Analysis of Inositol Mono- and Diphosphate Isomers Using High-Performance Ion Chromatography and Pulsed Amperometric Detection

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A rapid and sensitive high-performance ion chromatography method for separation and quantitative determination of inositol mono- and diphosphate (IP_1−IP_2) isomers using pulsed amperometric detection is described. The method involves extraction of samples with HCl, separation of inositol phosphates from the crude extract by anion-exchange chromatography, NaAc gradient elution in an NaOH environment to perform isomer separation on a high-performance anion-exchange column, and detection with pulsed amperometric detector. The applicability and sensitivity of the method is illustrated by measurement of the content of IP_1 and IP_2 in foods, human ileal contents, and enzymatic hydrolysis products of phytate (inositol hexaphosphate). The major IP_1 and IP_2 isomers formed during phytate hydrolysis with wheat phytase were shown to be Ins(2)P, DL-Ins(1)P, and DL-Ins(1,2)P_2 and with *Aspergillus niger* phytase, Ins(2)P and DL-Ins(1,2)P_2.

Keywords: *Inositol mono- and diphosphate isomers; high-performance ion chromatography; pulsed amperometric detection*

INTRODUCTION

Phytate (*myo*-inositol hexaphosphate), the major storage form of phosphorus in plants, can form insoluble mineral−phytate complexes at physiological pH values and thereby inhibit absorption of essential dietary minerals (Cheryan, 1980; Cosgrove, 1966). During processing of foods containing phytate, lower inositol phosphates are formed, that is, inositol phosphates with one, two, or three phosphate groups attached to the inositol ring. A reduction in the number of phosphate groups bonded to the inositol ring gives increased solubility and a decreased ability to form complexes (Jackman and Black, 1951; Kaufman and Kleinberg, 1971). Phytate can be degraded by enzymatic and nonenzymatic means. Enzymatic hydrolysis generally occurs during food bioprocesses, such as bread baking, malting, and fermenting of cereals and vegetables (Larsson and Sandberg, 1992; Nayini and Markakis, 1983; Türk et al., 1996), as a result of activity of the enzyme phytase (EC 3.1.3.26) naturally present in plants that contain phytate. Biotechnically produced phytase may be added in food processing to food considered to have low phytase activity to increase the mineral bioavailability. Fungal species, such as *Aspergillus* spp. and *Saccharomyces* spp., are used to produce phytase (EC 3.1.3.8) (Lyons, 1991). If food contains active phytase, inositol phosphates are further degraded in the alimentary tract of man (Sandberg et al., 1987; Sandberg and Andersson, 1988). Nonenzymatic hydrolysis usually takes place when foods are heat-treated (e.g. autoclaving, canning, extruding) (Phillippy et al., 1987; Sandberg et al., 1987; Tabekhia and Luh, 1980) or when foods are treated with a strong acid (Cosgrove, 1963).

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Biochemical interest in lower inositol phosphates has increased, owing to knowledge of their essential function in signal transmission in the cell (Streb et al., 1983). Furthermore, several isomers of inositol phosphates have shown important physiological functions, such as prevention of diabetes complications (Carrington et al., 1993; Ruf et al., 1991) and anti-inflammatory effects (Claxon et al., 1990). The position of the phosphate groups on the inositol ring is of great importance to their physiological function. If physiologically active lower inositol phosphates, or precursors to these, are absorbed in the alimentary tract of humans, one can expect processed food to exert various physiological effects of importance to health (Holub, 1987).

Difficulties in separating the isomers of inositol phosphates have been reported in connection with several analytic approaches. During the past few years, a number of isomer-specific, high-performance ion-exchange chromatography methods with gradient elution have been developed for use in the separation of inositol phosphates in biological tissues, foods, and intestinal contents (Guse et al., 1995; Mayr, 1988; Phillippy and Bland, 1988; Skoglund et al., 1997; Smith and MacQuarrie, 1988). Analysis has remained a problem, as the inositol phosphates do not absorb visible or ultraviolet light, nor can they be easily identified using specific colorimetric reagents. Electrochemical detectors, measuring current resulting from the application of potential across electrodes in a flow cell, are useful in ion chromatographic analysis. The conductivity of the solution or the current caused by reduction or oxidation of analytes (amperometry) can be measured, depending on how the potential is applied and the current measured. In the present paper, we describe a rapid and very sensitive HPIC method, using pulsed amperometric detection (PAD), for the separation and determination of isomers of inositol mono- and diphosphates in foods and intestinal contents. The method was furthermore applied to the analysis of inositol phosphates during enzymatic hydrolysis of phytate.

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MATERIALS AND METHODS

Materials. *myo*-Inositol 2-monophosphate, dicyclohexylammonium salt, D-*myo*-inositol 1-monophosphate, cyclohexylammonium salt, L-*myo*-inositol 5,6-diphosphate, cyclohexylammonium salt, D-*myo*-inositol 1,4,5-triphosphate, hexasodium salt, and D-*myo*-inositol 1,5,6-triphosphate, ammonium salt, were obtained from Sigma Chemical Co. (St. Louis, MO). *myo*-Inositol 2,4-diphosphate, tetraammonium salt, was obtained from Calbiochem Corp. (La Jolla, CA) and sodium phytate from BDH Chemicals Ltd. (Poole, England). Ins(1,2,6)P_3 was received as a gift from Perstorp Pharma (Perstorp, Sweden). Anion-exchange resin AG 1-X8, 200−400 mesh, was purchased from Bio-Rad (Richmond, CA). Type I deionized water for HPIC was purified by a Millipore water system to a specific resistance of 18 MΩ·cm or greater. Sodium hydroxide 50% solution was purchased from J. T. Baker B.V. (Deventer, Holland). Phytase from a mutant strain of *Aspergillus niger* (*A. niger*), Econase EP 433, was obtained from Alko Ltd. Biotechnology (Rajamäki, Finland) and wheat phytase from Sigma. All other reagents used were of analytical grade.

Sample Preparation. The preparation of foods and ileostomy samples was performed according to the method of Sandberg and Ahderinne (1986), as modified by Skoglund et al. (1997). Duplicate samples of 0.5 g of freeze-dried and ground sourdough fermented rye roll or the intestinal contents from an ileostomy subject consuming raw wheat bran were extracted with 20 mL of 0.5 M HCl for 3 h at 20 °C under agitation. Duplicate samples of 0.5 g of pea flour were soaked in 5 mL of buffer, at pH 7 and 45 °C, and duplicate samples of 0.5 g of malted oat were soaked in 5 mL of water, at pH 4.5 and 38 °C, for 20 h. The pea and oat samples were extracted with 15 mL of 0.67 M HCl for 3 h at 20 °C. The extracts were centrifuged, and the supernatant was decanted, frozen to precipitate gelatinous agents, and centrifuged again. Fifteen milliliters of the supernatant was taken out and evaporated to dryness and dissolved in 15 mL of water. The inositol phosphates were separated from the crude extract by ion-exchange chromatography. Plastic columns with porous polymer filters containing 2.5 mL of resin (AG 1-X8, 200−400 mesh) were used. The samples were washed with 5 and 10 mL of water, and inositol phosphates were removed from the resin with five 4 mL portions of 2 M HCl. The eluants were evaporated to dryness and diluted with 1−5 mL of water.

Recoveries of inositol monophosphate [Ins(2)P, 1 μmol] added to replicates of the rye roll sample were prepared and analyzed in duplicate and compared with nonspiked rye roll samples. Recoveries of replicates of inositol monophosphate [Ins(2)P, 1 μmol] from the AG 1-X8 column were investigated by comparison of peak areas before and after they were passed through the column. To study the repeatability of the method, five replicates of the pea flour and malted oat extracts were prepared and analyzed in duplicate. The linearity of inositol mono- and diphosphate concentrations versus peak area was investigated, and the response factors were determined by 25 μL injections of solutions containing 0.01−100 nmol/mL of Ins(2)P and L-Ins(5,6)P_2.

Enzymatic degradations of phytate were prepared with 25 mL of 1.5 μmol/mL sodium phytate and 20 mg of wheat phytase (0.05 U/mg) or 1.25 mL of *A. niger* phytase (23.8 U/mL). The solutions were stirred in a water bath at 55 °C after adjustment of the pH to 5.0 and 3.0, respectively, with 0.5 M HCl. During the enzymatic hydrolysis, aliquots of the reaction mixtures were taken at different times. The enzyme present in the samples was inactivated by adding 10 mL of 2 M HCl. The samples were evaporated to dryness, dissolved in 15 mL of water, and placed on plastic columns containing 2.5 mL of anion-exchange resin (AG 1-X8, 200−400 mesh) and a porous polymer filter. Inositol phosphates were removed from the resin with five 4 mL portions of 2 M HCl, after washing with 5 and 10 mL of water. The eluants were evaporated to dryness and diluted with 1−5 mL of water.

Reference samples for identification of peaks were prepared by dissolving 1.5 and 2.0 g of natrium phytate in 100 mL of 0.5 M HCl each. The solutions were reflux boiled for 12 and 22 h, respectively, and evaporated to dryness, after which 100 and 10 mL of water, respectively, were added to the hydrolyzed samples. The samples were mixed to obtain a reference sample with 25 nmol/mL of IP_1 and 215 nmol/mL of IP_2.

Table 1. Combination of Eluants for the Analysis of Inositol Mono- and Diphosphates

time (min)	NaAc (1 M) (%)	NaOH (1 M) (%)	water (%)
0	0	20	80
20	50	20	30
25	50	20	30
26	0	20	80

Table 2. Waveform Setting for the Cell

time		potential		
t	ms	E	V	integrate
t_1	0.00	E_1	+0.05	
	0.20		+0.05	begin
	0.40		+0.05	end
t_2	0.41	E_2	+0.75	
	0.60		+0.75	
t_3	0.61	E_3	−0.15	
	1.00		−0.15	

Sample Analysis. The chromatograph consisted of an HPLC pump (Merck Hitachi, Model L-6200A, Hitachi Ltd., Tokyo, Japan) equipped with a Reodyne injector loop, 25 μL (Reodyne, Cotati, CA), and an HPIC anion exchange CarboPac PA-10 (4 × 250 mm) analytical column (Dionex Corp., Sunnyvale, CA). IP_1−IP_2 were eluted with a gradient of 0−50% NaAc (1 M) in conjugation with water and NaOH (1 M). The NaOH eluant was prepared from 50% liquid NaOH, 19.1 M (J. T. Baker). The eluants were combined as listed in Table 1. The eluant flow rate was 0.8 mL/min. An equilibration time of 14 min was needed after each run. Inositol mono- and diphosphates were detected using PAD. A basic PAD cell with a gold electrode was used (Dionex ED 40 electrochemical detector). The waveform setting for the cell is shown in Table 2. All eluants were sparged with helium. Detector signals were processed by a laboratory data system (Borwin, Chromatography Software, JMBS Developpments, Grenoble, France). Ins(2)P and L-Ins(5,6)P_2 were used as external standards.

Statistical Evaluation. Data are presented as mean ± standard deviation (SD). For statistical comparisons, the means with their standard deviations were examined using Student's *t*-test (Box et al., 1978).

RESULTS AND DISCUSSION

Pulsed amperometry is a very sensitive electrochemical detection method. The technique is useful for the determination of sugars and alcohols that have a low specific conductance and are poor UV absorbers. A sequence of three different working potentials, E_1, E_2, and E_3, are rapidly repeated for times t_1, t_2, and t_3. In the case of carbohydrates, a −CHOH group is oxidized during t_1 to a C═O, which collects at an electrode of gold. The C═O is then cleaned off the surface of the gold electrode with a second pulse at a higher voltage. Because the gold electrode itself is partly oxidized to gold oxide, it is cleaned by applying a large negative voltage, E_3.

The most commonly used detection method in ion chromatography is electrical conductivity detection (Walton and Rocklin, 1990). With regard to determination of inositol phosphates in ion chromatography, several studies have been made using postcolumn derivatization and detection by UV absorbance (Guse et al., 1995; Mayr, 1988, Phillippy and Bland, 1988; Skoglund et al., 1997), as well as electrical conductivity detection (Skoglund et al., 1997; Smith and MacQuarrie, 1988). These detection methods give increasing sensitivity with an increasing number of phosphate groups attached to the inositol ring. The inverse relation is

valid for PAD, and thus an advantage of this detection method is its high sensitivity to the lower inositol phosphates. The interest in the determination of small amounts of inositol phosphates is great, both in the field of nutrition and in analyses of cells and tissues. Smith et al. (1989) discussed the importance of ion chromatography and the possibility of using PAD to detect IP_1−IP_3 in the study of metabolism. We, however, found the detector responses of inositol phosphates with more than two phosphate groups too small to give useful analyses. The detection limit of inositol triphosphates was 3 nmol/injection [calculated according to IUPAC (1978), $c_{L(k=3)}$].

Here we illustrate the usefulness of the present analytic method for inositol mono- and diphosphate isomers. The peak areas are linearly dependent on the concentrations of inositol monophosphate up to 10 nmol/mL (linear regression analysis gave a correlation coefficient of 1.0 for inositol monophosphates). For inositol diphosphates, a correlation coefficient of 1.0 was obtained at concentrations up to 90 nmol/mL. The slope of the linear calibration curve depends on the number of phosphate groups, owing to differences in detector responses. The correction factor for IP_2 was determined to be 9. The factor was calculated from an analysis of standard samples of IP_1 [Ins(2)P] and IP_2 [L-Ins(5,6)-P_2]. A chromatographic run takes 19 min and gives adequate separation of IP_1 isomers down to 0.04 pmol/injection and of IP_2 isomers down to 0.4 pmol/injection [calculated according to IUPAC (1978), $c_{L(k=3)}$].

There exist 6 possible inositol monophosphates and 1 cyclic and 15 noncyclic inositol diphosphates. Because the cyclic compound is acid-labile, it is difficult to measure and its proportion in various cells is thus unknown (Majerus et al., 1988). Some of the isomers are enantiomers and accordingly not separated on the achiral column. Four IP_1 isomers and nine IP_2 isomers may consequently be separated. Of these, we determined the retention of the four IP_1 isomers and seven IP_2 isomers. The elution orders of the different isomers of IP_1−IP_2 were established on the basis of commercially available inositol phosphate isomers [Ins(2)P, D-Ins(1)P, L-Ins(5,6)P_2, Ins(2,4)P_2] of phytate degraded by *Escherichia coli* phytase (Greiner and Konietzny, 1996) [Ins-(2,5)P_2] and of DL-Ins(1,2)P_2, DL-Ins(2,6)P_2, and DL-Ins(1,6)P_2, found as impurities in a preparation of Ins(1,2,6)P_3 obtained from Perstorp Pharma. The latter IP_2 isomers are the only ones formed from Ins(1,2,6)P_3. The elution orders of isomers Ins(5)P and DL-Ins(1,5)P_2 were determined from impurities in Ins(1,4,5)P_3 and Ins(1,5,6)P_3 (Sigma). The identification of isomers was also performed by determination according to the method of Skoglund et al. (1997) of Ins(2)P, DL-Ins(1)P, DL-Ins-(4)P, DL-Ins(1,4)P_2, DL-Ins(2,4)P_2, and DL-Ins(4,5)P_2. The reference sample was spiked with samples containing the different isomers, whereby it was possible to determine their positions in the chromatogram.

HPIC analysis of the reference sample shows the order in which the inositol phosphate isomers are eluted (Figure 1). The concentration of each peak in the chromatogram is indicated. The method does not resolve Ins(2)P from Ins(5)P, DL-Ins(1,4)P_2 from DL-Ins-(1,6)P_2, or DL-Ins(4,5)P_2 from DL-Ins(1,2)P_2 and Ins(2,5)-P_2. The repeatability of the method is shown in the pea and oat samples for inositol mono- and diphosphates (Table 3). When rye roll samples were spiked with inositol monophosphate standards [1 μmol of Ins(2)P], the recovery of added standards was 98 ± 4%. The

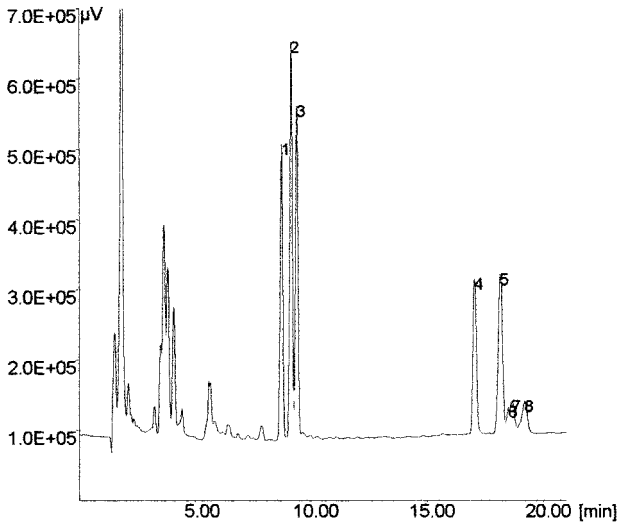

Figure 1. Isomers of inositol mono- and diphosphates in the reference sample. The peak assigned with a star (*) is not identified. Peaks: (1) Ins(2)P, Ins(5)P; (2) DL-Ins(1)P; (3) DL-Ins(4)P; (4) DL-Ins(1,4)P_2, DL-Ins(1,6)P_2; (5) DL-Ins(4,5)P_2, DL-Ins(1,2)P_2, Ins(2,5)P_2; (6) DL-Ins(2,4)P_2; (7) *; (8) DL-Ins(1,5)P_2. Concentrations (injected nmol): (1) 0.18; (2) 0.24; (3) 0.20; (4) 1.25; (5) 1.64; (6) 0.25; (7) 0.34; (8) 1.89.

Table 3. IP_1−FIP_2 Content (Micromoles per Gram) of Selected Samples[a]

IP_1 and IP_2[b]	pea	oat
IP_1	0.098 ± 0.011	0.230 ± 0.036
peak 1	0.059 ± 0.007	0.086 ± 0.012
peak 2	0.032 ± 0.008	0.127 ± 0.015
peak 3	0.007 ± 0.001	0.017 ± 0.03
IP_2	2.286 ± 0.119	5.975 ± 0.347
peak 4	0.932 ± 0.096	0.367 ± 0.042
peak 5	0.550 ± 0.037	4.172 ± 0.302
peak 6	0.390 ± 0.043	trace
peak 7	0.085 ± 0.021	0.453 ± 0.048
peak 8	0.329 ± 0.035	0.983 ± 0.158

[a] Mean ± SD of five replicate extracts of each sample were prepared and analyzed in duplicate with HPIC. [b] Peaks are numbered according to Figure 1.

recovery of IP_1 [1 μmol of Ins(2)P] on the anion-exchange resin (AG 1-X8, 200−400 mesh) was 98 ± 6%.

The choice of samples, to demonstrate the applications of the method, was made on the basis of their origin (food, ileostomy contents, and enzymatic hydrolysates), their amount of inositol mono- and diphosphates, and the differences between various isomers. Samples with peak concentrations above 10 nmol per inositol monophosphate and above 90 nmol per inositol diphosphate had to be diluted in order not to overload the system.

Enzymatic degradations of phytate are illustrated in Figure 2. The conditions were chosen for phytate hydrolysis with wheat phytase and *A. niger* phytase, respectively, according to the optimal conditions for enzymatic activity (Peers, 1953) and optimal degradation of phytate (Türk, 1995), respectively. The major pathways for hydrolysis of *myo*-inositol hexaphosphate by cereal phytase (Johnson and Tate, 1969; Lim and Tate, 1973; Tomlinson and Ballou, 1962; Phillippy, 1989) and microbial phytase (Cosgrove 1970) are indicated below.

We can see in Figure 2a that high amounts of Ins-(2)P/Ins(5)P and DL-Ins(1)P are formed in wheat phytase hydrolysates, with DL-Ins(1)P being dominant. Wheat bran phytase predominantly produces L-Ins(1,2,3,4,5)-P_5 in the initial dephosphorylation of phytic acid (Johnson and Tate, 1969; Lim and Tate, 1973). Further hydroly-

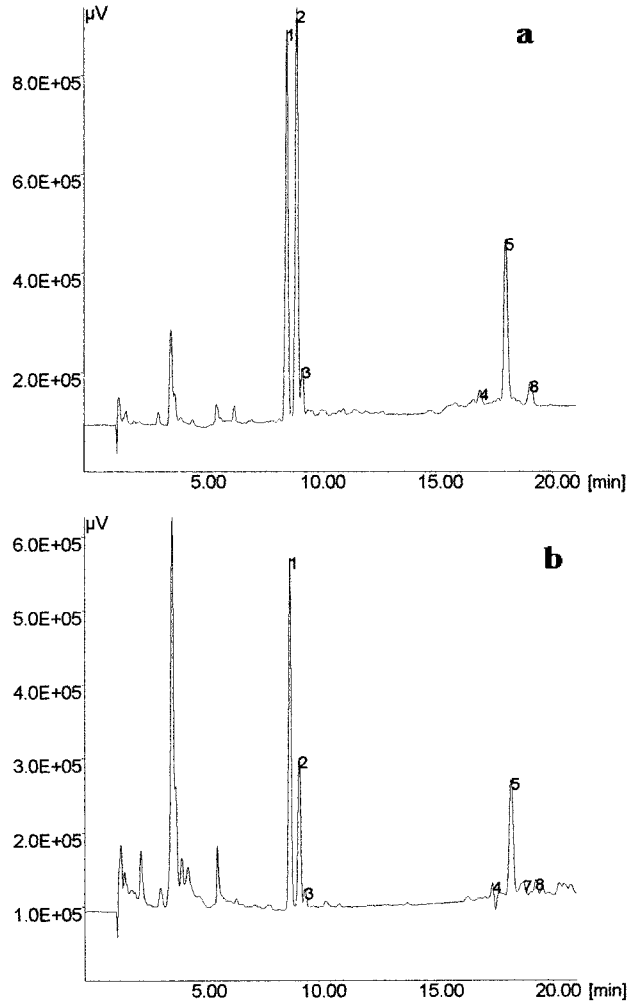

Figure 2. Chromatographic profiles of inositol mono- and diphosphates in enzymatic degradations of phytate and (a) wheat phytase, pH 5, 55 °C, and (b) *A. niger* phytase, pH 3, 55 °C. Peaks are numbered according to Figure 1.

cereal phytase:

$$\text{Ins}(1,2,3,4,5,6)P_6 \rightarrow \text{L-Ins}(1,2,3,4,5)P_5 \begin{cases} \rightarrow \text{L-Ins}(1,2,3,4)P_4 \rightarrow \text{Ins}(1,2,3)P_3 \\ \rightarrow \text{D-Ins}(1,2,5,6)P_4 \rightarrow \text{D-Ins}(1,2,6)P_3 \end{cases}$$

microbial phytase:

$$\text{Ins}(1,2,3,4,5,6)P_6 \rightarrow \text{D-Ins}(1,2,4,5,6)P_5 \rightarrow \text{D-Ins}(1,2,5,6)P_4 \begin{cases} \rightarrow \text{D-Ins}(1,2,5)P_3 \\ \rightarrow \text{D-Ins}(1,2,6)P_3 \end{cases}$$

sis results in L-Ins(1,2,3,4)P_4 and D-Ins(1,2,5,6)P_4 (Lim and Tate, 1973; Tomlinson and Ballou, 1962). Hydrolysis of L-Ins(1,2,3,4)P_4 gives only *myo*-inositol 1,2,3-triphosphate, and hydrolysis of D-Ins(1,2,5,6)P_4 gives D-Ins(1,2,6)P_3 as the major peak according to the results of Phillippy (1989). On basis of these IP_3 isomers formed, of the IP_1 isomers in Figure 2a and with the acceptance of earlier work (Johnson and Tate, 1969; Lim and Tate, 1973), we conclude that the main IP_2 isomer is DL-Ins(1,2)P_2. Figure 2b shows IP_1 and IP_2 isomers from phytate hydrolysis with *A. niger* phytase. The amount of Ins(2)P/Ins(5)P is approximately twice that of DL-Ins(1)P. The same peak of IP_2 isomers dominates when phytate is hydrolyzed with *A. niger* phytase as when it is hydrolyzed with wheat phytase. In contrast to wheat phytase breakdown, the initial dephosphorylation of phytate by microbial phytases gives D-Ins(1,2,4,5,6)P_5 (Cosgrove, 1970). Exceptions to this were found for *E. coli* phytase (Greiner et al., 1993) and for *Paramecium* phytase (Van der Kaay and Van Haastert, 1995), giving D-Ins(1,2,3,4,5)P_5 as the major IP_5. The IP_4 isomer generally produced in phytate degradations by microorganisms appears to be D-Ins(1,2,5,6)P_4 and the IP_3 isomers D-Ins(1,2,5)P_3 and/or D-Ins(1,2,6)P_3 (Cosgrove, 1970). From the IP_1 isomers generated during phytate hydrolysis by *A. niger* phytase (Figure 2b) and in view of earlier data (Cosgrove, 1970), the

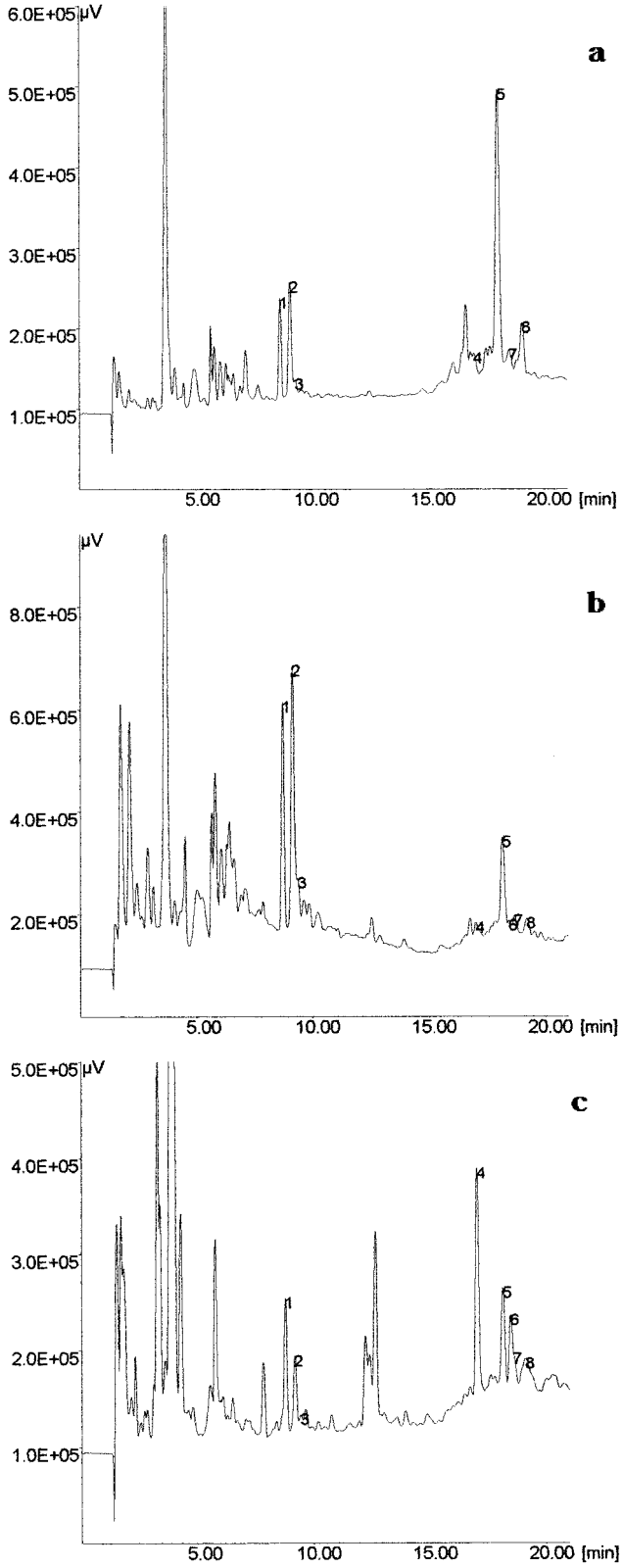

Figure 3. Chromatographic profiles of inositol mono- and diphosphates in (a) malted oat sample, (b) rye roll, and (c) dehulled pea flour. Peaks are numbered according to Figure 1.

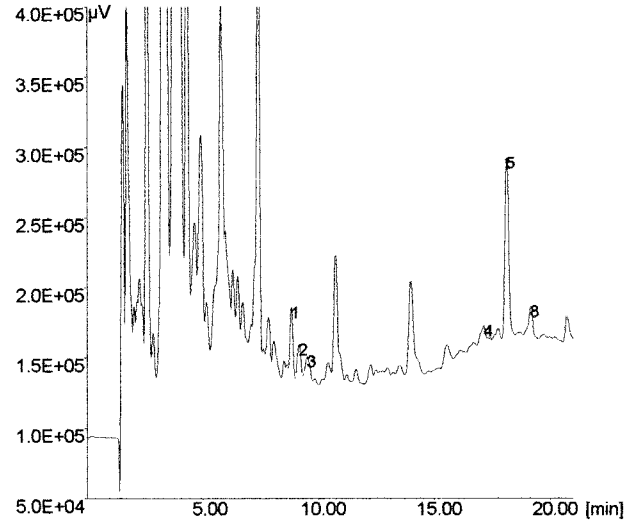

Figure 4. Chromatographic profile of inositol mono- and diphosphates in ileal contents from an ileostomy subject consuming raw wheat bran. Peaks are numbered according to Figure 1.

main IP_2 isomer formed was identified as DL-Ins(1,2)-P_2. We suggest the following pathways for hydrolysis of inositol triphosphate by cereal and microbial phytases:

cereal phytase:

$$\text{Ins(1,2,3,4,5,6)P}_6 \rightarrow \text{L-Ins(1,2,3,4,5)P}_5 \begin{cases} \rightarrow \text{L-Ins(1,2,3,4)P}_4 \rightarrow \text{Ins(1,2,3)P}_3 \\ \rightarrow \text{D-Ins(1,2,5,6)P}_4 \rightarrow \text{D-Ins(1,2,6)P}_3 \end{cases} \rightarrow \text{DL-Ins(1,2)P}_2 \begin{cases} \rightarrow \text{Ins(2)P} \\ \rightarrow \text{DL-Ins(1)P} \end{cases}$$

microbial phytase:

$$\text{Ins(1,2,3,4,5,6)P}_6 \rightarrow \text{D-Ins(1,2,4,5,6)P}_5 \rightarrow \text{D-Ins(1,2,5,6)P}_4 \begin{cases} \rightarrow \text{D-Ins(1,2,5)P}_3 \\ \rightarrow \text{D-Ins(1,2,6)P}_3 \end{cases} \rightarrow \text{DL-Ins(1,2)P}_2 \rightarrow \text{Ins(2)P}$$

Figure 3 shows chromatographic profiles of several food samples. The isomeric pattern in the oat sample (Figure 3a) and that in the rye roll (Figure 3b) are similar to that of wheat phytase (Figure 2a). The same phytate degradation pathway may be assumed in these cereal samples. In the pea flour (Figure 3c), a different combination of isomers is shown, and thus leguminous plants are likely to have a degradation pathway of phytate dissimilar to that of cereals.

The chromatographic profile of the intestinal contents of an ileostomy subject consuming raw wheat bran is shown in Figure 4. Small amounts of IP_1 isomers are present, and the dominating IP_2 isomer is DL-Ins(1,2)-P_2, as determined by retention time and according to wheat phytase degradation of phytate (Figure 2a). We found in Skoglund et al. (1997), as a result of the IP_4 and IP_5 isomers formed in the ileal content, that phytate was hydrolyzed by cereal phytase from the wheat bran.

The analytic method described permits the detection of inositol monophosphates down to 0.04 pmol/injection and of inositol diphosphates down to 0.4 pmol/injection. These low detection limits allow the analysis of inositol mono- and diphosphates present in small amounts in foods, intestinal contents, and tissues. In comparison with other methods for the analysis of lower inositol phosphates (Skoglund et al., 1997; Sun et al., 1990), the sensitivity of the present method is 10−100 times higher.

CONCLUSIONS

The HPIC method described was extremely sensitive to and reproducible for inositol mono- and diphosphate isomers in foods, intestinal contents, and enzymatic degradations of phytate. Elution orders of four IP_1 isomers and seven IP_2 isomers were established. The method will be used for further studies of the effect of food processing and digestion in the gut and the effect of different enzymes on the formation of lower inositol phosphates in cereals and legumes.

ABBREVIATIONS USED

HPIC, high-performance ion chromatography; PAD, pulsed amperometric detection; IPs, inositol phosphates; IP_1 and IP_2, inositol mono- and diphosphate; Ins, accepted NC−IUB abbreviation for *myo*-inositol with the numbering of the D configuration unless the prefix L is explicitly added [with regard to enantiomers not separated on the column, both possible isomers are denoted according to this rule (DL-Ins)]; U, one unit (U) liberates 1 μmol of inorganic P from sodium phytate per minute, at standard conditions.

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