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# Kynurenine Formamidase and Tetrahydrofolic Acid

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It has been found that kynurenine formamidase isolated from guinea-pig liver is unable to effect the transfer of a formyl group from *N*-formyl-L-kynurenine or formylanthranilic acid to tetrahydrofolic acid *in vitro*, but liberates the group and converts it into formic acid.

*N*-Formyl-L-kynurenine, which is formed from tryptophan by the action of tryptophan oxygenase, decomposes to L-kynurenine and formic acid in a reaction catalyzed by kynurenine formamidase (arylformylamine amidohydrolase, E.C. 3.5.1.9).[1] Santti and Hopsu-Havu have found that kynurenine formamidase isolated from guinea-pig liver functions as a transferase *in vitro*.[2] The enzyme is able to transfer a formyl group from a substrate not only to water, but also to an aliphatic alcohol, hydroxylamine and an aromatic amine (anthranilic acid or 1-naphthylamine).

The aim of the work described in this paper was to determine whether kynurenine formamidase is able to transfer a formyl group also to tetrahydrofolic acid. It had been observed that when tryptophan was given to rats suffering from folic acid deficiency, the amounts of formic acid secreted into the urine were greater in these animals than in normal rats.[3] One explanation for this observation is that kynurenine formamidase liberates the formyl group as formic acid and thus functions as a hydrolytic enzyme in tetrahydrofolic acid deficiency.

## METHODS AND RESULTS

Kynurenine formamidase was isolated from guinea-pig liver as described earlier.[4] The substrates used in experiments were *N*-formyl-L-kynurenine (Formyl-L-kynurenine x $H_2O$, B grade, Calbiochem) and formylanthranilic acid (a sample synthetized in the Department of Chemistry, University of Turku). Tetrahydrofolic acid was prepared by a method proposed by Kisliuk [5] with the modification that the filtrate was collected under nitrogen in 500 ml of peroxide-free diethyl ether that contained 1 % 2-mercaptoethanol and which was held at −20°C. The precipitated tetrahydrofolic acid was stored suspended in diethyl ether containing 1 % 2-mercaptoethanol at −20°C. For the experiments some of this suspension was added to a 0.1 M potassium phosphate buffer solution

of pH 7.4 that contained 2 % 2-mercaptoethanol. The concentration of the resulting solution was determined by measuring the absorbance of the solution at 298 mμ ($\varepsilon$=19 000). 10-Formyltetrahydrofolic acid was synthetized by a method that has been described earlier.[6]

Formyltetrahydrofolate synthetase (formate:tetrahydrofolate ligase (ADP), E.C. 6.3.4.3) was isolated from *Clostridium cylindrosporum*.[7] For use in the experiment this enzyme was dissolved in 0.05 M sodium maleate buffer solution of pH 7.0. The activity of the enzyme as determined by the method of Rabinowitz and Pricer [8] was 4.5 units/mg at 25°C.

After incubation the 5-formyl and 10-formyltetrahydrofolic acids formed were converted by treatment with acid into 5,10-methenyltetrahydrofolic acid which has an absorption peak at 350 mμ ($\varepsilon$=24 900).[8]

Kynurenine formamidase was incubated with its substrate and tetrahydrofolic acid at room temperature (22°C) for 10 min. The composition of the incubation solution was: 0.5 ml of a solution containing 0.5 μmole of *N*-formyl-L-kynurenine in one liter of water or 0.5 ml of a solution containing 0.5 μmole of formylanthranilic acid in one liter of 10 % methanol, 0.35 ml of a solution of kynurenine formamidase whose activity as determined with formylkynurenine at 22°C [4] was 67.5 units/ml, 0.15 ml of a solution of tetrahydrofolic acid (1.6 μmoles) and 1.0 ml of a 1.0 M triethanolamine buffer solution of pH 8.0. After the incubation, 2.0 ml of 0.36 N hydrochloric acid was added to each test tube and the absorbances of the solutions in the test tubes were measured at 350 mμ 10 min later against a solution of the same composition, but which did not contain any enzyme. No 5-formyl- or 10-formyltetrahydrofolic acid was formed during the incubation.

*N*-Formyl-L-kynurenine was incubated with kynurenine formamidase at 22°C for 10 min in a solution that was composed of 0.5 ml of an aqueous solution of *N*-formyl-L-kynurenine (0.5 μmole), 0.5 ml of kynurenine formamidase solution (67.5 units/ml) and 1.0 ml of a 1.0 M triethanolamine buffer solution of pH 8.0. After the incubation 0.5 ml of the mixture was added to a test tube that contained 0.1 ml of a 0.05 M ATP solution, 0.1 ml of 0.1 M magnesium chloride solution, 0.15 ml of a tetrahydrofolic acid solution (1.6 μmoles) and 20 μl of a formyltetrahydrofolate synthetase solution (1.9 units). The resulting mixture was then held at 22°C for 15 min, after which the 10-formyltetrahydrofolic acid content was determined spectrophotometrically. It was found that 80 millimicromoles of 10-formyltetrahydrofolic acid had been formed. This means that all of the formic acid liberated from the formylkynurenine had reacted with tetrahydrofolic acid. The reference solution was a mixture in which the formyltetrahydrofolate synthetase solution had been replaced by water.

It was confirmed that the sample of kynurenine formamidase did not contain any enzyme that deacylated 10-formyltetrahydrofolic acid. In this experiment a mixture composed of 10 μl of 10-formyltetrahydrofolic acid (0.12 μmole), 0.5 ml of kynurenine formamidase solution and 0.5 ml of 1.0 M triethanolamine buffer solution of pH 8.0 was incubated at 37°C for 15 min and the 10-formyltetrahydrofolic acid that had not decomposed was determined by measuring the absorbance of the mixture at 350 mμ 10 min after 2.0 ml of 0.36 N hydrochloric acid had been added to it.

## DISCUSSION

The results of these experiments show clearly that although kynurenine formamidase isolated from guinea-pig liver functions as a transferase in certain conditions, it is unable to transfer a formyl group from a substrate (*N*-formyl-L-kynurenine or formylanthranilic acid) directly to tetrahydrofolic acid but releases it in the form of formic acid. This is indicated by the observation that formyltetrahydrofolate synthetase catalyzes the formation of 10-formyltetrahydrofolic acid from tetrahydrofolic acid and formic acid liberated from formylkynurenine by the action of kynurenine formamidase. The conducted experiments showed also that the kynurenine formamidase preparation did not contain active 10-formyltetrahydrofolate deformylase (E.C. 3.5.1.10).[9]

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