The Effect of Thiol Compounds on the Glutamate Affinity of the Chloroplastic Glutamine Synthetase from Mustard Leaves

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The chloroplastic glutamine synthetase taken from mustard leaves shows a positive cooperative glutamate binding. The $S_{0.5}$-value depends on the DTE concentration in the homogenization buffer. Normal glutamate saturation kinetics have been found with the root enzyme and the enzyme of etiolated cotyledons. These are not influenced by thiol compounds. The possible function of the allosteric behaviour of GS_2 is discussed.

Introduction

Tischner und Schmidt [1] and Florenzio and Vega [2] found that algal GS can be activated by adding thiol compounds such as DTE, DTT, ME and cystein.

O'Neal and Joy [3] discovered that the purified GS of pea leaves could sometimes be activated by incubating it with thiol compounds. Investigations during the last years have shown that thiol compounds stabilize the GS in higher plants, mainly the chloroplastic enzyme [4, 5].

The saturation kinetic of the GS isoenzymes with glutamate was always found to be a normal Michaelis-Menten-kinetic with $K_m = 1-4$ mM for the root enzyme, 2–3 mM for the cytosolic leaf enzyme and 2–20 mM for the chloroplastic enzyme [6–11]. Only Guiz *et al.* [8] described a negative cooperative binding toward glutamate with GS_2 taken from rice leaves.

Materials and Methods

For plant culture of *Sinapis alba* L. and *Spinacia oleracea* L. var Matador and assay of the GS see Wild and Manderscheid [12]. ME was added just before homogenization. Enzyme activity was measured in the crude extract, which had not been desalted.

Before the isolation of the chloroplastic isoenzyme the crude extract was desalted. Then, the protein fraction was layered on the top of a DEAE-Sephacel column (10×2 cm) and eluted according to Guiz *et al.* [8].

Results and Discussion

The saturation kinetic of the chloroplastic enzyme with glutamate depends on the thiol concentration in the homogenization buffer (Fig. 1). The increase of the DTE concentration causes a shift of the $S_{0.5}$-value from about 60 mM to 13 mM. With thiol concentrations higher than 30 mM the glutamate affinity comes to an end. The Scatchard-Klotz-diagramm at 30 mM DTE clearly shows a positive cooperative binding (Fig. 2) with the Hill-coefficient being 1.8 and $R_s = 13$. Tests with 25 mM ME yielded the same results as with 30 mM DTE. Since ME is oxidized in the homogenization buffer [13] only DTE has been used for further investigations.

Up to now no publication has been made about positive cooperative glutamate binding with GS taken from higher plants. Only Guiz *et al.* [8] found a negative cooperativity for the GS_2 of rice leaves. Possibly, this was due to an inactivation of the enzyme by the oxidation of the SH-groups, as is possible also with the GS_2 of mustard leaves at a low thiol concentration.

An examination of the GS taken from etiolated cotyledons yielded a normal saturation curve with K_m (Glu) = 5 mM and the glutamate affinity is independent of the DTE concentration in the homogenization buffer. Additionally thiols had no effect on the glutamate saturation curve of the root enzyme.

As has been found out by the application of DEAE-sephacel chromatography, green leaves only possess the chloroplastic isoenzyme. Therefore a light-induced conformational change of the GS_2 in etiolated leaves can be assumed. The 'normal' enzyme is converted into an allosteric enzyme. The insertion of SH-groups seems to be responsible for the allosteric behavior.

With GS_2 from spinach, which also contains only GS_2 [14] we found the same positive cooperative

Abbreviations: DTE, 1,4 dithioerythritol; DDT, 1,4 dithiothreitol; Glu, glutamate; GS, glutamine synthetase; GS_1, cytosolic glutamine synthetase; GS_2, chloroplastic glutamine synthetase; ME, 2-mercaptoethanol.

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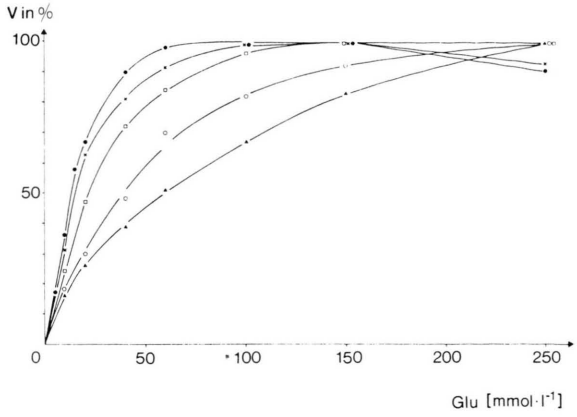

Fig. 1. The effect of different dithioerythritol concentrations contained in the homogenization buffer on the glutamate affinity of the GS_2 from mustard leaves. 1 mM DTE (▲), 5 Mm DTE (○), 10 mM DTE (□), 20 mM DTE (×), 30 mm DTE (●).

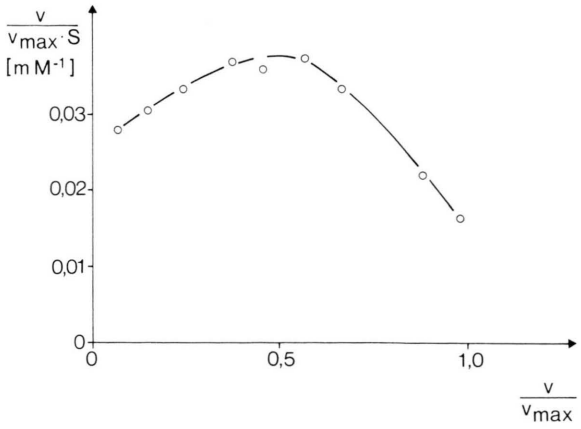

Fig. 2. The Scatchard-Klotz-diagramm showing the glutamate binding kinetic of GS_2 taken from mustard plants at a dithioerythritol concentration of 30 mM. Each value is the mean of 3 separate experiments.

glutamate binding, which was, however, independent of the thiol concentration used (data not shown). The positive cooperativity of the GS_2 in green leaves of mustard plants may be due to the complex functioning of the enzyme in ammonia assimilation. Many higher plants possess two isoenzymes in the shoot [15] which differ especially in their glutamate affinity. These differences can also be achieved by allosteric binding because of the change of the affinity by effectors. Possibly, these effectors can help to find out something about the different functions of GS_1 and GS_2.

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