A novel activity for a starch debranching enzyme

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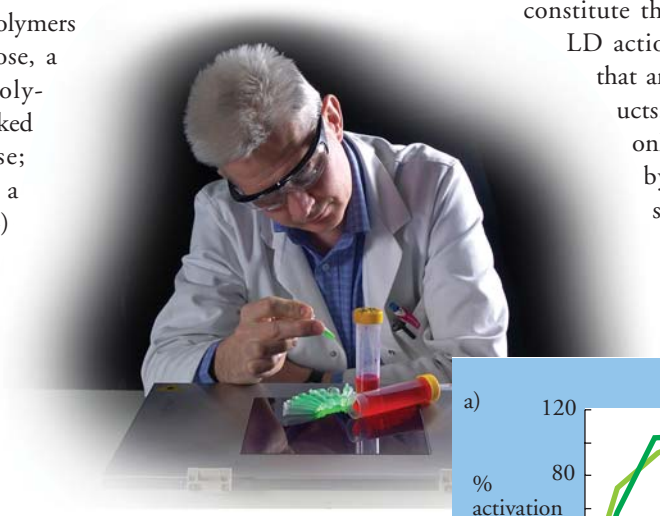
Starch is the major carbohydrate reserve in cereals and is an important source of dietary carbohydrate for humans and animals. Starch from barley is also important to brewers and distillers, as it is hydrolysed to sugars, which are then fermented to alcohol. Starch consists of two polymers of glucose; amylose, a straight chain polymer of α-(1-4) linked chains of glucose; and amylopectin, a polymer of α-(1-4) linked glucose polymer with α-(1-6) linked branch points (Fig. 1). During the germination of cereal grains, the starch reserves are broken down by the action of a battery of enzymes to support the growth of the emerging seedling. However, the α-(1-6) linked branch points can only be broken by the enzyme limit dextrinase (LD). Considering that barley starch consists of 75% amylopectin, debranching by LD is of crucial importance for complete starch degradation. LD activity has been confirmed as a rate limiting step in starch degradation in barley and much research has focused on selecting malting barley varieties with a high expression of LD to maximise fermentable carbohydrate for brewing and distilling[1].



During a study of the activity of LD in malting barleys of differing quality[2], we found that LD was increased by incubation with certain of the sugars that constitute the ultimate digestion products of LD action on starch. It is very unusual that an enzyme is activated by its products. However, this activation could only be detected if LD was measured by particular assays involving the solubilisation (Fig. 2a) or reduction in viscosity of substrates (Fig. 2b) but not if the cleavage events were measured directly (Fig. 2c).

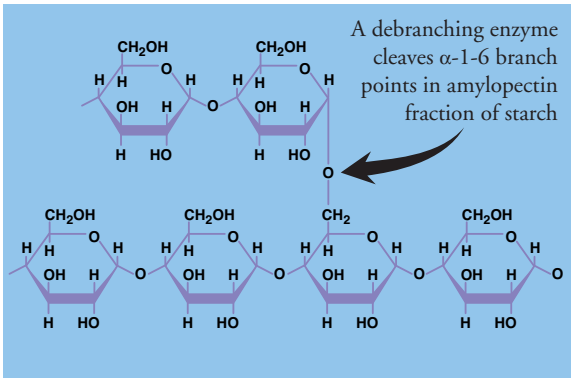


Figure 1 Action of limit dextrinase. LD is solely responsible for the hydrolysis of α-(1-6) branch points of amylopectin.

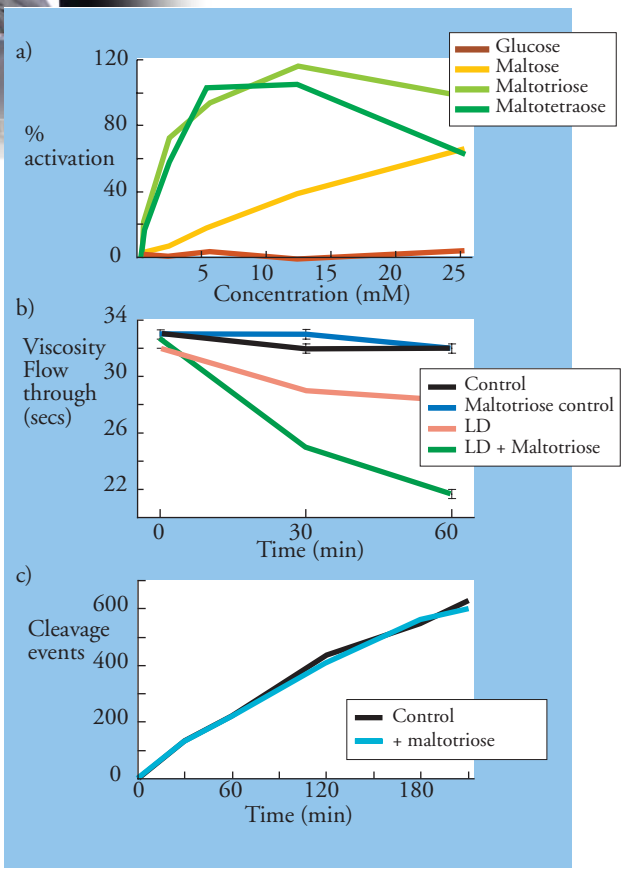


Figure 2 Activation of limit dextrinase by maltodextrins is dependent on the assay method. The activation is noted using solubilsation of dyed substrates (a), reduction of viscosity (b) but not when the cleavage events are measured directly (c).

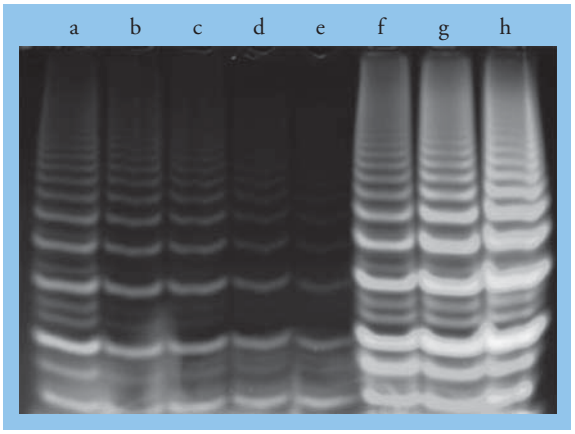


Figure 3 Fluorescent maltotriose is incorporated into higher molecular weight products by limit dextrinase. The figure shows the effect of increasing maltotriose concentration on transfer of fluorescent maltotriose into higher Mr products (lanes a-e). Lane a - no maltotriose; lane b - 2.5 mM; lane c - 5 mM; lane d - 12.5 mM; lane e - 25 mM. Lanes f-h show the effect of increasing enzyme content on transglycosylation.

Other experiments suggested that the degradation of substrates by LD in the presence of maltodextrins did not generate as many low molecular mass products as expected. Therefore, the activation of LD involved altering the pattern, rather than the rate of enzyme action.

To examine this phenomenon more closely the fate of the maltodextrins was monitored during the activation reaction. By synthesising a fluorescent derivative of maltotriose, the process could be traced using a novel form of electrophoresis, fluorophore-assisted carbohydrate electrophoresis (FACE) (Fig. 3). The fluorescent maltotriose was incorporated into higher molecular mass degradation products during the activation reaction (Fig. 3), but this was inhibited by the addition of unlabelled maltotriose. The extent of incorporation of fluorescence was directly linked to the purity and activity of LD.

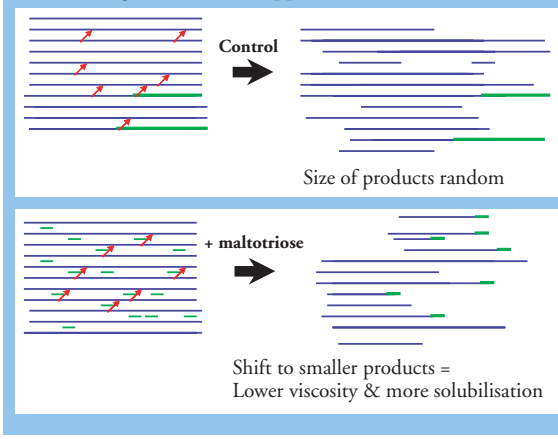


Figure 4 Scheme for transglycosylation reaction. In a proportion of cleavage events, LD transfers the cut end of one amylopectin chain to another glucan forming a new α-(1-6) linkage instead of transferring it to water and effecting hydrolysis.

The simplest explanation for these observations is that LD does more than simply cleave branch points. It can transfer the cut ends of the amylopectin molecule to maltodextrins, i.e. it can catalyse transglycosylation as well as transferring the cut ends of amylopectin to water hydrolysis (Fig. 4). Although structurally related enzymes that synthesise or degrade starch can catalyse transglycosylation reactions, this form of activity has never been recorded for LD. The ability of LD to transglycosylate may cause the reformation of certain, particularly resilient, branched dextrins during starch degradation in malting barley. In addition, the transglycosylation may be useful to modify industrial-scale saccharification to glucose- or maltose-rich syrups.

References

[1] MacGregor, A.W. (1996). *Journal of the Institute of Brewing* **102**, 97-102.

[2] Ross, H.A., Sungurtas, J., Ducreux, L., Swanston, J.S., Davies, H.V. & McDougall, G.J. (2003). *Journal of Cereal Science* **38**, 325-334.