Starch degradation by the mould *Trichoderma viride* II. Regulation of enzyme synthesis

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The synthesis of amylolytic enzymes by the maltose not-utilizing *Trichoderma viride* strain CBS 354.44 requires the presence of starch or dextrins. Several readily utilizable carbon sources such as glucose and glutamic acid were shown to exert a strong catabolite repression which completely inhibited enzyme induction by starch or dextrins.

Enzyme synthesis occurs in the exponential and in the stationary growth phase. In the latter, the ratio between saccharifying and dextrinizing enzyme activity is invariably high. In the exponential growth phase this ratio depends on the nature of the inducing substrate. Growth on starch results in an initially high production of dextrinizing activity, the saccharifying one becoming predominant in the course of exponential growth. The latter activity in dextrin DE 30 cultures is predominant from the very beginning. Thus, the amylolytic enzyme system of *T. viride* consists of at least two different enzymes, the synthesis of each being controlled specifically. The careful regulation of the synthesis of the dextrinizing enzyme is discussed with special reference to the production of non-utilizable maltose by the latter.

INTRODUCTION

In an earlier report (Schellart et al., 1976) the excretion and properties of the amylolytic enzymes of *Trichoderma viride* CBS 354.44 were described. It was concluded that α-amylase (α-1,4-glucan 4-glucanohydrolase, E.C.3.2.1.1.) plays only a minor rôle in starch degradation by the strain studied but that mainly glucoamylase (α-1,4-glucan glucohydrolase, E.C.3.2.1.3.) is produced. Thus, maltose is no intermediate in starch degradation. This sugar is hardly metabolized by the present strain. The same holds for many other strains of the genus *Trichoderma* (Aube and Gagnon, 1969; Danielson and Davey, 1973).

It is generally assumed that the synthesis of (gluco)amylases is induced by starch, dextrins or maltose. Enzyme activities in cultures of *Aspergillus niger* growing on these compounds are high; in cultures with glycerol, lactose and other sugars trace amounts of amylolytic enzymes are excreted (Barton, Lineback and Georgi, 1969; Barton, Georgi and Lineback, 1972). In cultures growing on a mixed carbon substrate consisting of an inducing compound and a non-inducing one (e.g. glucose, fructose, glycerol, 2-oxoglutarate, pyruvate, organic N compounds), low enzyme activities are found (e.g. Barton et al., 1972). Apparently, the induction of amylases by starch or its degradation products is counteracted by catabolite repression exerted by glucose and other readily metabolized carbon sources.

It has been shown (Schellart et al., 1976), that the production of glucoamylase and α-amylase by *T. viride* growing in a starch urea medium occurs to a considerable extent after maximum cell density has been reached. Growth on either starch, amylose or amylopectin results in about equal specific enzyme activities; growth at a low pH, which is unfavourable to the enzyme activity, resulted in the production of more enzyme. Preliminary experiments revealed that complex nitrogen sources (e.g. corn steep liquor, peptone) partly inhibit the induction of amylolytic enzymes (cf. Barton et al., 1972). These observations and the inability to metabolize maltose prompted us to a more detailed study of the regulation of the synthesis of amylolytic enzymes in *Trichoderma viride* CBS 354.44.

MATERIALS AND METHODS

For the description of the fungal strain, its cultivation, the composition of the growth medium, the carbon sources, and the analytical and biochemical methods see the previous report (Schellart et al., 1976).

In all cases the C/N ratio of the growth media was about 10 (2.5 g starch and 0.2 g urea per litre). In complex media corn steep liquor (CSL, kindly donated by Scholten-Honig Research B.V., Foxhol (Gr.), The Netherlands) or peptone (Oxoid Ltd., London, England) were added to a Kjeldahl nitrogen content equal to that of mineral medium (94 mg N per litre). In the case of complex media the concentration of the added carbohydrate was adjusted to give a C/N ratio of about 10, assuming that the Kjeldahl N in the complex nitrogen sources originated from a protein with a carbon content of 53%.

The amylolytic enzyme activities are expressed per mg of dry biomass per hour at 30 C under standard conditions (Schellart et al., 1976). Saccharifying activity (SA spec) is the increase, equivalent to mg maltose, of reducing carbohydrates in the reaction mixture. Dextrinizing activity (DA spec) is the decrease, in mg of soluble starch, in the reaction mixture, as determined with the iodine reagent.

RESULTS

Induction of amylolytic enzymes. Growth of *T. viride* on different C sources yields different amylolytic enzyme activities (Table 1). Very low activities are observed with glucose and lactose. The same holds for peptone, L-glutamic acid, ethanol, glycerol and several organic acids. A strong induction occurs with starch and dextrins. It appears that dextrin DE 30 and starch partially digested with *T. viride* enzymes are better inducers than whole soluble starch. The ratio of the saccharifying and dextrinizing enzyme activities is between 1.5 and 2.5. This does not hold for young cultures with starch as the sole carbon source (Fig. 1a). In these cultures dextrinizing activity is induced initially, the saccharifying activity becoming predominant in the course of exponential growth. In cultures with dextrin DE 30 (Fig. 1b) the saccharifying activity is predominant from the very beginning. Like in earlier experiments (Schellart et al., 1976) it appears that enzyme synthesis takes place to a considerable extent in the stationary growth phase. The same holds for the small activities observed with glucose as the sole C source (Table 1). Since synthesis of amylolytic enzymes were never observed in cultures with lactose as the sole C source, this post-exponential synthesis in glucose cultures is probably due to the presence of inducing maltodextrin contaminations in the glucose used and does not necessarily point to a depression of enzyme synthesis provoked by carbon limitation.

The induction of amylolytic enzymes either by starch or by dextrin DE 30 is inhibited completely by the presence of glucose or glutamic acid, substrates which are preferred by the organism to starch and dextrin DE 30. This points clearly to a role of catabolite repression in the regulation of amylolytic enzyme synthesis.

Table 1. Amylolytic activities in culture filtrates of *T. viride* grown on different C sources. The nitrogen source in the medium was urea: C/N ratio 10; total carbohydrate content 2500 mg/litre; initial pH 4.0. Growth period 48 h. Starch fragments were prepared by action of a *T. viride* culture filtrate on soluble starch. Growth yields and growth rates were about the same for all C sources tested.

C source	Initially present reducing compounds (as mg glucose/litre)	Specific amylolytic activities SA	DA
Glucose	2500	0.3	0.2
Lactose	1280	<0.2	<0.2
Starch	205	3.6	1.6
Dextrin DE 30	670	4.7	2.3
Maltotriose	805	2.0	1.3
Starch fragment	850	5.6	3.5
Starch fragment	1820	3.4	2.0
Starch fragment	2090	1.3	1.0

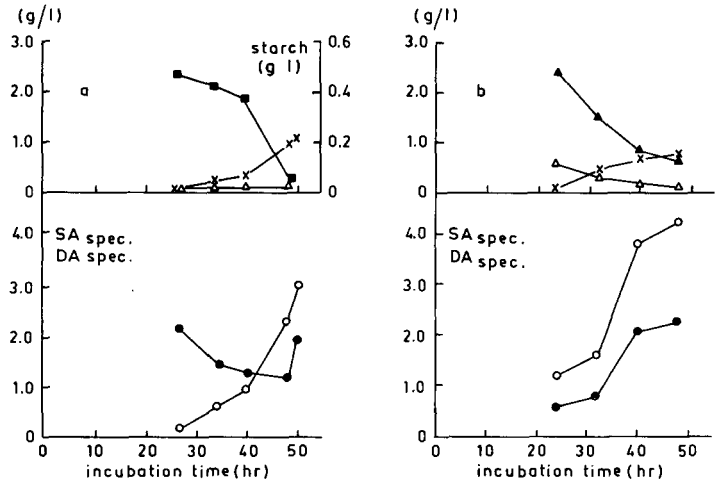
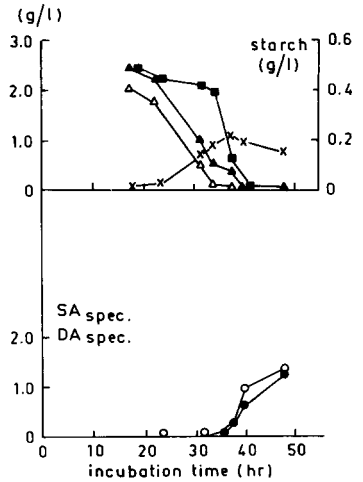


Fig. 1. Amylolytic activities of culture filtrates of *T. viride* during growth in media with different carbohydrates. The N source was urea; C/N ratio 10. The media were inoculated with a spore suspension. The pH was controlled at 4.0. Carbohydrate substrates were: a starch (2.5 g/litre): b dextrin DE 30 (2.5 g/litre)
× biomass (g/litre); ▲ total carbohydrates in glucose equivalents (g/litre); △ reducing compounds in glucose equivalents (g/litre); ■ starch (g/litre); ● DA spec; ○ SA spec.

Catabolite repression. Whereas synthesis of amylolytic enzymes in *T. viride* cultures with starch or dextrin DE 30 as the sole C source occurs from the early exponential growth phase, a marked delay in starch degradation and in enzyme synthesis is observed when besides starch either glucose (Fig. 2) or L-glutamate (Fig. 3) is present. The same holds for cultures in which starch is replaced with dextrin DE 30 (Schellart, 1975). Glucose and glutamate apparently are able to counteract induction by starch or dextrin DE 30. Such a repressing effect by preferential C sources is known as catabolite repression (Magasanik, 1961). Lactose represses enzyme synthesis only slightly when administered as a C source besides starch (Schellart, 1975). Complex nitrogen sources e.g. peptone or corn steep liquor, strongly repress the synthesis of amylolytic enzymes. Possibly, some amino acids different from L-glutamic acid exert a repressive action also. DL-Alanine and L-lysine are inactive in this respect, however.

DISCUSSION

The production of amylolytic enzymes by *Trichoderma viride* strain CBS 354.44 requires the presence of inducing medium constituents. In cultures with glucose, lactose or several other compounds as sole source of carbon and



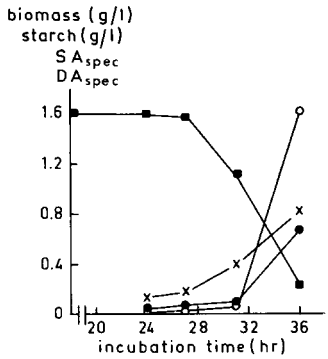


Fig. 3.

Fig. 2. Amylolytic activities of culture filtrates of *T. viride* during growth in a glucose- and starch-containing medium. The carbohydrate substrates were: glucose (2 g/litre) and starch (0.5 g/litre). For other conditions and explanation of symbols, see Fig. 1.
Fig. 3. Amylolytic activities of culture filtrates of *T. viride* during growth in starch glutamate medium. The C and N substrates were: starch 1.6 g/litre and L-glutamic acid 0.98 g/litre; C/N ratio 10. For other conditions and explanation of symbols, see Fig. 1.

energy at most traces of enzyme activity are detected.

Starch and its degradation products can serve as inducers of enzyme synthesis. Induction by starch is about the same as that by starch components amylose and amylopectin (Schellart et al., 1976). Dextrin DE 30 or starch partially digested with enzymes of *T. viride* are more effective inducers. Maltose is not utilized by most strains of *T. viride*.

The production of amylolytic enzymes induced either by starch or by dextrin DE 30 is inhibited completely by the presence of glucose or glutamic acid. This points clearly to a rôle of catabolite repression in the regulation of amylolytic enzyme synthesis. Enzyme synthesis takes place both during exponential growth and after maximum cell yield has been attained. Considerable differences in regulation of enzyme synthesis during these phases are observed.

During exponential growth the ratio of saccharifying and dextrinizing enzyme activities depends on the carbon source. In starch media the dextrinizing activity appears to precede the saccharifying one. In dextrin DE 30 media the latter is predominant from the very beginning, like it invariably is under all inducing conditions in the stationary growth phase. The variable ratio of the two enzyme activities strongly suggests that these are brought about by at least two enzymes: the dextrinizing activity mainly by an α-amylase, the saccharifying activity mainly by a glucoamylase.

The rôle of α-amylase in starch degradation by *T. viride* is questionable as the main low-molecular product of this enzyme, maltose, is not utilized by the fungus. Addition of a commercial fungal ß-amylase to cultures in starch medium is growth-inhibiting (results not presented). Possibly the function of α-amylase is the production of inducing dextrins from starch. The production of the right amount of α-amylase is the result of a careful regulation. An excess of α-amylase will liberate non-utilizable maltose from starch and decrease the yield of biomass.

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