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GLUTAMINE SYNTHETASE FROM THE PUMPKIN LEAF CYTOSOL

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Glutamine synthetase (L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2) from pumpkin leaf cytosol is an oligomer that consists of eight identical monomers. The enzyme has a partial specific volume of 0.720 ml/g. The pH optimum in the presence of Mg^{2+} is 7.2. It is shown by means of electron microscopy that the enzyme consists of elongated monomers, which are arranged with point 42 symmetry at the vertices of two squares. These squares are twisted about the 4-fold axis at 40° relative to each other.

Glutamine synthetase (L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2) plays a key role in cell nitrogen metabolism. Two molecular forms of this enzyme have recently been found in several higher plants [1–4]. We found that the first molecular form of glutamine synthetase in pea and pumpkin leaves is synthetized and localized in chloroplasts, and the second form is in the cell cytosol [1,5]. Homogeneous preparations were obtained for both glutamine synthetase forms from pumpkin leaves [6]. The molecular weight of pumpkin leaf cytosol glutamine synthetase was found to be 480000. The enzyme consisted of eight identical monomers. The ratio of α-helical structure in the enzyme molecule was 34%. The objective of this work was to study the quaternary structure and some properties of glutamine synthetase from the pumpkin leaf cytosol.

Seeds of pumpkin (*Cucurbita pepo* L., var. *Vitaminnaya*) were cultivated for 3 weeks in open ground, and the leaves were collected at 2 p.m. Glutamine synthetase of pumpkin leaf cytosol was isolated and purified according to the technique described previously [6]. The activity of the enzyme was assayed according to Elliott [7]. The incubation mixture (final volume 2.25 ml) contained 500 mM Tris-HCl buffer (pH 7.5)/60 mM $NH_2OH \cdot HCl$/50 mM ATP/250 mM sodium monoglutamate/0.5–1.0 μg enzyme. After 15 min incubation, the reaction was stopped by adding 0.75 ml 10% $FeCl_3 \cdot 6H_2O$ in 0.2 N HCl/24% trichloracetic acid/18.5% HCl. Amounts of γ-glutamylhydroxamate formed in the reaction

$$\text{Glutamate} + NH_2OH + ATP \rightarrow \gamma\text{-glutamylhydroxamate} + ADP + P_i$$

were calculated from the absorbance of the resulting solution measured at 540 nm, with a SF-16 spectrophotometer (LOMO, U.S.S.R.), against the control (containing the same components except for sodium glutamate). Protein was assayed according to Lowry et al. [8]. Glutamine synthetase preparations for electron microscopy were negatively stained with 2% uranyl acetate. The enzyme preparations were examined through the Philips 400 electron microscope operating at 80 kV accelerating voltage.

The reagents used were: sodium mono-L-glutamate from Ajinomoto, Japan; ATP, EDTA and Tris from Reanal, Hungary; other reagents were from Reachim (U.S.S.R.) and were of the highest purity.

The partial specific volume was estimated from the amino acid composition of the enzyme and was equal to 0.720 ml/g.

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The optimum for glutamine synthetase of pumpkin leaf cytosol in the presence of Mg^{2+} was pH 7.2, which is close to that of other glutamine synthetases (pH 7.0–8.0).

The quaternary structure of pumpkin leaf cytosol glutamine synthetase was determined by means of electron microscopy. Several characteristic forms of particles were observed. Particles of the first type were round-shaped and 10.0–10.2 nm in diameter. To determine the order of rotation axis, n, and to amplify major structural features related to rotational symmetry, the images turned with the angle $360°/n$ were photosummated [9]. Fig. 1 shows the superimposed pictures obtained with $n = 4$. With other values of n, for instance, with $n = 8$, the similarity with the original image was lost. In the resulting picture, the eight protrusion grouped pairs can be clearly seen. The images were characterized by 4 m symmetry. For the next three types of particle image, two longitudinal stripes of protein separated with stain were observed. All three types had the same width of 6.5–7.0 nm. Particles of the second and the third type were rectangular (Fig. 1b and c) and characterized by 2 m symmetry; their widths were 10.0–11.0 nm and 9.0 nm, respectively. Particles of the fourth type were trapezoid (Fig. 1d). Comparing these images one can deduce that the first type corresponds to the frontal projection of the molecule while the other types correspond to the lateral ones perpendicular to it. Taking these projections into consideration, a model of the molecule was constructed. It consisted of eight elongated monomers arranged with 42 point symmetry at the vertices of two squares twisted at an angle of approx. 40° about the 4-fold axis. Projections of the model shown at Fig. 1b and c correspond to the orientation of particles observed along the 2-fold axes crossed at an angle of 45° while the fourth type of particles (Fig. 1d) is seen along the bisector between these axes. Monomers in the glutamine synthetase molecule are arranged in two layers of 4 units each. Angle shift of the units in the layers results in the appearance of protrussion pairs seen at the particle images of the first type and in the trapezium-shaped images of the fourth type.

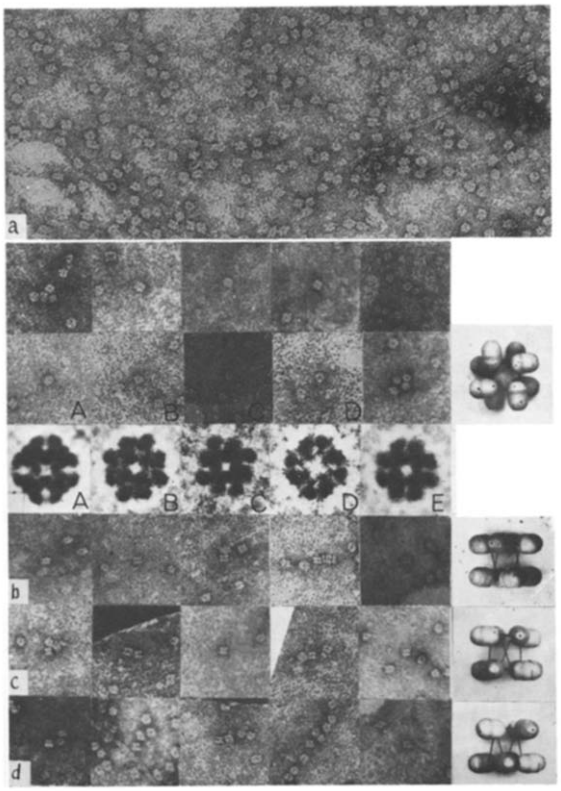

Fig. 1. Molecules of glutamine synthetase from pumpkin leaf cytosol negatively stained with uranyl acetate. Magnification, ×120 000. (a) General view of the preparation; images of the first type. Photosummated images with a 90° angle turn are given in the lower line (magnification, ×720 000). Particles averaged by phototechnique are marked A, B, C and D; E, the superimposed image of these particles. (b, c, d) Images of the second, third and fourth types, respectively. Different appearances of the model are shown on the right, corresponding to different projections of the particles.

The arrangement of monomers in the pumpkin leaf cytosol glutamine synthetase molecule appears to be essentially similar to that observed for glutamine synthetase from pea leaf chloroplasts [10]. At the same time, these glutamine synthetases differ from 12-unit glutamine synthetases of bacteria [11] and blue-green algae [12] in the number of monomers, and from 8-unit glutamine synthetases of animals [13] and yeasts [14] in the arrangement of monomers.

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