the enzymatic transamination of leucine. When one of the analogs at a concentration of 10 mM or 30 mM was added to the reaction mixture containing 5 mM L-leucine and 25 mM 2-oxoglutarate, the reaction rate was lower than in its absence. The results are tabulated in Table 1.

With all the 1-hydroxymethyl- and 1-methyl type analogs a sixfold amount of the analog, as compared with the leucine concentration, caused marked inhibition. The inhibition was higher with 1-methyl analogs than with 1-hydroxymethyl compounds. No marked differences were found with different side chains of alkylamines.

Inhibition constants and type of inhibition. Fig. 1 shows the graphical determination of inhibitor constants K_i and they were 2.0 mM for leucinol, 1.7 mM for 2-amino-4-methylhexane and 0.6 mM for isoleucine. The Dixon plot method was used to determine the constants, and the activity assays were made with the isotopic method.[7] The type of inhibition was competitive in
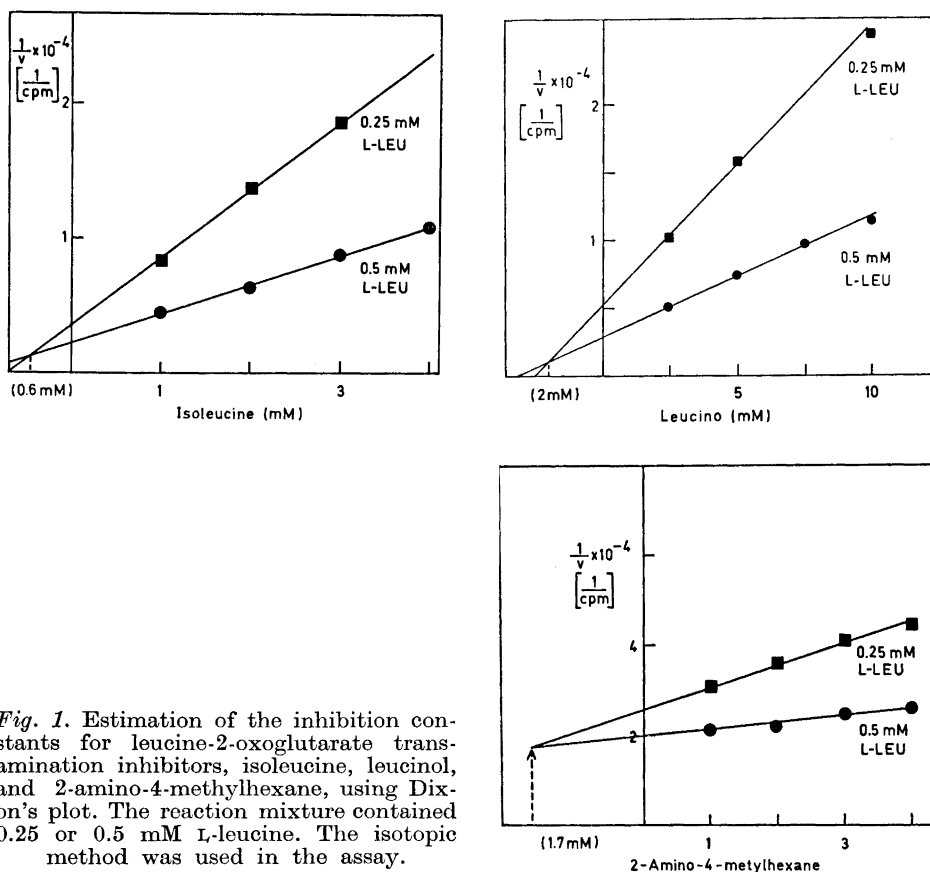

Fig. 1. Estimation of the inhibition constants for leucine-2-oxoglutarate transamination inhibitors, isoleucine, leucinol, and 2-amino-4-methylhexane, using Dixon's plot. The reaction mixture contained 0.25 or 0.5 mM L-leucine. The isotopic method was used in the assay.

all three cases studied. The K_m value for leucine was 0.3 mM with *E. coli* U5-41 enzyme.

Transamination of analogs. The deamination of the analogs used was studied by following the release of hydrogen from the α-carbon in the presence of 2-oxoglutarate by the NMR technique and also the formation of glutamate from 2-oxoglutarate. Fig. 2 shows the spectra obtained by the NMR technique when the reaction mixture contained L-leucine and isoleucinol as amino donor.
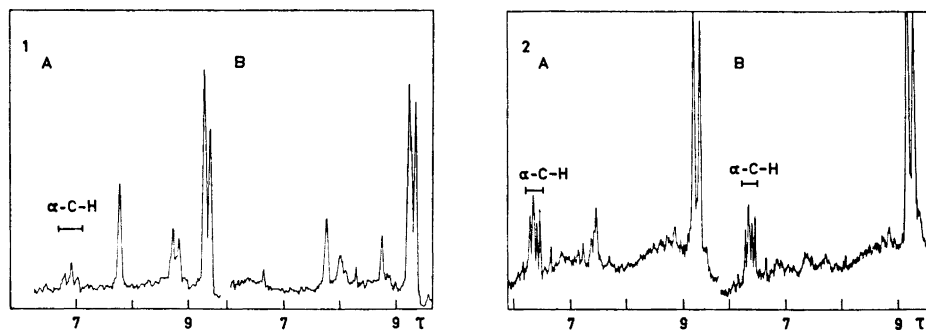

Fig. 2. NMR spectra of L-leucine (1) and isoleucinol (2) in D_2O before (A) and after (B) incubation of 30 mg of amino compound for 24 h at 37° in the presence of 30 mg 2-oxoglutarate and 30 mg of *E. coli* protein containing leucine aminotransferase per ml (pD was 8.0 and it was adjusted with NaOD). The protein was precipitated by heating and centrifuged off before NMR analysis. The position of the α-carbon hydrogen is marked as α-C-H. There was no release of hydrogen from the other analogs mentioned in Table 1.

The results showed that none of the analogs, except leucinol with an activity less than 10 % of that of leucine, acted as an amino donor to keto acid or was able to release hydrogen from the α-carbon. The concentration of the amino acid or amino compound in the reaction mixture was 30 mM in the chromatographic method, in which the formation of glutamate was followed. The composition of the reaction mixture in the NMR analysis is mentioned in Fig. 2.

Inhibition of E. coli growth by analogs. The effects of the analogs on the growth of a wild-type, branched-chain amino acid resistant *Escherichia coli* U5-41 were also studied. The additions were made at concentrations of 10^{-4} to 10^{-1} M to the medium, which contained glucose, ammonium chloride, and mineral salts.[5] The analogs were added to the medium aseptically before drop inoculation of 10 ml of the medium. The tubes were incubated overnight at 37° without shaking and the turbidities were determined with a Klett colorimeter, using filter No. 42 (400 – 450 nm). The 50 % inhibition concentrations of the analogs were as follows: 84 mM for 2-aminoethanol, 70 mM for 2-aminopropanol, 62 mM for 2-amino-1-butanol, 27 mM for leucinol, 38 mM for 2-aminobutane, 3 mM for 2-aminopentane, 21 mM for 2-aminohexane, 8 mM for 2-amino-3-methylpentane, 6 mM for 2-amino-4-methylhexane, and 2 mM for 2-amino-6-methylheptane. Isoleucinol and valinol up to the concentration of 10^{-1} M did not cause any growth inhibition. Very volatile compounds like 2-aminoethane and 2-aminopropane were not tested in the growth experiments.

The results showed again that 1-methyl type analogs are more efficient inhibitors than 1-hydroxymethyl type compounds.

DISCUSSION

It is supposed that in the present models of the transamination reaction mechanism the carboxyl group is needed to put the amino acid into the right position in the transaminase enzyme molecule, and thus that the group attachment on the enzyme determines which of the bonds in the Schiff base is broken in the reaction.

The results in Table 1 strongly support this evidence that the carboxyl group is essential for the enzymatic leucine-2-oxoglutarate transamination reaction. All the analogs tested were inhibitory and these analogs either had a 1-hydroxymethyl or a 1-methyl group instead of the carboxyl group in the amino compound. The change of the group resulted in inhibition with both types of analogs, although the 1-methyl group gave higher inhibition values than the 1-hydroxymethyl group. The study showed that the effect of the side chain was less strong. This is understandable if it is the carboxyl group, and not the side chain, that plays the key role in stabilizing the structure of the Schiff base or if the specificity of the leucine aminotransferase from $E.$ $coli$ is very low. Similar results were obtained with valyl-sRNA synthetase from $E.$ $coli$ when various amino acid analogs were studied.[8] The carboxyl group of the amino acid seems to be essential for the liberation of hydrogen from the α-carbon (Fig. 2). However, the K_i values in Fig. 1 show, that the binding of the analogs is of about the same order as the K_m values for L-leucine. This finding is consistent with the results of experiments with valyl-sRNA synthetase.[8] It is thus likely that the role of the carboxyl group in the binding process is very weak but may be important in the Schiff base structure, as suggested by Heinert and Martell [9] and by Martell.[10]

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