HYDROLYSIS OF INTACT LEAF STARCH GRAINS BY GLUCAMYLASE AND α-AMYLASE

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1. Introduction

Although considerable interest has been shown in the properties of starch grains (10–50 μm diam.) formed in storage organs such as tubers and seeds, leaf photosynthate starch grains (1–2 μm diam.) seldom appear to have been isolated or studied. Storage organ starch grains are built up over a relatively long period of time, whereas those in leaf tissues form each day and disappear almost completely during the night [1]. In view of this daily turnover of leaf starch *in vivo* it seems likely that the susceptibility of leaf starch to enzymic hydrolysis could differ from that of storage organ starch. These latter grains are hydrolysed very slowly and incompletely by amylases *in vitro* [2] and must be disrupted, usually by heat gelatinisation, for rapid, complete breakdown. The results in the present communication show that isolated leaf starch grains are completely hydrolysed by a mould glucamylase and by human salivary amylase without any prior gelatinisation treatment, indicating some difference in the structure of these grains as compared to plant storage organ grains.

2. Materials and methods

Storage starch grains were prepared by blending tissue in water and washing the settled grains by decantation (potato) or centrifugation (maize, *Amaranthus* seed). Leaf starch grains were isolated from white clover (*Trifolium repens*) or tobacco (*Nicotiana tabacum*) leaves by the following procedure. Leaves (500–1000 g) were macerated in an end-runner mill with a little (100 ml) water and sand, the pulp squeezed through cheese cloth and the liquid centrifuged at 5000 *g* for 20–30 min. The white starch layer in the centrifuged residue was dissected out and washed by repeated suspension and re-centrifugation. Electron micrographs confirmed the presence of uniform, small (1–2 μm diam.) round grains in both leaf starch preparations. All starch grain preparations contained > 85% of glucose polymer and were not further purified. β-Glucanase-free glucamylase was prepared from dialysed Agidex (Glaxo Ltd) by fractionation on DEAE-Sephadex 50 [3]. The diluted glucamylase used in the digests liberated 2.3 mg of glucose per hr at 60° in 10 ml of buffered digest containing 1 ml of enzyme and 20 mg of soluble starch. Human saliva was diluted with an equal volume of water, centrifuged at 10,000 rpm and used as salivary α-amylase; digests containing this enzyme were layered with toluene while glucamylase digests were layered with paraffin. When the salivary amylase was incubated at 37° in 10 ml of buffered digest containing 0.1 ml of enzyme and 200 mg of soluble starch it liberated 6 mg of glucose equivalent per hr. Digests contained starch grains (5 mg) in water (4 or 5 ml), intact or gelatinized by heating for 60 min at 100°, glucamylase (2.0 ml) or salivary α-amylase (0.1 ml) and buffer (4 or 4.9 ml). Buffer was sodium acetate–acetic acid (0.2 M, pH 4.5) for glucamylase and McIlvaine's citrate-phosphate (pH 6.5) for α-amylase. Except where stated, digests were incubated at 60° for glucamylase and 37° for α-amylase. Liberated glucose in the

glucamylase digests was measured with glucose oxidase [3] and total liberated reducing sugars in the α-amylase digests by a micro-cuprimetric method [4].

3. Results and discussion

The rates of glucamylase hydrolysis of intact and gelatinized clover leaf and potato starch grains are shown in fig. 1 as plots of liberated glucose in the total digest, uncorrected for impurities or moisture in the starch grains, against time. There was no hydrolysis of the starches, intact or gelatinized, when they were incubated in the buffer in the absence of glucamylase. Tobacco leaf starch and maize starch grains gave results similar to those from clover leaf starch and potato starch, respectively. In all of these results the rapid, complete hydrolysis of the leaf starch grains was in marked contrast to the characteristically slow hydrolysis of intact potato and maize starch grains. These results were obtained repeatedly.

Apart from an inherent difference between the two classes of starch grains these results could be due to several other possibilities. Firstly, the optimum incubation temperature for Agidex (60°) could be high enough to gelatinise the leaf starch grains. This seems unlikely as complete hydrolysis of the leaf starch grains was obtained after 24 hr incubation with the glucamylase at 37° or 25°. In addition, human salivary α-amylase also hydrolysed the leaf starch grains to the same extent as the gelatinised material compared with only 10% hydrolysis of potato starch grains. Secondly, the smaller size of the leaf starch grain with its greater surface area to mass ratio might permit more efficient enzymic attack. However, when the small starch grains (1–2 μm diam.) from *Amaranthus lividus* seed were incubated with the glucamylase, a similar result to those from the potato and maize starches was obtained. A third possibility that the grains had all been broken during preparation seems unlikely in view of the electron micrograph studies.

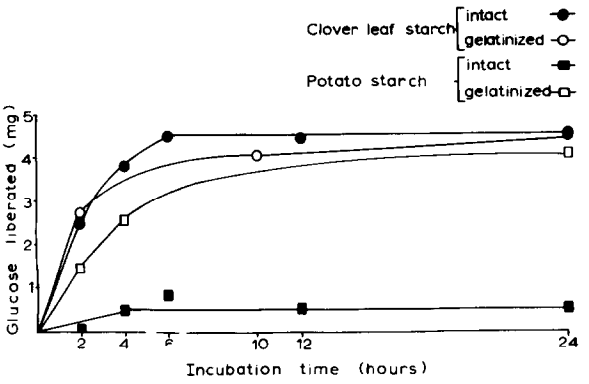

Fig. 1. Hydrolysis of intact (non-gelatinised) and gelatinised leaf and storage starch grains by glucamylase. Digests containing starch with glucamylase incubated at 60°; liberated glucose measured by glucose oxidase.

So far as susceptibility to hydrolysis by mould or animal amylases is concerned, these results indicate that leaf starch grains differ from storage starch grains in a way that may be relevant to their rapid diurnal breakdown in the leaf. A detailed investigation of the enzymes likely to be involved in this *in vivo* starch breakdown is being made.

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