Action of Transglutaminase on the Constitutive Polypeptides of Pea Legumin

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Transglutaminase catalyzes the polymerization of the constitutive polypeptides of legumin. This reaction was studied with the polypeptides in three conformations: organized in the native legumin, dissociated subunits, and separated subunits. In the native globular conformation, only the α polypeptides were polymerized, indicating that the β polypeptides were buried. Among the three types of α polypeptides, the two heavy α types were more involved in the polymerization reaction than the light type. This lesser reactivity was related to the small amount of Gln accessible and the absence of lysyl residues in the most flexible segments of this polypeptide. The dissociated forms were shown to be better substrates than the native form; however, polymers were obtained with both substrates. When native legumin was used as a substrate, only a few linkages were formed, mainly between the α polypeptides. In the case of separated polypeptides, a number of linkages were established between both α and β polypeptides. Dissociation induced by pH did not improve polymerization.

INTRODUCTION

Transglutaminase (TGase, EC 2.3.2.13) has been extensively studied (Folk, 1980) and is known to catalyze the transfer of the γ-carboxamide group of glutaminyl residues in proteins to primary amino groups in a variety of compounds. Among these, [^{14}C]putrescine and monodansylcadaverine were the acyl acceptors most frequently used to characterize *in vivo* the reactivity of peptides or proteins. A number of proteins were shown to be potential acyl donors in the amine incorporation reaction. Moreover, they are potential substrates; their glutaminyl (donor) residues and their lysyl (acceptor) residues simultaneously lead to the formation of polymers cross-linked through ϵ-(γ-glutamyl)lysyl isopeptidic bonds. From *in vitro* labeling experiments (Gorman and Folk, 1980, 1984; Berbers et al., 1983; Wold, 1985), it appears that both the local conformation and the amino acid sequence around a glutaminyl residue contribute to the substrate reactivity. More recently, taking into account the position and the surrounding of the reactive as well as the nonreactive glutaminyl residues in a group of 10 well-known proteins, Coussons et al. (1992) defined some rules concerning the reactivity of a glutaminyl residue. They applied these rules with success to account for the nonreactivity of some glutamines of high accessibility in the case of β-lactoglobulin, lysozyme, and phosphoglycerate kinase. When no amine is available in the reaction mixture, water acts as acyl acceptor, leading to the hydrolytic cleavage of the amide group of glutaminyl residues (Neidle et al., 1958; Mycek et al., 1959).

Ikura et al. (1980a,b) reported that transglutaminase can be used to polymerize bovine casein components and soybean proteins. The solubility, the emulsifying activity, and especially the hydration properties of polymerized proteins were greatly modified (Motoki et al., 1984). Such products might be interesting for the production of intermediate moisture protein food. Transglutaminase was also shown to be useful for preparing gels (Nio et al., 1986) with various breaking strengths and hardnesses (Nonaka et al., 1992). This enzymatic reaction is a promising method to modify proteins and to enlarge the application fields of seed storage proteins.

Legumin with a relative molecular weight (M_r) of about 360 000 is representative of seed storage proteins of 11S type. It is composed of six ($\alpha\beta$) subunits, each of which consists of disulfide-linked acidic (α) and basic (β) polypeptides (Derbyshire et al., 1976; Casey, 1979). Two types of constitutive subunits have been distinguished: the heavy subunits consisting of α and β polypeptides of respective molecular weights 40 000 and 22 000 and the light subunits consisting of α and β polypeptides of lower molecular weight, respectively, 24 500 and 21 000 (Matta et al., 1981). The complete structure of legumin has not yet been elucidated, but two models based on the $(\alpha\beta)_6$ oligomeric structure are generally accepted (Badley et al., 1975; Plietz et al., 1984).

Despite its relatively high content in glutaminyl residues, legumin was shown to be a rather poor substrate in its native conformation. One way to enhance its reactivity is to modify its lysyl residues by acylation (Larré et al., 1992).

In the present study, we were interested in comparing the types of polymers obtained when native or modified legumin was used as substrate, and the results were interpreted in terms of structure. The highest levels of polymerization were obtained with the modified protein. To avoid chemical modification, we tried to enhance the polymerization level of native legumin by inducing conformational changes by pH variations (Gueguen et al., 1988).

MATERIALS AND METHODS

Transglutaminase. Transglutaminase (TGase) was purified from fresh guinea pig liver according to the method of Brookhart et al. (1983) as modified by Larré et al. (1992).

Substrate Proteins. Native and citraconylated legumins (*Pisum sativum* L. cv. Amino) were prepared according to the methods of Larré and Gueguen (1986) and Larré et al. (1992).

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The citraconylated legumin used in this study had only 90% of its lysyl residues blocked and was completely dissociated into 3S components which were rather unfolded (Larré et al., 1992).

The various ($\alpha\beta$) subunits were obtained after fractionation of native legumin in 0.1 M phosphate buffer, pH 7.6, and 6 M urea by anion-exchange chromatography (Dudek, unpublished data). The sequences used for the different subunits were those established by Domoney et al. (1986), Gatehouse et al. (1988), and Lycett et al. (1984). The flexibility at each point of a selected protein sequence was predicted by the method of Karplus and Schultz (1985) using PCGENE software (Flexpro). The chain flexibility at an amino acid was measured from the average value of the atomic temperature factor (also called the B value) of the α carbon atom taking into account the seven neighboring amino acids. A normalized B value greater than 1 indicates a flexible amino acid. In the case of legumin polypeptides, the highest B value obtained was 1.143.

Transglutaminase Activity Assay. Kinetic reactions were performed in 0.2 M Tris-HCl buffer, pH 7.0, whereas the action of TGase depending on the pH was studied with the following buffer systems: citric acid–NaOH from pH 5 to 6.5 and Tris-HCl from pH 6.5 to 9. In every case, the enzymatic reaction was carried out in 0.2 M buffer, 7.5 mM $CaCl_2$, 1 mM DTT, 3.33 mg/mL protein substrate, and 0.2 unit of TGase/mg of substrate. Legumin was solubilized in 0.05 M Tris-HCl buffer, pH 7.5, and incubation was performed at 37 °C in the conditions described above. The reaction was stopped by adding EGTA at a final concentration of 20 mM. The zero time product was obtained by adding EGTA to the reaction mixture before the enzyme was added. The reaction was followed by the amount of ammonia released, and the reaction products were characterized by SDS–PAGE.

Determination of Ammonia. The amount ammonia released during the transglutaminase reaction was determined using glutamate dehydrogenase (EC 1.4.1.2) according to the method of Kun and Kearney (1974).

SDS–Polyacrylamide Gel Electrophoresis. SDS–PAGE was carried out in homogeneous polyacrylamide (13%) slab gels as described by Laemmli (1970) after reduction by mercaptoethanol of the TGase reaction products. The gels were stained for 2 h with 0.1% Coomassie blue G 250 solubilized in a methanol–acetic acid–water mixture (25/10/65 v/v/v). The destaining was performed overnight in 5% acetic acid. The gel was analysed by densitometry. For each scan, the baseline was subtracted and its total area was normalized to a standard value of 1. To analyze the action of the TGase on the substrates at various pH values, the pattern of the time zero assay was subtracted from each scan of the corresponding products. In this way the appearance and disappearance of component were calculated as positive or negative values. The resulting pattern was then integrated by sections. Four sections were retained: above 100 000 Da, from 100 000 to 45 000 Da, from 45 000 to 22 000 Da, and below 22 000 Da.

RESULTS AND DISCUSSION

Behavior of Native and Dissociated Legumin and the Separated Subunits during the Enzyme Reaction. In this study, the enzyme kinetics were performed on native legumin or on the constitutive subunits prepared by two different ways. The first type of modified substrate used was composed of subunits obtained after complete dissociation of the protein by partial acylation of its amino groups (Larré et al., 1992). The second type of substrate was composed of different subunits respectively separated by chromatography, the heavy (MW 62 000) or light (MW 46 500) subunits as described by Matta et al. (1981).

Kinetics were performed on native and dissociated legumins at pH 7 to keep the nonmodified protein with its globular conformation. The electrophoretic patterns of the reaction products revealed the main classical bands obtained for native legumin corresponding to the acidic and basic polypeptides (Figure 1) and also bands of higher

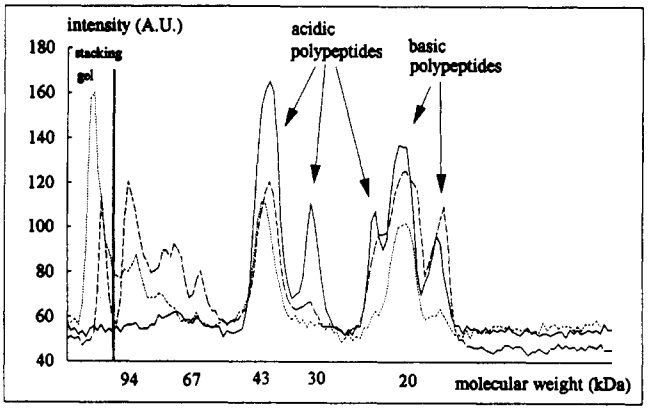

Figure 1. Densitometric profiles of control legumin (—) and native (- - -) and citraconylated (• • •) legumin products after 1 h of TGase reaction at pH 7. Experimental conditions: 0.2 M appropriate buffer, 10 mM $CaCl_2$, 1 mM DTT, 3.3 mg/mL protein substrate, and 0.2 unit of TGase/mg at 37 °C.

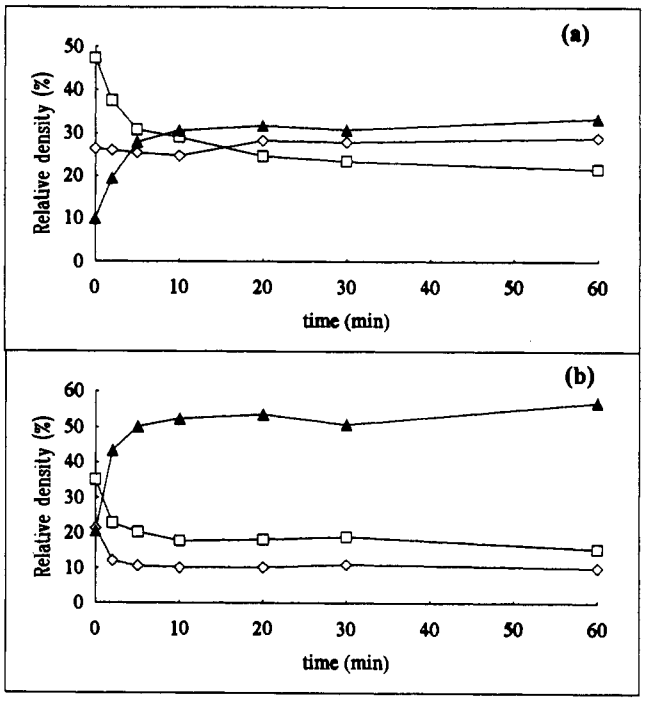

Figure 2. Kinetics of legumin polypeptide disappearance and polymer formation during the TGase reaction: (a) native legumin; (b) citraconylated legumin. (□) α polypeptides; (◇) β polypeptides; (▲) polymers.

molecular weights which are characteristic of polymerized forms. The bands observed in the molecular weight range of 67 000–90 000 should correspond to two polypeptides linked together through an isopeptidic bond, while those of molecular weight above 100 000 correspond to three or more cross-linked polypeptides. The bands observed for the reaction products obtained with the other substrates were analyzed by referring to these patterns. Quantitative analysis of polypeptide bands of the electrophoretic patterns gives evidence for different behaviors between native and citraconylated legumin in the polymerization reaction (Figure 2). When native legumin was substrate, the acidic polypeptides were clearly involved in the polymerization reaction; 54% of them were cross-linked after 1 h of reaction (Figure 2a). On the contrary, the basic polypeptides remained almost unchanged during the enzymatic reaction. This phenomenon did not occur for the citraconylated legumin in which both acidic and basic polypeptides participated in the cross-linking reaction (Figure 2b).

The same experiment was done with two separated ($\alpha\beta$) subunits, the heavy one (MW 62 000) and the light one (MW 46 500). Polymerization occurred in both cases, but the behaviors of the α and β polypeptides differed, depending on the type of subunit to which they belonged. In the case of the heavy subunit both polypeptides participated at the same rate in the polymerization process. In the case of the light subunit, the α polypeptide was more quickly involved in the polymerization than the β one.

The results obtained with citraconylated legumin suggested that its high reactivity should be related to the dissociation induced by the chemical modification or to a charge effect due to the introduction of citraconyl groups. Moreover, when the separated subunits were substrates, both acidic and basic polypeptides were involved in the polymerization reaction, indicating that some of their glutaminyl and lysyl residues can act in the TGase reaction as acyl donor and acyl acceptor, respectively. The behaviors of separated subunits strengthen the hypothesis that the increased reactivity is due to the dissociation phenomenon and not to sequence characteristics. It is interesting to point out that the 10% unblocked lysyl residues became highly reactive in the TGase reaction. Even with 90% lysyl residues blocked, the citraconylated legumin was highly polymerized; 69% of its β and 78% of its α polypeptides were involved in the reaction after 2 h (Figure 2b).

Our results established clearly that the participation of the constitutive polypeptides of legumin in the polymer formation is dependent on the legumin conformation. Information of two types can be deduced: on the substrate structure and on the type of polymers obtained. When the substrate was in its native conformation, the β polypeptides were not involved in the polymerization reaction, even though they were when legumin was dissociated or when purified subunits were substrates. We can therefore presume that this nonreactivity is related to the native legumin structure. This lower reactivity of the β polypeptides was also previously observed by chemical glycosylation of legumin (Caer et al., 1990). These results are in agreement with the model of Plietz (1984), who proposed that the β polypeptides are buried in the center of the structure while the α polypeptides are located in the periphery. Depending on the substrate conformation, different polymers should be obtained. In one case, the basic monomers are $6(\alpha\beta)$ globular hexameric forms (11S) with a quaternary structure and the isopeptidic bonds are established only between the peripheral α polypeptides. In the other case, the basic monomers are $\alpha\beta$ subunits (3S) with a tertiary structure and both polypeptide types (α and β) participate in the polymer formation. Moreover, it seems likely that these constituent monomers (3S) are simultaneously largely deamidated (Larré et al., 1991). The geometric arrangement of the constituent protein units of these two types of polymers was not characterized.

In the case of native legumin, some differences in the behavior of the various α polypeptide types during the polymerization reaction can be observed (Figure 3). Three α polypeptides of different molecular weights were distinguished (α_{H1}, 43 000, α_{H2}, 38 000; and α_L, 21 500). According to Matta et al. (1981) the first two are considered heavy polypeptides and the third one a light polypeptide. The heavy α polypeptides participated similarly in the polymerization. After 2 h of reaction, 75% and 86% of these two forms had disappeared, while only 30% of the light α polypeptide had polymerized.

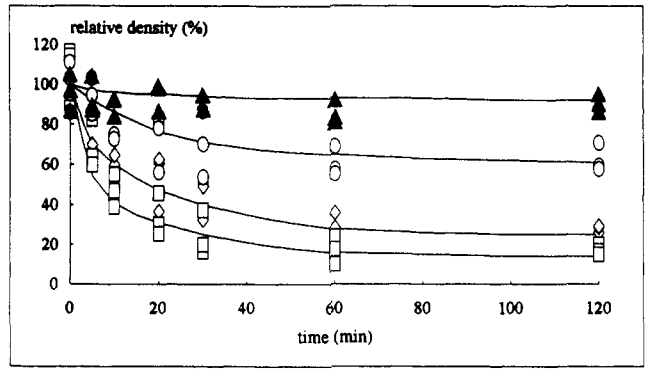

Figure 3. Quantitative analysis of the time-dependent decrease of the bands corresponding to the constitutive polypeptides of native legumin during the polymerization induced by TGase on three experiments. The 100% value was calculated for each band at the time zero of the first experiment. (▲) β polypeptides; (◇) α_{H1} polypeptides; (□) α_{H2} polypeptides; (○) α_L polypeptides.

The sequences of the three types of α polypeptides checked in this study are described in the literature (Domoney et al., 1986; Lycett et al., 1984; Gatehouse et al., 1988), but nothing is known on their position in the structure. Their relative proportions in the legumin structure varied with the genotype; in our case, the light α polypeptide represented about 20% of the total α fraction. From the different behaviors observed between the two α polypeptide types a hypothesis may be formulated. Assuming that the α polypeptides are equally distributed on the periphery of the native protein, it is possible to estimate the potential reactivity of a glutaminyl residue in the TGase reaction by using the rules proposed by Coussons et al. (1992). According to the first rule we have screened the sequences and obtained the list of flexible segments. Only the sequence segments with a B value above 1.1 were taken into account to analyze those of greatest flexibility. As we were interested in polymerization, the lysyl residues were considered to be reactive if located in flexible segments of the protein and if not preceded by a leucine residue in the sequence (Folk, 1980). Among the 18 flexible segments predicted, having the defined criteria ($B \geq 1.1$), 16 were located on the two heavy α polypeptides (8 on α_{H1} and 8 on α_{H2}) and only 2 on the light (α_L) α polypeptide sequence. Considering the amount of Gln and Lys potentially reactive in these segments, it appears that the α_{H1} polypeptide contained 8 Gln and 3 Lys potentially reactive, the α_{H2} 5 Gln and 5 Lys, and the α_L 1 Gln and no Lys. These results in addition to the low proportion of light α polypeptides in our legumin sample can explain the better reactivity of the heavy α polypeptides.

Effect of the Reaction pH on the Polymerization. The substrate effectiveness of native and modified legumin was examined at various pH values, first by measuring the amount of ammonia released and second by characterizing the obtained polymerized products.

Under the action of TGase, the amount of ammonia liberated from citraconylated legumin was higher than that from the native protein (Figure 4) for pH 6.5–9.0. The maximum reactivity, obtained at pH 7 (90 nmol of NH_3/mg), was 3.6 times higher than that of the native protein.

The ϵ amino groups of protein-bound lysine are known to act in the transglutaminase reaction as acyl acceptors and to participate to the formation of ϵ-(γ-glutamyl)lysyl cross-links. Because citraconylated legumin has 10% of its lysyl residues unblocked and potentially reactive, all reaction products were subjected to SDS electrophoresis

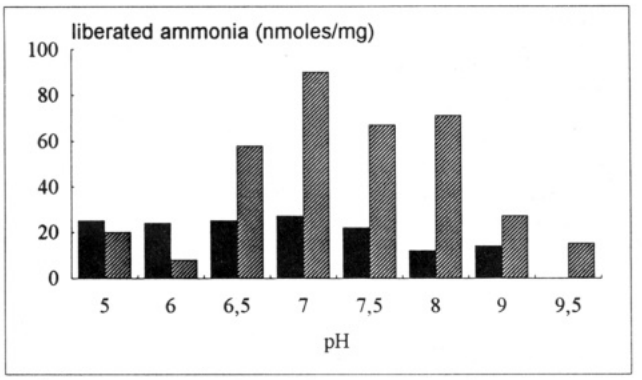

Figure 4. Ammonia liberated after 2 h of TGase reaction at various pH values. Experimental conditions: 0.2 M appropriate buffer, 10 mM $CaCl_2$, 1 mM DTT, 3.3 mg/mL protein substrate, and 0.2 unit/mg TGase at 37 °C. (■) Native; (▨) citraconylated.

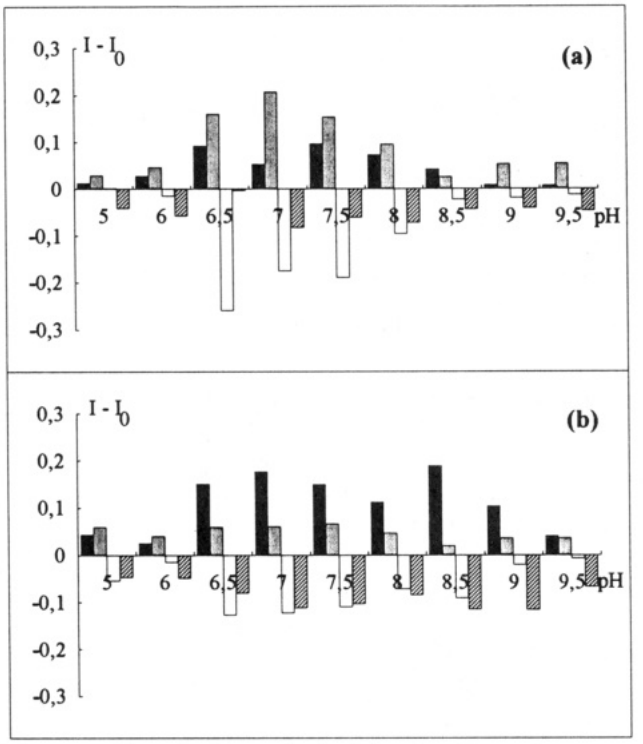

Figure 5. Quantitative analysis of the densitometric patterns obtained at each pH after 2 h of TGase reaction: (a) native legumin; (b) citraconylated legumin. (■) MW > 100 000; (▨) 100 000 > MW > 45 000; (□) α polypeptides; (▨) β polypeptides.

to evaluate the proportion of polymers resulting from the cross-linking. Two types of bands were distinguished, those that did not even penetrate the gel and those of apparent molecular weight from 50 000 to 95 000. The relative densities of the bands are reported in Figure 5. Polymerization occurred for both substrates over the whole range of pH, with a maximum between pH 6.5 and 7.5 for native legumin (Figure 5a). In the case of citraconylated legumin, polymerized products were obtained in a wide range of pH, from 6 to 8.5 (Figure 5b). The citraconylated protein is completely dissociated and soluble over the whole range of pH; therefore, the reaction was mainly dependent on the activity of the enzyme at the various pH values. The products obtained from native or modified legumin were quite different.

When native legumin was the substrate, most of the polymers were characterized by a main electrophoretic band of a molecular weight around 85 000 (Figure 5a). As far as the accessibility of β polypeptides was shown to be lower for the reaction, this band can be mostly attributed to two α polypeptides linked together through at least one isopeptide bond. Bands with a molecular weight higher than 95 000 were also detected on the electrophoretic pattern; they correspond to polymers resulting from the cross-linking of more than two polypeptides. The polymers obtained with citraconylated legumin were characterized by the same electrophoretic bands, but their relative proportions were different, showing mainly higher molecular weight components (Figure 5b).

Despite the blockage of most of the lysyl residues, a higher density of linkages was catalyzed for the citraconylated substrate in all pH conditions. The dissociation and the partial unfolding of the subunits allow a higher accessibility of the reactive sites. Many polypeptides can be bound together through isopeptidic bonds. In the case of native legumin most of the polymerized products characterized by a band around 80 000 MW may be attributed mainly to two α polypeptides linked together through an ε-(γ-glutamyl)lysyl bond. We can presume that the molecular size of native legumin added to its compact structure avoids the linkage of more than two polypeptides together in most cases. At pH <6.5 native legumin begins to dissociate but, unfortunately, did not give rise to an increased polymerization because of its partial insolubilization (Gueguen et al., 1988). At pH values higher than 8.5 dissociation also occurred but did not improve polymerization.

Conclusion. The analysis of the polymerized products supplies information on the structure of native legumin. In agreement with Plietz's model (1984), the nonaccessible β polypeptides should be buried in the structure and the α hydrophilic polypeptides mainly located at the periphery of the protein. The differences observed between the various types of polypeptides should be related to their amino acid composition rather than to their different locations on the surface of legumin. The β polypeptides should be buried in the structure and the α hydrophilic polypeptides mainly located at the periphery of the protein. The differences observed between the various types of α polypeptides should be related to their amino acid composition rather than to their different locations on the surface of legumin.

Polymerization by TGase occurred even if native legumin is a rather poor substrate. In the range of pH values used, dissociation was obtained for pH lower than 6.5 and pH higher than 8.5. At the acidic pH tested, TGase was highly reactive, but unfortunately the partial dissociation of legumin did not improve its ability to act as a substrate. This can be related to a partial insolubilization in these conditions. Basic pH induced dissociation and maintained a better solubility of the legumin; however, the enzyme was rapidly denatured in these conditions. Dissociation induced by pH did not improve polymerization, as was the case with dissociation obtained by chemical modifications. With native legumin as substrate, the polymers obtained were formed with very few bonds established through their α polypeptides, whereas those obtained with dissociated legumin were highly reticulated. It seems likely that the native legumin polymers are stabilized through the interactions involved in the quaternary structure as well as the isopeptidic bonds. The polymers obtained with the legumin subunits are stabilized by disulfide bridges between α and β polypeptides and isopeptidic bonds. High molecular weight polymers differing in their glutamine and glutamic acid content and presenting various geometric arrangements can be formed using this technique. Their functional properties, such as solubility, hydration properties, or interfacial behavior

should be quite different, and further investigations are needed before they can be put to practical use. Moreover, the introduction of covalent isopeptidic bonds into processing gels or films might be useful to obtain various textural properties.

LITERATURE CITED

Badley, R. A.; Athison, D.; Hauser, H.; Oldani, D.; Green, P.; Stubbs, J. M. The structure, physical properties of the soybean protein glycinin. *Biochim. Biophys. Acta* **1975**, *412*, 214–228.

Berbers, G. A. M.; Bentlage, H. C. M.; Brans, A. M. M.; Bloemendal, H.; de Jong, W. W. β Cristallin: Endogenous Substrate of lens Transglutaminase. Characterization of the Acyl-donor Site in the bBp Chain. *Eur. J. Biochem.* **1983**, *135*, 315–320.

Brookhart, P. P.; McMahon, P. L.; Takahashi, M. Purification of guinea pig liver transglutaminase using a phenyl sepharose 4B affinity column. *Anal. Biochem.* **1983**, *128*, 202–205.

Caer, D.; Baniel, A.; Subirade, M.; Gueguen, J.; Colas, B. In Vitro Glycosylation of Pea Legumin Effects on some functional Properties. *J. Agric. Food Chem.* **1990**, *38*, 1700–1705.

Casey, R. Immunoaffinity Chromatography as a Means of Purifying Legumin from Pisum (pea) Sativum Seeds. *Biochem. J.* **1979**, *177*, 509–520.

Coussons, P. J.; Price, N. C.; Kelly, S. M.; Smith, B.; Sawyer, L. Factors that Govern the Specificity of Transglutaminase-catalyzed Modification of Proteins and Peptides. *Biochem. J.* **1992**, *282*, 929–930.

Derbyshire, E.; Wright, D. J.; Boulter D. Legumin and Vicilin, Storage Proteins of Legume Seeds. *Phytochemistry* **1976**, *15*, 3–24.

Domoney, C.; Barker, D.; Casey, R. The Complete Deduced Amino Acid Sequences of Legumin β-Polypeptides from Different Genetic Loci in *Pisum*. *Plant Mol. Biol.* **1986**, *7*, 467–474.

Folk, J. E. Transglutaminases. *Annu. Rev. Biochem.* **1980**, *49*, 517–531.

Gatehouse, J. A.; Bown, D.; Gilroy, J.; Levasseur, M.;.Castleton, J.; Ellis, T. H.N. Two Genes Encoding "Minor" Legumin Polypeptides in Pea (Pisum Sativum L.). *Biochem. J.* **1988**, *250*, 15–24.

Gorman, J. J.; Folk, J. E. Structural features of Glutamine Substrates for Human Plasma XIIIa (Activated Blood Coagulation Factor XIII). *J. Biol. Chem.* **1980**, *255*, 419–427.

Gorman, J. J.; Folk, J. E. Structural features of Glutamine Substrates for Transglutaminases. Role of extended Interactions in the Specificity of Human Plasma Factor XIIIa and Guinea Pig Liver enzyme. *J. Biol. Chem.* **1984**, *259*, 9007–9010.

Gueguen, J.; Chevalier, M.; Barbot, J.; Schaeffer, F. Dissociation and Aggregation of Pea Legumin Induced by pH and Ionic Stength. *J. Sci. Food Agric.* **1988**, *44*, 167–182.

Ikura, K.; Kometani, T.; Sasaki, K.; Chiba, H. Cross-linking of Soybean 7S and 11S Proteins by Transglutaminase. *Agric. Biol. Chem.* **1980a**, *44*, 2979–2984.

Ikura, K.; Kometani, T.; Yoshikawa, M.; Sasaki, K.; Chiba, H. Crosslinking of Casein Components by Transglutaminase. *Agric. Biol. Chem.* **1980b**, *44*, 1567–1573.

Karplus, P. A.; Schulz, G. E. Prediction of chain flexibility in proteins. A tool for the selection of peptide antigens. *Naturwissenschaften* **1985**, *72*, 212–213.

Kun, E.; Kearney, E. B. Ammonia. In *Methods of Enzymatic Analysis;* Bergmeyer, H. U., Ed.; Academic Press: New York, 1974; Vol. 4, pp 1802–1806.

Laemmli, U. K. Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T4. *Nature* **1970**, *227*, 680–685.

Larré, C.; Gueguen, J. Large Scale Purification of Pea Globulins: Comparaison between Six Anion Exchangers in Medium-Pressure Liquid Chromatography. *J. Chromatogr.* **1986**, *361*, 169–179.

Larré, C.; Kedzior, Z. M.; Chenu, M. G.; Viroben G.; Gueguen, J. Action of Transglutaminase on an 11S Seed Protein (Pea Legumin): Influence of the substrate conformation. *J. Agric. Food Chem.* **1992**, *40*, 1121–1126.

Lycett, G. W.; Croy, R. R. D.; Shirsat, A. H.; Boulter, D. The Complete Nucleotide Sequence of a Legumin Gene from Pea (Pisum sativum L.). *Nucleic Acids Res.* **1984**, *12*, 4493–4506.

Matta, N.; Gatehouse, J. A.; Boulter, D. The structure of legumin of Vicia faba L. A reappraisal. *J. Exp. Bot.* **1981**, *32*, 1295–1307.

Motoki, M.; Nio, N.; Takinami, K. Functional properties of food proteins polymerized by transglutaminase. *Agric. Biol. Chem.* **1984**, *48*, 1257–1261.

Mycek, M. J.; Clarke, D. D.; Neidle, A.; Waelsch, H. Amine Incorporation into Insulin as catalyzed by Transglutaminase. *Arch. Biochem. Biophys.* **1959**, *84*, 528–533.

Neidle, A.; Mycek, M. J.; Clarke, D. D.; Waelsch, H. Enzymatic Exchange of Protein Amide Groups. *Arch. Biochem. Biophys.* **1958**, *77*, 227–229.

Nio, N.; Motoki, M.; Takinami, K. Gelation mechanism of protein solution by transglutaminase. *Agric. Biol. Chem.* **1986**, *50*, 851–855.

Nonaka, M.; Sakamoto, H.; Toiguchi, S.; Kawajiri, H.; Soeda, T.; Motoki, M. Sodium caseinate and skim milk gels formed by incubation with microbial transglutaminase. *J. Food Sci.* **1992**, *57*, 1214–1219.

Plietz, P.; Zirwer, D.; Schlesier, B.; Gast, K.; Damaschun, G. Shape, symmetry, hydratation and secondary structure of the legumin from vicia faba in solution. *Biochim. Biophys. Acta* **1984**, *784*, 140–146.

Wold, F. Reactions of the Amide Side-Chains of Glutamine and Asparagine in Vivo. *Trends Biochem. Sci.* **1985**, *10*, 4–6.

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