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PARTIAL PURIFICATION AND PROPERTIES OF L-GLUTAMINE: D-FRUCTOSE 6-PHOSPHATE AMINOTRANSFERASE FROM ZOOSPORES OF *BLASTOCLADIELLA EMERSONII*

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Key Word Index—*Blastocladiella emersonii*; zoospores; L-glutamine: D-fructose 6-phosphate aminotransferase; kinetics.

Abstract—L-Glutamine: D-fructose 6-phosphate aminotransferase (E.C. 2.6.1.16) was purified approx. 168-fold from zoospores of the fungus *Blastocladiella emersonii* by centrifugation and two successive fractionations on DEAE-cellulose columns. Specific activities up to 6·70 μmol glucosamine 6-phosphate × min^{-1} mg $protein^{-1}$ were achieved. Kinetics studies using 100000 *g* supernatants and column-purified fractions yielded K_ms for fructose 6-phosphate of 1·0 ± 0·3 mM and 0·7 ± 0·1 mM respectively. The corresponding K_ms for L-glutamine were 0·8 ± 0·3 mM and 0·7 ± 0·03 mM respectively. The feedback inhibitor UDP-*N*-acetylglucosamine was competitive with fructose 6-phosphate. K_is of 9·7 μM and 18·7 μM were calculated for the supernatant and column preparations, respectively. The inhibitor was uncompetitive with glutamine. The K_i for the supernatant enzyme was 0·19 mM.

INTRODUCTION

L-Glutamine: D-fructose 6-phosphate aminotransferase, a key enzyme in the synthesis of amino sugars and their derivatives, is subject to feedback inhibition by UDP-N-acetylglucosamine (UDP-GlcNAc) in mammals [1–11] and in higher plants [12, 13]. Studies of this enzyme have been hampered by its instability, and to our knowledge all efforts to purify it to homogeneity have failed. Partial purification of the aminotransferase from zoospores of *Blastocladiella emersonii* had been described in a preliminary report [14]. In this paper, we report a further increase in the specific activity of this enzyme and the results of kinetics studies performed with preparations of it at different stages in its purification.

RESULTS

The L-glutamine: D-fructose 6-phosphate aminotransferase from *B. emersonii* zoospores was further purified over previously reported [14] levels via a second step of column chromatography on DEAE-cellulose. A representative elution profile is illustrated in Fig. 1. Although the enzyme

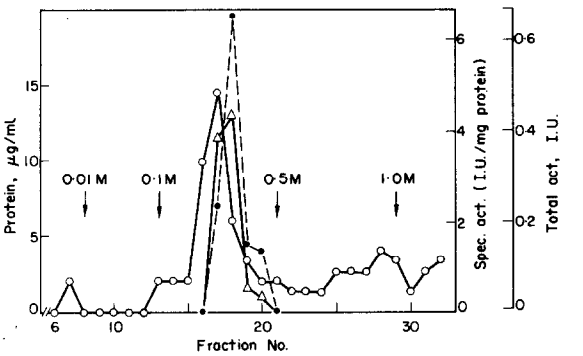

Fig. 1. Fractionation of a preparation of L-glutamine: D-fructose 6-phosphate aminotransferase on a second DEAE-cellulose column (2·0 × 19·5 cm). The sample (containing 2 mg protein) applied to the column consisted of a highly active fraction obtained from a first column of DEAE-cellulose and diluted with 4 vol. of buffer. Proteins were eluted stepwise with buffered KCl at increasing concentrations as indicated at arrows. The column flow rate was 70 ml/hr and fractions were 10 ml each. Protein (μg/ml), (○——○); sp. act. (●——●); total activity (△——△).

Table 1. Partial purification of L-glutamine:D-fructose 6-phosphate aminotransferase from zoospores of *Blastocladiellae mersonii*

Step	Fraction	Specific activity	Purification
1	Whole spore homogenate	0·04	1
2	110000 *g* supernatant	0·16	4
3	DEAE-cellulose I	3·50*	88
4	DEAE-cellulose II	6·70*	168†

* Peak values for sp. act.

† For additional information about the properties of the *B. emersonii* enzyme, see Lovett, J. S. and Cantino, E. C. (1960) *Am. J. Botany* **47**, 499.

and the major protein band were only partly separated, the specific activity was significantly increased. Results of the partial purification of the aminotransferase are summarized in Table 1. The enzyme collected from the second DEAE-cellulose column was very unstable and could not be used for kinetic studies.

The velocity of the aminotransferase catalysed reaction was linear for 30 min. Michaelis constants were determined for the two substrates. Enzyme preparations purified by centrifugation at 110000 *g* (Step 2 enzyme) yielded a K_m value of 1·0 ± 0·3 mM (s.d.) (seven experiments). Highly purified enzyme fractions from a DEAE-cellulose column (Step 3 enzyme) gave a K_m value of 0·7 ± 0·1 mM (s.d.) (three experiments).

The K_m values for L-glutamine were 0·8 ± 0·3 mM (s.d.) (five experiments) using Step 2 enzyme, and 0·7 ± 0·3 mM (s.d.) (three experiments) using Step 3 enzyme. An apparent decrease in GlcN-6-*P* formation with concentrations of L-glutamine over 12 mM was caused by the interference of glutamine in our analysis procedure.

As first reported by Kornfeld *et al.* [1], L-glutamine:D-fructose 6-phosphate amino-transferase can be inhibited by UDP-GlcNAc. Likewise, UDP-GlcNAc inhibited the *B. emersonii* aminotransferase (Fig. 2), but the level never reached 100%. As illustrated in Fig. 3, UDP-GlcNAc is a competitive inhibitor for Fru-6-*P*. The rate curve was apparently somewhat sigmoidal in the presence of 0·2 mM UDP-GlcNAc (Fig. 3, left); K_1 values of 9·7 μM for the Step 2 enzyme, and 18·7 μM for the Step 3 enzyme, were established from secondary plots.

The inhibitor was uncompetitive (terminology of Mahler and Cordes [15]) with L-glutamine (Fig. 4). Only Step 2 enzyme could be studied because all stable column fractions contained L-glutamine. A K_1 of 0·19 mM was calculated for the inhibition of glutamine (Fig. 4).

The Step 3 enzyme was free of phosphoglucose isomerase (E.C. 5.3.1.9) activity; a slight amount of it remained in the Step 2 enzyme. Both Step 2 and Step 3 enzymes were free of glucosamine 6-phosphate deaminase (E.C. 5.3.1.10) activity.

DISCUSSION

Further purification of the *B. emersonii* L-glutamine:D-fructose 6-phosphate aminotransferase has yielded a preparation with a higher specific activity than any thus far reported in the literature. Still, the enzyme was not purified to homogeneity. The Step 4 enzyme was very unstable, possibly due to the low concentration of protein in the eluate from the second DEAE-cellulose column.

A survey of the literature on the aminotransferase shows that the K_m values for Fru-6-*P* fall

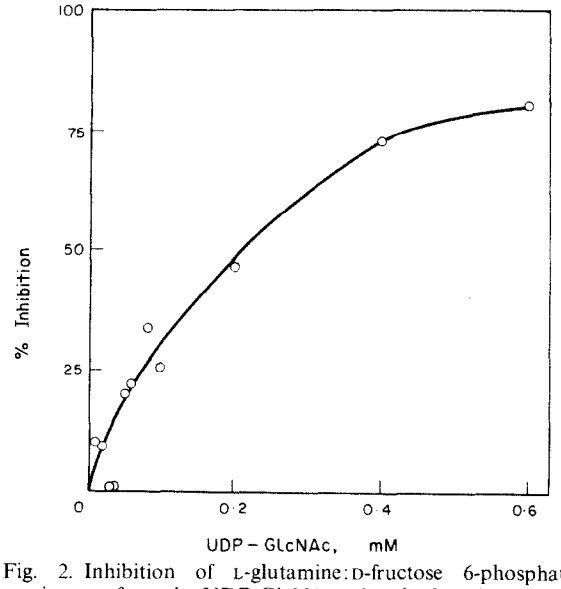

Fig. 2. Inhibition of L-glutamine:D-fructose 6-phosphate aminotransferase by UDP-GlcNAc using the Step 2 enzyme.

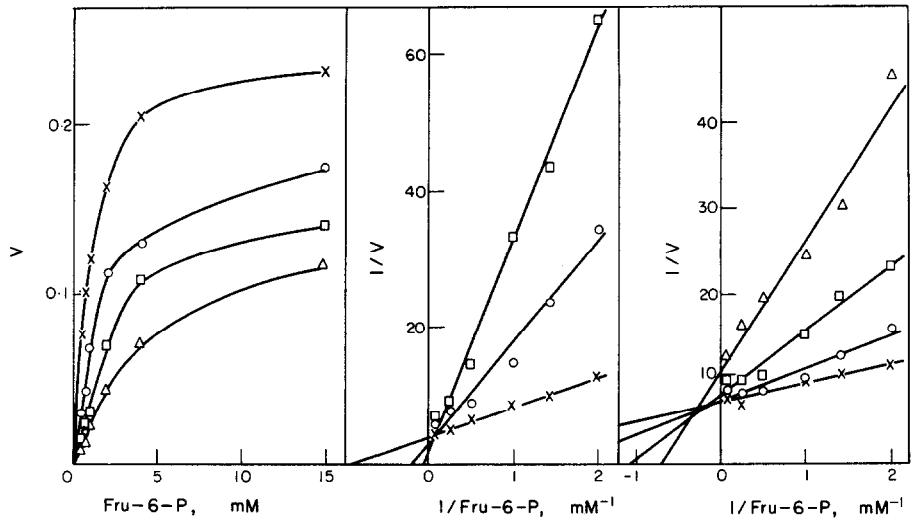

Fig. 3. Effect of UDP-GlcNAc on the velocity of L-glutamine:D-fructose 6-phosphate aminotransferase activity as a function of Fru-6-*P* concentration. Standard assay mixtures contained: No (×), 0·05 mM (○), 0·1 mM (□), or 0·2 mM (△) UDP-GlcNAc. Fig. 3, left; the velocity (v) vs substrate concentration. Fig. 3, center: double reciprocal plot of the data in Fig. 3, left, Step 2 enzyme being used. Fig. 3, right: a double reciprocal plot obtained using Step 3 enzyme.

within the range 0·22–3·8 mM, and those for L-glutamine between 0·2 and 1·6 mM. Our K_m values agree well with these findings. The aminotransferase from *B. emersonii* was inhibited by UDP-GlcNAc to a lesser extent than that from many other sources [2, 4, 6, 13]. It required 0·6 mM UDP-GlcNAc to cause 80% inhibition of the Step 2 enzyme, while Kornfeld [2] reported approx. the same inhibition in the presence of 0·1 mM UDP-GlcNAc for rat liver, mouse liver, and HeLa cell enzymes. On the other hand, the aminotransferase from bovine trachea [11] was inhibited in much the same way as the *B. emersonii* enzyme. Miyagi and Tsuiki [7] have shown that Glc-6-*P* enhances the inhibition caused by UDP-GlcNAc, hence differences in Glc-6-*P* concentration may explain the apparently variable responses to the inhibitor. Miyagi and Tsuiki [7] also pointed out that phosphoglucose isomerase (E.C. 5.3.1.9) converts some Fru-6-*P* to Glc-6-*P*. Our Step 3 enzyme was free of the latter activity. Assays employing Step 3 enzyme involved only 5 μM Glc-6-*P* (derived from the buffer). The fact that the K_i value for the Step 3 enzyme is twice that of the Step 2 enzyme indicates a decrease in aminotransferase sensitivity, possibly due to the removal of phosphoglucose isomerase.

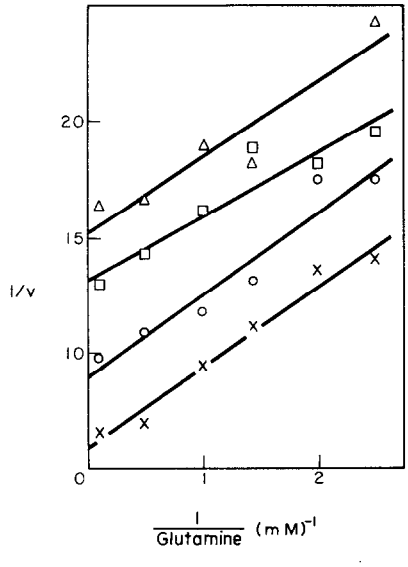

Fig. 4.

Kornfeld [2] noted that UDP-GlcNAc acts as a feedback inhibitor for the aminotransferase from mammals but not for that from bacteria. The same mechanism has been reported for higher plants [12, 13] and, now, also for *B. emersonii*. It may be of phylogenetic significance that this feedback mechanism is also present in a "primitive" water mold.

EXPERIMENTAL

The original strain of *Blastocladiella emersonii* Cantino and Hyatt [16] was used; zoospores were produced and the L-glutamine:D-fructose 6-phosphate aminotransferase extracted therefrom, all as previously described [14].

The standard assay mixture contained 25 mM potassium phosphate (pH 6·8), 10 mM L-glutamine, 15 mM D-fructose 6-phosphate (Fru-6-*P*), 1 mM ethylene-glycol-bis (B-aminoethyl-ether) -*N*,*N*'-tetraacetic acid (EGTA), 1 mM KCl, and 40–60 mM sucrose in a final vol. of 1 ml. The sucrose was carried over either from the original zoospore lysate or from column preparations where it had been added as a stabilizer. Incubations were started by addition of enzyme and terminated after 30 min (unless otherwise noted) at 30° by immersing the assay tubes in boiling H_2O for 1 min. D-Glucosamine 6-phosphate (GlcN-6-*P*) was determined according to Good and Bessman [17], the extinction being measured at 540 nm. Protein was determined according to Lowry *et al.* [18] whenever possible, bovine serum albumin being used as a standard. A tannin turbidimetric technique [19] was also used whenever dithiothreitol was present. One i.u. of enzyme was defined as the amount which catalyzed the formation of 1 μmol of GlcN-6-*P* per min. Sp. act. was expressed as i.u. per mg protein.

A DEAE-cellulose column (Whatman DE-11) was equilibrated with a soln of 25 mM potassium phosphate (pH 6·8), 1 mM EGTA, 1 mM KCl, 4 mM dithiothreitol, 0·05 mM D-glucose 6-phosphate (Glc-6-*P*), 10 mM L-glutamine and 600 mM sucrose. Elution was stepwise, using buffered KCl solns. Double reciprocal plots were derived by linear regression analysis. Unless otherwise noted, the linear correlation coefficient was >0·97. All K_is were calculated from secondary plots of slope (for Fru-6-*P*) or *y*-intercept (for L-glutamine) vs concn of UDP-GlcNAc according to Mahler and Cordes [15].

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