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Characterization of phosphorylated cross-linked resistant starch by ^{31}P nuclear magnetic resonance (^{31}P NMR) spectroscopy

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Abstract

The structure of phosphate esters in resistant starch (RS_4) is important to understand its digestibility by pancreatic α-amylase. Wheat starch was reacted at pH 11.5, 45 °C, and 3 h with 12% (starch basis, sb) of a 99/1 (w/w) mixture of sodium trimetaphosphate/sodium tripolyphosphate (STMP/STPP) to give modified starches with ~0.4% phosphorus (P). Exhaustive digestion of the phosphorylated starch with α-amylase followed by amyloglucosidase gave the α,γ-dextrin whose ^{31}P NMR spectrum indicated the presence of a ~1/1 molar mixture of distarch monophosphate (DSMP) and monostarch monophosphate (MSMP). Approximately one-half of STMP/STPP was recovered unreacted after the typical reaction, and it was reacted with a second batch of starch after replenishing with 7% (sb) STMP/STPP. RS_4 wheat starches prepared at pH 9.5–10.5 showed a decreasing ratio of DSMP/MSMP plus an increasing level of monostarch diphosphate (MSDP) and cyclic monostarch monophosphate as reaction pH decreased. Starch triphosphate ester was not detected in the RS_4 products, all of which had been prepared at reaction times greater than three hours. A series of RS_4 wheat starches were prepared where all contained ~0.4% P but with varying levels of DSMP from 41% to 80% of organophosphates. The level of DSMP was positively correlated with the level of RS_4 ($r = 0.96$) and with the level of total dietary fiber ($r = 0.90$).

Keywords: Phosphorylated resistant starch; ^{31}P NMR spectroscopy; Distarch monophosphate; Monostarch monophosphate

1. Introduction

Resistant starch (RS) may be desirable in the human diet principally because of its prebiotic effects and associated health benefits for the colon. RS is fermentable in the large intestine causing little, if any, discomfort, and producing mild laxation due to increased microbial mass. The principal products of fermentation are short-chain fatty acids, which lower colon pH by 1–2 U, plus some carbon dioxide and hydrogen. The acidic pH in the colon prevents the proliferation of pH-sensitive toxic bacteria and lowers the level of secondary bile acids that are cytotoxic at alkaline pH. Among the short-chain fatty acids, butyrate is the preferred energy source of the colonocytes lining the large intestine, and it induces cell death (apoptosis) in tumor cell lines. The high levels of butyric acid in the proximate colon where most fermentation of RS occurs may explain the prevalence of cancer and ulcerative colitis in the distal colon (Topping & Clifton, 2001). Starches rich in RS also contain elevated levels of slowly digestible starch and reduced levels of rapidly digestible starch. Thus, the glycemic index of RS_3 is ~44% compared to starch in bread (Vonk et al., 2000). Foods with a low glycemic index improve satiety (Roberts, 2000; Warren, Henry, & Simonite, 2003) and may help prevent obesity and Type-II diabetes. The reported satiety caused by RS may be explained by its low glycemic index.

RS is divided into physically inaccessible starch (Type I), granular starch with the B-type crystal pattern (Type II), retrograded starch (Type III), and chemically modified starch (Type IV). A general method to convert a granular

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starch to a food-grade RS was developed by Woo and Seib (2002). In that method starch is phosphorylated at alkaline pH with a 99/1 (w/w) mixture of STMP/STPP to give highly cross-linked starch. In one example, wheat starch was phosphorylated to ~0.4% phosphorus (P) and the cross-linked starch was found to have ~90% dietary fiber by the Prosky method and ~66% RS by the Englyst method (Woo & Seib, 2002). When assaying RS_4 by the Englyst method the level of RS_4 was determined by subtracting the rapidly and slowly digestible starch from 100%.

Sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), and phosphoryl chloride are used to prepare food-grade phosphorylated starch (CFR, 1995; EEC, 2000). Kerr and Cleveland (1957) pioneered the use of STMP to cross-link starch in an alkaline slurry. Those workers produced cross-linked starches with a phosphorus add-on of less than 0.04%. Such cross-linked starches gave improved thickening in foods because of their stability to heat, acid, and shearing. The reaction of starch with STMP/STPP at pH > 9.5 was shown to effect cross-linking, which was indicated by changes in the physical properties of starch (Woo & Seib, 1997). It is generally believed that the phosphorylation reaction also generated monostarch phosphate esters which theoretically could have been monostarch monophosphate, diphosphate or triphosphate. However, the chemical structures of the phosphate cross-links (distarch phosphates) and the monostarch phosphates in STMP-phosphorylated starches have not been determined. In addition, little is known about the mechanism of the reaction of STMP with starch.

The ^{31}P chemical shifts of model compounds are well known (Table 1). Therefore, the chemical structures of phosphorylated starches prepared with STMP can be determined by ^{31}P NMR. The objectives of this study were: (1) to investigate the phosphorylation reaction and the chemical forms of phosphate esters in the STMP phosphorylated starches by ^{31}P NMR; (2) to investigate the effect of reaction pH between 9.5 and 12.5 on the types of phosphate esters in the phosphorylated wheat starches; and (3) to determine the effect of the level of DSMP ester (cross-links) in phosphorylated wheat starches on their levels of resistant starch (RS) and total dietary fiber.

2. Materials and methods

2.1. Materials

Wheat starch (Midsol 50) was provided by MGP Ingredients Inc. (Atchison, KS). Three commercial phosphorylated/cross-linked wheat starches were examined; Products A, B and C.

Sodium trimetaphosphate (STMP), phosphoric acid (85%), nicotinamide adenine dinucleotide (NAD), ethylenediaminetetraacetic acid (EDTA) disodium salt and deuterium oxide were purchased from Sigma Chemical Company (St. Louis, MO). Thermally stable α-amylase (E-BLAAM) (3000 Ceralpha U/mL) and glucoamylase (E-AMGDF) (200 *p*-NP β-Maltoside U/mL) were purchased from Megazyme (Bray, Ireland). All chemicals were reagent-grade.

2.2. General methods

All assays were replicated twice unless otherwise stated. Moisture content was determined by drying at 130 °C for 1 h (AACC, 2000) and phosphorus content by the

Table 1
Phosphorus-31 chemical shifts of inorganic and organic phosphates at pH 7–8 with reference to external 85% phosphoric acid

Compounds	Chemical shift (ppm)	References
Inorganic phosphates		
Na_2HPO_4 (sodium phosphate dibasic)	3.3	Sojka and Wolfe (1978)
$Na_4P_2O_7$ (sodium pyrophosphate)	−5.3	Sojka and Wolfe (1978)
$Na_5P_3O_{10}$ (sodium triphosphate)		
α-P	−4.7	
β-P	−19.1	Sojka and Wolfe (1978)
$Na_3P_3O_9$ (sodium trimetaphosphate)	−21.0	Sojka and Wolfe (1978)
Mono-alcohol monophosphates		
D-Glucose 6-phosphate	4.2–4.7	Barany and Glonek (1982)
D-Glucose 1-phosphate	2.6	Barany and Glonek (1982)
Fructose 6-phosphate	2.6–4.1	Barany and Glonek (1982)
Di-alcohol monophosphates		
DNA, RNA	0–1	Barany and Glonek (1982)
Phospholipids	0–(−1)	Barany and Glonek (1982)
Mono-alcohol diphosphate		
ADP α-P	−10.3	Barany and Glonek (1982)
ADP β-P	−6.1	Barany and Glonek (1982)
Di-alcohol diphosphate		
Nicotinamide adenine dinucleotide (NAD)	−10.5–11.3	Barany and Glonek (1982)
Mono-alcohol triphosphate		
ATP α-P	−10.4	Barany and Glonek (1982)
ATP β-P	−21.2	Barany and Glonek (1982)
ATP γ-P	−5.7	Barany and Glonek (1982)