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Short Communication

Purification of glutamine synthetase by adenosine-affinity chromatography

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Abstract

The ability to purify insect flight muscle glutamine synthetase using various adenosine ligands was assessed. The enzyme bound most strongly to the ADP analogue (5′-ADP-agarose), followed by the NADPH analogue (2′,5′-ADP-Sepharose 4B), and least strongly to the cyclic AMP analogue (3′,5′-ADP-agarose). In all cases, binding was strongest in the presence of Mn^{2+} when compared to Mg^{2+}. These results suggest that the binding of glutamine synthetase to adenosine-affinity media is related to the participation of Mn · ADP in the γ-glutamyl transferase reaction that is catalyzed by glutamine synthetase.

1. Introduction

Glutamine synthetase has been purified from both procaryotic and eucaryotic sources using various types of affinity chromatography. For example, ADP-agarose has been used to purify glutamine synthetase from photosynthetic bacteria [1], while the related "Blue" chromatography media (*e.g.* Affigel Blue) have been used to purify glutamine synthetases from a variety of sources (*e.g.* ref. 2). In addition, 2′,5′-ADP-Sepharose 4B has been used to purify glutamine synthetase from procaryotes [3], plants [4] and insects [5]. However, this latter affinity ligand resembles NADP more than ADP, particularly with respect to the position of the phosphate moieties. This is reflected in the more general use of this affinity ligand in the purification of NADPH-dependent enzymes (*e.g.* refs. 6 and 7). In the present report, we characterize the ability of glutamine synthetase to be purified by three different adenosine-affinity ligands: 5′-ADP-agarose (an ADP analogue), 2′,5′-ADP-Sepharose 4B (an NADP analogue) and 3′,5′-ADP-agarose (a cyclic AMP analogue). We report conditions for the successful purification of insect glutamine synthetase using each of these three different affinity ligands.

2. Experimental

2.1. Insects

Parasarcophaga crassipalpis larvae were reared on liver; adults were fed sugar and water. Four days after emerging, adults were given a liver meal to allow development of sexual ma-

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turity and to provide a medium in which to lay eggs. All insects used in the present study were adults and were at least four days old.

2.2. Chemicals

EDTA and Triton X-100 were from BDH. β-Mercaptoethanol was from Calbiochem. 2',5'-ADP-Sepharose 4B, 3',5'-ADP-agarose, 5'-ADP-agarose, DEAE-Sepharose CL-6B and Sephadex G-15 were from Pharmacia. ADP, glutamine, γ-glutamyl hydroxamate, glutathione and imidazole were from Sigma.

2.3. Partial purification of insect glutamine synthetase

Glutamine synthetase was extracted from *P. crassipalpis* flight muscle, and purified by precipitation with 40–55% ammonium sulphate and ion-exchange chromatography on DEAE-Sepharose CL-6B, as previously described [5]. Glutamine synthetase was measured by the γ-glutamyl transferase assay (1 unit = 1 μmol γ-glutamyl hydroxamate produced per min), as outlined in ref. 5.

2.4. Affinity chromatography of glutamine synthetase

Active fractions from the DEAE ion-exchange chromatography fractionation were pooled and aliquots (0.2 transferase units) dialysed against 35 volumes of 10 m*M* imidazole · HCl buffer containing 10 m*M* β-mercaptoethanol prior to chromatography. Glutamine synthetase was then applied to pre-equilibrated columns of 2',5'-ADP-Sepharose 4B, 5'-ADP-agarose or 3',5'-ADP-agarose. Unbound protein was removed by washing the columns with five bed volumes of 10 m*M* imidazole · HCl buffer containing 10 m*M* β-mercaptoethanol, and bound protein eluted with 5 m*M* ADP in 10 m*M* imidazole · HCl buffer containing 10 m*M* β-mercaptoethanol.

3. Results

3.1. Affinity chromatography of glutamine synthetase

A typical purification of glutamine synthetase with each of the affinity media is shown in Fig. 1. Partially purified glutamine synthetase was equilibrated in 10 m*M* imidazole · HCl buffer (pH 6.3) containing 2.5 m*M* $MnCl_2$ and 10 m*M* β-mercaptoethanol. The enzyme was then applied to the various affinity columns, the column washed with five bed volumes of buffer and bound enzyme eluted with 5 m*M* ADP in buffer. Recoveries of glutamine synthetase were consistently high (70–100%), suggesting that the enzyme did not bind irreversibly to any of the affinity media.

The binding of glutamine synthetase to each of the affinity media was subsequently characterized. Aliquots of partially purified glutamine synthetase were equilibrated in 10 m*M* imidazole · HCl buffers of various pH containing either 2.5 m*M* $MnCl_2$ or 5 m*M* $MgCl_2$, and

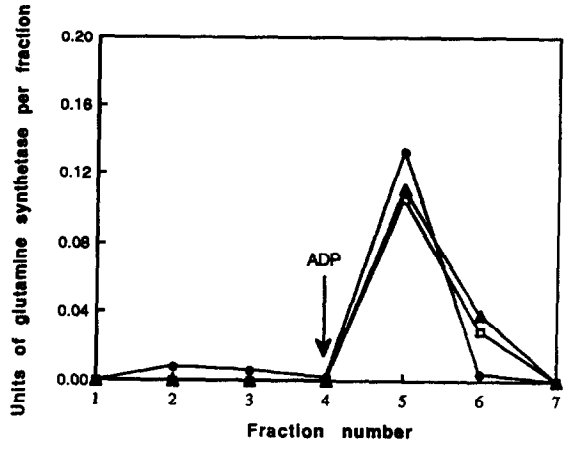

Fig. 1. The binding of insect glutamine synthetase to adenosine-affinity ligands. Partially purified glutamine synthetase (0.2 transferase units) was pre-equilibrated in 10 m*M* imidazole · HCl buffer (pH 6.3) containing 2.5 m*M* $MnCl_2$ and 10 m*M* β-mercaptoethanol. The enzyme was then applied to (□) 5'-ADP-agarose, (▲) 2',5'-ADP-Sepharose 4B, and (●) 3',5'-ADP-agarose, and bound enzyme eluted with 5 m*M* ADP in 10 m*M* imidazole · HCl buffer (arrow).

chromatographed. In the presence of Mn^{2+}, variation in pH over the range 6.3–8.3 had little effect on the binding of glutamine synthetase to either 5′-ADP-agarose or 2′,5′-ADP-Sepharose 4B (Fig. 2). By contrast, glutamine synthetase bound much less strongly to 3′,5′-ADP-agarose as the pH increased (Fig. 2). The latter trend was observed with all three forms of affinity media when the chromatography was performed in the presence of Mg^{2+} (Fig. 3).

The effect of the form of the divalent cation present during chromatography is evident when Fig. 2 is compared with Fig. 3. At pH 6.3, no effect of the form of the divalent cation was observed when the chromatography was carried out with 5′-ADP-agarose or 2′,5′-ADP-Sepharose 4B. However, in the case of 3′,5′-ADP-agarose, binding was much stronger in the presence of Mn^{2+} than it was with Mg^{2+}. At both pH 7.3 and 8.3, binding was stronger in the presence of Mn^{2+} to all three forms of affinity media. The effect of the form of the ADP-affinity media used can also be seen in Figs. 2 and 3. Throughout the range of conditions examined, glutamine synthetase bound most strongly to 5′-ADP-agarose, followed by 2′,5′-ADP-Sepharose 4B, and only weakly to 3′,5′-ADP-agarose.

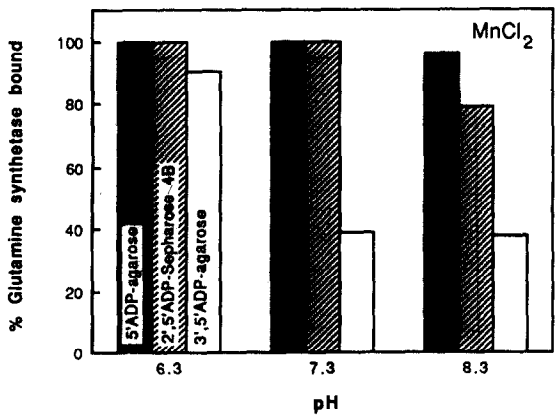

Fig. 2. The effect of pH on the binding of insect glutamine synthetase to adenosine-affinity ligands in the presence of Mn^{2+}. Partially purified glutamine synthetase was pre-equilibrated in 10 m*M* imidazole·HCl buffers of various pH containing 10 m*M* β-mercaptoethanol and 2.5 m*M* $MnCl_2$, and chromatographed. "% Glutamine synthetase bound" was calculated as the amount of glutamine synthetase that eluted from the column upon application of 5 m*M* ADP, expressed as a percentage of the amount of glutamine synthetase recovered during chromatography.

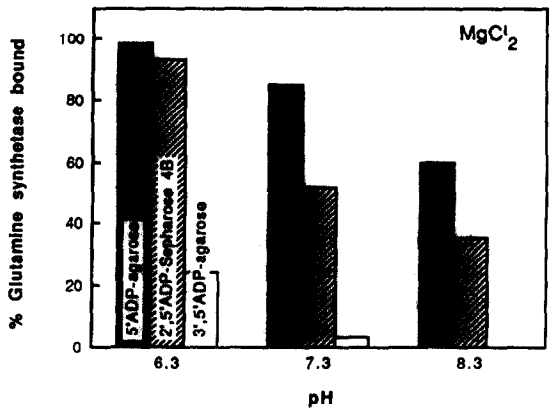

Fig. 3. The effect of pH on the binding of insect glutamine synthetase to adenosine-affinity ligands in the presence of Mg^{2+}. Partially purified glutamine synthetase was pre-equilibrated in 10 m*M* imidazole·HCl buffers of various pH containing 10 m*M* β-mercaptoethanol and 5 m*M* $MgCl_2$, and chromatographed. Calculation of % glutamine synthetase bound is outlined in the legend to Fig. 2.

4. Discussion

The three different forms of affinity media used in the present study have been used for the affinity purification of a number of enzymes. 2′,5′-ADP-Sepharose 4B has been used predominantly to purify NADP-dependent enzymes (*e.g.* refs. 6 and 7) because of the similarity of 2′,5′-ADP to NADP; the position of the monophosphate groups on the adenosine moiety are identical in these two molecules. Similarly, 3′,5′-ADP-agarose has been used to purify cAMP-dependent enzymes because of the similarity of these two molecules [8]; both have phosphate groups on the 3′ and 5′ carbon of the adenosine ring. In the present study, we found that glutamine synthetase bound most consistently to 5′-ADP-agarose; the position of a diphosphate group in this affinity ligand is identical to that found in ADP.

Our results are consistent with the hypothesis

that the binding of glutamine synthetase to adenosine-affinity media is related to the participation of Mn · ADP in the γ-glutamyl transferase reaction catalyzed by glutamine synthetase [9]. The γ-glutamyl transferase reaction shows a preference for Mn^{2+} over Mg^{2+} (*e.g* ref. 10), and has maximal activity at slightly acid pH (*e.g.* ref. 11). Similarly, the binding of insect glutamine synthetase to adenosine-affinity media also showed a preference for Mn^{2+} over Mg^{2+}, and highest binding at slightly acid pH. Furthermore, glutamine synthetase bound most consistently to the adenosine-ligand that most closely resembles ADP. These results suggest that insect glutamine synthetase has a Mn · ADP binding site similar to the one described on each sub-unit of bovine brain glutamine synthetase [12].

5. References

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