## SHORT COMMUNICATIONS

BBA 63357

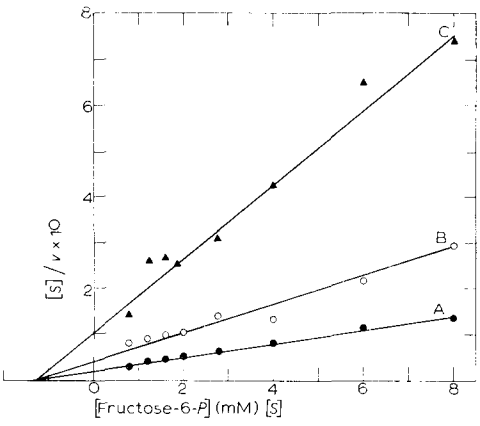
### The control of L-glutamine–D-fructose-6-phosphate amidotransferase in bovine retina

The enzyme L-glutamine–D-fructose-6-phosphate amidotransferase (EC 2.6.1.16), which catalyzes the formation of glucosamine 6-phosphate, was first described by LELOIR AND CARDINI[1] and further purified and studied by POGELL AND GRYDER[2] and by GHOSH *et al.*[3]. KORNFELD *et al.*[4] reported the feedback inhibition of rat liver amidotransferase by UDP-*N*-acetyl-D-glucosamine (UDP-GlcNAc), the end-product of the amino sugar pathway. KORNFELD[5] later showed that mouse liver and HeLa cell amidotransferases were similarly inhibited. The kinetics of inhibition of the rat liver amidotransferase are competitive with respect to fructose 6-phosphate and non-competitive with respect to glutamine[5]. The amidotransferase from bovine retina, reported here, exhibits a markedly different pattern of feedback inhibition.

Eyes were removed from cattle immediately after slaughter and kept on ice. Retinas were removed and washed by centrifugation in a buffer consisting of sucrose (0.35 M), Tris–HCl (pH 7.6, 50 mM), $MgCl_2$ (4 mM) and KCl (25 mM). In a typical preparation 100 washed retinas (50 g) were homogenized in 100 ml of the buffer used for washing. The homogenizer was washed with 50 ml buffer, and the wash was combined with the homogenate. The suspension was centrifuged for 15 min at 1000 $\times$ *g* and the supernatant was recentrifuged for 30 min at 165 000 $\times$ *g*. The high-speed supernatant was fractionated with ammonium sulfate. The fraction precipitating between 40 and 55% satn. was collected by centrifugation, dissolved in 2 ml 50 mM sodium phosphate buffer (pH 7.4), and dialyzed for 90 min against three 2-l changes of the same buffer. The resulting enzyme preparation was stable for several weeks at $-18°$.

The enzyme was assayed by measuring colorimetrically the formation of glucosamine 6-phosphate. Incubation mixtures contained L-glutamine, 3.0 $\mu$moles; fructose 6-phosphate, 1.5 $\mu$moles; phosphate buffer (pH 7.4), 7.5 $\mu$moles; 2-mercaptoethanol, 3.0 $\mu$moles; enzyme; and inhibitor (where indicated) in a final volume of 0.25 ml. Incubations were carried out at 37° and were stopped by heating for 2 min at 100°. After removal of coagulated protein by centrifugation, 0.15 ml was taken for hexosamine determination by a modification of the method of LEVVY AND MCALLAN[6]. All values were corrected by a zero-time control or by controls incubated either without enzyme or without substrates. Recoveries of added glucosamine 6-phosphate were better than 95%. $NH_4^+$ did not replace glutamine as a nitrogen donor.

The reaction rate was proportional to enzyme concentration in the range employed in these experiments and was linear for 3 h. 1 mg of enzyme was capable of catalyzing the formation of approx. 0.04 $\mu$mole of glucosamine 6-phosphate in 1 h. The amidotransferase was inhibited up to 90% by UDP-GlcNAc. The kinetics of this inhibition in the retinal enzyme are of the noncompetitive type with respect to fructose 6-phosphate (Fig. 1), *i.e.*, UDP-GlcNAc decreases the maximum velocity without changing the $K_m$. In this respect the retinal enzyme differs from the rat liver



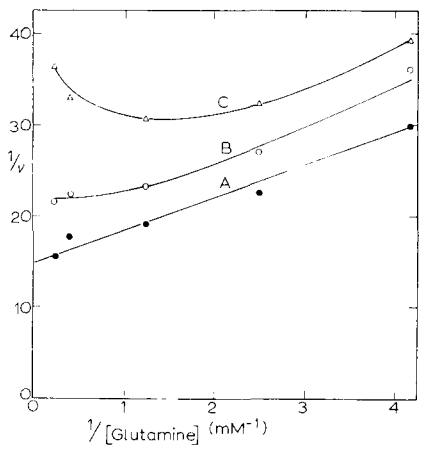




Fig. 1. Effect of UDP-GlcNAc concentration on the $K_m$ for fructose 6-phosphate. Velocity is expressed as nmoles glucosamine 6-phosphate formed in 2 h. Incubation mixtures were prepared and assayed as described in the text, except that they contained the indicated concentrations of fructose 6-phosphate, 0.65 mg enzyme and the following concentrations of UDP-GlcNAc: A, none; B, 6.4 $\mu$M; and C, 12.8 $\mu$M.

Fig. 2. Effect of UDP-GlcNAc on the reaction rate as a function of glutamine concentration. Velocity is expressed as $\mu$moles of glucosamine 6-phosphate formed in 2 h. Incubation mixtures were prepared and assayed as described in the text except that they contained the indicated concentrations of glutamine, 1.2 mg enzyme and the following concentrations of UDP-GlcNAc: A, none; B, 8 $\mu$M; and C, 16 $\mu$M.

enzyme which exhibits competitive inhibition[4,5]. From the data in Fig. 1, the $K_i$ for UDP-GlcNAc was calculated to be 3 $\mu$M.

The effect of UDP-GlcNAc on the reaction rate as a function of glutamine concentration is striking (Fig. 2). The end-product, UDP-GlcNAc, affects the enzyme in such a way that it becomes susceptible to inhibition by high concentrations of glutamine. The rat liver enzyme shows simple noncompetitive inhibition by UDP-GlcNAc with respect to glutamine[5].

In the presence of UDP-GlcNAc, variations in glutamine concentration do not affect the $K_m$ for fructose 6-phosphate, even with the enzyme inhibited up to 80%.

The conversion of the enzyme by UDP-GlcNAc to a form inhibitable by substrate represents an efficient use of the normally high level of glutamine in the retina, 4.29 mM (ref. 7). It is significant to note that the maximum inhibition *in vitro* is approached at this concentration (Fig. 2). The formation of *N*-acetyl-D-mannosamine from UDP-GlcNAc in rat liver is inhibited by CMP-*N*-acetylneuraminic acid in a similar way[4]. The enzyme becomes sensitive to the substrate, UDP-GlcNAc, which is present at concentrations of 0.2–0.3 mM. In both cases the effector apparently exerts a cooperative action with a substrate-binding site having a high $K_m$ and distinct from the catalytic site which has a lower $K_m$. Binding of the substrate to this high $K_m$ site produces inhibition only when the effector has also been bound. With this feedback control mechanism, the effect of rising end-product levels can be amplified by coupling with high substrate levels.

This type of mechanism may be the basis for the phenomenon reported by KINGDON, SHAPIRO AND STADTMAN[8] in their studies on the regulation of glutamine

synthetase in *Escherichia coli*. They observed that both AMP and L-alanine were considerably less effective inhibitors at nonsaturating substrate concentrations than they were at saturating levels of substrates. This would broaden the phenomenon to include effectors other than feedback inhibitors.

The spectrum of effectors must also be extended to include proteins. BREW, VANAMAN AND HILL[9] have shown that $\alpha$-lactalbumin produces a similar effect on the A protein of lactose synthetase. In the absence of $\alpha$-lactalbumin, the A protein transfers galactose to *N*-acetylglucosamine but not to glucose. In the presence of $\alpha$-lactalbumin, glucose becomes the preferred acceptor and transfer to *N*-acetylglucosamine is greatly diminished. The kinetics of this inhibition as a function of *N*-acetylglucosamine concentration are virtually identical to the amidotransferase kinetics when replotted in the manner shown here (Fig. 2).

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Received October 2nd, 1968

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