NOTE ON THE RAPID PROTEOLYSIS OF GLYCININ BY PEPSIN AND TRYPSIN

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The use of unconventional sources of protein for human consumption necessitates studies in regard to their enzymatic digestibility, especially by pepsin and trypsin. Glycinin—the major soybean storage protein (1–3) provides a suitable model for *in vitro* hydrolysis work since it can be obtained in homogeneous form and exhibits a complex structure (4–9) representative of other oilseed storage proteins. Demonstrated in this paper is the proof that, in spite of its structural complexity, glycinin can be hydrolyzed into large peptide fragments by pepsin within a few minutes. The acidic subunits of the protein are hydrolyzed faster than the basic subunits.

MATERIALS AND METHODS

In more detail, this work concerned the enzymatic hydrolysis of glycinin (prepared as described in refs. 4 and 10) with pepsin at pH 2.0 and 37°, 25°, and 4°C and trypsin at pH 8.0 at 25°C. The peptic hydrolysis was carried out in 0.01 *N* HCl, whereas, the tryptic hydrolysis was performed in a tris-HCl buffer (pH 8.0, 0.46 *M*). The enzyme-to-protein ratio was 1:100 in all the experiments. The concentration of glycinin was 0.1%. To stop the enzyme action, an aliquot (100 μl) of the hydrolysate was added to an equal volume of a solution containing 6 *M* urea, 0.3% SDSs, and 10 m*M* dithiothreitol, pH 8.9. The mixture was stored at −6°C until electrophoretic analysis was performed which involved sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a discontinuous buffer system (11). Equal amounts of material were applied to the gel.

RESULTS AND DISCUSSION

The SDS-PAGE electrophoretic patterns of the hydrolysis of glycinin by pepsin at pH 2.0 as a function of time are shown in Fig. 1. The results at both 25° and 37°C clearly indicate that the protein is cleaved into fragments of lower molecular weight than either the heavy (H) or light (L) chains (6, 11) of the protein within 2 min. The control glycinin solution incubated under identical conditions but without the enzyme exhibits no apparent change in its subunit composition. Since the hydrolysis proceeded at a very rapid rate at 25° and 37°C, the authors intentionally slowed down the reaction by incubation at 4°C. This latter experiment enabled observation of the differential rate of hydrolysis of H and L chains of glycinin by pepsin. As can be seen in Fig. 1, the H (acidic) subunits are digested at a faster rate than the L (basic) subunits. In an analogous experiment using trypsin at pH 8.0 (25°C), although the rate of hydrolysis was at least tenfold slower than the corresponding peptic digestion, the acidic subunits

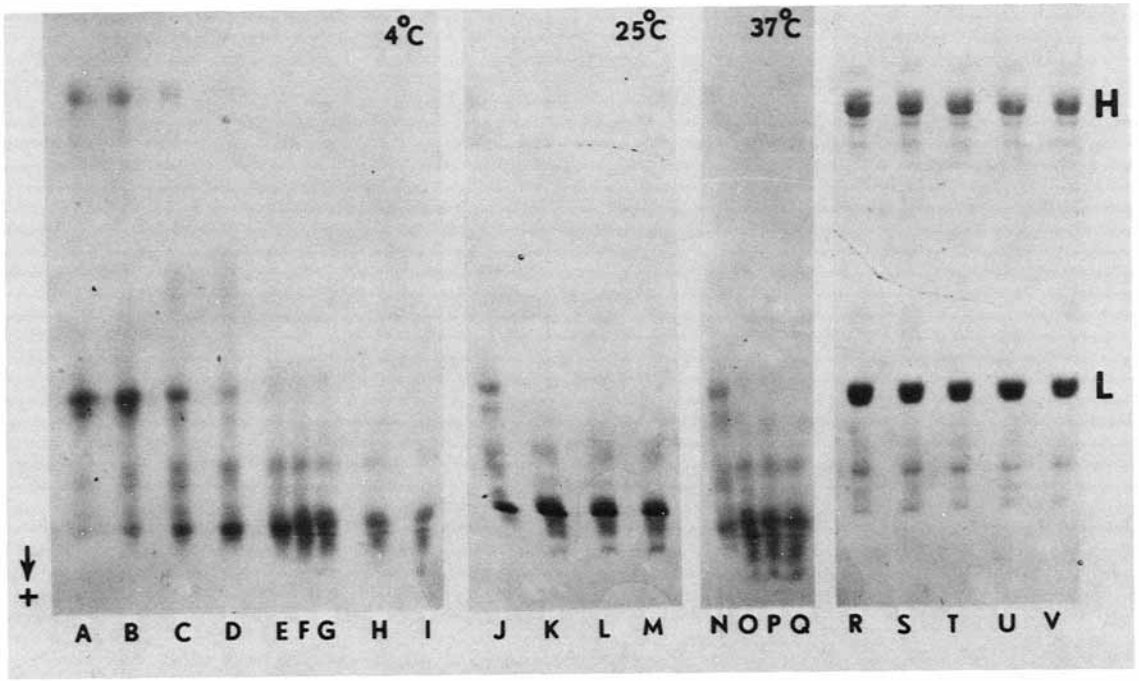

Fig. 1. SDS-PAGE patterns of the hydrolysates of glycinin by pepsin: A) zero time; B) 2 min; C) 5 min; D) 10 min; E) 20 min; F) 30 min; G) 45 min; H) 60 min; I) 90 min; J) zero time; K) 2 min; L) 4 min; M) 6 min; N) zero time, O) 2 min; P) 4 min; Q) 6 min; R) glycinin; S to V) are the controls at zero time, 2 min, 4 min, and 6 min, respectively. Large H and L indicate the heavy and light subunits. The zero time aliquot was taken immediately after dissolution of the protein in the enzyme solution.

were again preferentially cleaved at a faster rate (Fig. 2).

With low-molecular weight protein markers, peptide fragments were observed in Fig. 1 to be lower than 12,000 daltons and therefore fall outside the range of

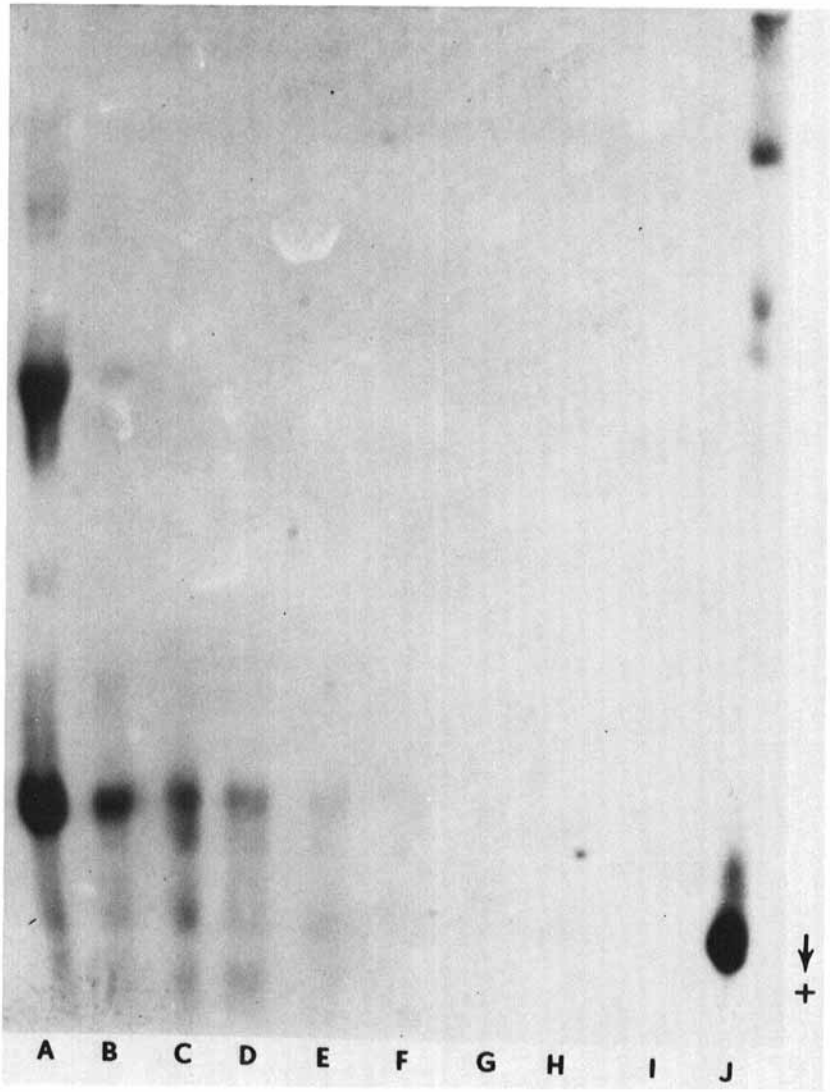

Fig. 2. SDS-PAGE patterns of the tryptic hydrolysates of glycinin at pH 8.0 at 25°C: A) native glycinin; B) zero time hydrolysate; C) 2 min; D) 30 min; E) 90 min; F) 150 min; G) 240 min; H) 300 min; I) 24 hr; J) standard proteins: albumin, ovalbumin, β-lactoglobulin, myoglobin, and lysozyme. The zero time aliquot was taken immediately after dissolution of the protein in the enzyme solution.

size estimated reliably by PAGE. However, it has been demonstrated using Sephadex G-50 gel filtration (12) that the weight-average molecular weight of peptides produced by the action of pepsin on glycinin is in the range of 5000 to 7000 daltons.

The fast hydrolysis of glycinin by pepsin at pH 2.0 is probably due to the acidic dissociation of the subunits with concurrent unfolding of the polypeptide chain. Evidence for such conformational changes at pH 2.0 have been presented previously by hydrogen ion titration and uv difference spectroscopy of glycinin (13–14). The unfolded polypeptide chains are then presumably subjected to rapid proteolytic cleavage by way of availability of susceptible bonds. The synergistic effect of the acidic pH denaturation with the optimum pH of pepsin activity aids to enhance the hydrolysis rate. One of the reasons for the observed slower hydrolysis rate by trypsin can probably be attributed to the fact that glycinin is conformationally stable at pH 8.0. In addition, trypsin is restricted to the hydrolysis of lysine and arginine residues only.

The apparent preferential enzymatic hydrolysis of the acidic over the basic subunits by both pepsin and trypsin is probably due to the greater hydrophobic character and therefore compactness of the basic subunits (6).

Acknowledgments

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