[20] *Kochetkov, N. K., L. I. Kudryashov, M. A. Chlenov* und O. *S. Chizhov:* Dokl. Akad. Nauk SSSR (Ber. Akad. Wiss. UdSSR) **179** (1968), 1385.
[21] *Phillips, G. O., W. Griffith* und *J. V. Davis:* J. Chem. Soc. [London] **1966**, 194.
[22] *Smith, D. R.*, und *W. H. Stevens:* Nature [London] **200** (1963), 66.
[23] *Scherz, H.:* Radiation Res. **43** (1970), 12.
[24] *Houminer, Y.*, und *S. Patai:* Isr. J. Chem. 7 (1969), 513.

Anschrift des Verfassers: Dr. *Heimo Scherz*, Institut für Strahlentechnologie der Bundesforschungsanstalt für Lebensmittelfrischhaltung, D-75 Karlsruhe 1, Engesserstr. 20.

(Eingegangen: 4. April 1971)

Studies on Starch Phosphate

Part 2. Isolation of Glucose 3-Phosphate and Maltose Phosphate by Acid Hydrolysis of Potato Starch.

By S. Tabata* and S. Hizukuri*, Kagoshima (Japan)

Three sugar phosphates, 4-0-(α-D-6-phosphoglucosyl)-D-glucose, glucose 3-phosphate, and glucose 6-phosphate, were isolated from the α-amylase limit phosphodextrin of potato starch by a partial acid hydrolysis and an ion exchange chromatography on Dowex-1×8 formate resin. From the quantitative yields of glucose 3-phosphate and glucose 6-phosphate, the distribution of esterified phosphate in potato starch was estimated to be 38 % on the C-3, and 61 % on the C-6 of glucose residues. It was suggested from a kinetic study that approximately 1 % of the phosphate in starch existed in a form more labile to acid than glucose 3-phosphate, possibly as glucose 2-phosphate.

(Zusammenfassung siehe Seite 271; Résumé à la page 271)

Introduction[1]

Posternak [1] isolated glc-6-P after acid hydrolysis of a phosphorylated dextrin which was prepared from potato starch with pancreatic α-amylase, indicating the presence of an esterified phosphate in potato starch. Since then, the fact has been confirmed by some workers and accepted widely as glc-6-P is the sole structure of the esterified phosphate in the starch [2]. In our previous work [3], however, not all but the majority (60—70 %, variable by specimens) of the bound phosphate in potato starch has been confirmed to exist as glc-6-P and the rest of it (Px) was found to exist as glc-3-P and/or glc-2-P by the quantitative determination of glucose after *Smith* degradation [4, 5] followed by the treatment with alkaline phosphatase.

The present study was undertaken to identify Px and it was concluded that the main portion of Px exists as glc-3-P and a trace of it may exist as a more acid labile phosphate than glc-3-P, possibly as glc-2-P.

* Dept. of Agricultural Chemistry, Faculty of Agriculture, Kagoshima University, Kagoshima (Japan).

1 The following abbreviations are used: α-LPD, α-amylase limit phosphodextrin; Pt, total phosphorus; Po, organic phosphorus; Pi, inorganic phosphorus; Px, unknown esterified phosphorus in starch; P(G6P), esterified phosphorus on C-6 hydroxyl of the glucose residue in starch and dextrin; glc-2-P, glucose 2-phosphate; glc-3-P, glucose 3-phosphate; glc-6-P, glucose 6-phosphate; mal-6-P, 4-0-(α-D-6-phosphoglucosyl)-D-glucose.

Materials and Methods

Materials

Potato starch was prepared in the laboratory from potatoes obtained in a local market by the usual method using just distilled water. α-Amylase limit phosphodextrin (α-LPD) was prepared from potato starch according to the procedure described previously [3].

Isolation of Px_1 and Px_2

α-LPD (500 mg, sodium salt) was hydrolyzed with 50 ml of 0.7 N H_2SO_4 in a flask fitted with a condenser, at 100 °C for 4 hrs. The temperature was maintained by steeping the flask in an oil (Evapoless G) bath (Thermonics Model M-4, Tokyo) at 100 ± 0.5 °C. After the hydrolysis, the solution was neutralized with 1 N $Ba(OH)_2$ (approximately 17.5 mmoles) to pH 5.5—6.0 with stirring in an ice bath. The resulting $BaSO_4$ was removed by centrifugation and was washed with 20 ml of 0.01 N H_2SO_4 by centrifugation. The pH of the combined supernatant solution of the centrifugations was adjusted to 6.0 with 0.2 N $Ba(OH)_2$ solution. The solution was centrifuged again to remove the precipitate. Most of Pi (85 %) in the hydrolysate was removed by the above treatment. The loss of Po was minor (24 %) and the ratio of glc-6-P to Po was constant (65.4 % in the hydrolysate and 65.8 % in the final supernatant solution). The supernatant solution (approximately 110 ml) from the centrifugation was applied on a Dowex-1×8 formate column (0.7×8 cm). The column was washed thoroughly with water to remove neutral sugars and was eluted with a linear gradient of formic acid up to 0.8 M (total

volumn was 240 ml). Three peaks of carbohydrate which coincided with phosphorus were separated by this elution (Fig. 1). The fractions of the first (Px_1) and the last (Px_2)

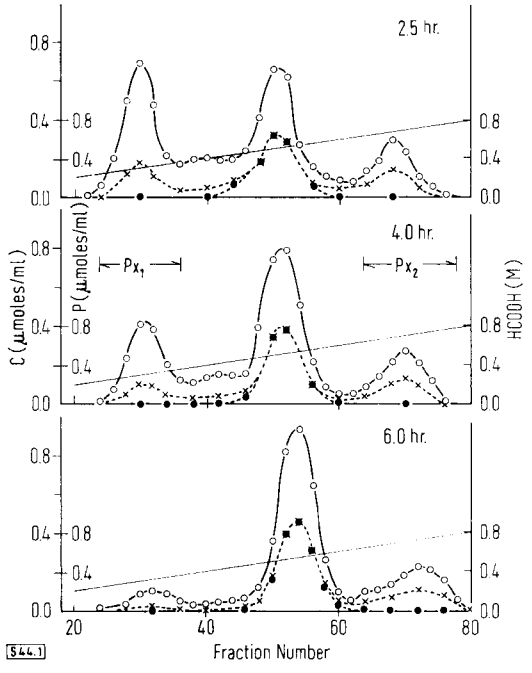

Figure 1. Ion exchange chromatogram of acid hydrolysate of α-LPD.

Five ml of hydrolysate of α-LPD (50 mg) with 0.7 N H_2SO_4 was chromatographed after removal of SO_4^{2-} as $BaSO_4$ on a Dowex-1×8-formate column (0.7×8 cm). The column was eluted with a linear gradient up to 0.8 M formic acid and each fraction was 3.0 ml.

Total carbohydrate (C) is indicated as glucose (○———○). Total phosphorus (×···×) and glc-6-P (●···●) are indicated as P.

peaks were collected separately. After an extraction of formic acid with an equal volume of ether, the aqueous layer was concentrated to dryness by a rotary evaporator under reduced pressure (at 37 °C).

Synthesis of glucose 3-phosphate

Twice recrystallized 1.2, 5.6-diisopropylidene glucose (9.5 g) from cyclohexane was treated with diphenyl phosphochloridate (9 ml) in dry pyridine (75 ml) according to the method of *Brown*, *Hayes*, and *Todd* [6].

The product, 1.2, 5.6-diisopropylidene 3-diphenyl phospho glucose, was hydrogenated in the presence of platinum oxide catalyst. After removal of the catalyst, the solution was treated with Dowex-50 (H^+) at 100 °C, for 5 min. Glucose 3-phosphate was obtained as barium salt in ethanol.

Analytical methods

Pi was measured by the method of *Fiske* and *Subbarow* [7] or of *Hurst* [8]. Glc-6-P was assayed by spectrophotometry (Hitachi Perkin-Elmar spectrophotometer Model 139) by using $NADP^+$ and glc-6-P dehydrogenase [3] in a small scale of total volume 360 μl. Po was measured as Pi after treatment with hot perchloric acid [9]. The esterified phosphorus on C-6 hydroxyl of the glucose residue, P(G6P), in starch and dextrin were estimated by the method reported previously [3]. Reducing sugar and total carbohydrate were determined colorimetrically according to the methods of *Somogyi* [10] and *Nelson* [11], and *Ough* [12], respectively. Glucose was assayed with glucose oxidase [3].

Chemicals and enzymes

Glc-6-P dehydrogenase (crystalline), peroxidase (POD-II), alkaline phosphatase (orthophosphate monoester phosphohydrolase, Purity-I) and $NADP^+$ were obtained commercially from C. F. Boehringer & Soehne (Germany). Glucose oxidase (Deoxin) and α-amylase (crystalline from *B. subtilis*) were kindly supplied by Nagase & Co. (Japan). Glucoamylase (crystalline from *Rhizopus delemar*) and isomaltose were kindly donated by Dr. *Tsujisaka* and Drs. *Matsuda* and *Watanabe*, respectively. Other chemicals were of the highest purity of Wako Chem. Co. (Japan).

Results

The posphorus content of potato starch was so low that the phosphorylated portion of the starch was enriched by using an ion exchange resin after an extensive hydrolysis with α-amylase. Thus prepared α-LPD conserved the same phosphorylated structure as potato starch as shown in Table 1. These values of α-LPD in the Table varied slightly

Table 1. Phosphorus in Potato Starch and α-LPD.

Materials	Potato starch	α-LPD
$\overline{D.P.}$[a]	—	5.20
Glucose residue/Po[b]	395	5.22
P(G6P)/Po	0.61	0.63
Px/Po	0.39	0.37

a) $\overline{D.P.}$ was calculated by the ratio of total carbohydrate to reducing sugar, both as glucose.

b) moles/mole.

in each preparation. The α-LPD in the Table was used in the following experiment: The potato starch and α-LPD contained 61 % and 63 %, respectively, of Pt on the C-6 hydroxyl of the glucose residue and the rest of it was esterified possibly on C-2 and/or C-3 hydroxyl(s) of the glucose residue as assayed by the method proposed previously [3].

Figure 2 shows the time courses of the hydrolysis of starch, α-LPD and glc-6-P with HCl and that starch and α-LPD have the same property in terms of phosphate but are different from glc-6-P. The faster liberation of Pi was noted in starch and α-LPD than glc-6-P. The sum of liberated glc-6-P and Pi from starch and α-LPD was considerably lower than Pt at any time of the hydrolysis. For example, 56 % and 22 % of Pt in these specimens were converted into glc-6-P and Pi, respectively, by the hydrolysis for 4 hrs. This indicates that phosphate(s) other than glc-6-P and Pi exists in the hydrolysate of the starch and α-LPD. Therefore, the isolation of phosphate(s) was attempted by an ion exchange chromatography.

For this purpose, α-LPD was hydrolyzed with H_2SO_4 instead of HCl and SO_4^{2-} was removed as $BaSO_4$. The desalted solution was applied on a Dowex-1×8 formate column and the column was washed with water to remove

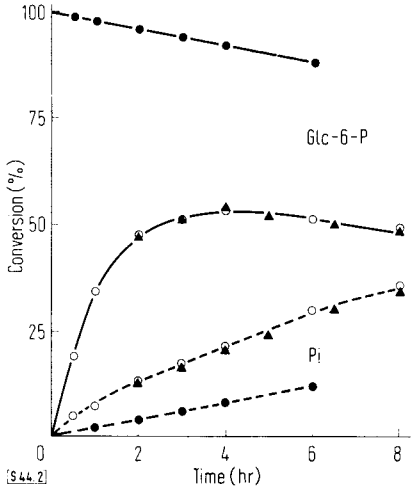

Figure 2. Time courses of the hydrolysis of potato starch, α-LPD, and glc-6-P.
Potato starch (60 mg) (▲), α-LPD (25 mg) (○) and glc-6-P (4.85 μmoles) (●) were heated with 3.3 ml of 0.7 N HCl at 100 °C in a separate tube. Glc-6-P (——) and Pi (- - - -) are indicated as percentage of Pt.

neutral sugars. Three peaks of phosphorylated carbohydrates were eluted from the column by a linear gradient of formic acid as shown in Figure 1. The first (Px_1) and the last (Px_2) peaks of the chromatogram were virtually free from glc-6-P. The second peak was identified as glc-6-P by carbohydrate and phosphorus content and by the enzymatic assay with glc-6-P dehydrogenase. Fraction of Px_1 decreased with the advance of hydrolysis and there were only a few percent of Po after 6 hrs. of hydrolysis (Fig. 1).

Table 2 shows some analytical data of Px_1. It was composed of 2 moles of glucose per mole of phosphorus and was hydrolyzed further with acid into glucose, glc-6-P and Pi. The amount of glc-6-P liberated by the hydrolysis agreed well with that of glucose. The above facts and the

Table 2. Properties of Px_1[a].

Acid hydrolysis[b]	Reducing power as glucose	Glucose	Glc-6-P	Pt
Before	0.47	0.00	<0.01	0.51
After	0.97	0.52	0.44	0.51

a) The values are indicated in moles per mole of Px_1 as glucose.

b) Px_1 was hydrolyzed with 0.7 N HCl for 4 hrs.

reducing power suggest that Px_1 is a disaccharide phosphate. By the action of alkaline phosphatase, it gave a single spot on a paper chromatogram which corresponded to maltose (Fig. 3). The dephosphorylated saccharide was converted quantitatively into glucose with glucoamylase same as maltose but not isomaltose (Table 3). These results indicate that Px_1 was maltose which was phosphorylated on the C-6 hydroxyl of either one of the glucose residues. For the determination of the position of the phosphate, Px_1 was hydrolyzed after reduction with sodium borohydride. By this treatment, glc-6-P but not glucose was recovered quantitatively as shown in Table 4. It is probable that less than 10 % of glucose found by the treatment is liberated mainly by the hydrolysis of the ester bond. It was hereby demonstrated that the reducing end of the disaccharide was glucose. Thus, Px_1 was established as 4-O-(α-D-6-phosphoglucosyl)-D-glucose (mal-6-P).

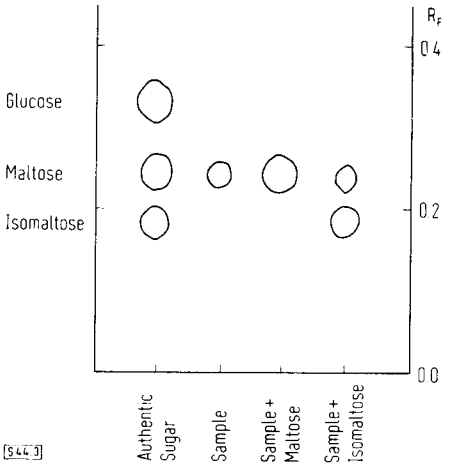

Figure 3. Paper chromatogram of Px_1 hydrolysate with alkaline phosphatase.
Px_1 (3.8 μmoles as P) was hydrolyzed with 3 units of alkaline phosphatase in 500 μl of 0.1 M ethanolamine-HCl buffer, pH 9.7 at 37 °C for 90 min. After the hydrolysis, the reaction mixture was evaporated to dryness *in vacuo* and was subjected to paper chromatography, which was carried out by the ascending method on Toyo Roshi No. 51 filter paper using a solvent system of pyridine-butanol-water (4 : 6 : 3, v/v). Color was developed by the method of *Trevelyan* et al. [13].

Table 3. Liberation of Glucose from Dephosphorylated Px_1[a] with Glucoamylase[b].

Incubation time (min)	Maltose	Dephosphorylated Px_1	Isomaltose
0	0.00	0.01	0.00
30	1.02	0.98	0.00
90	0.97	0.96	0.00

a) The same specimens as shown in Figure 3.

b) Dephosphorylated Px_1 (0.70 μmoles as glucose) was incubated with 3.5 units glucoamylase in 530 μl of 0.05 M acetate buffer of pH 4.8 at 37 °C. Maltose (0.53 μmoles) and isomaltose (0.39 μmoles) were treated in the same way as reference materials. The values are indicated in moles of glucose liberated per mole of these substrates as glucose.

Px_2 was composed of one mole of phosphate per mole of glucose as shown in Table 5 and it yielded equal amount of glucose and Pi by the action of alkaline monophosphatase (Fig. 4). From the following facts, it was determined as glc-3-P: 1.) Px was found to be glc-2-P and/or glc-3-P by *Smith* degradation [4, 5], followed by the action of alkaline phosphatase [3]. 2.) Px_2 was a reducing sugar, the

Table 4. Products from the Reduction of Px_1 with $NaBH_4$ and by Acid Hydrolysis[a].

Reduction time (min)	Reducing power (as maltose)	Glucose	Glc-6-P	
0	1.00	1.03	0.91	(0.98)[b]
10	0.71	—	—	—
60	0.00	0.10	0.93	(1.00)[b]
180	0.00	0.09	0.88	(0.95)[b]

a) Px_1 (3.08 μmoles as P, in 280 μl) was reduced by the addition of 20 μl of 30 mg/ml $NaBH_4$ solution at room temperature. The reaction mixture was hydrolyzed with 0.7 N HCl at 100 °C for 4 hrs. Reducing power was determined on 10 μl of the reaction mixture after an addition of 1 ml of 0.01 M acetate buffer (pH 4.8) to destory excess $NaBH_4$. The values are expressed per mole of Px_1 as maltose.

b) The values were corrected for the hydrolysis of the ester bond by the factor of 1.08 [3].

Table 5. Comparative Analytical Data of between Px_2 and Glc-3-P (in moles).

Materials	Total carbohydrate as glucose	Reducing power as glucose	Pt	Glc-6-P	Pi	Glucose
Px_2	1.00	0.325	0.98	0.001	0.02	0.001
Glc-3-P	1.00	0.318	0.98	—	0.01	0.000

reducing power of which was one-third of that of glucose. This value is good agreement with that of authentic glc-3-P (Table 5), while glc-2-P has been reported to be non-reducing against *Somogyi's* reagent [14]. 3.) The formation

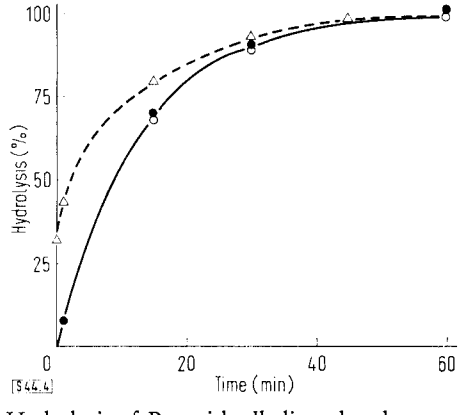

Figure 4. Hydrolysis of Px_2 with alkaline phosphatase.
Px_2 (6.5 μmoles as P) was incubated with 3 units of alkaline phosphatase in 800 μl of 0.1 M ethanolamine-HCl buffer (pH 9.7) at 37 °C. Glucose (● ——— ●) and reducing power (△ - - - - △) are indicated as percentage of total carbohydrate as glucose. Released Pi (○——○) is indicated as percentage of Pt.

of osazones with phenylhydrazine liberates phosphate which is located close to a carbonyl group and glc-2-P has been reported to release entire phosphate by the phenylhydrazine treatment [14]. However, Px_2 released only about 8.3 % of Pt by the above treatment. This value agreed with that (7.5 %) of authentic glc-3-P within an experimental error. According to *Percival* and *Percival* [15], glc-3-P forms a phospholylated osazone. 4.) Rate constant (k) of the ester hydrolysis of Px_2 with 1 N HCl at 100 °C was calculated as 2.82×10^{-3}/min. which was identical to that of authentic glc-3-P (Fig. 5). The value is a half of that of glc-2-P with

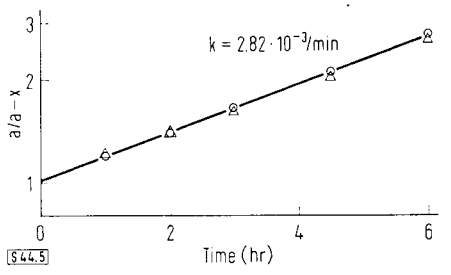

Figure 5. Time course of the acid hydrolysis of Px_2.
Px_2 (8 μmoles as P or glucose) or glc-3-P (5.3 μmoles) was heated at 100 °C with 1 ml of 1 N HCl. The hydrolysis rate constant (k) was calculated by $ln\,(a/a - x) = k\,t$, where a is concentration of total phosphate or total carbohydrate, x is released Pi (○) or glucose (△) at t (min). The plots of the rate constants of Px_2 and glc-3-P are the same.

0.1 N H_2SO_4 [16]. Therefore, Px_2 was more stable to acid than glc-2-P. 5.) Px_2 was found to be pure by the chromatographic (Fig. 2) and kinetic criteria (Fig. 5).

Discussion

The phosphate group of α-LPD has been considered to bind exclusively or at least mainly to non-terminal residues [17, 18]. By the partial acid hydrolysis of α-LPD, however, mal-6-P was the only isolated disaccharide phosphate and 4-O-(α-D-glucosyl)-6-phosphoryl-D-glucose was not isolated. This would be due to the controlling action of the bulky phosphate group for the formation of the intermediate half chair oxonium ion and its inductive effect [19]. In fact, the glucosidic linkage of mal-6-P was much more stable than that of maltose, since the latter was com-

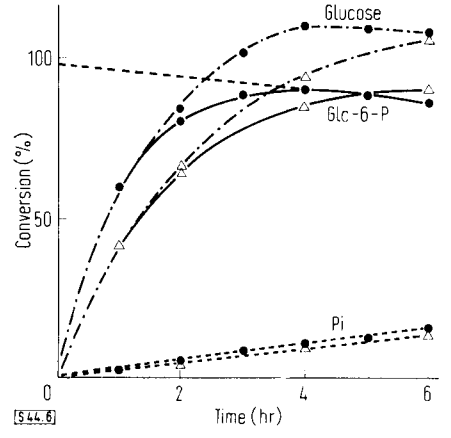

Figure 6. Time course of the hydrolysis of Px_1 with acids.
Px_1 (3.8 μmoles as P) in 2.2 ml of 0.7 N HCl (●) or 0.7 N H_2SO_4 (△) was heated at 100 °C.

pletely hydrolyzed in 2 hrs. under the same conditions as in Figure 6. The hydrolysis rate constant of maltose was 4.1×10^{-2}/min. with 0.7 N HCl at 100 °C and that of mal-6-P was 1.2×10^{-2}/min.

Px_1 contained 97.2 % of Pt as P(G6P) which agreed well with the value (97.0 %) found graphically by an extrapolation of the glc-6-P formation line to 0-time as shown in Figure 6. These values are slightly less than 100 % but they support the proposed method for the estimation of P(G6P) [3]. By this method, P(G6P) is measured as glc-6-P after the hydrolysis with 0.7 N HCl at 100 °C for 4 hrs. and the glc-6-P found was corrected for the hydrolysis of the ester bonds by the factor of 1.08 which was taken from the hydrolysis of glc-6-P under the same conditions. A slightly low estimation of P(G6P) might be due to the incomplete hydrolysis of glucosidic linkages under the estimation conditions, since the kinetic of the glucoside hydrolysis of mal-6-P suggested that approximately 3 % of mal-6-P would remain under these conditions and no difference was observed on the rates of the ester hydrolysis of glc-6-P and mal-6-P, and possibly that of polyglucose 6-phosphate. Further studies may be needed to improve the analytical method for the bound phosphate in starch.

A considerable amount of mal-6-P was found in the hydrolysate of α-LPD with 0.7 N H_2SO_4 at 100 °C for 4 hrs. as shown in Figure 1. This is ascribable to the slower hydrolysis with H_2SO_4 than with HCl as seen in Figure 6. Incidentally, it was noted that the hydrolysis rate constants of maltose with 0.7 N HCl and H_2SO_4 at 100 °C were 4.11×10^{-2}/min and 2.45×10^{-2}/min, respectively.

The yield of glc-3-P (Px_2) was 23 % of the chromatographed total Po. This yield agreed well with the value (22 %) calculated from Px content, the release of Pi during the hydrolysis (assuming the hydrolysis rate of the ester bond of polyglucose-3-P was same as that of glc-3-P) and the recovery (94.5 %) from the chromatography. The unknown phosphate (Px in Table 1) or at least main portion of it has been therefore concluded as glc-3-P.

Glc-3-P may be formed by the migration of phosphate from the C-6 position during the acid hydrolysis, but this possibility would be excluded by the fact that no detectable migration of phosphate was observed in the hydrolysis of mal-6-P and glc-6-P under the same conditions as P(G6P) estimation, since the sum of glc-6-P and Pi agreed well (more than 97.2 %) with Po in the hydrolysis of mal-6-P for more than 4 hrs. and that agreed exactly with Po in that of glc-6-P at any time of hydrolysis.

The presence of more acid labile phosphate than glc-3-P in α-LPD has been suggested by a kinetic study which is shown in Figure 7. The two different rates were observed

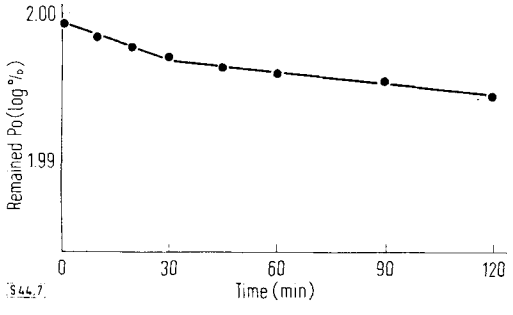

Figure 7. Time course of the acid hydrolysis of α-LPD.
α-LPD (4.0 μmoles as P) was hydrolyzed at 80° ± 0.02 °C with 5.5 ml of 0.7 N HCl.

under the milder conditions and short period as compared with the conditions of acid hydrolysis in Figure 2. The hydrolysis rate in the early period was greater than that of glc-3-P. The amount of the phosphate responsible for the faster rate was estimated as much as 1 % of Pt by an extrapolation of the line corresponding to the slower hydrolysis rate. This may suggest the presence of this amount of phosphate on C-2 position of the carbohydrate moieties.

In conclusion, glc-3-P and mal-6-P was first isolated by an acid hydrolysis of potato starch and it was demonstrated that the covalently bound phosphate of potato starch was distributed 38 % on the C-3, 61 % on the C-6, and a trace (1 %) may be on the C-2 hydroxyl of the glucose residues.

Aknowledgement

We wish to thank Dr. Y. Yamada (Osaka Univ.) for his help on the synthesis of glucose 3-phosphate.

Zusammenfassung

Studien über Stärkephosphate. Teil 2. Isolierung von Glucose-3-Phosphat und Maltosephosphat durch Säurehydrolyse von Kartoffelstärke.

Drei Zuckerphosphate 4-O-(α-D-6-phosphoglucosyl)-D-glucose, Glucose-2-phosphat und Glucose-6-phosphat wurden durch partielle Säurehydrolyse und Ionenaustauschchromatographie auf Dowex-1-8-Formiat-Harz aus α-Amylase-Phosphogrenzdextrin isoliert. Aus der quantitativen Ausbeute von Glucose-3-phosphat und Glucose-6-phosphat wurde die Verteilung von verestertem Phosphat in der Kartoffelstärke auf 38 % am C-3 und 61 % am C-6 des Glucoserestes geschätzt. Aus einer kinetischen Studie ergab sich, daß etwa 1 % des Phosphats in der Stärke in einer Form vorlag, die weniger stabil gegenüber Säure ist als Glucose-3-phosphat, möglicherweise auch als Glucose-2-phosphat.

Résumé

Etudes sur les amidons phosphatés. Partie 2. Isolement du glucose-3-phosphate et du maltosephosphate par hydrolyse acide de l'amidon de pomme de terre.

Trois sucres phosphatés, le 4-O-(α-D-6-phosphoglucosyl)-D-glucose, le glucose-3-phosphate et le glucose-6-phosphate ont été isolés d'une α-limite phosphodextrine de l'amidon de pomme de terre par hydrolyse acide partielle et par chromatographie d'échange d'ion sur une résine Dowex-1×8 sous forme de formiate. A partir des rendements quantitatifs en glucose-3-phosphate et en glucose-6-phosphate, la distribution du phosphate estérifié dans l'amidon de pomme de terre est estimée à 38 % sur le C-3 et à 61 % sur le C-6 des résidus glucose. Les résultats d'une étude cinétique suggèrent qu'environ 1 % du phosphate dans l'amidon existe sous une forme plus fragile vis-à-vis de l'acide que le glucose-3-phosphate, qui est probablement le glucose-2-phosphate.

References

[1] *Posternak, T.:* J. Biol. Chem. **188** (1951), 317.
[2] *Gracza, R.:* in "Starch: Chemistry and Technology", ed. by *R. L. Whistler* and *E. F. Paschall*, vol. 1 (1965), 105. Academic Press, New York and London.
[3] *Hizukuri, S., S. Tabata,* and *Z. Nikuni:* Stärke **22** (1970), 338.

[4] *Smith, F.*, and *J. W. Van-Cleve:* J. Amer. Chem. Soc. **77** (1955), 3091.
[5] *Goldstein, I. J., G. W. Hay, B. A. Lewis,* and *F. Smith:* in "Methods in Carbohydrate Chemistry", ed. by *R. L. Whistler* and *J. N. BeMiller*, vol. 5 (1965), 361. Academic Press, New York and London.
[6] *Brown, D., M. F. Hayes,* and *A. Todd:* Chem. Ber. **90** (1957), 936.
[7] *Fiske, C. H.*, and *Y. Subbarow:* J. Biol. Chem. **66** (1925), 375.
[8] *Hurst, R. O.:* Canad. J. Biochem. Physiol. **42** (1964), 287.
[9] *Allen, R. J. L.:* Biochem. J. [London] **34** (1940), 858.
[10] *Somogyi, M.:* J. Biol. Chem. **195** (1952), 19.
[11] *Nelson, N.:* J. Biol. Chem. **153** (1944), 375.
[12] *Ough, L. D.:* in "Methods in Carbohydrate Chemistry", ed. by *R. L. Whistler*, vol. 4 (1964), 91. Academic Press, New York and London.
[13] *Trevelyan, W. E., D. P. Procter,* and *J. S. Harrison:* Nature [London] **166** (1950), 444.
[14] *Paladini, A. C.*, and *L. F. Leloir:* Biochem. J. [London] **51** (1952), 426.
[15] *Percival, E. E.*, and *E. G. V. Percival:* J. Chem. Soc. [London] (1945), 874.
[16] *Farrar, K. R.:* J. Chem. Soc. [London] (1949), 3131.
[17] *Parrish, F. W.*, and *W. J. Whelan:* Stärke **13** (1961), 231.
[18] *McBurney, L. J.*, and *M. D. Smith:* Cereal Chem. **42** (1965), 161.
[19] *Leloir, L. F.*, and *C. E. Cardini:* in "Comprehensive Biochemistry", ed. by *M. Florkin* and *E. H. Stotz*, vol. 5 (1963), 113. Elsevier, Amsterdam, Netherland.

Address of authors: *Shiro Tabata*, Ph. D., Assistant Professor, and *Susumu Hizukuri*, Ph. D., Professor, Dept. of Agricultural Chemistry, Faculty of Agriculture, Kagoshima University, Uearatacho, Kagoshima (Japan).

(Received: April 24, 1971)

Untersuchung der Kinetik der Amylolyse*

Von J. Holló und E. László, Budapest (Ungarn)

Examination of the Kinetics of Amylolysis. *A new theory to explain the extraordinarily strong effectiveness of enzymatic hydrolysis has been developed from the kinetic data on the starch hydrolysis catalyzed by acid or respectively by β-amylase. According to this theory the β-amylase gradually fits itself to the substrate of spiral structure. This adaption induces the development of hydrogen bonds formed in stages between side chains of the enzyme molecule. By this development the space structure of the bond to be split up or the pyranose ring is several times more easily attacked and split up as it is the case with non-deformed structure.*

(Zusammenfassung siehe Seite 275; Résumé à la page 275)

Einleitung

Die amylolytischen Enzyme katalysieren die Aufspaltung der Bindungen zwischen den die Stärke bildenden Glucoseeinheiten. Die α-1,4-Glucosidbindungen werden durch die α- und β-Amylasen gespalten, während die γ-Amylasen (Glucoamylase, Amyloglucosidase) auch die α-1,6-Bindungen spalten können. Die durch Einwirkung der α-Amylasen entstandenen Produkte (Glucose, Maltose, Dextrine) verfügen über eine α-Konfiguration, während die β- und γ-Amylasen β-Maltose bzw. β-Glucose bilden. Natürlich entsteht in allen erwähnten drei Fällen sehr rasch eine Lösung mit Gleichgewichtskonfiguration infolge von Mutarotation; daher sind die oben angegebenen Konfigurationen nur für den Moment der Spaltung zutreffend.

Früher wurde die Bildung von Produkten mit verschiedener Konfiguration dadurch erklärt, daß sich die Enzyme an das Substrat aus verschiedenen Richtungen annähern [1]. Neuerdings wird jedoch die Entstehung einer α- oder β-Konfiguration der Angriffsrichtung des Wassers und nicht der des Enzyms eine wichtige Rolle zugeschrieben [2, 3].

Von den erwähnten drei Enzymen sind die α-Amylasen in der lebenden Natur am meisten verbreitet. Die β-Amylasen bilden sich nämlich nur in Pflanzen, die α-Amylasen in Mikroorganismen und tierischen Organismen, während die γ-Amylasen in allen drei Gruppen gebildet werden und dort den Stärkeabbau durchführen. Die Unterschiede im Vorkommen der Enzyme sind wahrscheinlich durch physiologische Ursachen erklärbar.

Alle drei Enzymtypen sind dadurch gekennzeichnet, daß sie die Aufspaltung der Glucosidbindungen irreversibel katalysieren. Diese Eigenschaft ist für verschiedene industrielle Verfahren sehr vorteilhaft und wird seit langer Zeit, z. B. in der Bierbrauerei, in der Spiritusindustrie usw. genutzt. In der letzten Zeit wurde aber hauptsächlich die Verwendung von auf mikrobiologischem Wege billig und in großen Mengen herstellbaren amylolytischen Enzymen zur Grundlage zahlreicher großindustrieller Prozesse. Insbesondere die Anwendung der wärmeresistenten α-Amylasen zur Verflüssigung konzentrierter Stärkesuspensionen sowie die Anwendung von Amyloglucosidasen zur Herstellung kristalliner Dextrose hat wesentliche technologische Erfolge erzielt.

Die Vorteile der enzymatischen Verfahren (spezifische Enzymwirkung, erhöhte Ausbeuten usw.) sind allgemein bekannt. Die zur Zeit verwendeten Enzyme haben jedoch auch einige technologische Nachteile, denen zufolge die auf

* Vortrag, gehalten von Prof. Dr. *J. Holló* auf der Stärke-Tagung 1971 der Arbeitsgemeinschaft Getreideforschung, Detmold.