## Arsenolysis and Phosphorolysis of the Amylose and Amylopectin Fractions of Starch

Doudoroff, Barker and Hassid[1] showed that the addition of arsenate to sucrose phosphorylase from *Pseudomonas saccharophila* catalyses the decomposition of sucrose to glucose and fructose, the reaction being:

$$\text{sucrose} + H_2O \xrightarrow[\text{arsenate}]{\text{sucrose phosphorylase}} \text{glucose} + \text{fructose}.$$

We have found that the addition of inorganic arsenate to potato phosphorylase will also cause the liberation of glucose from starch according to the reaction:

$$\text{starch} + H_2O \xrightarrow[\text{arsenate}]{\text{potato phosphorylase}} \text{glucose}.$$

Apparently an intermediate glucose-1-arsenate is formed which does not accumulate but is decomposed rapidly to glucose and arsenate.

However, there is a distinct difference in the limit to which arsenolysis of amylose and of amylopectin proceeds when potato phosphorylase is used. Whereas amylose is practically completely decomposed to glucose by this enzyme in the presence of arsenate, amylopectin is degraded in this process only to approximately 54 per cent (see table).

Likewise, it has been observed that potato phosphorylase acts towards amylose and amylopectin in a similar manner in the process of phosphorolysis. Under conditions in which the amount of amylose is insufficient to allow equilibrium to be reached between glucose-1-phosphate and inorganic phosphate, the amylose is almost completely phosphorolysed to glucose-1-phosphate, but only about 57 per cent of the amylopectin is converted to this ester.

It is interesting to note that such behaviour is characteristic also of β-amylase[2]. In the well-known hydrolytic reaction, amylose is broken down by β-amylase almost completely to maltose, while amylopectin is degraded by this enzyme only to the extent of about 55 per cent. It can therefore be postulated that, like β-amylase, potato phosphorylase acts upon the branched amylopectin through the process of arsenolysis or phosphorolysis, attacking the non-reducing ends, splitting off successive terminal glucose fragments (β-amylase splits off maltose), until it encounters an obstruction. The obstruction is considered to be a modified structure, which is a 1,6-glucosidic linkage at or near the point of branching. With amylose, which has an unbranched structure and therefore no such linkages, arsenolysis or phosphorolysis continues until the whole molecule is degraded. This difference in behaviour of potato phosphorylase towards the amylose and amylopectin components of starch constitutes further evidence that the former component is linear while the latter is branched.

PHOSPHOROLYSIS AND ARSENOLYSIS OF AMYLOSE AND AMYLOPECTIN

Enzyme digest for phosphorolysis consists of 0·05 *M* sodium phosphate and 0·05 *M* sodium maleate buffers at *p*H 6·65, 2 ml. potato phosphorylase preparation. Total volume of digest, 8 ml. Incubation at 30°.

Enzyme digest for arsenolysis consists of 0·05 *M* sodium arsenate and 0·05 maleate buffers at *p*H 6·65, 6 ml. of potato phosphorylase (different preparation than used above). Total volume of digest 36 ml. Incubation at 30°.

| Substrate | Starch added (mgm./ml.) | Starch phosphorolysed (as percentage of total glucose esterified, ± 3) after | | Starch arsenolysed (as percentage of total glucose esterified, ± 3) after | |
|---|---|---|---|---|---|
| | | 4½ hr. | 7 hr. | 12 hr. | 24 hr. |
| Potato amylose | 1·0 | 102 | 100 | 88 | 89† |
| ,, | 2·0 | 66 | 65* | 85 | 88† |
| ,, | 4·0 | 35 | 35* | — | — |
| Potato amylopectin | 0·5 | 56 | 60 | | |
| ,, | 1·0 | 57 | 58 | 51 | 54 |
| ,, | 2·0 | | | 48 | 53 |
| Tapioca amylopectin | 0·5 | 56 | 58 | | |
| ,, | 1·0 | 55 | 56 | | |

* Starch incompletely phosphorolysed because equilibrium between glucose-1-phosphate and inorganic phosphate had been reached. Excess of starch does not affect the equilibrium.

† The fact that the reaction does not go to completion is probably due to the small amount of retrogradation of amylose that occurs during the experiment.

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[1] Doudoroff, M., Barker, H. A., and Hassid, W. Z., *J. Biol. Chem.*, **170**, 147 (1947).
[2] Samec, M., and Waldschmidt-Leitz, Z., *Physiol. Chem.*, **203**, 16 (1931). Hanes, C. S., *New Phyt.*, **36**, 189 (1937). Meyer, K. H., and Bernfeld, P., and Press, J., *Helv. Chim. Acta*, **23**, 1465 (1940). Hassid, W. Z., and McCready, R. M., *J. Amer. Chem. Soc.*, **65**, 1154 (1943).

## Reaction of Boric Acid with Polysaccharides

MANY polyhydroxy compounds of low molecular weight are known to form complexes with boric acid in aqueous solutions, as numerous investigations (Böeseken *et al.*) have shown. These complexes are much more easily obtained from cyclic glycols possessing the *cis* configuration than from the *trans* isomers. One boric acid molecule may react with either one or two 1,2-glycol molecules; in the latter case a 'boron spirane' is formed.

No systematic and theoretical investigations concerning boric acid – polysaccharide compounds have been published. Irany[1] studied the effect of boric acid on various substances of high molecular weight in the absence of water. Cross-linking was observed especially in substances in which the macromolecules carried non-adjacent hydroxyl groups. The Pharmacopœia Helvetica V mentions the incompatibility of borax with gum arabic (gel formation). The gelification of aqueous solutions of *Ceratonia siliqua* mucilage by borax has been observed repeatedly[2].

It is to be expected that polysaccharides characterized by adjacent hydroxyl groups in the *cis* configuration will form boron spiranes in the presence of water. If chain molecules are employed, this reaction should lead to the formation of three-dimensional networks (gelification). Experiments with numerous polysaccharides of known composition[3] have shown that only those substances possessing the postulated configuration tend to form chelate esters: mannan from tubers of *Orchis morio*, mono-glycol ester of alginic acid (polymannuronic acid) and galacto-mannans from the endosperm of *Ceratonia siliqua* and *Trigonella fœnum grœcum*. Aqueous solutions containing even less than 0·3 per cent polysaccharide are transformed into jellies by the addition of borax, provided that the degree of polymerization of the chain molecules is sufficiently high. Because of its more spherical macromolecules and its low content of rhamnose[4], gum arabic, even