ISOLATION OF GLYCININ SUBUNITS BY ISOELECTRIC FOCUSING IN UREA-MERCAPTOETHANOL

Nicholas CATSIMPOOLAS
Protein Research Laboratory, Central Soya-Chemurgy Division, Chicago, Illinois 60639, USA

Received 25 July 1969

1. Introduction

Glycinin is the major reserve protein of soybean seeds (*Glycine max.*) stored in subcellular particles called "protein bodies". The protein has been isolated in homogeneous form [1], and some of its immunochemical properties [2], association-dissociation phenomena [3], and changes during germination [4,5] have been studied in this laboratory. N-terminal amino acid analysis [1] and disc electrophoresis data [3] have suggested that glycinin in its monomeric form is composed of six subunits. The present report describes the isolation of dissociated glycinin subunits by isoelectric focusing in sucrose-ampholyte medium containing 6 M urea and 0.2 M mercaptoethanol. The method may be applicable to the separation of subunits of other proteins where the presence of interchain disulfide bonds is indicated.

2. Materials and methods

Glycinin was isolated by DEAE-Sephadex chromatography as described previously [1]. Isoelectric focusing [6,7] in the pH region between pH 3 and pH 10 was performed for 48 hr at 10° using the LKB 8102 electro-focusing column and LKB ampholine. The experimental procedure was similar to that described in other reports [8–10] with the exception that all the solutions used to prepare the column were made 6 M in urea and 0.2 M in mercaptoethanol. Glycinin (100 mg) was dissolved in fractions Nos. 23–25 of the density gradient and was left for 2 hr before applying the sample to the column in order to ensure complete dissociation into subunits. After electrofocusing, 3 ml fractions were collected and the absorbance of each fraction was measured at 280 nm, using 1 cm cell, with a Gilford 2000 spectrophotometer. The pH of each fraction was also measured at 25° with a Beckman Expandomatic pH meter. Selected fractions were pooled, dialyzed against water in the cold for several days to remove sucrose, ampholytes and reagents, and freeze-dried.

Disc electrophoresis of the dissociated glycinin and of the isolated subunits in the solvent system phenol-acetic acid-0.2 M mercaptoethanol, 2:1:1, w/v/v, made 5 M in urea (PAMU) was performed as described previously [5]. Improved resolution of components was achieved by dissolving the samples first in the urea-mercaptoethanol portion of the solvent for 2 hr and then adding the calculated amount of phenol and acetic acid to the specified proportions. The other technical details of the technique [5] were not changed.

3. Results

Glycinin dissociated into subunits in 6 M urea containing 0.2 M mercaptoethanol and subjected to isoelectric focusing (pH 3–10) in the presence of 6 M urea-0.2 M mercaptoethanol exhibits the typical pattern shown in fig. 1. The protein subunits were recovered in six fractions designated I through VI. Fractions I–III contained the acidic subunits (called A) which were focused between pH 4.50 and pH 5.75. The basic subunits (called B) were focused between pH 7.50 and pH 8.50, and were eluted in fractions IV–VI.

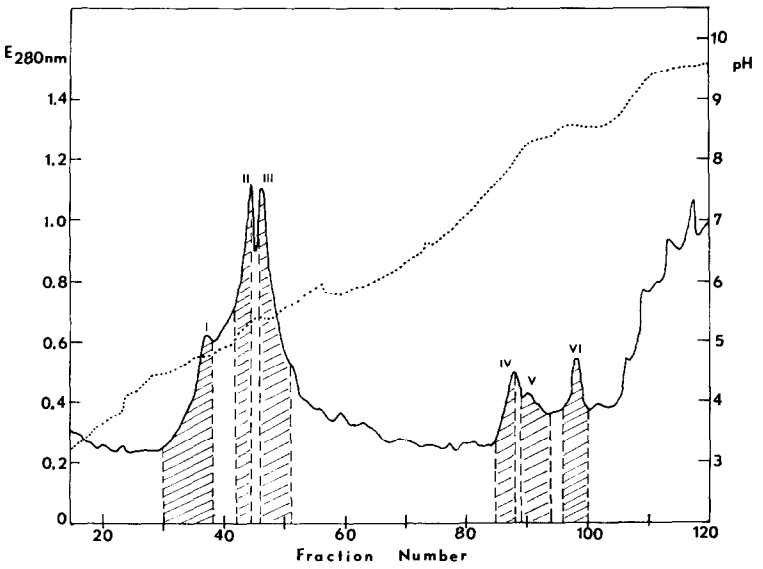

Fig. 1. Isoelectric focusing of dissociated glycinin (100 mg) in the region between pH 3 and pH 10 in the presence of 6 M urea and 0.2 M mercaptoethanol. The solid line represents absorbance at 280 nm (1 cm cell). The dotted line shows the pH gradient measured at 25°. Shaded areas indicate fractions pooled (I through VI).

Fig. 2. Disc electrophoresis in PAMU solvent of dissociated glycinin (a), fraction I (b), fraction II (c), fraction III (d), fractions IV or V (e), fraction VI (f), pooled fractions I–III (g), and pooled fractions IV–VI (h). Acidic subunits have been designated "A", and basic subunits "B".

Comparison of the disc electrophoresis patterns in PAMU solvent of dissociated glycinin and of the isolated subunits is shown in fig. 2. The six subunits of glycinin have been designated A1, A2, A3 and B1, B2, B3, according to their relative electrophoretic mobility toward the negative electrode (fig. 2a). The acidic subunits (pooled fractions I–III) exhibit three bands A1, A2 and A3 (fig. 2g). Similarly, the basic subunits (pooled fractions IV–VI) also exhibit three bands B1, B2 and B3 (fig. 2h). Fraction I contains subunit A3; fraction II,

subunit A1; fraction III, subunit A2; fraction VI, subunit B3; and fractions IV and V subunits B1 and B2, respectively. The latter two subunits were heavily contaminated with each other and are shown together in the disc electrophoresis patterns. The other subunits were contaminated with adjacent fractions to a minor extent.

The mean values of the isoelectric points (25°) of the isolated subunits measured at the peak were as follows: A3, pH 4.75; A1, pH 5.15; A2, pH 5.40; B1, pH 8.00; B2, pH 8.25; and B3, pH 8.50.

4. Discussion

The results presented in this paper show that dissociated glycinin subjected to isoelectric focusing in urea-mercaptoethanol is separated into three acidic and three basic subunits. The acidic subunits appear to be present in larger amount than the basic subunits. The separation of these subunits by isoelectric focusing supports previous suggestions [1–3] that glycinin in its monomeric form consists of six subunits. The dimer (11S component, m.w. 350,000) which is the most stable form of the protein contains twelve subunits. Preliminary electron microscopy experiments (unpublished) show that the dimer of glycinin is composed of two annular-hexagonal structures each composed of six subunits. The two annular-hexagonal structures are packed the one on top of the other. An attractive hypothesis would be that the acidic and basic subunits are alternating within the annular structure thus contributing to the stability of the molecule in terms of ionic interactions. Arachin, a protein closely related to glycinin, also consists of two monomers each composed of six subunits [11]. Possible differences in the biological function of the acidic and basic subunits of glycinin during germination may shed light on the utilization of storage proteins by seeds. Understanding of the basic structure of the protein may also lead to improved utilization in human nutrition by appropriate chemical or physical modifications.

A more detailed chemical and physical characterization of the subunits of glycinin is in progress. The reported method may be applicable to the isolation of subunits of other proteins where recombination of the dissociated subunits through sulfhydryldisulfide interactions is possible. The presence of mercaptoethanol prevents such reassociation leading to a clearer separation of subunits.

References

[1] N.Catsimpoolas, D.A.Rogers, S.J.Circle and E.W.Meyer, Cereal Chem. 44 (1967) 631.
[2] N.Catsimpoolas and E.W.Meyer, Arch. Biochem. Biophys. 125 (1968) 742.
[3] N.Catsimpoolas, T.G.Campbell and E.W.Meyer, Arch. Biochem. Biophys. 131 (1969) 577.
[4] N.Catsimpoolas, T.G.Campbell and E.W.Meyer, Plant Physiol. 43 (1968) 799.
[5] N.Catsimpoolas, C.Ekenstam, D.A.Rogers and E.W. Meyer, Biochim. Biophys. Acta 168 (1968) 122.
[6] H.Svensson, Arch. Biochem. Biophys. Suppl. 1 (1962) 132.
[7] O.Vesterberg and H.Svensson, Acta Chem. Scand. 20 (1966) 820.
[8] N.Catsimpoolas, C.Ekenstam and E.W.Meyer, Biochim. Biophys. Acta 175 (1969) 76.
[9] N.Catsimpoolas, Arch. Biochem. Biophys. 131 (1969) 185.
[10] N.Catsimpoolas and E.W.Meyer, Arch. Biochem. Biophys. 132 (1969) 279.
[11] M.P.Tombs and M.Lower, Biochem. J. 105 (1967) 181.