Mercier, J. C.; Grosclaude, F.; Ribadeau Dumas, B. "Structure Primaire de la Caséine α_{S1} Bovine". *Eur. J. Biochem.* **1971**, *23*, 41–51.

Mercier, J. C.; Brignon, G.; Ribadeau Dumas, B. "Structure Primaire de la Caséine κ_B Bovine: Séquence Complète". *Eur. J. Biochem.* **1973**, *35*, 222–235.

Monti, J. C.; Jost, R. "Enzymatic Solubilization of Heat-Denatured Cheese Whey Protein". *J. Dairy Sci.* **1978**, *61*, 1233–1237.

Morr, C. V. "Functional Properties of Milk Proteins and Their Use as Food Ingredients". In *Developments in Dairy Chemistry-1-Proteins*; Fox, P. F., Ed.; Applied Science: London, 1982; pp 375–399.

Olsen, H. S.; Adler-Nissen, J. "Industrial Production and Applications of a Soluble Enzymatic Hydrolyzate of Soya Protein". *Process Biochem.* **1979**, *14*, 6–11.

Pearce, K. N.; Kinsella, J. E. "Emulsifying Properties of Proteins: Evaluation of a Turbidimetric Technique". *J. Agric. Food Chem.* **1978**, *26*, 716–723.

Pearson, A. M.; Spooner, M. E.; Hegarty, G. R.; Bratzler, L. J. "The Emulsifying Capacity and Stability of Soy Sodium Proteinate, Potassium Caseinate and Nonfat Dry Milk". *Food Technol.* **1965**, *19*, 103–107.

Phillips, R. D.; Beuchat, L. R. "Enzyme Modification of Proteins". In *Protein Functionality in Foods*; Cherry, J. P., Ed.; ACS Symposium Series 147; American Chemical Society: Washington, DC, 1981; pp 275–298.

Preaux, G.; Braunitzer, G.; Schrank, B.; Stangl, A. "The Amino-acid Sequence of Goat's β-Lactoglobulin". *Hoppe Seyler's Z. Physiol. Chem.* **1979**, *360*, 1595–1604.

Ribadeau Dumas, B.; Brignon, G.; Grosclaude, F.; Mercier, J. C. "Structure Primaire de la Caséine β Bovine". *Eur. J. Biochem.* **1972**, *25*, 505–514.

Saint-Paul, F.; Humbert, G.; Pâquet, D.; Linden, G. "Hydrolyse Enzymatique de Protéines Laitières Industrielles: Incidences sur leur Solubilité". *Sci. Aliments* **1984**, *4*, 259–264.

Shimizu, M.; Takahashi, T.; Kaminogawa, S.; Yamauchi, K. "Adsorption onto an Oil Surface and Emulsifying Properties of Bovine α_{S1}-Casein in Relation to Its Molecular Structure". *J. Agric. Food Chem.* **1983**, *31*, 1214–1218.

Shimizu, M.; Lee, S. W.; Kaminogawa, S.; Yamauchi, K. "Functional Properties of a Peptide of 23 Residues Purified from the Peptic Hydrolysate of α_{S1}-Casein: Changes in the Emulsifying Activity during Purification of the Peptide". *J. Food Sci.* **1986**, *51*, 1248–1252.

To, B.; Helbig, N. B.; Nakai, S.; Ma, C. Y. "Modification of Whey Protein Concentrate to Simulate Whippability and Gelation of Egg White". *Can. Inst. Food Sci. Technol.* **1985**, *18*, 150–157.

Volkert, M. A.; Klein, B. P. "Protein Dispersibility and Emulsion Characteristics of Flour Soy Products". *J. Food Sci.* **1979**, *44*, 93–96.

Wang, J. C.; Kinsella, J. E. "Functional Properties of Novel Proteins: Alfalfa Leaf Protein". *J. Food Sci.* **1976**, *41*, 286–292.

Webb, N. B.; Ivey, F. J.; Craig, H. B.; Jones, V. A.; Monroe, R. J. "The Measurement of Emulsifying Capacity by Electrical Resistance". *J. Food Sci.* **1970**, *35*, 501–504.

Whitaker, J. R., Ed. *Food related enzymes.* Advances in Chemistry Series 136; American Chemical Society: Washington, DC, 1974; 365 pp.

Whitaker, J. R. "Enzymatic Modification of Proteins Applicable to Foods". In *Food Proteins: Improvement through Chemical and Enzymatic Modification*; Feeney, R. E., Whitaker, J. R., Eds.; Advances in Chemistry Series 160; American Chemical Society: Washington, DC, 1977; pp 95–155.

Whitaker, J. R.; Puigserver, A. J. "Fundamentals and Applications of Enzymatic Modifications of Proteins—an Overview". In *Modifications of Proteins: Food, Nutritional and Pharmacological Aspects*; Feeney, R. E., Whitaker, J. R., Eds.; Advances in Chemistry Series 198; American Chemical Society: Washington, DC, 1982; pp 57–87.

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Soybean Metal-Binding Proteins: Isolation of a Phosphatase That Inhibits Calmodulin Activity

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Soybean seeds and seedlings contain monophosphatase (MP), phosphodiesterase (PDE), and a calmodulin (CAM) that stimulates PDE activity. Interactions of these proteins likely regulate phosphate metabolism in the seed. This paper identifies proteins that interact with CAM and defines the function of CAM in soybeans. We find that an MP enzyme can inhibit CAM activity and is associated with CAM in soybean whey. MP enzyme is separated into a single peak by DEAE chromatography in the presence of a calcium chelator, EGTA, and further chromatography on a calmodulin affinity column. Its binding to the calmodulin affinity column is reversible and depends on the presence of Ca^{2+} ions. HPLC, gel electrophoresis, and gel filtration analyses of the MP enzyme show that it contains two subunits (24 and 20 kDa), but neither of the subunits alone exhibits phosphatase activity. The 20-kDa subunit retains the ability to inhibit CAM stimulation of PDE. Recombination of the two subunits yields MP activity that can be further enhanced by the addition of CAM. MP activity appears to be less stable than the CAM inhibitor activity.

As part of studies on CAM in soybeans, we isolated the protein from soybean whey by chromatographing extracts on DEAE and then subjecting collected fractions to affinity chromatography on W-7 agarose to obtain pure CAM. The purified protein is similar to calmodulin from spinach and bovine brain in terms of ability to stimulate PDE activity and electrophoretic mobility as a 18-kDa protein (unpublished result). Its behavior during purification suggests that it is associated with other calcium-binding proteins in the whey, particularly in the presence of Ca^{2+} ions. A calcium chelator (EGTA) is required during DEAE chromatography to separate CAM from other whey proteins. We now have evidence that the proteins to which CAM binds include an MP enzyme that hydrolyzes *p*-nitrophenyl phosphate. This MP enzyme also inhibits the stimulatory action of CAM on PDE. It is a multisubunit

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enzyme that is inactivated by dissociation. Furthermore, it is interesting that a 20-kDa subunit retains CAM inhibitor activity and that reconstituted enzymatic activity can be enhanced by the addition of CAM.

Soybeans contain various nonspecific phosphatases, among which phytase is prominent. Partially purified soybean phytase has the ability to hydrolyze phytate and β-glycerophosphate at neutral pHs (Peng and Wang, 1954). The enzyme, however, has not been purified or characterized fully. Another nonspecific acid phosphatase that hydrolyzes β-glycerophosphate was also partially purified from soybean by Mayer et al. (1961). Since then, various attempts to obtain a single highly active phytate-specific enzyme from soybeans have been unsuccessful (Wang, unpublished data). Phytase recently purified 100-fold from soybeans still contained nonspecific phosphatase activity (Sutardi and Buckle, 1986).

The question may be raised whether phytase in soybeans is a single enzyme or a cascade of enzymes that collectively behave like a phytase but individually function as various nonspecific phosphatases. Upon purification, a protein complex might lose components, which could disrupt sequential enzymatic activity and cause a decrease in specific phytase activity. Reports on nonspecific phosphatases in soybeans are thus of particular interest, but, to the best of our knowledge, the literature on soybean phosphatases is quite limited.

A more recent report on phosphatase in soybeans by Ravi et al. (1983) includes dolichyl phosphate phosphatase. This enzyme is present in the cotyledons at a level of about 9 mg/100 g of dry seeds. It hydrolyzes dolichyl phosphate at a K_m 5 μM, does not require divalent metal ions, and in fact is slightly enhanced by EDTA. Of various enzyme substrates tested, phosphatidylcholine and phosphatidylethanolamine were slightly stimulating at 0.1 mM substrate concentration, but phosphatidic acid and lysophosphatidic acid inhibited. Phosphatidic acid showed competitive inhibition. At 1.0 mM substrate concentration all phospholipids tested were inhibitory.

Here we report the isolation of an MP enzyme that may regulate phosphate mobilization through its ability to inhibit CAM stimulation of PDE activity.

MATERIALS AND METHODS

Materials. Soybeans of Amsoy variety were purchased locally; full-fat soybean flour available commercially was obtained from Archer Daniels Midland, Decatur, IL; DEAE cellulose (DE-52) was from Whatman Co.; calmodulin coupled to Sepharose 4B was either purchased from Sigma Chemical Co., St. Louis, MO, or synthesized in our laboratory according to the procedure of Hart et al. (1983); 5′-nucleotidase, cAMP, and PDE were obtained from Sigma. All reagents used were of analytical grade. Deionized water was used throughout.

Methods. *Preparation of MP Enzyme.* The MP enzyme was prepared from either germinated beans or full-fat soybean flour.

A. From Germinated Beans. Soybean seeds, usually about 50 g, were soaked in 0.1% Clorox solution under vacuum until all beans were floating and then transferred to a crystal dish filled with a 5-cm-thick layer of moist vermiculite. The dish was covered with a glass plate and placed in a growth chamber (29 °C) for 3 days. Sprouts were added with 10× w/v buffer A [Tris-HCl (20 mM), pH 7.2, containing 1 mM imidazole, $MgCl_2$, and EGTA] and homogenized with a Polytron for 2 min with the sample chilled in an ice bucket. The homogenate was acidified to pH 4.5, allowed to stand for 30 min in the cold, and then centrifuged at 10000*g* for 10 min. The clear supernatant was collected and neutralized to make a whey solution for chromatography on DEAE.

B. From Soybean Full-Fat Flour. Soybean flour (100 g) was homogenized and fractionated the same way as sprouts to obtain whey proteins.

DEAE Chromatography. Usually the full amount of the sample was pumped onto the column at a rate of about 10 mL/min. The column was washed with buffer A containing 0.05 M NaCl until the effluent OD at 280 reached a minimum. The washing was discard. Phosphatase was eluted with buffer A containing 0.2 M NaCl, and the eluate was collected in 10-mL fractions. The column was then eluted with buffer A containing 0.5 M NaCl to collect the CAM fraction. A column size of 2.6 × 36 cm was used, and the operation was in a cold chamber.

Affinity Chromatography. The MP enzyme collected from the DEAE chromatography was concentrated by lyophilization and then equilibrated with buffer B (Tris-HCl (20 mM), pH 7.2, containing 1 mM imidazole, $MgCl_2$, and $CaCl_2$). A column of 1 × 11 cm filled with calmodulin cross-linked to Sepharose 4B was preequilibrated with the same buffer. After the sample was added, the column was washed with buffer B containing 0.05 M NaCl until the effluent OD fell to a minimum. The enzyme was eluted with buffer A (same as buffer B except with 1 mM EGTA, no Ca). The enzyme activity was monitored, and the protein peak with enzyme activity was collected for further analyses by HPLC, gel electrophoresis, and gel filtration. All operations were carried out at 4 °C.

Gel Filtration. A Sephadex G-200 column of 2.6 × 40 cm size was used in the cold. The enzyme collected from affinity chromatography was again lyophilized and equilibrated with buffer A. A flow rate of 15 mL/30 min was used. Protein peaks detected at 280 nm were collected and pooled for assay of enzymatic activity and gel electrophoresis.

HPLC Analyses. A Spectra Physics 8100 HPLC unit equipped with a Vydac 218TP analytical column was used. Separation of protein was done at a flow rate of 1 mL/min with a 90-min gradient of 20–45% acetonitrile versus water containing 0.1% trifluoroacetic acid followed by a 20-min holding period. The column was operated at 60 °C and monitored with a UV detector at 210 nm.

Gel Electrophoresis. A 10–20% gradient acrylamide gel with added SDS was prepared, loaded with samples, and run according to the procedure given by Fling and Gregerson (1986).

Enzyme Assays. A. MP enzymatic activity was assayed according to a modified procedure described by Pallen and Wang (1983). Essentially, a reaction mixture of 700 μL containing 3.5 mM $NiCl_2$, 3.0 mM Tris-HCl, pH 7.0, 0.25 mg of BSA, and 50 μL of MP enzyme solution was incubated for 30 min at 37 °C. The reaction mixture was treated with 150 μL of freshly prepared *p*-nitrophenyl phosphate solution (5 mg/mL) and incubated for another 10 min. The reaction was stopped by adding 150 μL of 13% K_2HPO_4 solution. The yellow color was measured spectrophotometrically at 405 nm after 10 min. The protein concentration of the enzyme solution was determined by the Bradford (1976) method.

B. CAM stimulation of PDE activity was assayed as described by Sharma and Wang (1979). The inhibitory effect of MP enzyme on CAM was measured by comparing CAM-stimulated PDE activity in the presence of MP enzyme with that of a control.

RESULTS

Stepwise elution of the DEAE column separated MP enzyme from CAM and other proteins as shown in Figure

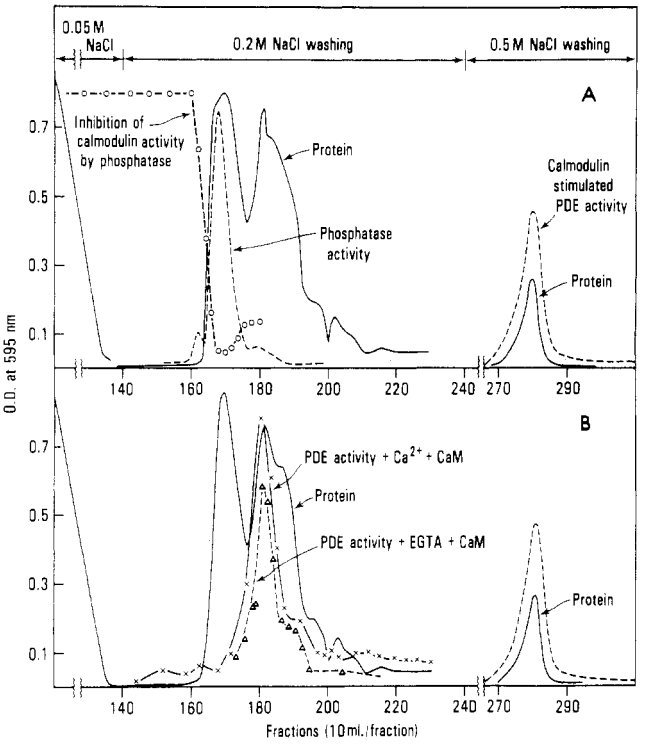

Figure 1. DEAE column separation of soybean monophosphatase from phosphodiesterase and calmodulin. Conditions were as described in the text. (a) Monophosphatase activity and calmodulin inhibitory activity. (B) Phosphodiesterase activity.

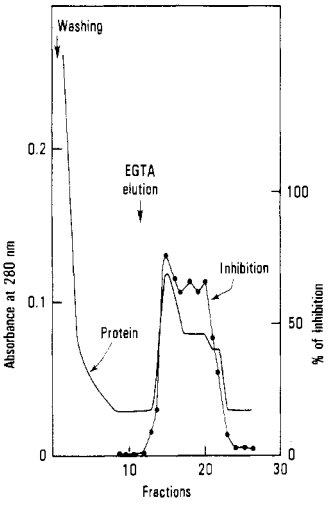

Figure 2. Calmodulin affinity chromatography of soybean monophosphatase enzyme obtained from DEAE chromatography. See text for details.

1A,B. The MP enzyme eluted ahead of PDE with 0.2 M NaCl in Tris buffer, and CAM eluted after both enzymes with Tris buffer containing 0.5 M NaCl. Figure 1A presents the elution profile and the inhibiting effect of MP enzyme on the CAM stimulation of PDE activity. PDE activity, given in Figure 1B, was obtained from the same experiment and is plotted separately for clarity. MP enzyme overlapped slightly the PDE peak, which contained a mixture of Ca^{2+} ion and CAM-dependent and -independent enzymes. Data in Figure 1A,B indicate that more soybean whey protein associates with MP and PDE enzymes than with CAM.

The MP enzyme was further purified by calmodulin affinity chromatography as shown in Figure 2, which traces elution of the enzyme's CAM inhibitory activity. The association of MP enzyme with calmodulin was Ca^{2+} ion

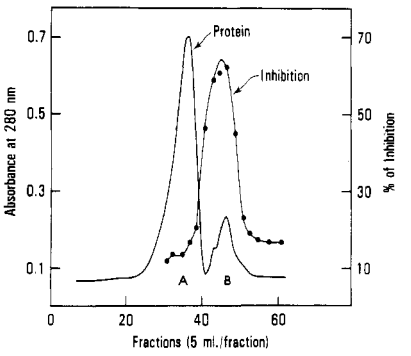

Figure 3. Sephadex G-200 gel filtration of soybean monophosphatase obtained from affinity chromatography in Figure 2.

Table I. Monophosphatase Enzyme Activity in Soybean Fractions after Affinity Chromatography and Gel Filtration

fraction	relative activity: monophosphatase,[a] μM P/μg prot/10 min	relative activity: calmodulin inhibn,[b] %
fraction A, Figure 3	0.10	[c]
fraction B, Figure 3	0.10	86.7
A + B	0.45	35.4
A + B + calmodulin + Ca^{2+}	0.76	[c]

[a] Monophosphatase activity was determined by a modified procedure of Pallen and Wang (1983). [b] Calmodulin inhibition measured by the reduction of phosphodiesterase activity after addition of MP enzyme fraction and comparison to a control according to Sharma and Wang (1979). [c] Not determined.

dependent and reversible. Binding to the calmodulin column occurred when Ca^{2+} ions were present in the buffer. EGTA in the eluting buffer released the enzyme from the column. MP enzyme, at this stage, retained both phosphatase and CAM inhibiting activity.

When the MP enzyme obtained from affinity chromatography was pooled, lyophilized, and chromatographed further on Sephadex G-200 (Figure 3), two protein peaks were obtained (at 180 and 230 mL). Neither of these peaks contained phosphatase activity, but CAM inhibiting activity remained in one of the peaks. Recombination of the two separated protein peaks produced a 4-fold increase in phosphatase activity (Table I). Moreover, mixing the recombined enzyme with CAM and Ca^{2+} ions produced an additional 2-fold increase in phosphatase activity. Proteins in the second peak alone (Figure 3) suppressed CAM activation of PDE up to 87%, whereas the combined protein from both peaks caused only 35% inhibition. At least two protein subunit components are needed for MP activity, but not for CAM inhibiting activity.

The MP enzyme was also separated by HPLC into two distinct protein peaks emerging at 47 and 74 min (Figure 4A). There were other peaks in Figure 4A absorbing strongly at 210 and 250 nm but weakly at 280 region. They were probably nonprotein materials. The MP enzyme was denatured after HPLC. Data in Figure 4A are consistent with our belief that the active MP enzyme contains at least two subunits.

Figure 4B presents an HPLC pattern of CAM prepared from soybean by DEAE and W-7 affinity chromatographies. This pattern with a predominate peak at 78 min matched patterns of calmodulin from animal and spinach with only slight differences in minor peaks (results are not shown here). However, the peak at 78 min in Figure 4B did not match the major peak at 74 min in Figure 4A. We thus assumed that CAM was not a component of the MP enzyme, but another experiment was preformed to pursue

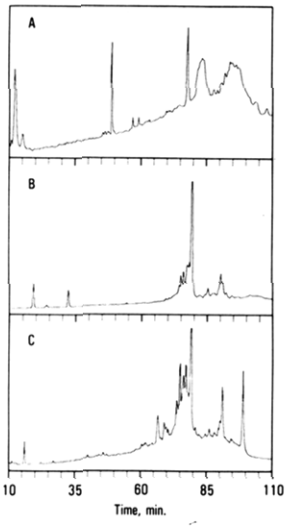

Figure 4. HPLC: (A) soybean monophosphatase, (B) calmodulin from soybean whey prepared by DEAE and W-7 affinity chromatographies, (C) calmodulin from soybean whey prepared by the same method as B but with heat treatment. See text for HPLC conditions.

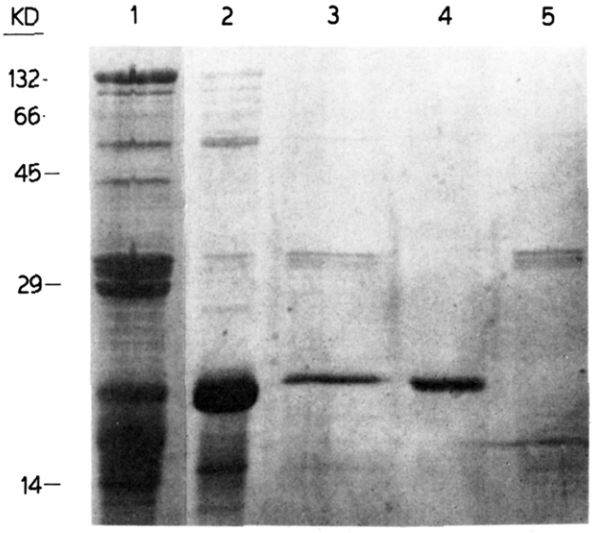

Figure 5. SDS gel electrophoresis of soybean monophosphatase in 10–20% gradient acrylamide. Samples: whey, lane 1; MP enzyme obtained from DEAE chromatography, lane 2; MP enzyme after calmodulin affinity, lane 3; peaks A and B from Figure 3, lanes 4 and 5. Protein markers (Sigma): urease, 132 kDa; BSA, 66 kDa; egg albumin, 45 kDa; carbonic anhydride, 29 kDa; α-lactalbumin, 14 kDa.

this issue further. Results are given in Figure 4C. The sample used in Figure 4C was heated at 95 °C for 5 min prior to DEAE chromatography on the assumption that heat denaturation would leave heat-stable low molecular weight proteins in solution and make it easier to identify CAM-like molecules. Indeed, in Figure 4C, multiple protein components were present in the specially prepared CAM sample. Perhaps, the small subunit of the MP enzyme (the 74-min peak in Figure 4A) is one of these proteins. We are collecting enough of these components to investigate further.

Figure 5 gives results from gel electrophoresis of protein samples obtained at different stages of MP enzyme purification. DEAE chromatograpy of soybean whey (lane 1) concentrated the MP enzyme at 24 kDa (lane 2). After CAM affinity chromatography (lane 3), the enzyme was about 90% pure. Small amounts of contaminants may include high molecular weight proteins such as 7S (>45 kDa) and 11S (29-kDa region) proteins and low molecular weight proteins such as trypsin inhibitors (Kunitz 22 kDa, and Burk 8 kDa) and CAM (18 kDa). Lane 4 is the inactive MP enzyme pooled from the first peak after gel filtration (Figure 3) and lane 5 the protein from the second peak (Figure 3). Interestingly, lane 5 contains small amounts of high molecular weight proteins in addition to a distinct low weight molecular protein of about 20 kDa. It seems that the MP enzyme includes, at least, proteins of 24 and 20 kDa.

Table II. Monophosphatase Activity in Enzyme Preparations from Different Soybean Sources

enzyme source	relative activity: monophosphatase, μM P/mg prot/10 min	relative activity: calmodulin inhibn,[a] %
hypocotyl	0.60	*b*
cotyledon	1.49	45.1
soaked beans[c]	0.39	*b*
germinated beans (3 days)	1.35	48.6
commercial flour	0.65	17.5

[a] See Table I for their measurements. [b] Not determined. [c] Beans washed with distilled water and 0.1% Clorox and then soaked for 8 h.

Table II presents MP enzyme activities in samples from different sources. Cotyledons and germinated beans contained the highest activity, and hypocotyls, soaked beans, and commercial flour contained much less. Ability to inhibit CAM stimulation of PDE activity was also estimated. Cotyledons and germinated beans were equally effective in terms of CAM inhibiting activity. Commercial flour was much less.

Whereas Ni^{2+} ions are essential for legumes and possibly all higher plants (Eskew, 1983), we assayed the MP enzyme in the presence of Ni^{2+} ions. Nickel deprivation in soybeans results in urea accumulation to toxic levels, delayed nodulation, and reduced early growth. In our assays of MP activity, we noticed that crude enzyme preparation required less nickel for optimum activity than did purified preparations. During purification of the MP enzyme we continuously eliminated white precipitates, which appeared to be phytates because of insolubility and high phosphorus content of approximately 12% by weight. Gradual elimination of nickel in these precipitates might account for the observed effect of nickel supplementation on purified MP enzyme.

DISCUSSION

An MP enzyme was isolated from soybean seeds as well as from germinated soybeans. The enzyme appears to be located more in cotyledons than in hypocotyls (Table II). The purified MP enzyme, which is estimated over 90% pure, contains at least two different size subunits (24 and 20 kDa) and binds CAM reversibly in the presence of Ca^{2+} ions. Subunits of each size are required for phosphatase activity (Table I), but the ability to inhibit CAM stimulation of PDE activity resides in 20-kDa subunits. Addition of CAM to the reaction mixture can stimulate the phosphatase activity.

It is not surprising to find various calcium-binding proteins in soybeans. Rather, it is interesting to find intricate interrelationships between these proteins. In soybeans, CAM can stimulate PDE activity with or without added Ca^{2+} ions.

CAM can also stimulate MP activity in soybeans, and the MP enzyme can, in turn, inhibit CAM stimulation of PDE activity. All of these proteins might interact in soybean to regulate phosphate-dependent metabolic processes. It is also interesting to speculate that the 20-kDa

subunit of the MP enzyme is enough like the CAM molecule to compete with it in such protein interactions.

ABBREVIATIONS

CAM, calmodulin (soybean calcium-binding protein); cAMP, cyclic adenosine monophosphate; DEAE, (diethylamino)ethyl; EGTA, ethylene glycol bis(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid; HPLC, high-pressure liquid chromatography; MP, monophosphatase; PDE, phosphodiesterase; W-7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide.

Registry No. MP, 9013-05-2; PDE, 9025-82-5.

LITERATURE CITED

Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248-254.
Eskew, D. *Science (Washington, D.C.)* **1983**, *222*, 621-623.
Fling, S. P.; Gregerson, D. S. *Anal. Biochem.* **1986**, *155*, 83-88.
Hart, C. R.; Hice, R. E.; Charbonneau, H.; Putnam-Evans, C.; Cormier, M. *J. Anal. Biochem.* **1983**, *135*, 208-220.
Mayer, F. C.; Campbell, R. E.; Smith, A. K.; McKinney, L. L. *Arch. Biochem. Biophys.* **1961**, *94*(2), 301-307.
Pallen, C. J.; Wang, J. H. *J. Biol. Chem.* **1983**, *258*, 8550-8553.
Peng, C. M.; Wang, Y. L. *Chin. J. Physiol. (P.R.C.)* **1954**, *19*, 249-267.
Ravi, K.; Rip, J. W.; Carroll, K. K. *Biochem. J.* **1983**, *213*, 513-518.
Sharma, R. K.; Wang, J. H. In *Advances in Cyclic nucleotides*; Brooker, G., Greengard, P., Robinson, G. A., Eds.; Raven: New York, 1979; pp 187-189.
Sutardi; Buckle, K. A. *J. Food Biochem.* **1986**, *10*, 197-216.

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Comparative Digestibility of Legume Storage Proteins

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Native and heated legume storage proteins were digested with various proteinases. Gel electrophoresis patterns indicated that phaseolin (dry bean) was most resistant to digestion, vicilin (pea) was most susceptible, and glycinin and β-conglycinin (soybean) were intermediate in susceptibility to various proteinases. The native proteins were cleaved by trypsin and chymotrypsin in only limited areas of the molecule, but they were all readily degraded upon heating. N-Terminal sequence analysis of the major breakdown products from phaseolin digestion and hydrophilicity and surface probability determinations indicated that trypsin, chymotrypsin, and papain cleave native phaseolin near the center of the protein molecule at a hydrophilic region predicted to be on the surface. This region in phaseolin is highly homologous in sequence with hydrophilic regions in vicilin and β-conglycinin.

The resistance of native legume proteins to proteolysis by mammalian digestive enzymes is an important factor contributing to the poor nutritive value of the unheated protein (Romero and Ryan, 1978; Liener and Thompson, 1980). Considerable attention in this regard has focused on phaseolin, the major storage protein of dry beans, *Phaseolus vulgaris*. While heated phaseolin is readily susceptible to proteolysis (Liener and Thompson, 1980; Bradbear and Boulter, 1984; Deshpande and Nielsen, 1987), native phaseolin has been shown to be largely resistant to complete hydrolysis by trypsin, chymotrypsin, and pepsin (Romero and Ryan, 1978; Liener and Thompson, 1980; Bradbear and Boulter, 1984; Deshpande and Nielsen, 1987). The inaccessibility of phaseolin to enzymes has been attributed to its structural properties (Romero and Ryan, 1978), particularly its compact structure (Chang and Satterlee, 1981). Native phaseolin is much more resistant to pepsin than trypsin and is more rapidly hydrolyzed by trypsin than chymotrypsin (Romero and Ryan, 1978; Vaintraub et al., 1979; Liener and Thompson, 1980; Deshpande and Nielsen, 1987). Trypsin and chymotrypsin have been shown to cleave native phaseolin in such a way that the halves of the molecule remain intact (Liener and Thompson, 1980; Bradbear and Boulter, 1984; Deshpande and Nielsen, 1987). Previous studies have also observed that the patterns of native phaseolin disappearance and the appearance of degradation products suggest each subunit is cleaved in a similar position near the center of the subunit (Romero and Ryan, 1978; Deshpande and Nielsen, 1987). However, enzyme cleavage sites for native or heated phaseolin have not been determined. Information on the nucleotide sequence of phaseolin subunits (Slightom et al., 1983) now allows these determinations to be made.

Legume proteins are known to differ in their nutritive value. When the nutritive value of unheated dry beans, peas, and soybeans is compared, that of dry beans is lowest, that of peas highest, while that of soybeans intermediate (Evans and Bandemer, 1967). While the nutritive value of dry beans and soybeans is greatly increased by heat treatment, that of peas is not appreciably increased (Evans and Bandemer, 1967). The described differences in nutritive value exist between the three legumes despite the fact that they contain nearly equal quantities of methionine and/or cystine (Kakade, 1974). It has not been determined whether these differences in nutritive value between legume proteins can be accounted for by the digestibility of the proteins.

Digestion of native and heated phaseolin by various proteinases has been studied (Deshpande and Nielsen, 1987), but essentially no information is available on the digestion of vicilin (*Pisum sativum*). While tryptic hydrolysis of glycinin and β-conglycinin (*Glycine max*) has been examined (Lynch et al., 1977; Kamata and Shibasaki, 1978; Kamata et al., 1979a,b, 1982), little is known about the degradation of these proteins by other proteinases. One research group (Vaintraub et al., 1976, 1979) observed

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