

Radioactively Labelled Phytic Acid from Maturing Seeds of *Sinapis alba*

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Maturing seeds of *Sinapis alba* were incubated with D-[U-¹⁴C]glucose, sodium [1-¹⁴C]acetate or *myo*-[U-¹⁴C]inositol in order to prepare radioactively labelled phytic acid with high specific activity. Although each substrate was utilized for the biosynthesis of phytic acid, maximum incorporation of radioactivity into phytic acid was found with *myo*-inositol. Radiochemical purity of the [U-¹⁴C]phytic acid preparations was confirmed by chromatographic techniques. Such preparations should be useful for the study of interaction of phytic acid with metal ions and proteins and may serve as substrate in the assay of phytase.

Introduction

Phytic acid, *myo*-inositol-1,2,3,4,5,6-hexakis-(di-hydrogen phosphate), occurs in protein bodies of plant seeds [1] and serves as major storage form of phosphate [2]. The presence of phytic acid in diets has been shown to cause mineral deficiency in experimental animals, because at intestinal pH phytic acid and nutrient minerals form insoluble salts which cannot be absorbed [3]. It is also known that phytic acid or its salts are bound to proteins [4, 5], which results in relatively high levels of phytates in protein isolates and concentrates derived, for example, from oilseeds [6, 7].

Radioactively labelled phytic acid should be eminently suitable for studies concerned with its interaction with metal ions and proteins. Moreover, radioactive phytic acid should serve as substrate in the assay of phytase, EC 3.1.3.8.

We have studied in the maturing seeds of *Sinapis alba* the formation of phytic acid from ¹⁴C-labelled substrates, such as glucose, acetate and *myo*-inositol, in order to prepare radioactively labelled phytic acid having high specific activity. *S. alba* was chosen since seeds of this plant are known to be rich in phytic acid [8]. Thus, the maturing seeds can be expected to be abundant in enzymes involved in phytic acid biosynthesis.

Experimental

The radiochemicals, D-[U-¹⁴C]glucose (230 mCi/mm), sodium [1-¹⁴C]acetate (60.2 mCi/mm) and *myo*-[U-¹⁴C]inositol (225 mCi/mm) were obtained from

Amersham Buchler, D-3300 Braunschweig. Sodium phytate, obtained from Sigma Chemie GmbH, D-8000 München, was purified via ferric phytate [9]. Maturing seeds of field-grown *S. alba*, cv. Albatros, were collected 6 weeks after flowering, and the seed coats were removed. The tissue from 30 seeds was used in each incubation.

The seed tissue was incubated in 0.5 ml sodium phosphate buffer (0.1 M, pH 6.0) with 5 μ Ci, of either D-[U-¹⁴C]glucose, sodium [1-¹⁴C]acetate or *myo*-[U-¹⁴C]inositol for 6 h or with 100 μ Ci D-[U-¹⁴C]glucose for 22 h. All incubations were carried out by gentle shaking in loosely stoppered test tubes under diffuse light at 28 °C. At the end of the incubation the contents of the tubes were heated with 1 ml isopropanol at 80 °C for 10 min, cooled to room temperature and homogenized with 8 ml chloroform-methanol (2:1). Lipids were extracted following an established procedure [10]. The aqueous phase obtained after extraction and partitioning of the lipids was mixed with the solid residue after lipid extraction. Phytic acid was extracted from these "nonlipids" with perchloric acid (0.4 M), sodium phytate (3.5 mg) was added as carrier and labelled phytic acid was isolated as its sodium salt via ferric phytate [9].

All radioactivity measurements were carried out in a liquid scintillation counter (Packard Instrument Company, Downers Grove, Illinois, USA) using Aquasol-2 (NEN, D-6072 Dreieichenhain). Gel chromatography was carried out in columns of Sephadex G-25 and Sephadex G-75 using Tris buffer (0.2 M, pH 8.0) containing EDTA (0.04 M). Paper chromatography was performed on strips (Schleicher & Schüll, 2043 b) which were developed with the

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Table I. Distribution of radioactivity in fractions of *S. alba* after incubations with ^{14}C -substrates.

Substrate	Radio-activity added [μCi]	Distribution of radioactivity [%] ^a			Specific activity of phytic acid [$\mu\text{Ci}/\text{mm}$]
		Lipids	Nonlipids	Sodium-phytate	
D-[U- ^{14}C]glucose	5	12	88	0.6	6.7
Sodium [1- ^{14}C]acetate	5	48	52	0.2	1.7
<i>myo</i> -[U- ^{14}C]inositol	5	4	96	2.3	18.8
D-[U- ^{14}C]glucose	100	12	88	0.4	79.2

^a Per cent of radioactivity added.

solvent mixture given in ref. [11]. The spots were stained with molybdenum blue reagent [12]. The paper chromatograms were assayed with a radio TLC scanner (BF-Vertriebsgesellschaft, D-7547 Wildbad).

Results and Discussion

The distribution of radioactivity in the lipid and nonlipid fractions of seed tissue after incubation of the maturing seeds of *S. alba* with 5 μCi of either D-[U- ^{14}C]glucose, sodium [1- ^{14}C]acetate or *myo*-[U- ^{14}C]inositol is given in Table I. After isolation of sodium phytate from the nonlipid fraction, maximum incorporation of radioactivity into phytic acid was observed with *myo*-[U- ^{14}C]inositol (2.3%), followed by D-[U- ^{14}C]glucose (0.6%) and sodium [1- ^{14}C]acetate (0.2%). These findings are consistent with the generally accepted view that *myo*-inositol is the immediate precursor of phytic acid [2]. In contrast to earlier findings in ripening rice grains [13] we have found in *S. alba* seeds considerable incorporation of radioactivity into phytic acid from D-[U- ^{14}C]glucose.

It should be noted that among the substrates tested least incorporation of radioactivity into lipids occurred with *myo*-inositol.

For the preparation of [U- ^{14}C]phytic acid having high specific activity, 100 μCi D-[U- ^{14}C]glucose was

incubated with maturing seeds of *S. alba*. Although considerably more radioactivity was incorporated into phytic acid from *myo*-[U- ^{14}C]inositol than from D-[U- ^{14}C]glucose, the latter, being far less expensive, was chosen.

The chemical and radiochemical purity of the [U- ^{14}C]phytic acid preparation was confirmed by gel chromatography and paper chromatography. On columns of Sephadex G-25 and Sephadex G-75 authentic sodium phytate exhibited distribution coefficients (K_{av}) of 0.23 and 0.62, respectively. When the labelled phytic acid preparation was eluted from these columns, a single radioactive peak was observed, with either column, which coincided with the peak of authentic phytic acid. Paper chromatography of the labelled phytic acid preparation yielded a single radioactive spot having an R_f identical to that of authentic phytic acid.

The specific activity of the [U- ^{14}C]phytic acid preparation was found to be 79.2 $\mu\text{Ci}/\text{mm}$. In comparison, labelled phytic acid having a specific activity of only 4.9 $\mu\text{Ci}/\text{mm}$ has been isolated from wheat kernels by injecting radioactive *myo*-inositol into the maturing kernels [14]. The advantages of the method described in the present communication are thus obvious.

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