Enzymatic Phosphorylation by a Casein Kinase II of Native and Succinylated Soy Storage Proteins Glycinin and β-Conglycinin

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A casein kinase II purified from the lipolytic yeast *Yarrowia lipolytica* has been used to phosphorylate purified soy storage proteins and their isolated constitutive subunits. β-Conglycinin appeared to be a good substrate for the enzyme, glycinin being much poorer. Phosphate was incorporated specifically in the α and α′ subunits of β-conglycinin and in the acidic subunits of glycinin. Phosphorylation stochiometries of 0.73 mol of P/mol for β-conglycinin and 0.20 mol of P/mol for glycinin were determined. To improve the accessibility of the phosphorylation sites of glycinin and β-conglycinin for the enzyme, these proteins were succinylated at different rates. Both glycinin and β-conglycinin became better substrates after extensive succinylation. Phosphate incorporation after succinylation was still occurring in the α and α′ subunits of β-conglycinin but also in an unknown low molecular weight fraction. The acidic subunits of succinylated glycinin were still mainly phosphorylated, but the basic subunits became substrates as well.

Keywords: *Casein kinase II; soy; glycinin; β-conglycinin; phosphorylation; succinylation*

INTRODUCTION

The two major soy storage proteins, glycinin (11S) and β-conglycinin (7S), have been isolated and purified by numerous authors (Wolf and Sly, 1967; Kitamura and Shibasaki, 1975; Thanh and Shibasaki, 1976, 1977). Glycinin is an oligomeric protein having a molecular weight of approximately 350 000 and consisting of six subunits $(AB)_6$ (Catsimpoolas *et al.*, 1971). The polypeptides are of two types: acidic (M_r = 37 000−42 000) (A) and basic (M_r = 17 000−20 000) (B) linked by disulfide bridges. β-Conglycinin is a trimer composed of three major subunits (α, α′, and β) associated in various combinations by noncovalent interactions (Thanh and Shibasaki, 1977, 1978a,b; Coates *et al.*, 1985). The molecular weight of β-conglycinin is in the range 150 000−175 000 (Thanh and Shibasaki, 1978a,b) (with $M_r \sim$ 57 000 for α and α′ and $M_r \sim$ 42 000 for β subunits).

Native soy proteins exhibit good functional properties and can be used as food ingredients (Kinsella, 1979; Kinsella *et al.*, 1985; Nielsen, 1985). However, a roadblock to the large-scale use of soy proteins is their poor solubility and emulsification properties under mildly acidic (pH 3−6) conditions (Hirotsuka *et al.*, 1984; Kinsella *et al.*, 1985; Ross and Bhatnagar, 1989). This excludes their use in acidic foods such as coffee whiteners, acidic beverages, yogurts, and pourable and nonpourable dressings. The introduction of negatively charged phosphate groups, by lowering the isoelectric point of soy proteins, could improve their solubility and emulsification properties under mildly acidic conditions.

Chemically phosphorylated soy proteins have been reported to exhibit improved functional properties at slightly acidic pH in terms of aqueous solubility, emulsification, and gelling ability (Hirotsuka *et al.*, 1984). More recently, enzymatic phosphorylation of soy proteins has been described (Ross, 1989; Seguro and Motoki, 1989; Ross and Bhatnagar, 1989; Seguro and Motoki, 1990; Campbell *et al.*, 1992). Most of these authors used the commercially available catalytic subunit of a cAMP-dependent protein kinase which is most active toward serine and threonine residues located one or two amino acid residues away from basic amino acids (Kennelly and Krebs, 1991). The nature of the subunits preferentially phosphorylated is not totally clear yet, but glycinin seems to be a better substrate than β-conglycinin for this kinase.

We now report on the enzymatic phosphorylation of soy proteins by a ubiquitous cAMP-independent type II casein kinase (CKII) isolated and purified from the yeast *Yarrowia lipolytica* (Chardot and Meunier, 1994). CKII recognizes phosphorylatable serine and threonine residues in the consensus sequence Ser/Thr-X-X-Asp/Glu (Kuenzel *et al.*, 1987; Meggio *et al.*, 1984; Perich *et al.*, 1992). β-Conglycinin has been shown to be a better substrate than glycinin (Chardot and Meunier, 1994). Succinylation is well-known to lead to both dissociation and unfolding of glycinin (Rao and Rao, 1979; Kim and Kinsella, 1986, 1987). These structural changes could result in a better accessibility of the phosphorylation sites of both glycinin and β-conglycinin. We examine here the phosphorylation by CKII of native and succinylated glycinin, β-conglycinin, and their constitutive subunits.

EXPERIMENTAL PROCEDURE

Materials. β-Conglycinin and glycinin were a generous gift of Drs. W. J. Wolf and J. A. Bietz (USDA−ARS, Peoria, IL). Succinic anhydride was from Prolabo. Cold ATP was from Boehringer. [^{32}P]ATP was from Isotopchim (France). Scintillation liquid (Picofluor 40) was from Packard. Sephacryl S-300 superfine chromatography medium and Superose S-200 prepacked column were from Pharmacia. All other materials were reagent grade products.

Methods. *Cell Culture.* The yeast *Y. lipolytica* (wild strain W-29) was grown as described by Chardot and Meunier (1994). The cells were harvested in midlog phase by centrifugation (10 min at 3000*g*), washed twice with distilled water to remove any traces of the culture medium, and stored at −20 °C until use.

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Enzyme Purification. Casein kinase II purification was performed as described earlier (Chardot and Meunier, 1994) with slight improvements. A Pharmacia FPLC system equipped with Hi-Trap heparin and Superose S-200 columns was used instead of heparin−Sepharose and Sephacryl S-300, respectively. The enzyme exhibited higher specific activity (~3000 units/mg, i.e. 3−4 times by comparison with previous purifications) and was homogeneous as judged from SDS−PAGE. One unit is the amount of enzyme that transfers 1 pmol of P to 0.25 μg of dephosphocasein per minute at 22 °C at pH 7.8, casein concentration being 0.5 mg/mL. The batch of enzyme used in these experiments contained 88 μg of protein/mL as determined with the Bradford method (Bradford, 1976) using bovine serum albumin as standard.

Soy Proteins Purification. Soy proteins have been separated into subunits as described by Coates *et al.* (1985) and Wolf and Tamura (1969).

Succinylation. Succinylation of soy proteins was performed as described by Kim and Kinsella (1986). To the protein solution (7.5 mL at 12.5 mg/mL) in 0.1 M phosphate buffer, pH 8, were added known amounts of succinic anhydride in small increments over 1 h. The solution was stirred and the pH maintained above 8 by adding 3 N NaOH. After the pH stabilized at 8 following addition of all the succinic anhydride, the solution was extensively dialyzed against distilled water and then lyophilized.

The extent of succinylation was quantified by determining the free amino groups with TNBS (Habeeb, 1966). The percent succinylation was calculated as

$$\% \text{ succinylation} = [(A_{420\ \text{native}} - A_{420\ \text{succinylated}})/A_{420\ \text{native}}] \times 100$$

Chromatography. Native and succinylated glycinin samples were chromatographed on a Sephacryl S-300 column (2.6 × 83 cm) eluted with 0.05 M phosphate buffer, pH 7, containing 0.2 M NaCl at a flow rate of 60 mL/h. Native and succinylated β-conglycinin samples were chromatographed on a Pharmacia FPLC system Superose S-200 column (1 × 30 cm) eluted with 0.05 M phosphate buffer, pH 7, containing 0.2 M NaCl at a flow rate of 30 mL/h.

Soy Proteins Phosphorylation. The kinase activity was assayed by monitoring the incorporation of ^{32}P into soy proteins. The assay medium contained [^{32}P]ATP (5 μL at 100 μM), substrate solution in alkalinized, pH 8, distilled water (2−15 μL at 5 mg/mL), CKII (5 μL, i.e. 0,44 μg of protein), 50 mM TEA buffer, pH 7.8, containing 100 mM NaCl and 10 mM $MgCl_2$ (25 μL), and distilled water (up to 50 μL). The assays were performed at room temperature (always close to 22 °C). After a 30 min incubation, 500 μL of 20% ice-cold TCA containing 100 mM PP_i was added and the mixture centrifuged for 5 min at 13500*g* with a bench-top centrifuge. The supernatant was carefully removed and the pellet washed with 500 μL of a 10% ice-cold TCA solution containing 50 mM PP_i. The mixture was centrifuged for 5 min at 13500*g* and the supernatant discarded. The pellet was then washed twice with 500 μL of 90% ethanol. After removal of ethanol, scintillation liquid was added and the tubes were counted in a Packard scintillation counter. Kinetic studies were carried out with 475 μL of reaction mixture (142.5 μL of substrate solution at 5 mg/mL, 42.5 μL of 100 μM ATP, 50 μL of enzymatic solution, and 235 μL of buffer). At the desired times, aliquots (50 μL) were pipetted, mixed with 500 μL of 20% ice-cold TCA containing 100 mM PP_i, and treated as described above.

Electrophoreses. SDS−PAGE analyses performed on Novex ready-to-use 4−20% gradient gels and stained with Coomassie Brilliant Blue (R-250). The protein standards were phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, and α-lactalbumin for the Pharmacia kit and myosin, β-galactosidase (*Escherichia coli*), phosphorylase *b* (rabbit muscle), bovine serum albumin, glutamic dehydrogenase (bovine liver), lactate dehydrogenase (porcine muscle), carbonic anhydrase, trypsin inhibitor (soybean), lysozyme (chicken egg white), aprotinin (bovine lung), and unresolved insulin B chain and A chain for the Novex kit.

Autoradiographies were performed using a Kodak X-Omat film in direct contact with the gel. Exposures of the films were for 30 min to 24 h depending on the amount of ^{32}P incorporated.

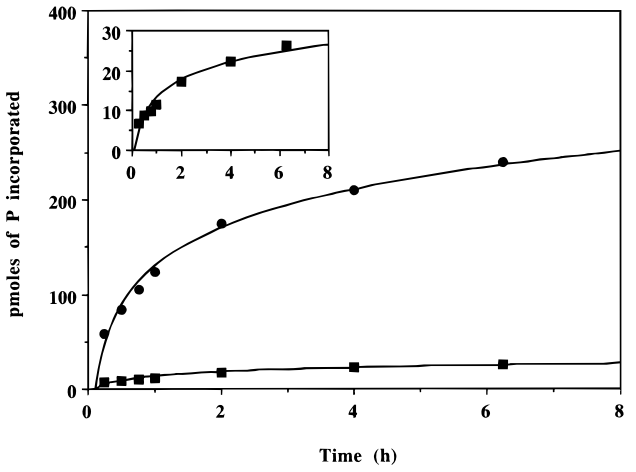

Figure 1. Effect of reaction time on the phosphorylation of glycinin (11S globulins) (■) and β-conglycinin (7S globulins) (●).

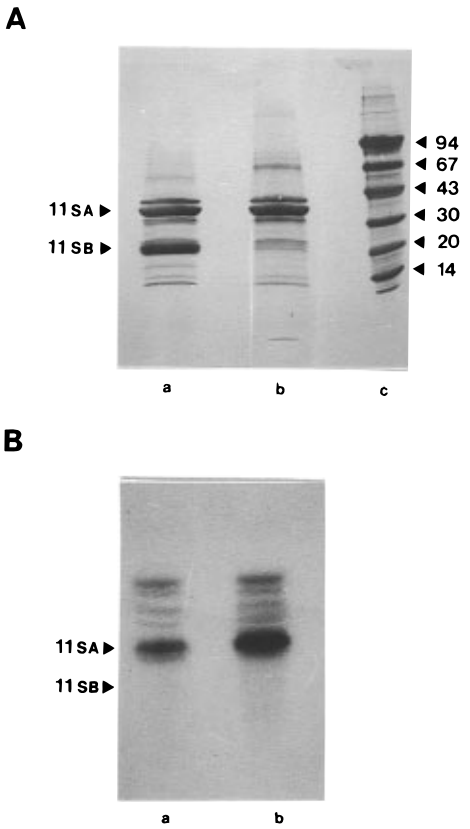

Figure 2. Gradient SDS−PAGE of 24 h phosphorylated glycinin: (A) Coomassie Brilliant Blue R-250 staining; (B) autoradiogram (contact 16 h); (lane a) glycinin (11S); (lane b) glycinin enriched in acidic subunits (11S A); (lane c) molecular weight standards (Pharmacia).

RESULTS

Phosphorylation of Glycinin. Glycinin is a poor substrate for CKII (Figure 1). The amount of ^{32}P incorporated increased with incubation time (Figure 1, insert) up to 42.5 pmol per assay (i.e. 0.57 nmol of P/mg of glycinin) after 24 h at 22 °C. Phosphorylated glycinin has been subjected to a SDS electrophoresis. Molecular weights of ~40 000 and ~20 000 were found for acidic (A) and basic (B) subunits, respectively (Figure 2A), leading to an average molecular weight of 360 000 for

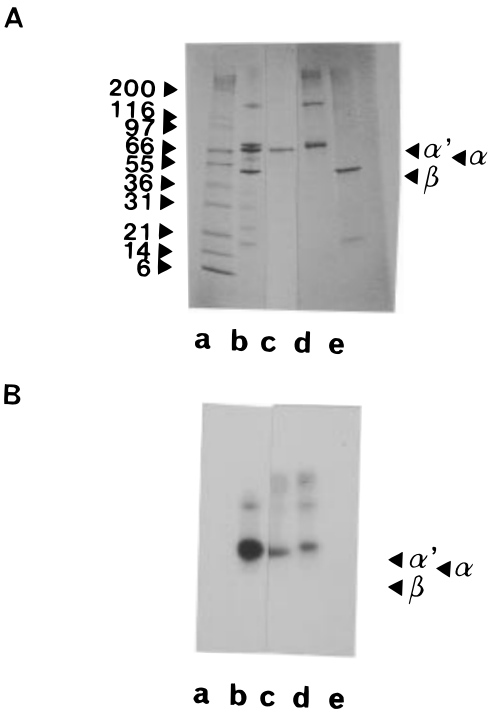

Figure 3. Gradient SDS−PAGE of 24 h phosphorylated β-conglycinin: (A) Coomassie Brilliant Blue R-250 staining; (B) autoradiogram (contact 30 min for lanes a and b and 1 h for lanes c−e); (a) molecular weight standards (Novex); (lane b) β-conglycinin (7S); (lane c) β-conglycinin α subunits (7S α); (lane d) β-conglycinin α′ subunits (7S α′); (lane e) β-conglycinin β subunits (7S β).

glycinin $(AB)_6$, in agreement with the findings of Catsimpoolas *et al.* (1971). Autoradiography of the gel showed that ^{32}P was incorporated into the glycinin acidic subunits (Figure 2B, lane a). Acidic and basic subunits have been separated after heating at pH 8 in the presence of β-mercaptoethanol as described by Wolf and Tamura (1969). No differences could be observed in the phosphorylation of the isolated fraction enriched in acidic subunits (87% of acidic and 13% of basic subunits) (Figure 2B, lane b) and glycinin (Figure 2B, lane a). No phosphorylation experiment could be conducted on isolated basic subunits due to their lack of solubility at the working pH.

Phosphorylation of β-Conglycinin. β-Conglycinin is a much better substrate for CKII than glycinin (Figure 1). The amount of ^{32}P incorporated increased with incubation time to reach 295 pmol per assay (i.e. 3.9 nmol of P/mg of β-conglycinin) after 24 h at 22 °C. Phosphorylated β-conglycinin has been subjected to a SDS electrophoresis. Molecular weights of ∼70 000, 66 000, and 50 000 were found for α′, α, and β subunits, respectively, leading to an average molecular weight of 186 000 for β-conglycinin (αα′β) (Figure 3A, lane b), in agreement with previously published data (Thanh and Shibasaki, 1978a,b). Autoradiography of the gel showed that ^{32}P was mainly incorporated into the α and/or α′ subunits and also, to a much lower extent, into some high molecular weight aggregates ($M_r \sim 140\,000$) that might be composed of dimers of α and/or α′ subunits (Petruccelli and Añón, 1995) (Figure 3B, lane b). On the other hand, no ^{32}P seemed to be incorporated into β subunits.

α, α′, and β subunits, separated by ion-exchange chromatography as described by Coates *et al.* (1985), were phosphorylated. Assays were performed with 403 pmol of each substrate, as for native β-conglycinin. After 24 h at 22 °C, 98.3, 149.5, and 13.6 pmol of ^{32}P were incorporated into α, α′, and β subunits, respectively. The sum of the picomoles of ^{32}P incorporated into the isolated subunits (261) is close to the picomoles of ^{32}P incorporated into the native β-conglycinin in the same conditions (295), 38, 57, and 5% being incorporated into the α, α′, and β subunits, respectively. Autoradiography of the gel showed that ^{32}P was incorporated almost exclusively into the isolated α and α′ subunits (Figure 3B, lanes c and d). The α′ fraction contained some high molecular weight aggregates, probably composed of dimers ($M_r \sim 140\,000$) and trimers or tetramers ($M_r > 200\,000$) of α′, as reported by Petruccelli and Añón (1995), which were also phosphorylated. Isolated β subunits did not seem to be phosphorylated (Figure 3B, lane e).

Succinylation of Glycinin and β-Conglycinin. The succinylation increased progressively with amounts of succinic anhydride added; 29, 51, 66, 87, and 94% succinylation were achieved at ratios of 0.025, 0.05, 0.1, 0.25, and 0.5 succinic anhydride to glycinin (g/g), respectively. Treatment of β-conglycinin at the same ratios led to 22, 31, 52, 79, and 87% succinylation, respectively.

To study the molecular weight distribution of native and succinylated glycinin, gel filtration chromatography was carried out on Sephacryl S-300 (Figure 4). The native glycinin (11S0) appeared as a main peak eluting at $K_{av} = 0.19$, which corresponds to a molecular weight of 360 000 $(AB)_6$, in agreement with SDS−PAGE experiments. A minor peak at $K_{av} = 0.42$, corresponding to a molecular weight of 47 000, was also detected. This peak could correspond to residual β-conglycinin β subunits. After succinylation and whatever the succinylation rate, a minor peak appeared at the void volume, which suggests the appearance of high molecular weight aggregates. The 29 and 51% succinylated samples (11S29 and 11S51, respectively) showed a major peak at $K_{av} = 0.25$, consistent with the presence of a trimer composed of three acidic and three basic polypeptides $(AB)_3$, and a minor peak at $K_{av} = 0.37$, consistent with the occurrence of a monomer formed by one acidic and one basic polypeptide $(AB)_1$. The 66% succinylated sample (11S66) exhibited the same peaks, at the void volume, at $K_{av} \sim 0.25$ and at $K_{av} \sim 0.37$, the latter being present in larger amounts than for 11S29 and 11S51. For 87 and 94% of succinylation (11S87 and 11S94, respectively), the peak at $K_{av} \sim 0.37$ became largely predominant. As already pointed out by various authors (Rao and Rao, 1979; Kim and Kinsella, 1986; Schwenke *et al.*, 1986; Gueguen *et al.*, 1990), some dissociation of the native glycinin hexamer occurred after progressive succinylation. Some high molecular weight components, probably due to aggregation by hydrophobic interactions (Kim and Kinsella, 1986; Schwenke *et al.*, 1986), were also detected.

Native and succinylated β-conglycinin samples were chromatographed on Superose S-200 (Figure 5). The native β-conglycinin (7S0) appeared as two main peaks eluting at $K_{av} \sim 0.07$ and ~ 0.19, which corresponds to molecular weights of ∼395 000 and ∼186 000, respectively. As already reported by Thanh and Shibasaki (1978a,b), there seems to be a dimerization of the 7S trimeric form ($M_r \sim 186\,000$) into a 9S hexameric form ($M_r \sim 395\,000$). Two other peaks were eluted at $K_{av} \sim 0.62$ and ~ 0.78, which correspond to molecular weights lower than 30 000. Succinylated samples, whatever the succinylation rate, exhibited similar elution patterns, very close to that obtained with the untreated sample

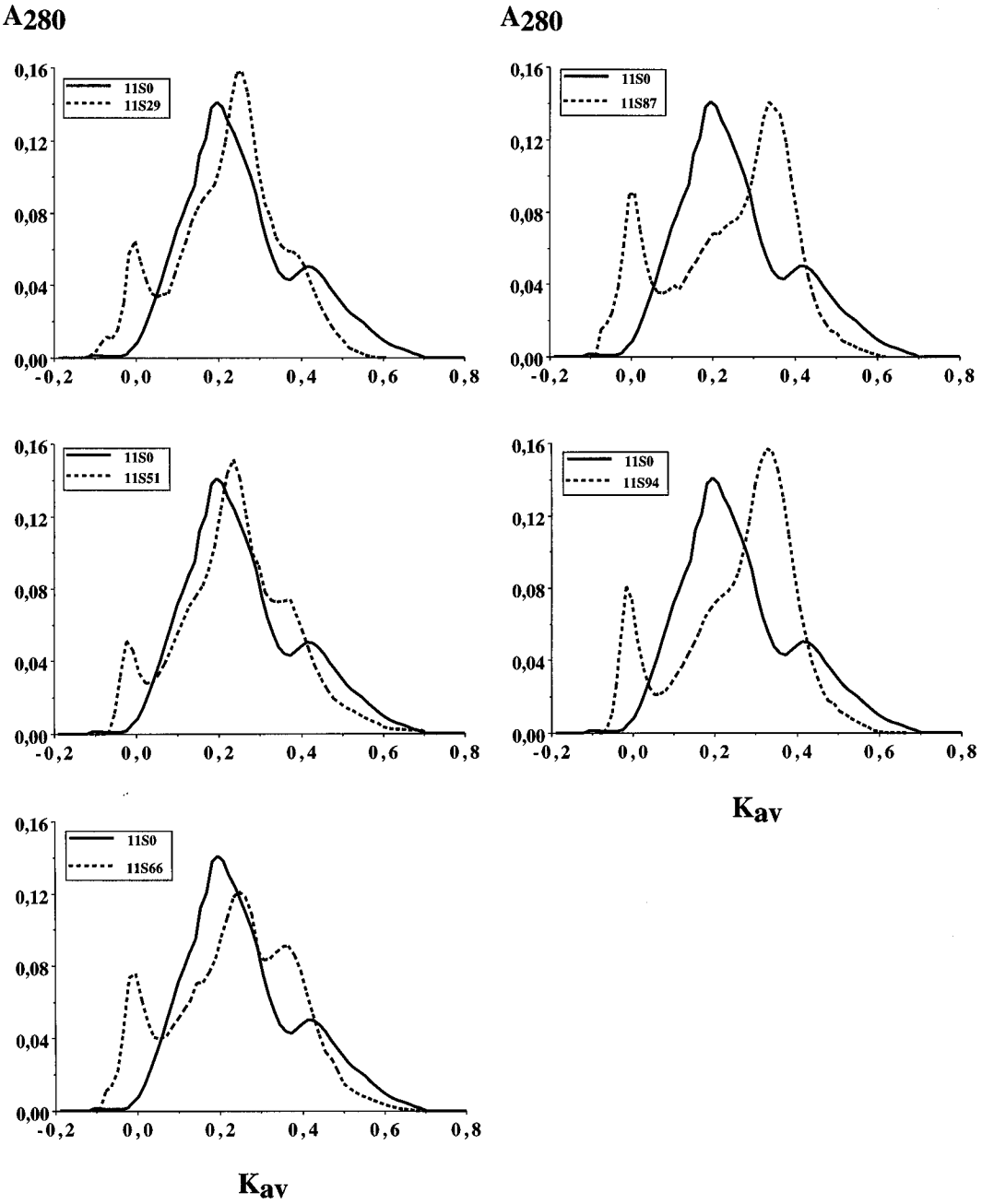

Figure 4. Sephacryl S-300 elution patterns of native (—) and succinylated (- - -) glycinin.

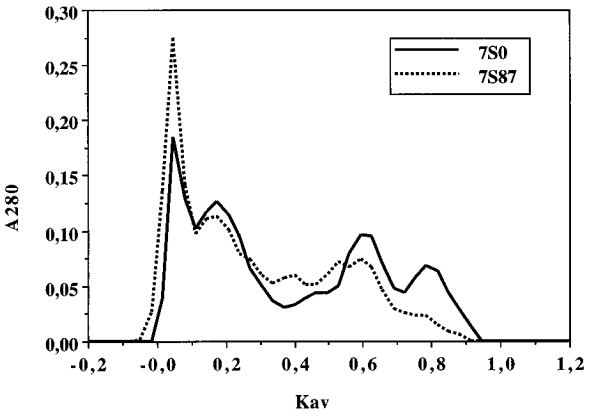

Figure 5. Superose S-200 elution patterns of native (—) and succinylated (- - -) β-conglycinin.

(7S0). This would suggest that no extensive dissociation or aggregation occurred after succinylation of β-conglycinin.

Phosphorylation of Succinylated Glycinin and β-Conglycinin. For both glycinin and β-conglycinin, high rates of succinylation led to increased phosphorylation (Figure 6A,B). For 87 and 94% succinylated glycinin, phosphorylation was increased 1.3- and 1.6-fold, respectively (1.5 mg/mL samples). For 79 and 87% succinylated β-conglycinin, phosphorylation was increased 1.4- and 1.7-fold, respectively (1.5 mg/mL samples). Lower succinylation rates did not influence phosphorylation rates. It can be noted, however, that low succinylation rates (<50%) seemed to imply slightly decreased phosphorylation of glycinin and β-conglycinin.

After phosphorylation by CKII, succinylated glycinin and β-conglycinin were subjected to a SDS electrophoresis (Figures 7 and 8). For both samples, the mobility of the subunits toward the anode was reduced as a function of the extent of succinylation (Figures 7A and 8A). This certainly reflects an increase of molecular weight due to succinate moieties. For glycinin, autoradiography of the gel (Figure 7B) showed that more ^{32}P was incorporated into the acidic subunits after

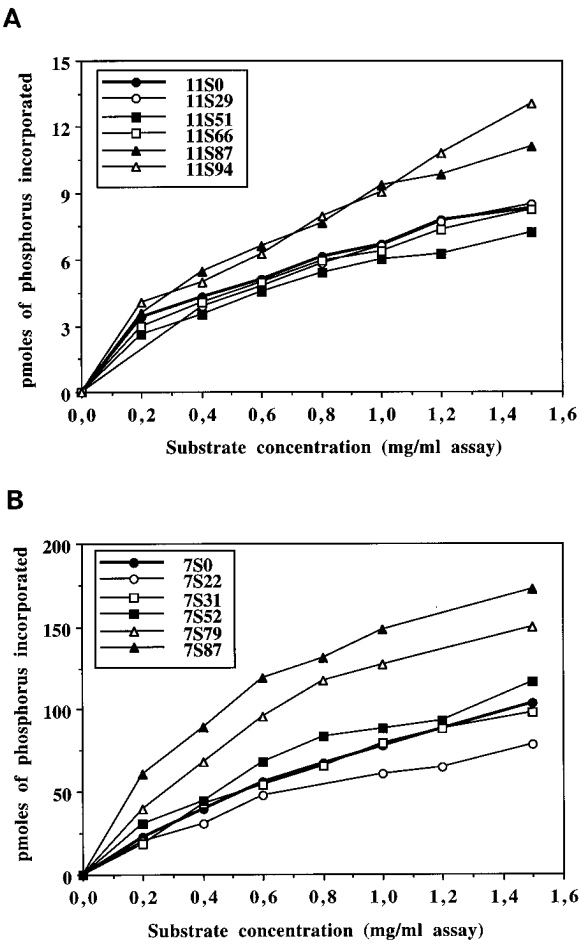

Figure 6. Enzymatic phosphorylation of native and succinylated glycinin (A) and β-conglycinin (B).

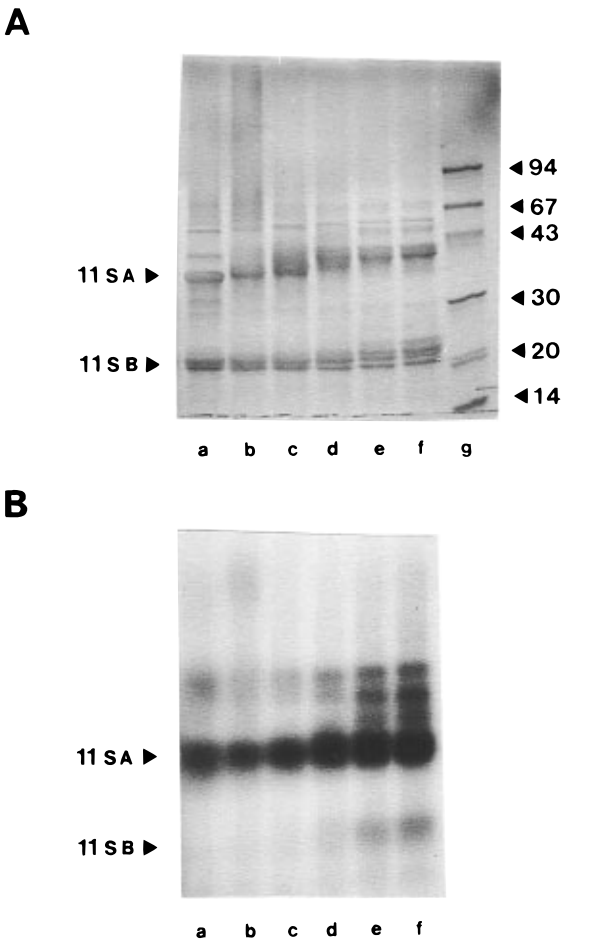

Figure 7. Gradient SDS−PAGE of 24 h phosphorylated native and succinylated glycinin: (A) Coomassie Brilliant Blue R-250 staining; (B) autoradiogram (contact 16 h); (lane a) native glycinin (11S0); (lane b) 29% succinylated glycinin (11S29); (lane c) 51% succinylated glycinin (11S51); (lane d) 66% succinylated glycinin (11S66); (lane e) 87% succinylated glycinin (11S87); (lane f) 94% succinylated glycinin (11S94); (lane g) molecular weight standards (Pharmacia).

progressive succinylation. For 11S87 and 11S94, basic subunits and undetermined minor high molecular weight fractions became substrates (Figure 7B, lanes e and f). However, ^{32}P was still mainly incorporated into acidic subunits for these extensively succinylated samples. For β-conglycinin, autoradiography of the gel (Figure 8B) showed that the α and/or α′ subunits became slightly better substrates after extensive succinylation. A low molecular weight (~30 000) undetermined fraction, which was a poor substrate in 7S0 (Figure 8B, lane a), became a much better substrate after progressive succinylation (Figure 8B, lanes b−f). There seemed to be equal amounts of ^{32}P incorporated into the α and α′ subunits and into this low molecular weight fraction for highly succinylated samples (7S79 and 7S87) (Figure 8B, lanes e and f). β subunits did not seem to be phosphorylated after moderate succinylation but seemed to be slightly phosphorylated in the 87% succinylated sample.

DISCUSSION

The two major soy storage proteins glycinin and β-conglycinin could be enzymatically phosphorylated by the cAMP-independent type II casein kinase from the yeast *Y. lipolytica*. Glycinin was phosphorylated to a lower extent than β-conglycinin, and it can be calculated that 0.20 and 0.73 mol of P were incorporated per mole of glycinin and β-conglycinin after 24 h, respectively. According to the sequences published by Xue *et al.* (1992) for glycinin and by Doyle *et al.* (1986) (α), Harada *et al.* (1989) (β), and Sebastiani *et al.* (1990) (α′) for β-conglycinin, there are 36 and 25 mol of potential phosphorylation sites (Ser/Thr-X-X-Asp/Glu for CKII) per mole of glycinin and β-conglycinin, respectively. It seems that the only consensus sequence criteria is not sufficient to explain the ability of a protein to be a substrate for CKII. The nature of the neighboring amino acids and the globular compact structure of the protein have to be considered too. Glycinin phosphorylation sites might be less accessible than β-conglycinin ones.

Of the 36 mol of serine and threonine in the consensus sequences for CKII in glycinin, 0.20 mol (0.6%) is phosphorylated and exclusively into acidic polypeptides of glycinin. This is understandable considering the difference in the number of potential phosphorylation sites between acidic (5) and basic (1) polypeptides. Of the 25 mol of serine and threonine in the consensus sequences for CKII in β-conglycinin, 0.73 mol (3.0%) is phosphorylated and only into the α and α′ subunits. An examination of the known primary sequences of β-conglycinin subunits reveals that there are approximately the same number (from 7 to 10) of potential phosphorylation sites in α, α′, and β subunits. The number of potential phosphorylation sites cannot explain the lack of phosphorylation of β-conglycinin β subunits. Here again, the consensus sequence criteria are not sufficient to predict the ability of a polypeptide to be phosphorylated. The sites present in β subunits must be very poorly accessible to CKII.

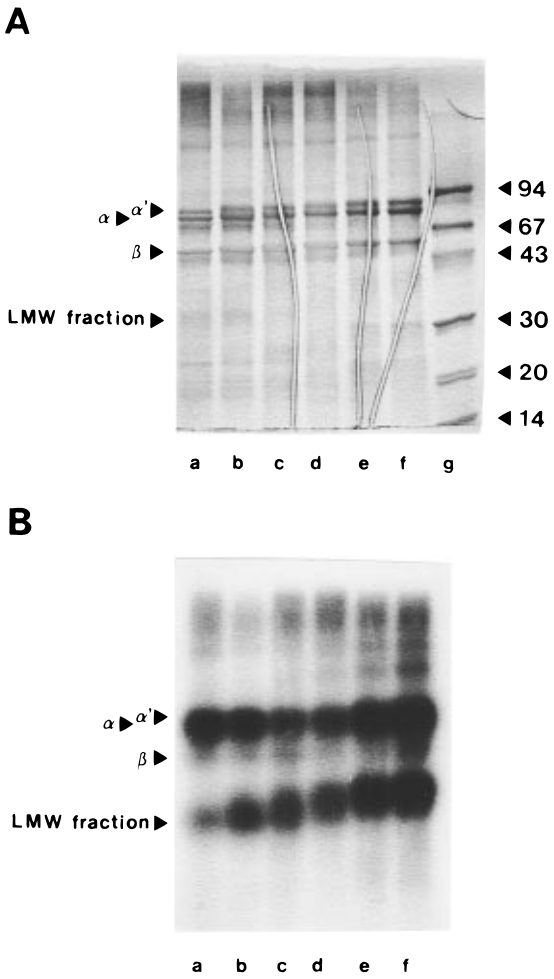

Figure 8. Gradient SDS−PAGE of 24 h phosphorylated native and succinylated β-conglycinin: (A) Coomassie Brilliant Blue R-250 staining; (B) autoradiogram (contact 2 h); (lane a) native β-conglycinin (7S0); (lane b) 22% succinylated β-conglycinin (7S22); (lane c) 31% succinylated β-conglycinin (7S31); (lane d) 52% succinylated β-conglycinin (7S52); (lane e) 79% succinylated β-conglycinin (7S79); (lane f) 87% succinylated β-conglycinin (7S87); (lane g) molecular weight standards (Pharmacia).

Levels of phosphorylation (between 0.20 and 0.73 mol of P/mol of native protein) obtained with CKII were similar to levels (between 0.2 and 2.0 mol of P/mol of native protein) obtained with cAMP-dependent protein kinases (Ross, 1989; Seguro and Motoki, 1989, 1990; Ross and Bhatnagar, 1989; Campbell *et al.*, 1992), although the consensus sequences for these enzymes are quite different (Ser/Thr-X-X-Asp/Glu and Arg/Lys-Arg-X-Ser/Thr for CKII and cAMP-dependent protein kinase, respectively) (Kennelly and Krebs, 1991). In contrast to our results, glycinin was found to be phosphorylated to a greater extent than β-conglycinin by cAMP-dependent protein kinases. This has been explained by the difference in the number of potential phosphorylation sites for cAMP-dependent protein kinase between glycinin and β-conglycinin.

An increase in the phosphorylation of glycinin and β-conglycinin after heat denaturation has been reported by Ross and Bhatnagar (1989) and Ross (1989). The authors suggested that heat denaturation for a short time could induce changes in the structure of glycinin and β-conglycinin and therefore increase the accessibility of some phosphorylation sites. We tried here to improve the accessibility of the phosphorylation sites of glycinin and β-conglycinin for CKII by succinylation. This method has been indeed proved to induce dissociation and unfolding of glycinin and other 11S globulins (Rao and Rao, 1979; Kim and Kinsella, 1986; Schwenke *et al.*, 1986, 1990; Gueguen *et al.*, 1990). In agreement with those previously published data, we showed that succinylation of glycinin caused dissociation of the protein. The extent of dissociation depends upon the degree of succinylation, and the following dissociation scheme can be deduced from our results:

subunit structure:	hexamer	→ trimer	→ monomer
	$(AB)_6$	$(AB)_3$	$(AB)_1$
molecular weight:	360 000	180 000	60 000
degree of succinylation:	low	→ medium	→ high

Similar results have been reported for various 11S globulins from different plant families (Schwenke *et al.*, 1990). In contrast, succinylation was not efficient in dissociating β-conglycinin into its constitutive subunits (α, α′, and β). The way the different subunits are held together differs considerably between glycinin and β-conglycinin. In glycinin, electrostatic interactions between A and B polypeptides are important, while in β-conglycinin the trimeric structure is mainly due to hydrophobic interactions.

Succinylation increased phosphorylation of both glycinin and β-conglycinin. In the case of glycinin, the changes in the tertiary and quaternary structure induced by succinylation can explain this increase; some phosphorylation sites in the protein must become more accessible to the enzyme. The acidic subunits incorporated more phosphate groups after progressive succinylation. Furthermore, basic subunits, which were not substrates at all, were slightly phosphorylated after extensive succinylation of glycinin. The only consensus sequence present in the basic subunits seems to become accessible to the enzyme. The increased phosphorylation observed after succinylation of β-conglycinin cannot be explained by partial dissociation into subunits as in the case of glycinin. Although β subunits are rich in consensus sequences for the enzyme, they were not, or only very slightly, phosphorylated, even after extensive succinylation. As for glycinin acidic subunits, β-conglycinin α and/or α′ subunits incorporated more phosphate groups after progressive succinylation. This could be related to a slight unfolding of the molecule leading to a better accessibility of at least one of the potential phosphorylation sites. An undetermined minor low molecular weight fraction became an excellent substrate after extensive succinylation. This material, because of its low molecular weight, could be largely unfolded by the succinylation treatment. One or multiple phosphorylation sites could therefore become accessible. Further work is needed to elucidate the structure of this low molecular weight fraction and to determine whether it is a degradation product of β-conglycinin or not. Seguro and Motoki (1989) also found radiolabeled P_i incorporated into some uncharacterized low molecular weight fractions of soy protein.

CONCLUSION

The ability of type II casein kinases to phosphorylate vegetable proteins had not, to our knowledge, been investigated. We have shown that a significant degree of phosphorylation could be achieved by CKII on both glycinin and β-conglycinin. The recognition sites for kinases are different depending on the type of kinase (histone-type or casein-type). In contrast to the results obtained with cAMP-dependent histone-type kinases (Ross, 1989; Seguro and Motoki, 1989, 1990; Ross and Bhatnagar, 1989; Campbell *et al.*, 1992), β-conglycinin

was phosphorylated to a much greater extent than glycinin. Further work is needed to optimize enzymatic reaction parameters and to determine the precise positions of the phosphorylation sites in both glycinin acidic subunits and β-conglycinin α and α′ subunits before and after succinylation.

ABBREVIATIONS USED

cAMP, cyclic adenosine monophosphate; CKII, type II casein kinase; PP_i, inorganic pyrophosphate; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TCA, trichloracetic acid; TEA, triethanolamine; TNBS, trinitrobenzenesulfonic acid.

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