protein kinase with decreasing substrate concentration has been reported by Lin and Key (1976) and was attributed to a heterogeneous substrate with one of the proteins in the mixture inhibiting the kinase.

Using more enzyme will increase phosphate incorporation for up to 3 units of the enzyme. As seen in Figure 8 the phosphate incorporation levels off with additional levels of the enzyme. As mentioned previously, there are only so many sites on the soy proteins with the appropriate sequence that can be phosphorylated with the enzyme so the presence of more enzyme will not increase the degree of phosphorylation after all available sites are phosphorylated and a steady state is attained.

The effects of enzymatic phosphorylation of soy proteins by protein kinase on alterations in the isoelectric points of these proteins is under investigation. Initial studies to determine whether the soy proteins and storage proteins like glycinin and β-conglycinin could be carried out enzymatically have proven successful. This study represents the optimization of methodology and reaction conditions for the phosphorylation of soy protein substrates with a commercial preparation of cAMPdPK. Future studies will focus on scaling up the reaction and characterizing the phosphorylated proteins in relation to their solubility under acidic conditions with a view to utilization of the economical soy proteins in various food products such as beverages, coffee whiteners, or mayonnaise.

ACKNOWLEDGMENT

I gratefully acknowledge the technical assistance and contributions of S. M. Guillard.

LITERATURE CITED

Coates, J. B.; Medeiros, J. B.; Thanh, V. H.; Nielsen, N. C. Characterization of the subunits of β-conglycinin. *Arch. Biochem. Biophys.* **1985**, *243*, 184–194.

Hirotsuka, M.; Taniguchi, H.; Narita, H.; Kito, M. Functionality and Digestibility of a Highly Phosphorylated Soybean Protein. *Agric. Biol. Chem.* **1984**, *48*, 93–100.

Hoshi, Y.; Yamauchi, F. Determination of Sulfhydryl and Disulfide Contents of Soybean 11 S Globulin and Their Change by Lyophilization. *Agric. Biol. Chem.* **1983**, *47*, 2435–2440.

Kinsella, J. E.; Damodaran, S.; German, B. Physicochemical and Functional Properties of Oilseed Proteins with Emphasis on Soy Proteins. *New Food Proteins* **1985**, *5*, 107–178.

Krebs, E. B.; Beavo, J. A. Phosphorylation-Dephosphorylation of Enzymes. *Annu. Rev. Biochem.* **1979**, *48*, 923–959.

Lin, P. P. C.; Key, J. L. Lysine-Rich Histone H1 Kinase from Soybean Hypocotyl. *Biochem. Biophys. Res. Commun.* **1976**, *73*, 396–403.

Moreira, M. A.; Hermodson, M. A.; Larkins, B. A.; Nielson, N. C. Comparison of the Primary Structure of the acidic Polypeptides of Glycinin. *Arch. Biochem. Biophys.* **1981**, *210*, 633–642.

Roskoski, R. Assay of Protein Kinase. *Methods Enzymol.* **1983**, *99*, 3–6.

Ross, L. F.; Bhatnagar, D. *J. Agric. Food Chem.* **1989**, in press.

Staswick, P. E.; Hermodson, M. A.; Nielson, N. C. The Amino Acid Sequence of the A_2B_{1a} Subunit of Glycinin. *J. Biol. Chem.* **1984**, *259*, 13424–13430.

Sung, H. Y.; Chen, H. J.; Liu, T. Y.; Su, J. C. "Improvement of the Functionalities of Soy Protein Isolate through Chemical Phosphorylation. *J. Food Sci.* **1983**, *48*, 716–721.

Sykes, G. E.; Gayler, K. E. Detection and Characterization of a New β-Conglycinin. *Arch. Biochem. Biophys.* **1981**, *210*, 525–530.

Received for review November 23, 1988. Accepted May 8, 1989. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of this product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

Identification by Two-Dimensional NMR of *myo*-Inositol Tris- and Tetrakis(phosphates) Formed from Phytic Acid by Wheat Phytase

Brian Q. Phillippy

The pathway of hydrolysis of phytic acid (*myo*-inositol hexakis(phosphate)) by wheat phytase from the tetrakis(phosphate) to the tris(phosphate) level was determined. Individual tetrakis(phosphate) isomers were hydrolyzed to tris(phosphates), which were purified by ion-exchange chromatography. Chemical shifts were determined by one-dimensional ^1H NMR, and structural assignments were confirmed by two-dimensional ^1H–^1H NMR. Hydrolysis of D-*myo*-inositol 1,2,5,6-tetrakis(phosphate) produced a mixture of D-*myo*-inositol 1,2,6-tris(phosphate) and D-*myo*-inositol 1,5,6-tris(phosphate). Hydrolysis of L-*myo*-inositol 1,2,3,4-tetrakis(phosphate) gave only *myo*-inositol 1,2,3-tris(phosphate). The role of phytic acid in inositol phosphate mediated calcium metabolism may depend on whatever functions its hydrolysis products are found to perform.

The recent discoveries of IP_6 (*myo*-inositol hexakis(phosphate), phytic acid) in a variety of animal cells (Heslop et al., 1985; Morgan et al., 1987; Jackson et al., 1987; Tilly et al., 1987) bring the possibility that it plays a role in the inositol phosphate pathway regulating calcium metabolism in plant (Schumaker and Sze, 1987; Rincon and Boss, 1987) and animal (Streb et al., 1983) cells. D-$I(1,4,5)P_3$ has shown more potency than any other inositol phosphate in mobilizing calcium from intracellular stores (Irvine et al., 1984, 1986), specifically identified as endoplasmic reticulum (Delfert et al., 1986). D-$I(1,3,4,5)P_4$ can control entry of calcium into cells (Irvine and Moor, 1986) and can be dephosphorylated to D-$I(1,3,4)P_3$, which has

Southern Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, 1100 Robert E. Lee Boulevard, New Orleans, Louisiana 70124.

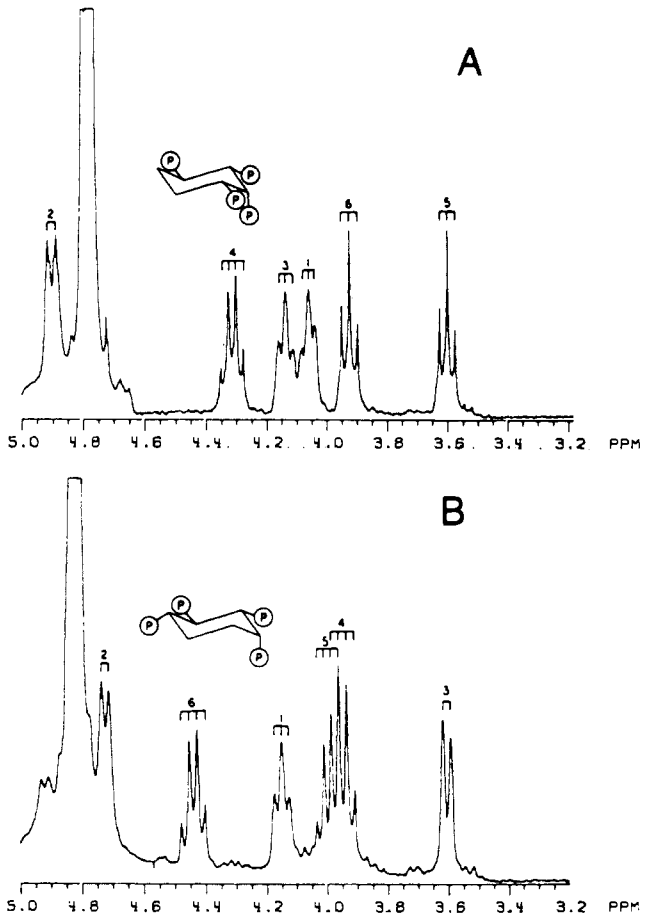

Figure 1. ^1H NMR of L-I(1,2,3,4)P_4 (A) and D-I(1,2,5,6)P_4 (B).

a positive polarizing effect on membrane potential opposite to that of D-I(1,4,5)P_3 (Tertoolen et al., 1987). Compared to the other IPs, relatively large amounts of IP_5 have been found in cancer cells (Jackson et al., 1987; Tilly et al., 1987). This IP_5, assumed to be the I(1,3,4,5,6)P_5 isomer (Irvine and Moor, 1987), could be the precursor of IP_6.

Since IP_6 is present in large amounts in seeds, an appropriate enzyme to use in studying its breakdown is wheat phytase, a nonspecific acid phosphatase (Courtois, 1947). Wheat phytase produces predominantly L-I(1,2,3,4,5)P_5 in the initial dephosphorylation of phytic acid (Johnson and Tate, 1969). Tomlinson and Ballou (1962) used this enzyme to prepare IP_3 and IP_4 fractions from IP_6, which were identified by periodate oxidation, borohydride reduction, and dephosphorylation to alditols. They concluded that the IP_3 fraction contained I(1,2,3)P_3 and D-I(1,2,6)P_3 (configurations have been changed to correspond to currently accepted nomenclature). The IP_4 fraction contained D-I(1,2,5,6)P_4, and another IP_4 that was later identified as L-I(1,2,3,4)P_4 (Lim and Tate, 1973).

With use of improved separation techniques all of the above plus an additional IP_3 have been isolated and precursor–product relationships have been defined. Structural designations were made by two-dimensional ^1H–^1H correlation spectroscopy (COSY) based on data reported for other IP isomers (Cerdan et al., 1986; Lindon et al., 1987).

EXPERIMENTAL PROCEDURES

Materials. Sodium phytate and wheat phytase were obtained from Sigma Chemical Co. (St. Louis, MO). AG1-X8 200–400-mesh anion-exchange resin was from BioRad Laboratories (Richmond, CA).

Preparation of IPs. L-I(1,2,3,4)P_4 and D-I(1,2,5,6)P4 were prepared as described previously (Phillippy et al., 1987). A 30-mg portion of each IP_4 was dissolved in 10 or 20 mL of H_2O at room temperature, the pH was adjusted to 5.0, and 10 or 15 mg of

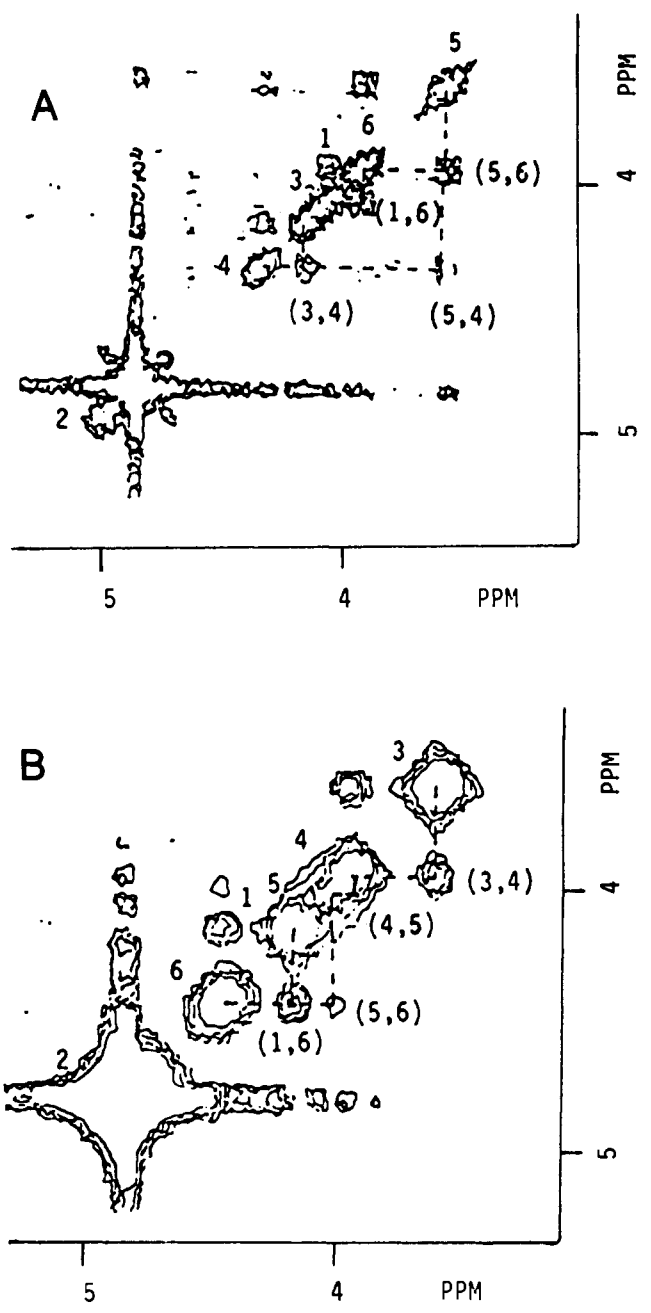

Figure 2. ^1H–^1H COSY of L-I(1,2,3,4)P_4 (A) and D-I(1,2,5,6)P_4 (B).

phytase was added. At hourly intervals, 0.2 mL of the mixture was added to 0.2 mL of 1.2% HCl, passed through a 0.45-μm Millex HV-4 filter, and monitored by gradient ion chromatography (Phillippy and Bland, 1988). Additional phytase was added until most of the IP_4 had been converted to IP_3. The sample was then diluted to 100 mL and applied to a 2 × 25 cm column of AG1-X8 200–400-mesh anion-exchange resin. IPs were eluted with a gradient of 2 L of 0–1 N HCl at a flow rate of 2 mL/min, and 20-mL fractions were collected in 100 test tubes. To obtain the elution profile, 1 mL from each tube was combined with 2 mL of 0.05% $Fe(NO_3)_3 \cdot 9H_2O$ in 1% $HClO_4$, and the absorbance was read at 290 nm. Five tubes corresponding to the center of each IP_3 peak were pooled, diluted to 500 mL, and applied to a 2-g column of AG1-X8, 200–400 mesh, at approximately 0.5 mL/min. IP_3 was eluted with 10 mL of 1 N HCl and lyophilized to remove the HCl. Samples were then dissolved in 3 mL of H_2O, and the pH was adjusted to 7.0. Following lyophilization, samples were dissolved in 99.8% ^2H_2O and lyophilized once again.

NMR. ^1H NMR spectra were obtained by Spectral Data Services (Champaign, IL) on a Spectral Data Services 360-MHz NMR spectrometer at 25 °C. Approximately 5–10-mg portions of individual IPs were dissolved in 1 mL of 99.96% ^2H_2O and

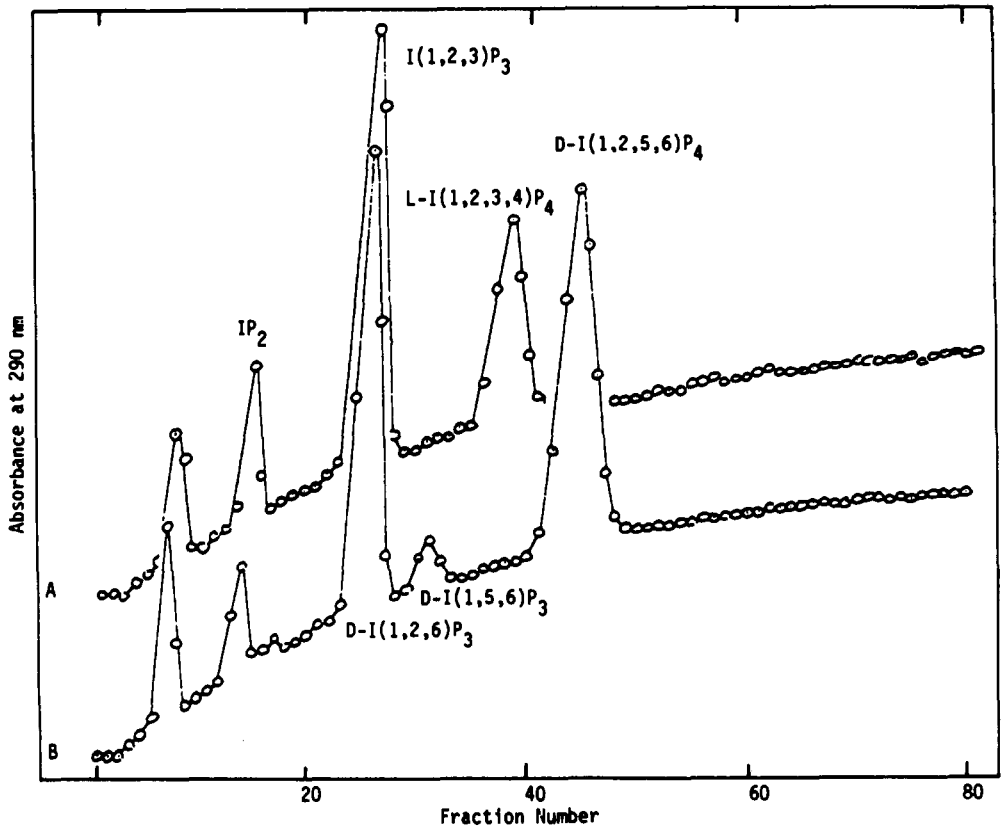

Figure 3. Formation of IP_3s from L-I(1,2,3,4)P_4 (A) and D-I(1,2,5,6)P_4 (B) by hydrolysis with wheat phytase.

transferred to 5-mm NMR tubes. A 16 000-point data set was obtained for the one-dimensional spectrum, while a 512-point data set with 256 increments and 32 transients/increment was obtained for the two-dimensional COSY. For the latter, the recycle time was 3 s. Sweep width was 2000 Hz.

RESULTS

In these studies one-dimensional ^1H NMR was used to confirm the purity of the IPs, to determine the chemical shifts of the six ring proton resonances, and to observe couplings. Correlated two-dimensional ^1H–^1H NMR was used to confirm the structural assignments. The one-dimensional spectrum of each IP_4 clearly indicated the presence of only one species (Figure 1). The two-dimensional spectra in Figure 2 confirmed the structural designations of L-I(1,2,3,4)P_4 and D-I(1,2,5,6)P_4 made previously (Tomlinson and Ballou, 1962; Lim and Tate, 1973). There were no cross peaks from the proton at position 2 possibly due to its distance downfield from protons 1 and 3 or interference from the large water shift at 4.8 ppm.

The fractionation patterns of the IP_3s obtained by the phytase hydrolysis of L-I(1,2,3,4)P_4 and D-I(1,2,5,6)P4 are shown in Figure 3. One peak was obtained from the former, whereas two were obtained from the latter. The one-dimensional spectra (Figures 4) indicate the purities and identifications of the three IP_3s. In Figure 5 are shown the two-dimensional spectra from which the structural assignments were made. The IP_3 from L-I(1,2,3,4)P_4 was identified as I(1,2,3)P_3. The larger IP_3 peak formed from D-I(1,2,5,6)P_4 was D-I(1,2,6)P_3 while the smaller peak proved to be D-I(1,5,6)P_3.

The chemical shifts of the IP_4s and IP_3s are listed in Table I. By comparison, it can be clearly seen that phosphorylation at any position caused the proton shift at the same position to move downfield several tenths of a ppm. Protons adjacent to phosphorylated positions also tended to move downfield. One exception, also observed by Cerdan et al. (1986), was that when position 2 was phosphorylated, the chemical shift of the adjacent proton was decreased. In addition, when position 2 was phosphorylated, phosphorylation at position 3 caused the proton at position 4 to move upfield.

Table I. ^1H NMR Chemical Shifts of Inositol Phosphates at pH 7

	proton position no.					
	1	2	3	4	5	6
L-I(1,2,3,4)P_4	4.07	4.91	4.14	4.32	3.61	3.93
D-I(1,2,5,6)P_4	4.15	4.72	3.60	3.95	4.00	4.44
I(1,2,3)P_3	3.90	4.96	3.90	3.68	3.19	3.68
D-I(1,2,6)P_3	4.11	4.72	3.52	3.81	3.52	4.30
D-I(1,5,6)P_3	4.09	4.23	3.67	3.84	4.01	4.40

Couplings between trans-axial protons at adjacent positions produced the expected doublet of doublets, which collapsed into triplets with J_{H-H} of ≈10 Hz. However, couplings of the equatorial proton at position 2 with adjacent cis-axial protons on the order of J_{H-H} of ≈2 Hz (Cerdan et al., 1986) were too weak to observe. Phosphorylation at any position caused additional splitting similar to that of adjacent trans protons with J_{H-P} of approximately 10 Hz.

DISCUSSION

These results were consistent with the pattern that this enzyme displays by first removing the phosphates at positions 1 and 5 of L-I(1,2,3,4,5)P_5. In every case the phosphorylated positions were consecutive and either of the phosphates neighboring a free hydroxyl position was removed. This pattern allowed the IP_3s formed from D-I(1,2,5,6)P_4 to be assigned the D configuration with a fairly high degree of certainty. The absence of detectable D-I(1,2,6)P_3 (L-I(2,3,4)P_3) as a product from L-I(1,2,3,4)P_4 was unexpected. From Figure 3 it is evident that D-I(1,2,6)P_3 would have, if present, coeluted with I(1,2,3)P_3. Although there did appear to be some contaminant in the I(1,2,3)P_3 isolate (Figure 4A), it did not correspond to either of the other IP_3s in Figure 4B,C.

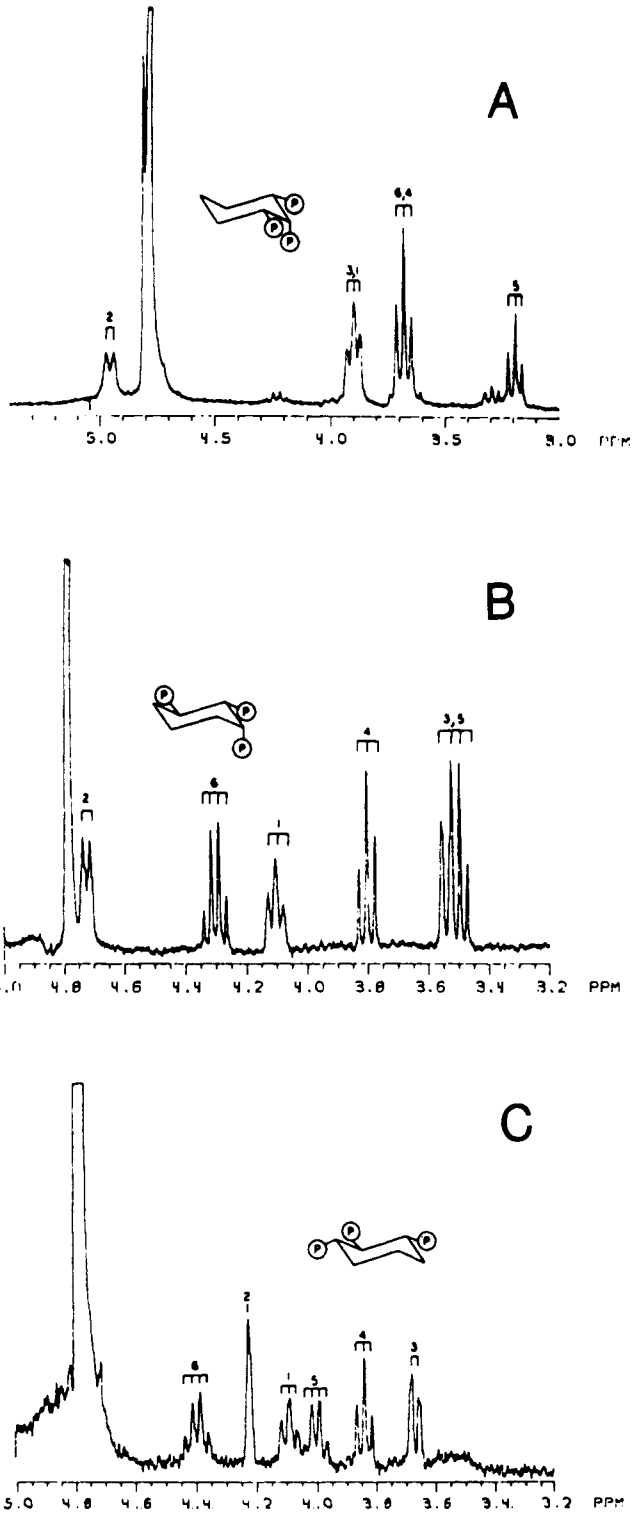

Figure 4. ^1H NMR of I(1,2,3)P_3 (A), D-I(1,2,6)P_3 (B), and D-I(1,5,6)P_3 (C).

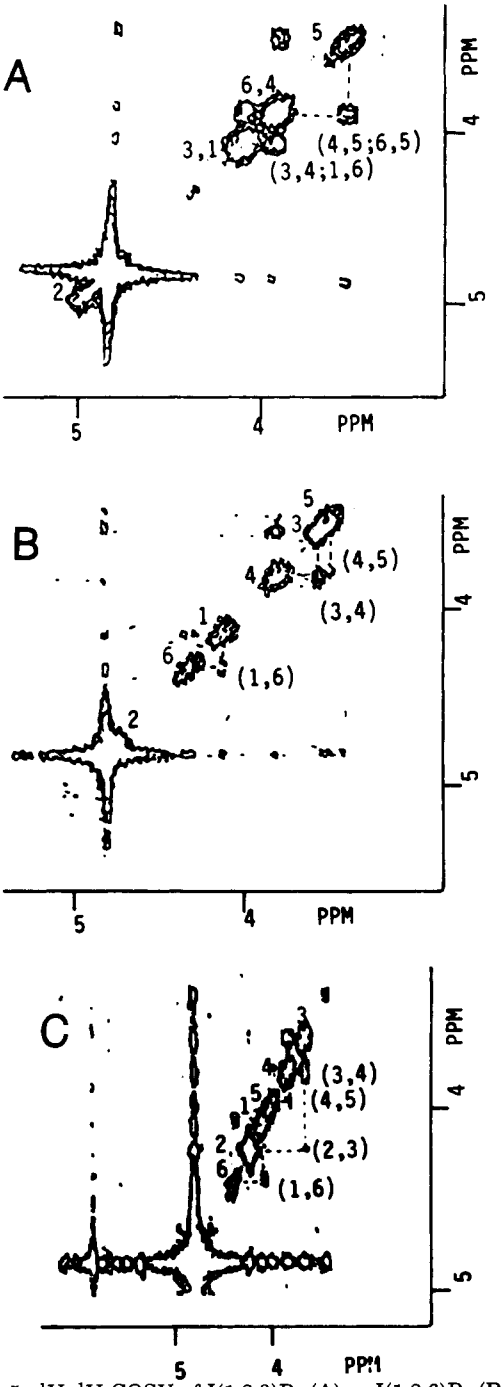

Figure 5. ^1H–^1H COSY of I(1,2,3)P_3 (A), D-I(1,2,6)P_3 (B), and D-I(1,5,6)P_3 (C).

Although the biosynthetic pathway to IP_6 has yet to be determined, there is a good possibility that the intermediate IP_3s and IP_4s will include nonconsecutively phosphorylated isomers such as D-I(1,3,4,5)P_4 and I(1,3,4,6)P_4, which have been identified in animal cells (Balla et al., 1987). From a metabolic standpoint, separating the anabolic and catabolic pathways of IP_6 into those involving nonconsecutively and consecutively phosphorylated IPs, respectively, would seem logical.

While the levels of IP_5 and IP_6 do not appear to change as rapidly as the other IPs in cancer (Jackson et al., 1987; Tilly et al., 1987) cells, in pituitary gonadotrophs their responses were similar to that of I(1,4,5)P_3 (Morgan et al., 1987). The authors of the latter report suggested that a biphasic rise of I(1,4,5)P_3 might be caused by the coelution of an unknown isomer produced from higher IPs. If the IP_5 is predominantly I(1,3,4,5,6)P_5 as suggested (Irvine and Moor, 1987), the products should be different from those identified here. Hydrolysis according to the pattern observed above would lead to I(1,4,5,6)P_4, which has been found in turkey blood (Mayr and Dietrich, 1987), I-(1,5,6)P_3, and I(4,5,6)P_3.

The role of IP_6 in IP-mediated calcium metabolism may depend on whatever function its hydrolysis products are found to perform. Hydrolysis of the IPs derived from IP_6 could increase the levels of other isomers by increasing the levels of IP_2s and IP_1s, which could in turn be phosphorylated in different positions. A remote possibility is the conversion of D-I(1,2,5,6)P_4 to L-I(1,3,4,5)P_4 and

D-I(1,5,6)P_3 to L-I(1,4,5)P_3 by the acid-catalyzed migration of cis phosphates (Tomlinson and Ballou, 1961). At this time it is unknown whether the L forms of the second messengers possess the biological activities of the D enantiomers. However, L-I(1,4,5)P_3 has recently been shown to display little if any second messenger activity (Polokoff et al., 1988; Strupish et al., 1988).

In germinating seeds, where abundant IP_6 hydrolysis occurs (Scott and Loewus, 1986), it would seem unlikely for its products not to participate to some degree in mediating calcium fluxes. If they are not restricted through compartmentation, they could compete for the kinases and phosphatases that regulate the concentrations of the biologically active isomers. It remains to be determined to what extent the isomers identified in this study accumulate in germinating seeds. Surprisingly, the most abundant IP_5 in ungerminated soybean seeds is I(1,2,4,5,6)P_5 of unknown enantiomeric composition (Phillippy and Bland, 1988). This IP_5, which tends to coelute with I(1,3,4,5,6)P_5 on ion-exchange columns (Phillippy et al., 1987; Cosgrove, 1969), could be formed from I(1,2,4,5)P_4 in the biosynthetic pathway of IP_6 (Igaue et al., 1982).

ACKNOWLEDGMENT

I thank Michael S. Fisher for help in interpreting the NMR data.

ABBREVIATIONS USED

IP, inositol phosphate; IP_1, IP_2, IP_3, IP_4, IP_5, and IP_6, *myo*-inositol mono-, bis-, tris-, tetrakis-, pentakis-, and hexakis(phosphates), respectively, with isomeric lettering and numbering as appropriate; COSY, correlation spectroscopy.

LITERATURE CITED

Balla, T.; Guillemette, G.; Baukal, A. J.; Catt, K. J. Formation of Inositol 1,3,4,6-Tetrakisphosphate during Angiotensin II Action in Bovine Adrenal Glomerulosa Cells. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 199–205.

Cerdan, S.; Hansen, C. A.; Johanson, R.; Inubushi, T.; Williamson, J. R. Nuclear Magnetic Resonance Spectroscopic Analysis of myo-Inositol Phosphates Including Inositol 1,3,4,5-Tetrakisphosphate. *J. Biol. Chem.* **1986**, *261*, 14676–14680.

Cosgrove, D. J. Ion-exchange Chromatography of Inositol Polyphosphates. *Ann. N.Y. Acad. Sci.* **1969**, *165*, 677–686.

Courtois, J. Recherches sur la Phytase III. Essais de Separation de l'Activite Glycerophosphatasique et de l'Activite Phytasique du son de ble. *Biochim. Biophys. Acta* **1947**, *1*, 270–276.

Delfert, D. M.; Hill, S.; Pershadsingh, H. A.; Sherman, W. R.; McDonald, J. M. myo-Inositol 1,4,5-Trisphosphate Mobilizes Ca^{2+} from Isolated Adipocyte Endoplasmic Reticulum but not from Plasma Membranes. *Biochem. J.* **1986**, *236*, 37–44.

Heslop, J. P.; Irvine, R. F.; Tashjian, A. H., Jr.; Berridge, M. J. Inositol Tetrakis- and Pentakisphosphates in GH_4 Cells. *J. Exp. Biol.* **1985**, *119*, 395–401.

Igaue, I.; Miyauchi, S.; Saito, K. Formation of myo-Inositol Phosphates in a Rice Cell Suspension Culture. In *Proceedings, 5th International Congress on Plant Tissue & Cell Culture*; Fujiwara, A., Ed.; Maruzen: Tokyo, Japan, 1982.

Irvine, R. F.; Moor, R. M. Micro-injection of Inositol 1,3,4,5-tetrakisphosphate Activates Sea Urchin Eggs by a Mechanism Dependent on External Ca^{2+}. *Biochem. J.* **1986**, *240*, 917–920.

Irvine, R. F.; Moor, R. M. Inositol (1,3,4,5)tetrakisphosphate-induced Activation of Sea Urchin Eggs Requires the Presence of Inositol Trisphosphate. *Biochem. Biophys. Res. Commun.* **1987**, *146*, 284–290.

Irvine, R. F.; Brown, K. D.; Berridge, M. J. Specificity of Inositol Trisphosphate-induced Calcium Release from Permeabilized Swiss-mouse 3T3 Cells. *Biochem. J.* **1984**, *222*, 269–272.

Irvine, R. F.; Letcher, A. J.; Lander, D. L.; Berridge, M. J. Specificity of Inositol Phosphate-stimulated Ca^{2+} Mobilization from Swiss-mouse 3T3 Cells. *Biochem. J.* **1986**, *240*, 301–304.

Jackson, T. R.; Hallam, T. J.; Downes, C. P.; Hanley, M. R. Receptor Coupled Events in Bradykinin Action: Rapid Production of Inositol Phosphates and Regulation of Cytosolic Free Ca^{2+} in a Neural Cell Line. *EMBO J.* **1987**, *6*, 49–54.

Johnson, L. F.; Tate, M. E. The Structure of Myo-Inositol Pentaphosphates. *Ann. N.Y. Acad. Sci.* **1969**, *165*, 526–532.

Lim, P. E.; Tate, M. E. The Phytases II. Properties of Phytase Fractions F_1 and F_2 from Wheat Bran and the myo-Inositol Phosphates Produced by Fraction F_2. *Biochim. Biophys. Acta* **1973**, *302*, 316–328.

Lindon, J. C.; Baker, D. J.; Williams, J. M.; Irvine, R. F. Confirmation of the Identities of Inositol 1,3,4-trisphosphate and Inositol 1,3,4,5-tetrakisphosphate by the Use of One-dimensional and Two-dimensional N.M.R. Spectroscopy. *Biochem. J.* **1987**, *244*, 591–595.

Mayr, G. W.; Dietrich, W. The Only Inositol Tetrakisphosphate Dectable in Avian Erythrocytes is the Isomer Lacking Phosphate at Position 3: a NMR Study. *FEBS Lett.* **1987**, *213*, 278–282.

Morgan, R. O.; Chang, J. P.; Catt, K. J. Novel Aspects of Gonadotropin-releasing Hormone Action on Inositol Polyphosphate Metabolism in Cultured Pituitary Gonadotrophs. *J. Biol. Chem.* **1987**, *262*, 1166–1171.

Phillippy, B. Q.; Bland, J. M. Gradient Ion Chromatography of Inositol Phosphates. *Anal. Biochem.* **1988**, *175*, 162–166.

Phillippy, B. Q.; White, K. D.; Johnston, M. R.; Tao, S.-H.; Fox, M. R. S. Preparation of Inositol Phosphates from Sodium Phytate by Enzymatic and Nonenzymatic Hydrolysis. *Anal. Biochem.* **1987**, *162*, 115–121.

Polokoff, M. A.; Bencen, G. H.; Vacca, J. P.; deSolms, S. J.; Young, S. D.; Huff, J. R. Metabolism of Synthetic Inositol Trisphosphate Analogs. *J. Biol. Chem.* **1988**, *263*, 11922–11927.

Rincon, M.; Boss, W. F. myo-Inositol Trisphosphate Mobilizes Calcium from Fusogenic Carrot (*Daucus carota* L.) Protoplasts. *Plant Physiol.* **1987**, *83*, 395–398.

Schumaker, K. S.; Sze, H. Inositol 1,4,5-Trisphosphate Releases Ca^{2+} from Vacuolar Membrane Vesicles of Oak Roots. *J. Biol. Chem.* **1987**, *262*, 3944–3946.

Scott, J. J.; Loewus, F. A. Phytate Metabolism in Plants. In *Phytic Acid Chemistry and Applications*; Graf, E., Ed.; Pilatus Press: Minneapolis, MN, 1986.

Streb, H.; Irvine, R. F.; Berridge, M. J.; Schulz, I. Release of Ca^{2+} from a Nonmitochondrial Intracellular Store in Pancreatic Acinar Cells by Inositol-1,4,5-trisphosphate. *Nature* **1983**, *306*, 67–69.

Strupish, J.; Cooke, A. M.; Potter, B. V. L.; Gigg, R.; Nahorski, S. R. Steriospecific Mobilization of Intracellular Ca^{2+} by Inositol 1,4,5-Trisphosphate. *Biochem. J.* **1988**, *253*, 901–905.

Tertoolen, L. G. J.; Tilly, B. C.; Irvine, R. F.; Moolenar, W. H. Electrophysiological Responses to Bradykinin and Microinjected Inositol Polyphosphates in Neuroblastoma Cells. *FEBS Lett.* **1987**, *214*, 365–369.

Tilly, B. C.; van Paridon, P. A.; Verlaan, I.; Wirtz, K. W. A.; de Laat, S. W.; Moolenar, W. H. Inositol Phosphate Metabolism in Bradykinin-stimulated Human A431 Carcinoma Cells – Relationship to Calcium Signalling. *Biochem. J.* **1987**, *244*, 129–135.

Tomlinson, R. V.; Ballou, C. E. Complete Characterization of the Myo-inositol Polyphosphates from Beef Brain Phosphoinositide. *J. Biol. Chem.* **1961**, *236*, 1902–1906.

Tomlinson, R. V.; Ballou, C. E. Myoinositol Polyphosphate Intermediates in the Dephosphorylation of Phytic Acid by Phytase. *Biochemistry* **1962**, *1*, 166–171.

Received for review August 24, 1988. Accepted May 1, 1989. Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.