Inducible Sucrase Activity in *Bacillus subtilis* Distinct from Levan-sucrase

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SUMMARY

An inducible sucrose hydrolysing enzyme has been found in *Bacillus subtilis*. The enzyme is intracellular, unstable in the absence of EDTA and its formation is repressed by glucose. These properties contrast with those of the levan-sucrase enzyme previously investigated in this organism.

INTRODUCTION

The occurrence of a sucrase without associated levan or dextran synthesizing activity has not been reported in *Bacillus subtilis* although separate enzymes for these two functions were suggested some time ago in *Aerobacter* strains (Hestrin, Avineri-Shapiro & Ascher, 1943) and in *B. megaterium* (Forsyth & Webley, 1950).

In this report evidence is presented for an inducible invertase-like activity in *Bacillus subtilis* which differs from the levan-sucrase (E.C. 2.4.1.10) extensively studied by Delobbe & Dedonder (1966), Dedonder (1960), Joyeux & Seys-Borrel (1966) and Delobbe (1966). The lability, EDTA stabilizing effect, intracellular location and glucose repressibility of this sucrose hydrolysing activity differ from those reported for levan-sucrase.

METHODS

Bacterial strains. *Bacillus subtilis* 168 *try*-2, (designated '168' in the text) was from the collection of this laboratory while BS5 and MC2, kindly provided by Dr Dedonder, were mutants obtained from the 168 strain carried in his laboratory. These three strains were, respectively, low level inducible, high level inducible, and constitutive with regard to levan-sucrase production (personal communication from Dr Dedonder). They behave identically with respect to the newly detected sucrose hydrolyzing activity reported in this note.

Growth conditions and experimental techniques. Cultures were grown in either basic salts composition A (Dedonder, 1960) or B (Anagnostopoulos & Spizizen, 1961), containing 0.5 % carbon source, 20 µg./ml. L-tryptophan and 0.05 % casein hydrolysate. Inducers were added to exponential growth-phase cultures and samples removed at intervals into iced tubes containing chloramphenicol sufficient to give a final concentration of 25-50 µg./ml. and EDTA, when used, at a final concentration of 0.01-0.015 M. Suspensions were broken before assay either by lysozyme treatment or by sonic oscillation. Where necessary to eliminate compounds reacting in the assay, dialysis was carried out before determination of sucrose hydrolysis or levan synthesis activity.

Assays. Sucrose hydrolysis activity was measured by glucose formation after incubation with 0.3–0.4M-sucrose. Glucose was then determined by a modification of the method used by Messer & Dahlqvist (1966) as follows. After incubation with sucrose a total volume of 0.5 ml. diluted sample was equilibrated at 37° and 2.0 ml. assay reagent added. (The assay reagent contained 10 µg./ml. glucose oxidase, 1 µg./ml. peroxidase and 50 µg./ml. *o*-dianisidine in 0.25M-PO₄, pH 6). After a suitable incubation period, the reaction was terminated and purple colour developed by the addition of 1.0 ml., 50 % H₂SO₄. Glucose concentrations of the samples were obtained by comparison of optical density readings at 530 mµ with those of glucose standards run under the same conditions.

Levan production was estimated from turbidity readings of incubated samples in which levans of mol. wt 5×10^4 or larger were precipitated by the addition of 2 volumes of 95 % EtOH.

Chemicals. Lysozyme grade I, glucose oxidase (*Aspergillus niger*) purified type II, peroxidase (horse-radish) type I, and *o*-diansidine were products of Sigma Chemical Company. Chloramphenicol was a gift of Parke Davis and Company. Other analytical grade reagents used were products of Fisher, Mallinkrodt, or Matheson Coleman and Bell.

RESULTS

Preliminary experiments of levan-sucrase induction in *Bacillus subtilis* strain BS5 done in the presence of 0.5 % glucose in a medium of salts composition 'A' were in qualitative agreement with data presented by Dedonder (1960) for the induction of levan-sucrase in a medium utilizing glycerol as a carbon source. Both sucrose hydrolysis and levan synthesis activity were optimally induced at the highest inducer concentration tested (5×10^{-2} M), while comparatively negligible activities were found at inducer concentrations of 1×10^{-4} M or lower.

However, when lactate was substituted for glycerol or glucose as carbon source in the same salts 'A' medium, it was found that both *Bacillus subtilis* 168 and BS5 were capable of synthesizing significant levels of sucrose hydrolysis activity at inducer concentrations below 1×10^{-4} M.

Levan synthesis activity could not be demonstrated in either strain by turbidity assay of samples induced at 1×10^{-4} M-sucrose although appreciable activities were found in Strain BS5 at inducer concentrations of 0.5×10^{-2} M or higher.

Induction of sucrose hydrolysis activity at low sucrose concentration was dramatically increased in both BS5 and 168 in lactate medium when salts 'B' was substituted for salts 'A'. However, regardless of the composition of the medium, the activity induced at low concentrations of sucrose proved to be quite unstable (unlike levan-sucrase), and various compounds were added in an attempt to stabilize the enzyme. Of the additions tested (β -mercaptoethanol, cysteine, vitamin mixtures, trace metals, yeast extract, EDTA), only EDTA was found to have an appreciable effect on sucrose hydrolysis activity. Addition of 0.01–0.015M-EDTA increased activity and stabilized extracts for a short time during storage at 4°.

Studies of the EDTA-activated sucrase (which will henceforth be referred to as 'sucrase B') were then repeated in *B. subtilis* 168 and BS5 under experimental conditions found optimal for induction and assay (i.e. the medium contained salts B and lactate as carbon source, low sucrose inducer concentrations were employed and

EDTA was added to broken-cell samples). The results contrasted strikingly with those previously obtained for levan-sucrose induction. A higher initial rate of enzyme induction was obtained with decreasing inducer concentration. The lowest concentration of sucrose tested ($1.46 \times 10^{-5} \text{ M}$) acted as a more effective inducer than either of the two higher sucrose concentrations ($1.17 \times 10^{-4} \text{ M}$ or $2.92 \times 10^{-4} \text{ M}$) when early time samples prior to 30 min. were compared.

The role of carbon source on induction suggested a catabolite repression effect. Addition of a low concentration of glucose (40 $\mu\text{g./ml.}$) was found sufficient to cause a lag period in initiation of sucrase B synthesis in BS5 and 168 during growth on lactate. Addition of glucose to cultures in which induction was already in progress

Fig. 1. A culture of *Bacillus subtilis* strain BS5 in exponential growth phase at 30° on salts A, 0.1 % glycerol, 0.1 % casein hydrolysate and 20 $\mu\text{g.}$ L-tryptophan/ml. was divided among several flasks to which various concentrations of sucrose were added at 0 time. At intervals samples were removed and the organisms collected by centrifugation. Pellets were washed and resuspended in 0.02 M-sodium phosphate buffer (pH 6) + 0.01 M-EDTA. (Resuspension volumes were adjusted to make all samples to the same concentration based on Klett Summerson readings made at the time of sampling.) After addition of 75 $\mu\text{g./ml.}$ lysozyme/ml., 15 $\mu\text{g.}$ RNase/ml. and 12 $\mu\text{g.}$ DNase/ml., a 30 min. incubation was carried out at 37° in order to lyse the organisms prior to subsequent determination of sucrose hydrolysis activity. No levan synthesis activity was detectable by turbidity assay.

caused abrupt and complete cessation of further enzyme production for a period of time was related to the amount of glucose added. This contrasts with levan-sucrase which is non-glucose repressible.

Sucrase B also differs from the predominantly extracellular levan-sucrase in that it appears to be completely intracellular. When sucrase B is induced by low concentration of inducer, and the cells and medium separated by centrifugation are assayed indepen-

dently, all of the activity is found with the resuspended cells. Lysis by lysozyme or sonic treatment is required for full expression of the intracellular enzyme.

Finally, sucrase B can be most clearly distinguished by its different pattern of induction under conditions comparable to those used by Dedonder to study total levan-sucrase in the culture (i.e. salts A with glycerol carbon source). When BS5 organisms were separated from the supernatant medium and maintained in the presence of EDTA their sucrose hydrolysis activity pattern was the reverse of that ascribed to levan-sucrase. Thus, at the lowest sucrose concentration ($1.2 \times 10^{-4} \text{ M}$) maximum sucrose hydrolysis activity was produced without associated levan-synthesis activity. Figure 1 illustrates these results.

DISCUSSION

Two distinct sucrose hydrolysing activities can be induced in the same culture; one (sucrase B) being intracellular and produced with greatest activity at a lower inducer concentration ($1.17 \times 10^{-4} \text{ M}$ or less), and the other (levan-sucrase) found by Dedonder predominantly in the supernatant medium and present with maximal activity at high inducer concentrations ($5 \times 10^{-2} \text{ M}$ or higher).

The two enzymes are presumably subject to different genetic controls. The MC2 mutant strain of *Bacillus subtilis* 168 (classified as levan-sucrase 'constitutive' by Dedonder) produced only a low level of levan-sucrase under conditions where levan-sucrase is fully induced in BS5. The induction pattern of sucrase B is, however, identical in both MC2 and BS5.

These observations, together with observed differences in the properties of levan-sucrase and sucrase B with respect to the effects of EDTA and glucose repression provide strong evidence for the existence of two separate inducible sucrose hydrolysing enzymes in *Bacillus subtilis*.

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