Inhibition by 5'-Adenylate of Glucosamine 6-Phosphate Formation in Chick Cartilage

Glucosamine-6-phosphate biosynthesis, the first step in the pathway leading to UDP-N-acetyl-aminosugars, precursors of the glycosaminoglycans, is catalyzed by the enzyme L-glutamine-D-fructose-6-phosphate amidotransferase (E.C. 2.6.1.16).

This enzyme has been partially purified from several sources[1,2] and its activity has been demonstrated in rabbit[3] and shark[4] cartilage.

KORNFELD[5] has shown the feed-back inhibition by UDP-N-acetyl-D-glucosamine of the rat liver and HeLa cells enzyme, and considers that the step catalyzed by it controls the biosynthesis of aminosugar nucleotides.

Since this transamidation determines a derivation of a glycolytic intermediate (fructose-6-phosphate) to a synthetic pathway, and thus 5'-adenylate is one of the main regulating factors of glycolysis[6], we have studied the effect of 5'-nucleotides on the amidotransferase activity in the chick cartilage.

A number of chickens were killed the day of hatching, the epiphyses of the long bones were removed and homogenated in a solution of 0.154 *M* potassium chloride, 20 m*M* potassium phosphate buffer pH 7.0, 5 m*M* sodium ethylene diamine tetraacetate (EDTA), 2.5 m*M* dithithreitol (Calbiochem), 100–200 mg of cartilage (wet weight) was used for each ml of homogenating solution. Homogenation was carried out in a Sorvall Omni-Mixer at 35,000 r.p.m. for 3 min, and the preparation was centrifuged at 37,000 *g* for 60 min at 4 °C. 1 ml of mixture containing 0.4 ml of crude extract was incubated at intervals of 30 or 60 min at 38 °C. This mixture contained: 10 m*M* L-glutamine, 5 m*M* EDTA, 2.5 m*M* dithiothreitol, 10 m*M* potassium phosphate buffer pH 6.35, and variable amounts of fructose-6-phosphate or nucleotides (Figure). In some experiments glucose-6-phosphate was used instead of fructose-6-phosphate.

The enzyme reaction was stopped by immersion in a boiling water bath during 3 min. The hexosamines were determined by the method of GHOSH[2].

Incubations were carried out at pH 6.35 because the enzyme shows a maximum activity within the pH range 6.2–6.4.

The addition of 5'-adenylate to the incubation mixture determined an inhibition of the enzyme activity. When this inhibition was studied as a function of fructose-6-phosphate concentration, the results in the Figure were obtained. The inhibition follows a competitive-type kinetics, with an increase of the apparent Km for fructose-6-phosphate which, in the absence of the inhibitor, is nearly 1 m*M*.

The Ki calculated for 5'-adenylate was 1.5 m*M*; this value was found independent of L-glutamine concentration.

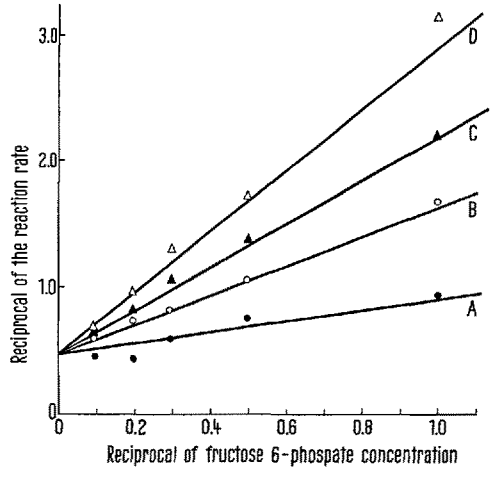
5'-adenosine diphosphate inhibits the enzyme also but only at a higher concentration. 2'- and 3'-isomers of adenylic acid were no inhibitors; ribose 5-phosphate was likewise unable to compete with the sugar-phosphate substrate.

Other 5'-nucleotides tested were inhibitors. Inosine and guanosine 5'-phosphates inhibited the enzyme at the same rate as 5'-adenylate. The 5'-phosphates of cytidine and uridine were also inhibitors – although less effective ones – with Ki values of 2.5 and 5.4 m*M*, respectively.

This inhibition by 5'-nucleotides of glucosamine-6-phosphate-forming enzyme should be able to play some role in metabolic regulation, as the Ki values obtained are of the same order of magnitude as the Km for the fructose-6-phosphate.

However, taking into account the high values found for this Km, the main factor controlling fructose-6-phosphate usage for glucosamine synthesis could be the pool size of this metabolite.

The possible regulatory effect of 5'-adenylate that could be postulated from the experiments described here, may be regarded as a part of the adenylate control system[6] and its function could be that of avoiding the loss of hexose phosphates to the aminosugars formation when the AMP/ATP ratio is high[7].



Double reciprocal plot for the amidotransferase reaction, in the presence of variable concentrations of sodium 5'-adenylate. Reaction mixture as described, and the following amounts of nucleotide: A, none; B, 2.5 m*M*; C, 5.0 m*M*; D, 7.5 m*M*.

Riassunto. Gli autori hanno studiato l'inibizione della L-glutamina-D-fruttosio-6-fosfato amidotransferasa per 5'-nucleotidi in estratti crudi di cartilagine di pollo. L'inibizione per 5'-adenilato e altri 5'-nucleotidi segue una cinetica competitiva. Il 2'- e 3'-adenilato, così come il ribosio 5-fosfato non sono inibitori. Gli autori prospettano una possibile partecipazione nelle regolazione della sintesi degli aminozuccheri.

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7 Acknowledgment. The authors are indebted to Mr. L. MORO, from 'Criadero San Luis', who supplied the chickens for this research.