BBA 66419

EFFECT OF PHOSPHOGLUCOSE ISOMERASE AND GLUCOSE 6-PHOSPHATE ON UDP-*N*-ACETYLGLUCOSAMINE INHIBITION OF L-GLUTAMINE:D-FRUCTOSE 6-PHOSPHATE AMINOTRANSFERASE

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(Received April 26th, 1971)

SUMMARY

1. L-Glutamine:D-fructose 6-phosphate aminotransferase (EC 2.6.1.16) was purified about 30-fold from rat liver extracts with a recovery of about 70%. Fractionation involved $(NH_4)_2SO_4$ precipitation and DEAE–Sephadex column chromatography. Further purification (240-fold) was possible by use of hydroxylapatite chromatography.

2. The sensitivity of aminotransferase to UDP-*N*-acetylglucosamine inhibition was markedly reduced when the $(NH_4)_2SO_4$ precipitate was purified by DEAE–Sephadex column chromatography. The original high sensitivity, however, was restored by addition of phosphoglucose isomerase (D-glucose 6-phosphate ketol-isomerase, EC 5.3.1.9) or glucose 6-phosphate.

3. Glucose 6-phosphate enhanced the UDP-*N*-acetylglucosamine inhibition of aminotransferase purified by DEAE–Sephadex chromatography. The kinetics of UDP-*N*-acetylglucosamine inhibition with respect to fructose 6-phosphate were of the competitive type in the purified preparation but of the mixed type in the ammonium sulfate precipitate. The former preparation, however, exhibited the mixed-type kinetics when fructose 6-phosphate/glucose 6-phosphate ratio was maintained at 0.32, the equilibrium of phosphoglucose isomerase reaction.

4. It was concluded that the higher sensitivity to UDP-*N*-acetylglucosamine observed in crude preparations of aminotransferase is due to phosphoglucose isomerase which converts fructose 6-phosphate added as substrate to glucose 6-phosphate.

5. Comparison of the rat liver and Yoshida sarcoma aminotransferases purified by DEAE–Sephadex chromatography confirmed the previous observation that the tumor enzyme is more sensitive to feedback inhibition than is the liver enzyme.

INTRODUCTION

L-Glutamine:D-fructose 6-phosphate aminotransferase (EC 2.6.1.16) catalyzes the formation of glucosamine 6-phosphate (GlcN-6-*P*) from fructose 6-phosphate (Fru-6-*P*) and glutamine[1,2]. The enzyme is subject to feedback inhibition by UDP-

N-acetylglucosamine (UDP-GlcNAc)[3,4] and plays an important regulatory role in the synthesis of UDP-GlcNAc and hence of glycoproteins[5].

Previous work from this laboratory has shown that the aminotransferase from rat and mouse tumors is more sensitive to UDP-GlcNAc inhibition than is the rat liver enzyme[5]. Crude $(NH_4)_2SO_4$ precipitate was used for these earlier studies. For both the liver and tumor enzymes, however, unexpected reduction in the sensitivity to UDP-GlcNAc inhibition was encountered during subsequent purification. Elucidation of the mechanisms underlying this phenomenon seemed necessary in order to determine if the liver and tumor aminotransferases were mutually different proteins.

The present communication deals with these investigations. It was found that in crude preparations, phosphoglucose isomerase (D-glucose 6-phosphate ketol-isomerase, EC 5.3.1.9) potentiates UDP-GlcNAc inhibition of aminotransferase by converting Fru-6-*P* to glucose 6-phosphate (Glc-6-*P*), the actual enhancer for the inhibition. These findings are of physiological importance since phosphoglucose isomerase occurs in tissues in amounts sufficient to maintain the interconversion reaction of Fru-6-*P* and Glc-6-*P* near its equilibrium.

After this work was completed, WINTERBURN AND PHELPS[6–8] published their studies on purification and kinetics of rat liver aminotransferase. They also showed that Glc-6-*P* and AMP potentiate the UDP-GlcNAc inhibition of aminotransferase.

MATERIALS AND METHODS

Assay of aminotransferase

The standard assay mixture contained the following components in a final volume of 1 ml: sodium phosphate (pH 7.5), 55 μmoles; glutamine, 15 μmoles; Fru-6-*P*, 3 or 10 μmoles; EDTA, 0.2 μmole; dithiothreitol, 4 μmoles; and enzyme. Preliminary studies have shown that three factors, *i.e.* phosphate ion, EDTA and sulfhydryl compounds such as dithiothreitol are required for maximum aminotransferase activity. The requirement as for dithiothreitol was increased with the progress of purification.

After incubation for 1 h at 37°, the reaction was stopped by heating for 2 min at 100°. The mixture was centrifuged and GlcN-6-*P* was determined in the supernatant. One unit of enzyme was defined as the amount which catalyzed the formation of 1 nmole of GlcN-6-*P* per h.

Analytical methods

GlcN-6-*P* was determined by the method of GHOSH *et al.*[2] using free glucosamine as standard. Although GlcN-6-*P* has a molecular extinction coefficient that is approx. 85% that of glucosamine[2], no correction was made for this difference. Glc-6-*P* was determined enzymatically[9]. UDP-*N*-Acetylglucosamine 2'-epimerase (UDP-2-acetamido-2-deoxy-D-glucose 2-epimerase, EC 5.1.3.7) which was purified about 20-fold from rat liver extract by $(NH_4)_2SO_4$ fractionation and DEAE-Sephadex chromatography (K. KIKUCHI, H. KIKUCHI AND S. TSUIKI, unpublished observations) was used for the determination of UDP-GlcNAc. After incubation for 1 h at 37°, the amount of *N*-acetylmannosamine liberated was measured by the method of REISSIG *et al.*[10]. Protein concentrations were measured by the phenol reagent[11].

Chemicals and commercial enzymes

Glc-6-*P*, Fru-6-*P* and Glc-6-*P* dehydrogenase (D-glucose 6-phosphate: NADP oxidoreductase, EC 1.1.1.49) were purchased from Boehringer. UDP-GlcNAc was the product of Sigma. Phosphoglucose isomerase was a purified crystalline preparation (specific activity 350 I.U. (μmoles GlcN-6-*P* formed per min) per mg protein) obtained from Boehringer. Hydroxylapatite was prepared according to TISELIUS *et al.*[12].

RESULTS

Preparation and purification of aminotransferase from rat liver

All operations were conducted at 0–4°. Typical results of each purification step are summarized in Table I.

Step 1. Extraction. Male Donryu rats weighing 180–250 g and fed *ad libitum* were used. Livers were quickly removed from freshly slaughtered rats, rinsed in cold physiological saline, blotted on filter paper and cut into small pieces. The tissue was homogenized for 1 min in a glass tube with Teflon pestle using 2 vol. of 0.154 M KCl–1 mM EDTA–12 mM Glc-6-*P* adjusted to pH 7.5. The homogenate was centrifuged successively at 2000 × *g* for 10 min and at 105 000 × *g* for 1 h and the precipitate was removed and discarded.

TABLE I

PURIFICATION OF L-GLUTAMINE: D-FRUCTOSE 6-PHOSPHATE AMINOTRANSFERASE FROM RAT LIVER

Step	*Fraction*	*Total protein (mg)*	*Specific activity (units/mg)*	*Purification (-fold)*	*Recoverty (%)*
1	105 000 × *g* supernatant	897	25.3	(1)	(100)
2	$(NH_4)_2SO_4$ precipitate	305	58.8	2.3	80
3	Combined fractions from DEAE–Sephadex column	23.8	663	26	71
4	Combined fractions from hydroxylapatite column	1.11	6063	240	30

Step 2. $(NH_4)_2SO_4$ precipitation. The 105 000 × *g* supernatant was brought to 40% saturation with saturated $(NH_4)_2SO_4$ solution and the precipitate was removed and discarded after centrifugation at 18 000 × *g* for 10 min. The supernatant was adjusted to 60% saturation with saturated $(NH_4)_2SO_4$ solution and the precipitate was collected by centrifugation. The precipitate could be stored at — 25° for a month at least without losing activity.

Step 3. DEAE–Sephadex chromatography. The pooled $(NH_4)_2SO_4$ precipitate (305 mg in protein) was dissolved in a minimum volume of 50 mM sodium phosphate (pH 7.5)–50 mM KCl–1 mM EDTA and passed through a desalting column (2.5 cm × 30 cm) of Sephadex G-25. The protein eluates (Step 2 enzyme) were combined and applied to a column (1.5 cm × 25 cm) of DEAE–Sephadex A-50 previously equilibrated with 50 mM sodium phosphate (pH 7.5)–50 mM KCl–1 mM EDTA. The column was washed with approx. 150 ml of the same buffer; no aminotransferase activity was eluted in these breakthrough fractions. Gradient elution was then begun with 200 ml of the above buffer in the mixing chamber and 200 ml of 50 mM sodium

phosphate (pH 7.5)–500 mM KCl–1 mM EDTA in the reservoir, and fractions of 5 ml were collected at a flow rate of 15 ml/h. As shown in Fig. 1, aminotransferase activity emerged from the column as a single peak in a volume of 85–120 ml. These active fractions were pooled (Step 3 enzyme). Purification from the initial 105 000 × *g* supernatant was 26-fold with a recovery of 71%. The purified enzyme was unstable and lost 50% of its activity in 3 days at 2°. Neither Fru-6-*P*, GlcN-6-*P*, UDP-GlcNAc nor dithiothreitol protected the purified enzyme from inactivation. Glc-6-*P* and glutamine, however, exhibited a slight protecting effect.

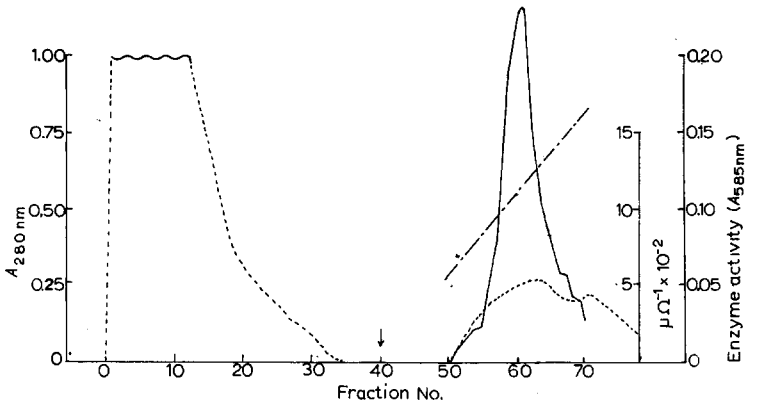

Fig. 1. Purification of rat liver aminotransferase on a DEAE-Sephadex column. Gradient started at the arrow. The fractions were assayed for the following: – – –, $A_{280\,nm}$; ———, aminotransferase activity; and —·—, conductivity (expressed as Ω^{-1}.

Step 4. Hydroxylapatite chromatography. Further purification of aminotransferase was possible by use of hydroxylapatite chromatography. The active fractions from the Step 3 were combined (20–30 ml) and brought to 70% saturation with solid $(NH_4)_2SO_4$. The resulting precipitate was collected by centrifugation, dissolved in a minimum volume of 0.1 M potassium phosphate (pH 7.5) containing 1 mM EDTA and passed through a desalting column of Sephadex G-25. The protein eluates were combined and applied to a column (1.5 cm × 20 cm) of hydroxylapatite previously equilibrated with 0.1 M potassium phosphate (pH 7.5) containing 1 mM EDTA. The column was first washed with approx. 60 ml of the above buffer and a gradient of potassium phosphate (pH 7.5, 1 mM EDTA) from 0.1 to 1 M in a total volume of 100 ml was applied. Fractions of 3 ml were collected at a flow rate of 5 ml/h; activity was eluted in a volume of 50–70 ml. The active fractions were pooled (Step 4 enzyme).

For the studies described below, Step 2 and Step 3 enzymes were mainly used. The properties of Step 4 enzyme were not different from those of Step 3 enzyme except that a number of contaminating enzyme activities had been removed.

Properties of purified enzyme

Step 2 enzyme contained a high level of phosphoglucose isomerase thereby exhibiting aminotransferase activity in the presence of either Fru-6-*P* or Glc-6-*P*. Step 3 enzyme differed from Step 2 enzyme in that Glc-6-*P* could not replace Fru-6-*P* in supporting GlcN-6-*P* synthesis. Moreover, when incubated with Glc-6-*P* (7 mM)

under otherwise standard assay conditions, Step 3 enzyme failed to utilize Glc-6-*P*. The DEAE–Sephadex chromatography thus effected a complete separation of aminotransferase from phosphoglucose isomerase, the bulk of which was recovered in the breakthrough fraction.

The 105 000 × *g* supernatant contained high level of UDP-GlcNAc 2'-epimerase. The difficulty of detecting an effective inhibition by UDP-GlcNAc in these supernatants is due to the occurrence of this enzyme. The epimerase, however, was readily separated from aminotransferase by $(NH_4)_2SO_4$ fractionation and therefore not present in Step 2 enzyme. Incubating Step 3 enzyme with 0.2 mM UDP-GlcNAc (under otherwise standard assay conditions) and determining the level of UDP-GlcNAc before and after the incubation revealed that the enzyme preparation was incapable of utilizing UDP-GlcNAc. Step 3 enzyme was also free from glutaminase (L-glutamine aminohydrolase, EC 3.5.1.2.) and glutamate dehydrogenase (L-glutamate:NAD^+ oxidoreductase (deaminating) E,C 1.4.1.2) but retained phosphofructokinase (ATP:D-fructose 6-phosphate 1-phosphotransferase, EC 2.7.1.11) activity. The latter enzyme was not detected in Step 4 enzyme.

The effects of substrate concentrations on the reaction velocities were studied by using Step 3 enzyme. The K_m values derived from these data were $3.1 \cdot 10^{-4}$M for Fru-6-*P* and $6.9 \cdot 10^{-4}$M for glutamine.

Apparent "desensitization" of aminotransferase to UDP-GlcNAc inhibition that accompanies the progress of purification

KORNFELD[4] has stated that "on several occasions the aminotransferase fractions recovered from DEAE-cellulose chromatography have had drastically reduced sensitivity to UDP-GlcNAc with good recovery of enzyme activity". In our studies, this has been a consistent finding: as shown in Fig. 2, the sensitivity to UDP-GlcNAc inhibition of Step 3 enzyme was drastically reduced as compared with Step 2 enzyme*. This reduction may be attributed to the "spontaneous" desensitization of enzyme to UDP-GlcNAc inhibition[4], but may also be explained well by postulating the removal

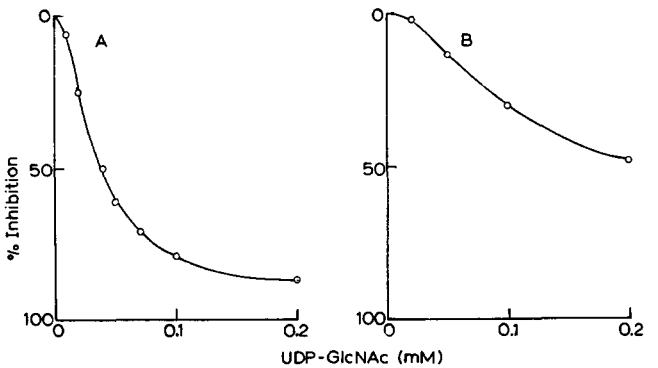

Fig. 2. UDP-GlcNAc inhibition of rat liver aminotransferase at different steps of purification. (A) $(NH_4)_2SO_4$ precipitate (Step 2 enzyme). (B) combined active fractions from DEAE-Sephadex column (Step 3 enzyme). The concentration of Fru-6-*P*: (A) 10 mM; (B)3 mM.

* The extent of reduction varied somewhat with the different preparations. This indicates that desensitization may be involved to some extent in the reduction of UDP-GlcNAc inhibition.

TABLE II

SEARCH FOR THE COMPOUND THAT POTENTIATES UDP-*N*-ACETYLGLUCOSAMINE INHIBITION OF RAT LIVER AMINOTRANSFERASE

Enzyme and addition	*Inhibition by 0.1 mM UDP-N-acetylglucosamine (%)**
Step 2 enzyme (4.4 mg) (A)	74.8
Step 3 enzyme (0.16 mg) (B)	40.0
B (0.16 mg) + A (4.4 mg)	77.4
+ 0.05 M KCl eluate from DEAE-Sephadex column (1.02 mg)	74.0
+ phosphoglucose isomerase (0.01 mg)**	69.1
+ Glc-6-*P* (5 mM)	76.5

* Fru-6-*P*, 10 mM.
** Crystalline yeast enzyme obtained from Boehringer.

of compound(s) that potentiates UDP-GlcNAc inhibition. In order to test this latter possibility, a series of experiments were performed; the results obtained are summarized in Table II.

When Step 2 and 3 enzymes were mixed in equal enzyme activities, the extent of UDP-GlcNAc inhibition attained was similar to that of Step 2 enzyme alone. This result was just as expected if the above-mentioned compound was present in Step 2 enzyme. It was subsequently found that the UDP-GlcNAc inhibition of Step 3 enzyme was markedly enhanced by the addition of the breakthrough fraction after DEAE-Sephadex chromatography or simply by the addition of crystalline phosphoglucose isomerase. On mg protein basis, the latter appears to be much more effective. The

TABLE III

EFFECT OF VARIOUS COMPOUNDS ON UDP-*N*-ACETYLGLUCOSAMINE INHIBITION OF RAT LIVER AMINOTRANSFERASE

Compounds	*Concentration (mM)*	*% Inhibition by 0.1 mM UDP-N-acetylglucosamine**
None	0	40.0
Glucose	5	37.5
Glucose 6-phosphate	5	79.2
Glucose 1-phosphate	5	48.0
Glucosamine 6-phosphate	1	40.0
6-Phosphogluconate	5	39.5
UDP-glucose	5	41.3**
UDP-xylose	0.2	45.6
UDP-glucuronic acid	0.2	63.0
ATP	1	38.5
ADP	1	38.5
AMP	1	41.0
UTP	1	38.7
UDP	1	30.0
NADP	1	46.5
NADPH	1	34.5**

* Fru-6-*P*, 3 mM.
** The data may not be very accurate since the method of GHOSH *et al.*[2] gave an absorption spectrum somewhat different from that of GlcN-6-*P*.

idea that the compound missing in Step 3 enzyme might be phosphoglucose isomerase itself was further supported by the following observations: (1) after DEAE-Sephadex chromatography, the bulk of phosphoglucose isomerase activity was recovered in the breakthrough fractions; and (2) fractionation of Step 2 enzyme on Sephadex G-200 column (not reported) gave the aminotransferase fractions that retained phosphoglucose isomerase activity and almost unaltered sensitivity to UDP-GlcNAc inhibition. However, the possibility of phosphoglucose isomerase potentiating the inhibition merely by decreasing Fru-6-*P* concentration was unlikely, since the enhancement obtained by decreasing Fru-6-*P* concentration was not as extensive as that caused by phosphoglucose isomerase.

Clue to the possible mechanism for the action of phosphoglucose isomerase

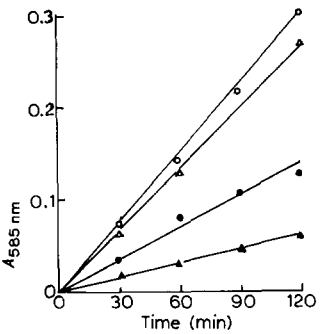

Fig. 3. Time course of aminotransferase reaction under various conditions. Step 3 enzyme from rat liver was incubated under the standard conditions in the presence of the following compounds: ○, none; △, 7 mM Glc-6-*P*; ●, 0.1 mM UDP-GlcNAc; ▲, 7 mM Glc-6-*P* and 0.1 mM UDP-GlcNAc. The incubation was terminated at indicated times and the GlcN-6-*P* formed was determined. Fru-6-*P*, 3 mM.

regarding UDP-GlcNAc inhibition was revealed when Glc-6-*P* was found to be an effective enhancer for the UDP-GlcNAc inhibition of Step 3 enzyme (Table II). Phosphoglucose isomerase (3.5 I.U./incubation mixture) and Glc-6-*P* (5 mM) enhanced the UDP-GlcNAc inhibition to comparable extents.

A wide variety of sugar phosphates and nucleotides were tested to see if they would potentiate the UDP-GlcNAc inhibition of Step 3 enzyme. As shown in Table III, except Glc-6-*P*, only UDP-glucuronic acid had a significant effect. Fig. 3 shows that the degree of inhibition by UDP-GlcNAc was constant throughout the period of incubation both in the absence and presence of Glc-6-*P*.

Taking all these results together suggested strongly that the reduction of UDP-GlcNAc inhibition was due to the removal of phosphoglucose isomerase that was capable of enhancing the inhibition by providing Glc-6-*P* from Fru-6-*P*.

Effect of Glc-6-P on UDP-GlcNAc inhibition of aminotransferase

Fig. 4A compares the effects of increasing concentrations of UDP-GlcNAc on Step 3 enzyme in the absence and presence of Glc-6-*P*. Although UDP-GlcNAc alone caused a progressive inhibition, the inhibition was markedly pronounced in the presence of Glc-6-*P*. In the experiments shown in Fig. 4B, the concentration of Glc-6-*P* was varied. Although Glc-6-*P* alone had little inhibitory effect, it behaved as if it

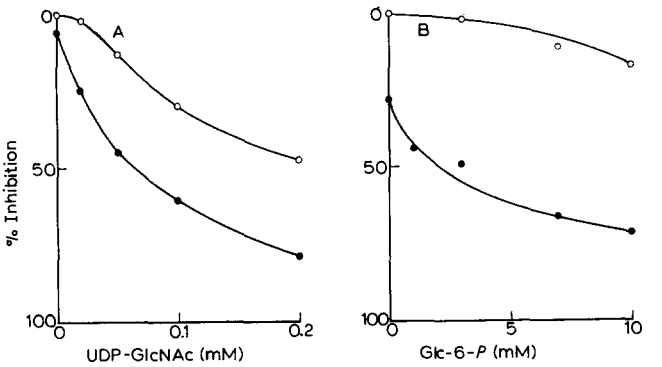

Fig. 4. Effect of Glc-6-*P* on UDP-GlcNAc inhibition of rat liver aminotransferase (Step 3 enzyme). (A) The enzyme was incubated with varying concentrations of UDP-GlcNAc in the absence (○) or presence (●) of 7 mM Glc-6-*P*. (B) The enzyme was incubated with varying concentrations of Glc-6-*P* in the absence (○) or presence (●) of 0.1 mM UDP-GlcNAc. Fru-6-*P*, 3 mM.

were a powerful inhibitor of aminotransferase when 0.1 mM UDP-GlcNAc was present.

The kinetics of UDP-GlcNAc were studied with Step 3 enzyme as a function of Fru-6-*P* concentration (Fig. 5). Using 0.1 mM UDP-GlcNAc, the inhibition curve was somewhat sigmoidal and its double reciprocal plots were slightly curved. The inhibition was of the competitive type, *i.e.* UDP-GlcNAc increased the K_m for Fru-6-*P* without changing the v_{max}. KORNFELD[4] also reported competitive kinetics for the UDP-GlcNAc inhibition of rat liver enzyme purified by DEAE-cellulose chromatography. The presence of Glc-6-*P* (7 mM) did not affect the nature of inhibition appreciably.

Similar experiments were performed with Step 2 enzyme and the results are

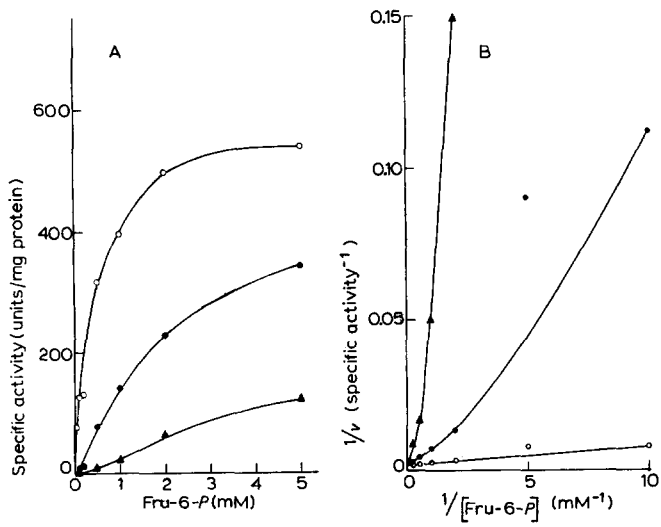

Fig. 5. Effects of UDP-GlcNAc and Glc-6-*P* on the velocity of rat liver aminotransferase (Step 3 enzyme) as a function of Fru-6-*P* concentration. ○, + none; ●, + 0.1 mM UDP-GlcNAc; ▲, + 0.1 mM UDP-GlcAc, 7 mM Glc-6-*P*.

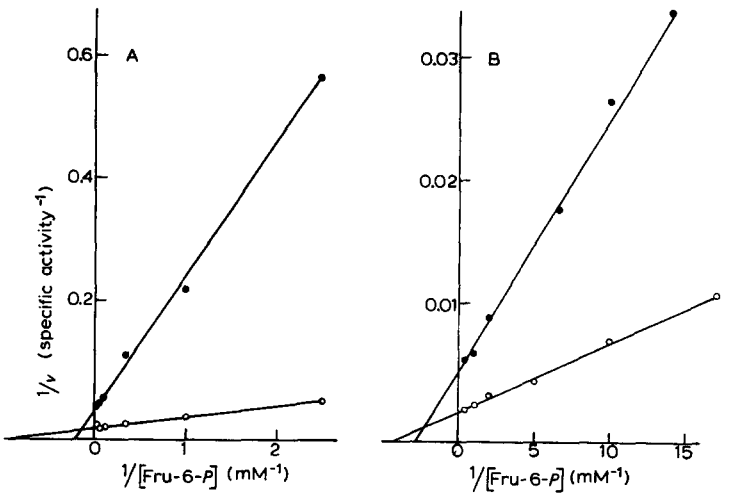

Fig. 6. Effects of UDP-GlcNAc on the velocity of rat liver aminotransferase (A, Step 2 enzyme; B, Step 3 enzyme) as a function of Fru-6-*P* concentration. In the experiments given in B, Glc-6-*P* was included in the incubation mixture so that the Fru-6-*P*/Glc-6-*P* ratio of 0.32 was attained at every Fru-6-*P* concentration indicated. ○, + none; ●, + 0.05 mM UDP-GlcNAc.

shown in Fig. 6A. Several differences could be noted; the most striking was that the inhibition was of the mixed type with respect to Fru-6-*P*, *i.e.* UDP-GlcNAc increased the K_m for Fru-6-*P* and simultaneously decreased the v_{max}. A sigmoid character of the curve was not apparent as indicated by a linear character of the double reciprocal plots. In these experiments, however, a lower concentration (0.05 mM) of UDP-GlcNAc was used and this might be sufficient to account for the lack of cooperative effects.

Step 2 enzyme contained the amount of phosphoglucose isomerase capable of converting the Fru-6-*P* added as substrate to the equilibrated mixture of Fru-6-*P* and Glc-6-*P* within a few minutes. Under the standard assay conditions for aminotransferase and using rat liver phosphoglucose isomerase, the equilibrium constant for Fru-6-*P*/Glc-6-*P* was found to be 0.32. In the experiments shown in Fig. 6B, the enzyme used was that of Step 3, but Glc-6-*P* was included in the incubation mixtures so that the Fru-6-*P*/Glc-6-*P* ratio of 0.32 was attained at every Fru-6-*P* concentration studied. The UDP-GlcNAc inhibition became of the mixed type. It is therefore apparent that the different kinetics observed for Step 2 (Fig. 6A) and Step 3 enzymes (Fig. 5) was due to the occurrence of phosphoglucose isomerase in the former.

Comparison of purified rat liver and Yoshida sarcoma aminotransferases

The sensitivity to UDP-GlcNAc inhibition of aminotransferase from rat Yoshida sarcoma or mouse Ehrlich ascites carcinoma was also markedly reduced after purification on DEAE-Sephadex column; the reduction was overcome by phosphoglucose isomerase or Glc-6-*P*. While the general pattern was thus similar to that obtained with the rat liver enzyme, the UDP-GlcNAc inhibition of Step 3 enzyme from Yoshida sarcoma and its enhancement by Glc-6-*P* (Fig. 7) were more extensive than in the rat liver enzyme (Fig. 4B). Thus the results confirmed the previous observation

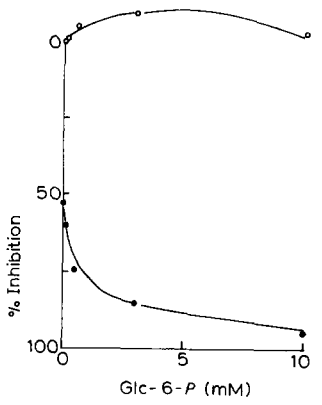

Fig. 7. Effect of Glc-6-*P* on UDP-GlcNAc inhibition of purified Yoshida sarcoma enzyme. Preparation of $(NH_4)_2SO_4$ precipitate (Step 2 enzyme) from rat Yoshida sarcoma has been described[5]. Further purification was performed on DEAE-Sephadex column. The procedures employed were similar to those for rat liver except that 0.05 mM GlcN-6-*P* and 20 mM glutamine were present throughout the purification procedures. Step 3 enzyme which was entirely free from phosphoglucose isomerase activity was incubated with varying concentrations of Glc-6-*P* in the absence (○) or presence (●) of 0.1 mM UDP-GlcNAc. Fru-6-*P*, 3 mM.

made with Step 2 enzyme[5]. Step 3 enzyme from Ehrlich carcinoma was also much more sensitive to feedback inhibition than rat liver enzyme.

DISCUSSION

Owing to the great difficulty of purifying aminotransferase[1–5,13–15], a number of earlier studies on this enzyme employed relatively crude preparations such as $(NH_4)_2SO_4$ precipitate [1,3,5,13,15]. The present studies as well as those reported previously[4], however, demonstrated that UDP-GlcNAc inhibition was markedly reduced when the $(NH_4)_2SO_4$ precipitate was further purified by DEAE-Sephadex or DEAE cellulose chromatography. This may be in part due to "spontaneous desensitization", but it is now evident that the reduction in UDP-GlcNAc inhibition is caused largely by the removal of phosphoglucose isomerase. The enzyme is capable of enhancing the UDP-GlcNAc inhibition of aminotransferase because of its capacity to convert Fru-6-*P* added as substrate to Glc-6-*P*. The actual enhancer is Glc-6-*P*; its effect appears to be specific both for aminotransferase and UDP-GlcNAc. AMP, which was reported to have a similar effect[7], was ineffective at least under our experimental conditions (Table III).

In rat liver, the concentration of Glc-6-*P* is maintained about 3 times as high as that of Fru-6-*P* (ref. 16) because of the high level of phosphoglucose isomerase (approx. 100 I.U./g wet weight[17]). It is therefore apparent that intracellularly, phosphoglucose isomerase constitutes an essential part of the feedback mechanism for aminotransferase, as depicted in Fig. 8.

WINTERBURN AND PHELPS[6], however, reported that the sensitivity to UDP-GlcNAc inhibition of rat liver aminotransferase did not change significantly during the course of purification. This is puzzling since it implies that phosphoglucose isomerase does not potentiate the UDP-GlcNAc inhibition. The 105 000 × *g* supérna-

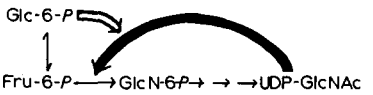

Fig. 8. Feedback control system for aminotransferase. →, inhibition; ⇒, enhancement of inhibition.

tant prepared by these authors contained phosphoglucose isomerase and its level was high enough to attain the equilibrium between Fru-6-*P* and Glc-6-*P* almost instantly[6]. Using the supernatant as the source of aminotransferase, therefore, the incubation would have started with 1.2 mM Fru-6-*P* *plus* 3.8 mM Glc-6-*P* instead of 5 mM Fru-6-*P* and these conditions were more than enough to lower the control ratio (the ratio of the maximum activities in the presence and absence of 0.1 mM UDP-GlcNAc according to ref. 6) from 0.47 to 0.16 (Table I of ref. 7). This apparent discrepancy may be explained by our finding that the 105 000 × *g* supernatant contains UDP-GlcNAc 2'-epimerase in much greater amount than aminotransferase (K. KIKUCHI, H. KIKUCHI AND S. TSUIKI, unpublished results). As also noted by KORNFELD *et al.*[3], this cytosol enzyme suppresses the UDP-GlcNAc inhibition by removing the inhibitor rapidly. In the course of purification given in Table I, the extent of UDP-GlcNAc inhibition was first increased by the removal of the epimerase (the $(NH_4)_2SO_4$ precipitation) and then decreased by the removal of phosphoglucose isomerase (the DEAE-Sephadex chromatography).

It is interesting to note that the type of UDP-GlcNAc inhibition with respect to Fru-6-*P* changed from the competitive to mixed type in the presence of phosphoglucose isomerase (Figs. 5 and 6). When the Glc-6-*P* concentration was held constant, the inhibition remained competitive (Fig. 5). Using rat liver aminotransferase purified by DEAE-cellulose chromatography, KORNFELD[4] showed the type of UDP-GlcNAc inhibition to be competitive with respect to Fru-6-*P*, whereas the inhibition of bovine retinal enzyme reported by MAZLEN *et al.*[15] was of the noncompetitive type. Since the latter preparation was just equivalent to our Step 2 enzyme, it is possible that the inhibition was under the influence of phosphoglucose isomerase.

The present data, however, differ from those of WINTERBURN AND PHELPS[7] who showed that the type of UDP-GlcNAc inhibition was noncompetitive when Glc-6-*P* or AMP was absent. Although the reasons for the difference is obscure, we are inclined to consider that the assay conditions may be an important factor since, as just described, our data are rather similar to those of KORNFELD[4] and of MAZLEN *et al.*[15], whose assay conditions were similar to those of ours. The conditions employed by WINTERBURN AND PHELPS[6] were different from others in that Tris–HCl buffer rather than phosphate buffer was used and that no thiols were deliberately added to the assay mixture. In our experience the aminotransferase activity measured in Tris–HCl buffer (pH 7.5) is rather low but increases considerably on addition of 20–40 mM phosphate. In the presence of 4 mM dithiothreitol, the increase is 100–150%.

Although it is true that no exogenous thiols are required for the maximum activity when the 105 000 × *g* supernatant is the test enzyme, they are needed to compensate the losses of activity during the course of purification[5]. The thiol concentration of the assay mixture of WINTERBURN AND PHELPS[6] may not be sufficiently

high since its only possible source appeared to be the enzyme solution (the purification medium contained 5 mM glutathione). We have also found that AMP acts as a weak inhibitor of aminotransferase when dithiothreitol is omitted from the incubation mixture. However, whether or not the ineffectiveness of AMP in the present work is really due to the use of dithiothreitol remains to be seen.

Using enzyme preparations of much higher purity, the present studies confirmed the previous observation that the aminotransferase of tumors is more sensitive to UDP-GlcNAc inhibition than is the liver enzyme[5]. In addition to this difference, the tumor enzyme appears to differ from the liver enzyme in its stability, affinity to DEAE-Sephadex column and immunochemical behavior[18]. Studies are now in progress to determine whether or not the two enzymes represent a set of isozymes of aminotransferase.

ACKNOWLEDGEMENT

This work was supported by scientific research fund from the Ministry of Education of Japan (S. T.).

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