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INDUCTION AND CATABOLITE REPRESSION OF  $\alpha$ -GLUCOSIDASE SYNTHESIS IN PROTOPLASTS OF *SACCHAROMYCES CARLSBERGENSIS*

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## SUMMARY

1. Kinetic data on the repression, the derepression and the induction of  $\alpha$ -glucosidase synthesis in protoplasts of *Saccharomyces carlsbergensis* suggested that some site other than the stereospecific site for the induction by maltose was involved in the repression by glucose.

2. A study of the effect of actinomycin D on the induced and noninduced (derepressed) synthesis of  $\alpha$ -glucosidase showed that induction by maltose caused mRNA to be synthesized. The mRNA coding for  $\alpha$ -glucosidase synthesized during the first 120 min of induction appeared to be stable under conditions of both repression and derepression.

3. During the phase of synthesis in which translation occurred only *via* the mRNA, glucose strongly affected  $\alpha$ -glucosidase synthesis. Maltose did not exert any effect on translation.

4. When both transcription and translation occurred, glucose inhibited transcription. However, translation and transcription were not affected to the same extent by glucose.

## INTRODUCTION

In microorganisms, the synthesis of several enzymes is repressed by adding glucose to the medium. For example enzyme repression in yeast by glucose has been described<sup>1-8</sup>, and the terms "glucose effect"<sup>9,10</sup> and "catabolite repression"<sup>11</sup> have been introduced to describe this phenomenon. However, neither the chemical nature of the compound(s) which is (are) responsible for the glucose effect nor its mode of action is known.

For *Escherichia coli* it has been reported that catabolite repression decreases the rate of synthesis of the mRNA specific for  $\beta$ -galactosidase<sup>12</sup>, but it is not known if it is dependent on the repressor as defined by PARDEE *et al.*<sup>13</sup>. It appears that part of the catabolite repression operates independently of the *i*-gene repressor<sup>14-18</sup>. Moreover, the possibility that glucose also exerts its repressing effect at the ribosomal level in protein synthesis cannot be excluded. After studying the  $\beta$ -glucosidase synthesis in yeast, HAUGE *et al.*<sup>19</sup> suggested this alternative proposition concerning the nature of the glucose effect.

Abbreviation: PNPG, *p*-nitrophenyl- $\alpha$ -D-glucoside.

The experimental work described in this paper investigates the level(s) of action of the regulatory mechanism(s) involved in the induction and the glucose repression of  $\alpha$ -glucosidase synthesis in protoplasts of *S. carlsbergensis*. The synthesis of  $\alpha$ -glucosidase is induced by maltose and repressed by glucose. Moreover in the absence of maltose, the synthesis of  $\alpha$ -glucosidase is derepressed at low glucose concentrations<sup>20</sup>. Under special incubation conditions, both with and without maltose the transition from the repressed to the derepressed state of  $\alpha$ -glucosidase synthesis can be studied. In addition, the effects of inhibitors of RNA and protein syntheses (actinomycin D, puromycin, and cycloheximide, respectively) on  $\alpha$ -glucosidase synthesis were studied in the same protoplasts<sup>21,22</sup>. In this manner the effects of induction and glucose repression on both transcription and translation could be investigated.

#### MATERIALS AND METHODS

##### *Chemicals*

Glucose and glucose oxidase were obtained from British Drug Houses, Poole; maltose came from Fluka A.G., Buchs; casamino acids were produced by Difco Laboratories, Detroit; actinomycin D was donated by Merck Sharp and Dohme Research Laboratory, Rahway; puromycin dihydrochloride was purchased from Nutritional Biochemicals Corp., Cleveland; and cycloheximide (actidione) was purchased from Koch-Light Laboratories, Colnbrook. The chromogenic substrate *p*-nitrophenyl- $\alpha$ -D-glucoside (PNPG) was prepared according to AIZAWA<sup>23</sup>.

##### *Yeast strain*

*S. carlsbergensis* strain No. 74, N.C.Y.C., England, was used. Yeast culturing was performed as previously described<sup>20</sup>, and protoplasts were prepared according to EDDY AND WILLIAMSON<sup>24</sup>.

##### *Induction of $\alpha$ -glucosidase*

Induction or derepression of  $\alpha$ -glucosidase synthesis in yeast protoplasts was effected at 30° in a medium containing per ml: 10 mg casamino acids, 50  $\mu$ moles potassium/sodium phosphate buffer (pH 6.2), and 120 mg mannitol. Because the concentrations of glucose and maltose varied greatly, they are mentioned separately in RESULTS. The total amount of protoplast protein present in most experiments was 1 mg/ml of incubation medium.  $\alpha$ -Glucosidase activity and protein determinations were performed as previously described<sup>20</sup>. The specific enzyme activity is expressed as  $\mu$ moles PNPG split  $\cdot$  min<sup>-1</sup>  $\cdot$  mg<sup>-1</sup> protein.

Under these incubation conditions, the increase in the total protein of protoplasts of *S. carlsbergensis* was 2–5 % per h and never exceeded 12 % in the 4-h experiments. Therefore, the values reported for the specific  $\alpha$ -glucosidase activity are approximately proportional to those for the absolute  $\alpha$ -glucosidase activities.

##### *Determination of glucose and maltose*

The concentrations of glucose and maltose in the incubation medium were determined by the cysteine-sulfuric acid method according to ASHWELL<sup>25</sup>. Glucose in the presence of maltose was determined according to the method of HUGGETT AND NIXON<sup>26</sup>.

## RESULTS

*Effects of various glucose and maltose concentrations on the rate of  $\alpha$ -glucosidase synthesis*

The induced synthesis of  $\alpha$ -glucosidase is dependent on the concentration of glucose in the medium, as shown in Fig. 1. The lag-time, *i.e.* the time lapse between the addition of the inducer maltose and the initiation of enzyme synthesis, appears to be independent of the glucose concentration used in the experiment. However, the various glucose concentrations affect significantly both the increase in the rate of synthesis and the maximal rate of synthesis. As illustrated in Fig. 2, during the latter stages in all experiments the change in the rate of synthesis with incubation time ( $\Delta(\text{rate})/\Delta(\text{time})$ ) increases linearly.

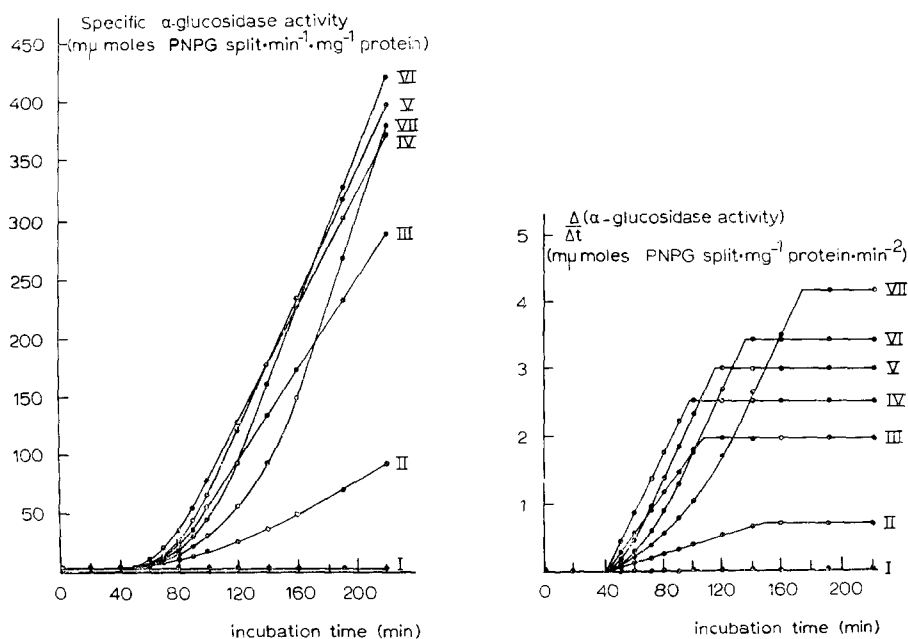


Fig. 1. The effect of various glucose concentrations on induced  $\alpha$ -glucosidase synthesis in protoplasts of *S. carlsbergensis*. The inducer maltose was added at zero time; its concentration was 1 %. Glucose concentrations: I, 0 mg/ml; II, 1 mg/ml; III, 2 mg/ml; IV, 2.5 mg/ml; V, 3 mg/ml; VI, 4 mg/ml; VII, 6 mg/ml. Other experimental conditions as described in MATERIALS AND METHODS.

Fig. 2. The effect of various glucose concentrations on the change in the rate of induced  $\alpha$ -glucosidase synthesis with incubation time. In this figure, the differential curves,  $\Delta(\text{rate})/\Delta t$ , are plotted from the experiment illustrated in Fig. 1.

This increase, which reached a maximum at a 0.25 % glucose concentration, appeared to be smaller when the glucose concentration present was higher; the time of apparent inhibition depended on the glucose concentration<sup>20</sup>. Periodically during the induction of the  $\alpha$ -glucosidase synthesis in the presence of 0.25, 0.3, 0.4 and 0.6 % glucose, the amount of glucose in the incubation medium was determined and the maximal increase in the rate of synthesis appeared to occur at a glucose concentration of about 0.15 %. Moreover, no further increase was observed when the glucose was exhausted from the medium.

At glucose concentrations of less than 0.25 %, an opposite effect is observed. The increase in the rate of  $\alpha$ -glucosidase synthesis with incubation time is lower than the previously mentioned extreme value reached at 0.25 % glucose. The cause of this effect is not the subject of this study; presumably it reflects a starvation of the protoplasts rather than a specific inhibition of  $\alpha$ -glucosidase synthesis.

In Fig. 3, the effect of various glucose concentrations on the noninduced synthesis of  $\alpha$ -glucosidase is demonstrated. Moreover, the change in the rate of noninduced  $\alpha$ -glucosidase synthesis with incubation time is shown in Fig. 4.

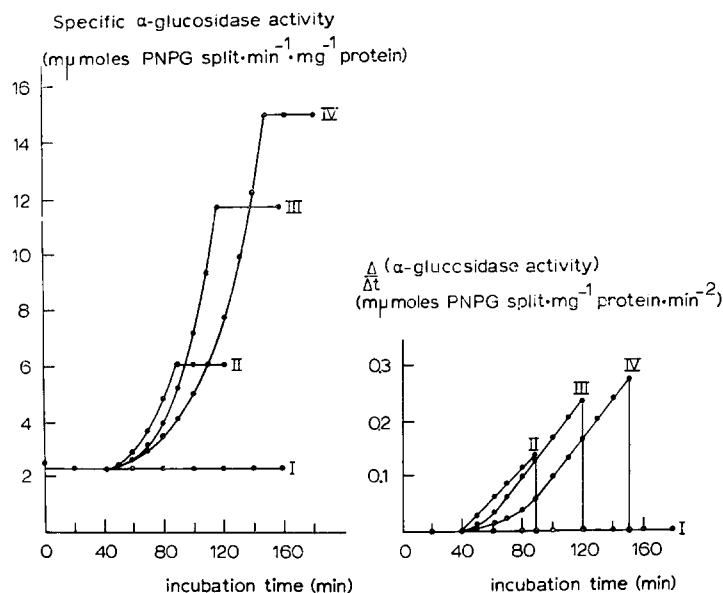


Fig. 3. The effect of various glucose concentrations on noninduced (derepressed)  $\alpha$ -glucosidase synthesis in protoplasts of *S. carlsbergensis*. Glucose concentrations: I, no glucose; II, 2 mg/ml; III, 3 mg/ml; IV, 4.5 mg/ml. Other experimental conditions are described in MATERIALS AND METHODS.

Fig. 4. The effect of various glucose concentrations on the change in the rate of noninduced  $\alpha$ -glucosidase synthesis with incubation time. In this figure, the differential curves,  $\Delta(\text{rate})/\Delta t$ , are plotted from the experiment illustrated in Fig. 3.

As presented in Figs. 1–4, the effects of glucose on noninduced synthesis appear to be qualitatively the same as on induced  $\alpha$ -glucosidase synthesis. The maximal increase in the rate of  $\alpha$ -glucosidase synthesis, during incubation in a medium containing 0.3 and 0.45 % glucose respectively, occurs again when the glucose concentration is lowered to about 0.15 %, moreover,  $\alpha$ -glucosidase synthesis stops abruptly when glucose, being the only carbon source, is exhausted in the medium.

The results reported above suggest that the kinetic characteristics of  $\alpha$ -glucosidase synthesis are determined mainly by the glucose concentration in the medium. The presence of the inducer maltose appeared to prolong and to increase considerably the rate of  $\alpha$ -glucosidase synthesis, as determined by the glucose in the medium. This assumption was substantiated by the experimental results illustrated in Figs. 5A and B. In these figures is shown the effect of different maltose concentrations on the rate of  $\alpha$ -glucosidase synthesis in the presence of 0.2 and 0.3 % glucose.

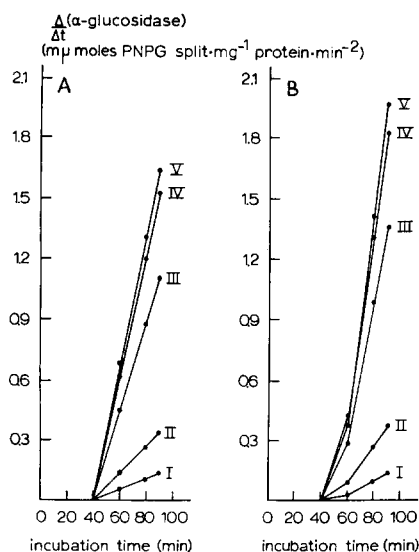


Fig. 5. The effect of various maltose concentrations on the change in the rate of  $\alpha$ -glucosidase synthesis  $\Delta(\text{rate})/\Delta t$ , with incubation time. In the experiment illustrated in Fig. 5A 0.2 % glucose, in that presented in Fig. 5B 0.3 % glucose was present in the incubation medium. Maltose concentrations: I, no maltose; II, 1 mg/ml; III, 3 mg/ml; IV, 10 mg/ml; V, 30 mg/ml.

It appears that the brief repression of enzyme synthesis in the medium containing 0.3 % glucose cannot be abolished even by a high maltose concentration of 3 %. Moreover, depending on its concentration, maltose appears to have a simple "multiplying effect" on the rate of  $\alpha$ -glucosidase synthesis as determined by the glucose concentration. As calculated from the data presented in Figs. 5A and 5B, the enhancing effects exerted by the maltose concentrations of 0, 0.1, 0.3, 1 and 3 % were proportional to 1 : 3 : 11 : 15 : 16. The kinetic data lead to the tentative conclusion that no competition between the inducing effect of maltose and the repressing effect of glucose occurs in the  $\alpha$ -glucosidase synthesis. This conclusion was supported by the results of experiments designed to study the level(s) of protein synthesis at which induction by maltose and catabolite repression act, using inhibitors of protein synthesis at the level of transcription and that of translation, *viz.* actinomycin D, puromycin and cycloheximide.

#### *Effect of maltose on the synthesis of mRNA for $\alpha$ -glucosidase*

Induced  $\alpha$ -glucosidase synthesis by the yeast protoplasts can be inhibited by actinomycin D. This inhibition is strongly dependent on the time at which actinomycin D is added<sup>21</sup>. The data presented in Fig. 6 show that the inhibitory effect of actinomycin D is stronger when it is added soon after induction, *i.e.* after the addition of maltose. These results suggest that a phase in the induced  $\alpha$ -glucosidase synthesis may exist during which the inhibition by actinomycin D becomes minimal. This hypothesis was confirmed by the experiment illustrated in Fig. 7 which demonstrates that if actinomycin D is added when  $\alpha$ -glucosidase synthesis occurs at its near maximal rate, a significant inhibitory effect is no longer observed. Although insensitive to the addition of actinomycin D,  $\alpha$ -glucosidase synthesis is sensitive to

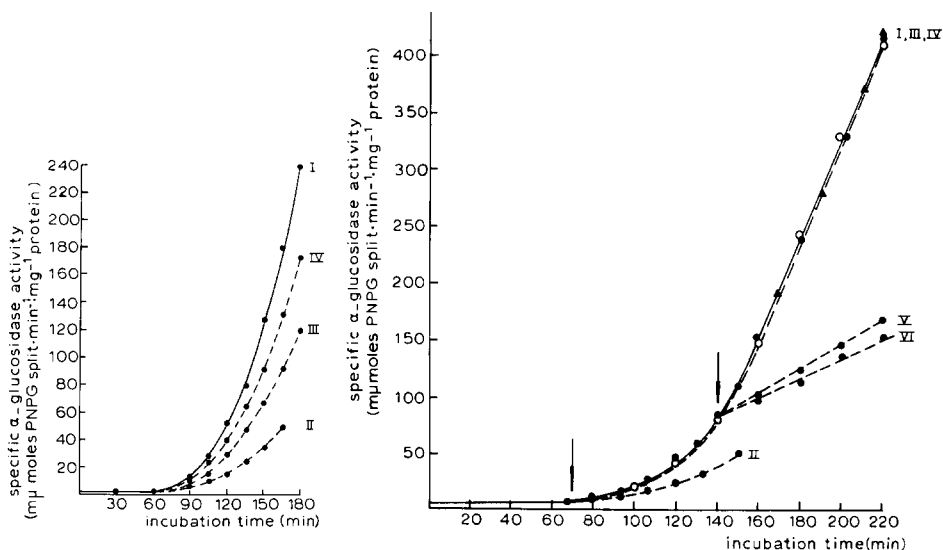


Fig. 6. The effect of actinomycin D ( $80 \mu\text{g/ml}$ ) on induced  $\alpha$ -glucosidase synthesis in protoplasts of *S. carlsbergensis*, when added at different times during incubation in a medium containing 0.2 % glucose and 2 % maltose. I, no actinomycin D (blank experiment); II, actinomycin D added at 0 min, together with the inducer maltose; III, actinomycin D added at 30 min; IV, actinomycin D added at 60 min.

Fig. 7. The effect of actinomycin D ( $80 \mu\text{g/ml}$ ), puromycin ( $400 \mu\text{g/ml}$ ) and cycloheximide ( $1 \mu\text{g/ml}$ ), respectively, on induced  $\alpha$ -glucosidase synthesis by the yeast protoplasts in a medium containing 0.2 % glucose and 2 % maltose. I, no inhibitor added; II, actinomycin D added at 0 min; III, actinomycin D added at 75 min; IV, actinomycin D added at 140 min; V, puromycin added at 140 min; VI, cycloheximide added at 140 min.

inhibitors *i.e.* puromycin and cycloheximide operating at the level of translation (Fig. 7). The variation in the inhibition by actinomycin D on induced  $\alpha$ -glucosidase synthesis was not due to a difference in the permeability of the protoplasts because, as was shown previously the degree of inhibition under all experimental conditions used remains constant<sup>21</sup>. Moreover, it was found that during the actinomycin D-insensitive phase of the induced  $\alpha$ -glucosidase synthesis, mRNA synthesis (*i.e.* the synthesis of pulse-labeled RNA) was inhibited to almost the same extent as during the earlier sensitive phase<sup>21,22</sup>. The complete inability of actinomycin D to inhibit  $\alpha$ -glucosidase synthesis during the later phase of incubation in the presence of maltose suggests that the mRNA coding for  $\alpha$ -glucosidase is mainly synthesized during the early phase of induction. Thereafter, enzyme synthesis occurs *via* the translation of the mRNA.

The effect of actinomycin D on noninduced or derepressed  $\alpha$ -glucosidase synthesis is shown in Fig. 8. As can be seen from the data presented in this figure, derepressed  $\alpha$ -glucosidase synthesis is not inhibited at all. On the contrary, a slight stimulation by actinomycin D was repeatedly observed. However, because enzyme synthesis is decreased by puromycin and cycloheximide, it must be concluded that for the derepression of  $\alpha$ -glucosidase synthesis by low concentrations of glucose mRNA synthesis is not obligatory. Thus, the induction of  $\alpha$ -glucosidase synthesis by maltose appears to depend on the synthesis of new mRNA.

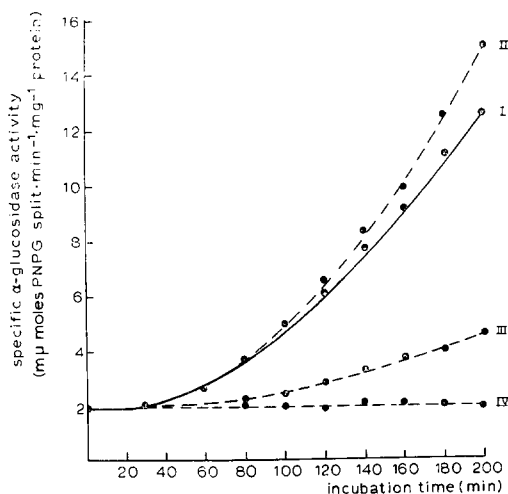


Fig. 8. The effects of actinomycin D ( $80 \mu\text{g/ml}$ ), puromycin ( $400 \mu\text{g/ml}$ ) and cycloheximide ( $1 \mu\text{g/ml}$ ) on noninduced (derepressed)  $\alpha$ -glucosidase synthesis in the presence of 0.2 % glucose. I, no inhibitor added; II, actinomycin D; III, puromycin; IV, cycloheximide; all added at the beginning (0 min) of the experiment.

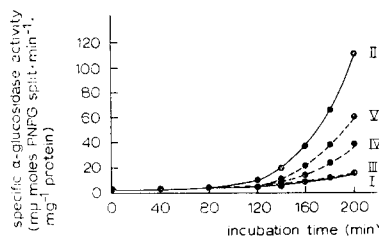


Fig. 9. The effect of actinomycin D ( $80 \mu\text{g/ml}$ ) on  $\alpha$ -glucosidase synthesis induced by maltose. Derepressed synthesis starts at 0 min in the presence of 0.2 % glucose; maltose (2 %) was added at 80 min. Actinomycin D was added at various times before the inducer. I, no maltose added; II, no actinomycin D added; III, actinomycin D added at 40 min; IV, actinomycin D added at 60 min; V, actinomycin D added at 80 min.

The effect of maltose on the synthesis of mRNA coding for  $\alpha$ -glucosidase could be either an all-or-none effect or a concentration-dependent effect. In order to distinguish between these two possibilities, the effect of maltose on the synthesis of the mRNA necessary for enhanced  $\alpha$ -glucosidase synthesis was studied in more detail. As previously stated, the rate of  $\alpha$ -glucosidase synthesis depends on the concentration of the inducer (Fig. 5). It was found (see ref. 20) that maltose exerts a direct stimulating effect (*i.e.* no time lag occurs) when it is added to the incubation medium at least 40 min after the addition of glucose. Therefore, similar experiments on the induction of  $\alpha$ -glucosidase synthesis were executed using various suboptimal maltose concentrations. During the later phase of enzyme synthesis, which was shown to be insensitive to actinomycin D (Fig. 7), an additional amount of maltose was added, and the effect of actinomycin D on the subsequent additional stimulation of  $\alpha$ -glucosidase synthesis was determined. Assuming that the rate of enzyme synthesis during this later phase reflects the amount of mRNA coding for  $\alpha$ -glucosidase, no further stimulation should occur when mRNA synthesis is blocked. On the contrary, when it is assumed that the same amounts of mRNA are present independently of the concentration of the inducer and that the different rates of synthesis, as observed when various maltose concentrations are present, reflect a concentration-dependent effect of the inducer on the rate of translation of the mRNA, stimulation will be obtained even when actinomycin D completely inhibits mRNA synthesis. As shown in Fig. 9, inhibition of  $\alpha$ -glucosidase synthesis was almost complete when actinomycin D was added about 40 min before the sequential addition of maltose. Summarized in Table I are the results of the experiments in which induction was performed with various

TABLE I

THE EFFECT OF ACTINOMYCIN D ON THE STIMULATION OF  $\alpha$ -GLUCOSIDASE SYNTHESIS BY THE SEQUENTIAL ADDITION OF MALTOSE

Protoplasts were induced with suboptimal maltose concentrations. At 150 min after induction, a second quantity of maltose was added. The rate of synthesis was determined in the absence and in the presence of actinomycin D (80  $\mu$ g/ml, added at 90 min). All rates of enzyme synthesis reported were determined from the increase in specific  $\alpha$ -glucosidase activity during the phase of a constant rate of synthesis, *i.e.* 210 min after the addition of the inducer maltose.

Maltose concentration at 0 min (%)	$\Delta(\alpha\text{-glucosidase})/\Delta t$ ( $\mu\text{moles PNPG split}\cdot\text{mg}^{-1}\text{protein}\cdot\text{min}^{-2}$ )		Maltose concentration at 150 min (%)	$\Delta(\alpha\text{-glucosidase})/\Delta t$ ( $\mu\text{moles PNPG split}\cdot\text{mg}^{-1}\text{protein}\cdot\text{min}^{-2}$ ) at 210 min	
	at 120 min	at 150 min		With actinomycin D	Without actinomycin D
0.1	0.6	0.5	3	0.7	3.2
0.3	2.2	2.2	3	2.4	3.0
1	3.0	3.1	3	3.2	3.4
0.1	0.6	0.5	0.3	0.7	2.3
0.3	2.2	2.2	1	2.3	3.0

maltose concentrations and in which the effects of actinomycin D (added 90 min after induction) on the stimulation of  $\alpha$ -glucosidase synthesis by an additional amount of maltose (added 150 min after the first portion) were determined.

From the data in Table I, it can be concluded that after the sequential addition of more maltose during the phase of induction where actinomycin D no longer affected the original rate of (suboptimal)  $\alpha$ -glucosidase synthesis, the synthesis of new mRNA must occur before enhanced synthesis of  $\alpha$ -glucosidase occurs. Consequently, the results suggest that the final constant rate of  $\alpha$ -glucosidase synthesis, which was dependent on the maltose concentration, reflects the final amounts of mRNA present in protoplasts to which various amounts of inducer were added. No significant effect of maltose on  $\alpha$ -glucosidase synthesis was observed when actinomycin D was present. The hypothesis that maltose cannot exert any significant effect on the rate of translation is corroborated by the following experimental results.

#### *Effects of maltose and glucose on the level of translation*

The mRNA coding for  $\alpha$ -glucosidase is apparently rather stable because in the presence of high concentrations of actinomycin D for a long period, enzyme synthesis can occur only *via* translation of the preexisting mRNA. Therefore, the effects of both maltose and of glucose were studied at the translation level by varying their concentrations during the actinomycin D-insensitive phase of  $\alpha$ -glucosidase synthesis. The effect of high concentrations of glucose on the rate of maltose-induced synthesis was determined in the presence of maltose. As seen in Fig. 10 (Curve VI), glucose (final concentration 1 %) causes a strong inhibition of the  $\alpha$ -glucosidase synthesis. This inhibition does not occur immediately because for about 20 min after the addition of glucose,  $\alpha$ -glucosidase synthesis continues at a normal rate before repression occurs.

It also seemed of interest to determine the effect of glucose on the stability of the existing mRNA. As reported in the preceding section, it was found that the final



rate of induced  $\alpha$ -glucosidase synthesis is determined by the content of the corresponding mRNA. Therefore, the stability of this mRNA was studied by comparing the original rate of this synthesis with the rate of  $\alpha$ -glucosidase synthesis determined after 50 min of glucose repression under optimal conditions of derepressed synthesis in the presence of 2 % maltose and actinomycin D. As seen in Fig. 10 (Curves VII and VIII)  $\alpha$ -glucosidase synthesis continues at its original rate without being affected by actinomycin D. It is concluded that the mRNA remains stable during glucose repression of  $\alpha$ -glucosidase synthesis.

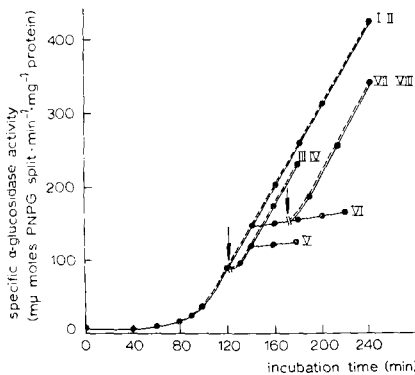


Fig. 10. The effects of glucose and of maltose during the actinomycin D-insensitive phase of induced  $\alpha$ -glucosidase synthesis. I, blank induction curve; II, actinomycin D added at 90 min. The protoplasts were first incubated in a medium containing 0.1 % glucose and 2 % maltose. After 120 min of incubation, half of the protoplast suspension was rapidly centrifuged; the protoplasts were washed and resuspended in the same medium containing no maltose but various glucose concentrations. III, 0.1 % glucose; IV, 0.1 % glucose and 80  $\mu$ g/ml actinomycin D; V, 1 % glucose. In the other half of the protoplasts, which were not centrifuged, the effect of a high glucose concentration in the presence of maltose was determined. VI, 1 % glucose added at 120 min. The capacity to synthesize  $\alpha$ -glucosidase after glucose repression (at 170 min) was also tested by centrifuging and washing the protoplasts and resuspending them in a fresh medium containing 0.1 % glucose and 2 % maltose. VII, no actinomycin D, VIII, actinomycin D added at 170 min.

Another series of experiments was performed in order to study the effect of maltose on  $\alpha$ -glucosidase synthesis during the actinomycin D-insensitive period (Curves III–V). In Fig. 10 are presented the results of an experiment in which protoplasts were transferred to an inducer free medium after rapid centrifugation and after a subsequent washing in an incubation medium containing 0.1 % glucose. As seen in Fig. 10,  $\alpha$ -glucosidase synthesis continues for a long time at a rate equal to that at the time of transfer. A 0.1 % glucose concentration was experimentally found to be optimal. A higher glucose concentration causes, even in the absence of maltose, a strong repression after about 20 min (Fig. 10, Curve V). Moreover,  $\alpha$ -glucosidase synthesis as determined in the absence of inducer appeared to be insensitive to actinomycin D.

The results of the previous experiments led us to conclude that the translation of mRNA coding for  $\alpha$ -glucosidase is inhibited when high glucose concentrations are present in the incubation medium. However, the addition (Table I) or omission (Fig. 10) of maltose is not effective at the translation level. Moreover, maltose was shown

to exert a direct stimulatory effect on the synthesis of the mRNA needed for  $\alpha$ -glucosidase synthesis.

The effect of glucose on the synthesis of this mRNA was also of interest. Unfortunately, it appeared to be impossible to study the effect of glucose on mRNA synthesis in the absence of inducer (compare Fig. 3). Therefore in the next section, the experimental results concerning the effect of glucose on mRNA synthesis as stimulated by maltose are presented.

*Changes in the maximal capacity of protoplasts to synthesize  $\alpha$ -glucosidase during induced synthesis under various conditions of repression*

The maximal capacity of protoplasts to synthesize  $\alpha$ -glucosidase ultimately depends on the amount of specific mRNA present. In the following series of experiments, conditions were chosen which led to a variation in the maximal capacity to synthesize  $\alpha$ -glucosidase (even in the absence of maltose) after various induction periods. The aim of these experiments was to compare the changes in maximal synthetic capacity with the changes in the specific mRNA content during the induction period.

Fig. 11 provides an illustration of induced  $\alpha$ -glucosidase synthesis under conditions of nearly optimal derepression.

Moreover, at various times after induction with maltose, the protoplasts were used to determine their maximal capacity to synthesize  $\alpha$ -glucosidase in the absence of inducer in a medium containing 0.1 % glucose.

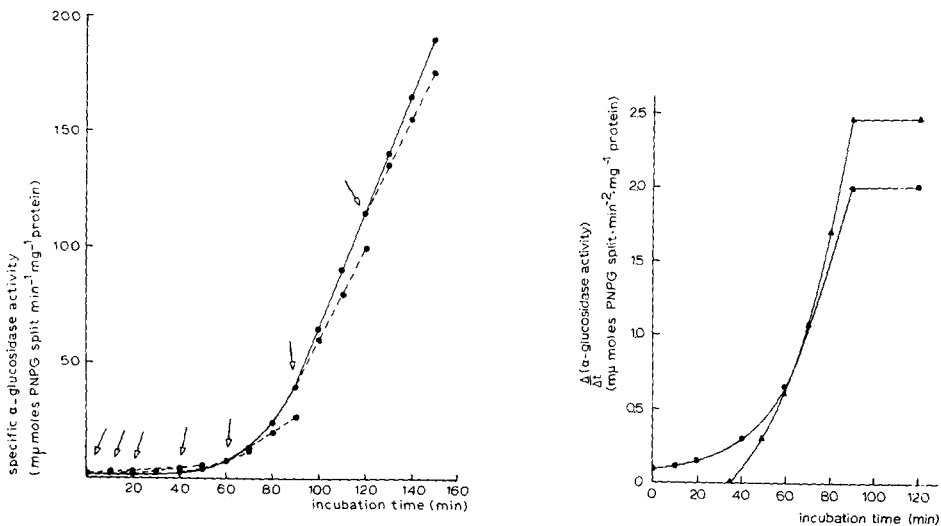


Fig. 11. The rate of  $\alpha$ -glucosidase synthesis and the maximal capacity of the yeast protoplasts to continue synthesizing  $\alpha$ -glucosidase in the absence of the inducer maltose after induction during varying lengths of time under derepressed conditions. Protoplasts were incubated in a medium containing 0.1 % glucose and 2 % maltose (●-●). At various times after induction, as indicated by the arrows, part of the protoplasts was centrifuged, washed and resuspended in a medium containing 0.1 % glucose (●--●).

Fig. 12. In this figure, the differential curve  $\Delta(\text{rate})/\Delta t$  is plotted from the experiment illustrated in Fig. 11 (▲-▲). Moreover, the change in the maximal capacity to synthesize  $\alpha$ -glucosidase in the absence of the inducer maltose is also plotted (●-●).

In Fig. 12, the change in the rate of enzyme synthesis with incubation time ( $\Delta(\text{rate})/\Delta t$ ) during induction is compared with the change in the maximal capacity of protoplasts to continue synthesizing  $\alpha$ -glucosidase after the removal of the inducer maltose. This synthesis was found to be insensitive to actinomycin D. Therefore, from the data in Figs. 11 and 12 it can be concluded that only during the first 40 min the actual rate of  $\alpha$ -glucosidase synthesis is lower than the potential capacity. This finding suggests that an inhibition of translation occurs during this period.

A similar experiment to study induced enzyme synthesis under conditions of strong repression has been performed. In Fig. 13, induced  $\alpha$ -glucosidase synthesis under such conditions is illustrated. Moreover, the rate of synthesis occurring during optimal (derepressing) conditions after removal of the inducer was also determined. The curves obtained were not linear; the rate of  $\alpha$ -glucosidase synthesis in the protoplasts shows a diauxic curve which, moreover, was found to be insensitive to actinomycin D. In Fig. 14 are presented data on both the change in  $\Delta(\text{rate})/\Delta t$  during induced synthesis and the change in the maximal capacity to continue  $\alpha$ -glucosidase synthesis after removal of the maltose, as determined from the linear rates of synthesis after the 15–20-min inhibition period (see Fig. 13). During 120 min the capacity to synthesize  $\alpha$ -glucosidase is higher than that observed during incubation in the presence of maltose and of high glucose concentrations.

The experimental data presented in Figs. 11–14 led to the conclusion that changes in the maximal capacity to synthesize  $\alpha$ -glucosidase during and after induction strongly differ during derepression and repression, respectively.

As previously stated, the aim of these experiments was to compare the changes in maximal synthetic capacity with the changes in the increase in the content of

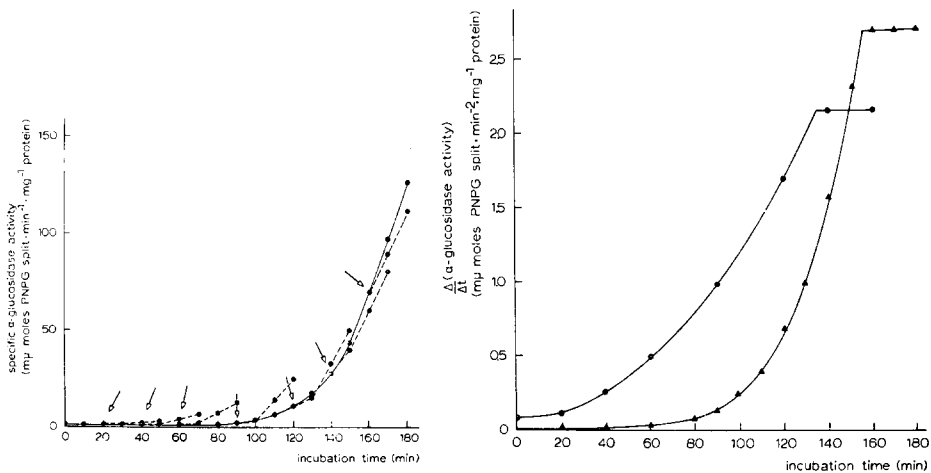


Fig. 13. The rate of synthesis and the maximal capacity to synthesize  $\alpha$ -glucosidase during and after induction under repressed conditions. Protoplasts were incubated in a medium containing 1 % glucose and 2 % maltose (●-●). At various times after induction (indicated by the arrows), aliquotes of the protoplasts were centrifuged, washed and resuspended in a medium containing 0.1 % glucose (○-○).

Fig. 14. In this figure the differential curve,  $\Delta(\text{rate})/\Delta t$ , is plotted from the experiment illustrated in Fig. 13 (▲-▲). Moreover, the change in the maximal capacity to synthesize  $\alpha$ -glucosidase as determined from the rate of this synthesis after transfer of the protoplasts to a medium containing a low glucose concentration (Fig. 13) is plotted (●-●).

mRNA coding for  $\alpha$ -glucosidase during the induction period. Thus, in another series of experiments under varying conditions of glucose repression, the inhibitory effect of actinomycin D on induced  $\alpha$ -glucosidase synthesis was determined.

From the data in the foregoing sections, it followed that actinomycin D inhibits  $\alpha$ -glucosidase synthesis during derepression *via* transcription only during an early and relatively short period, and that the synthesis of mRNA coding for  $\alpha$ -glucosidase must be (almost) finished after 60 to 75 min (Fig. 7). In Table II are presented data on the inhibitory effect of actinomycin D added at various times during induction under conditions of derepression and repression, respectively.

TABLE II

THE PERCENTAGE OF INHIBITION OF  $\alpha$ -GLUCOSIDASE SYNTHESIS BY ACTINOMYCIN D, WHEN ADDED AT VARIOUS TIMES DURING INDUCED SYNTHESIS DURING BOTH REPRESSION AND DEREPRESSION

At different times after the induction of the enzyme synthesis by maltose (2 %) actinomycin D (80  $\mu$ g/ml) was added in a medium containing 0.1 % glucose and 1 % glucose, respectively. From the differential curves  $\Delta(\text{rate})/\Delta t$ , derived from the experiments, the percentage of inhibition was calculated.

Time at which actinomycin D was added after the start of induction (min)	Inhibition (%) of $\alpha$ -glucosidase synthesis in a medium containing	
	0.1 % glucose	1 % glucose
No actinomycin D	0	0
120	0	0
90	0	15
60	0	50
30	56	76
0	87	90
30 min before the inducer	96	96

The percentages of inhibition, as calculated from the rates of synthesis, are presented for derepression with 0.1 % glucose and for repression with 1 % glucose. The combined data presented in Figs. 11–14 and in Table II show that during induction both the maximal capacity to synthesize  $\alpha$ -glucosidase and the content of mRNA coding for  $\alpha$ -glucosidase increase for a longer period during repression by 1 % glucose.

Furthermore, as shown in Fig. 15, the change in the maximal synthetic capacity (expressed as a percentage of the blank, *i.e.* as the percentage of the maximal synthesis in the absence of the inhibitor actinomycin D) parallels the change in the maximal synthetic capacity for both types of induction curves (*i.e.* for induction during derepression (Curves a and b) and during repression (Curves c and d)). However, it can be seen that Curves a and c precede b and d by about 30 min. This, together with the results reported earlier in Fig. 9, indicates that the inhibition by actinomycin D is complete only about 30 min after its addition.

The experimental results suggest that the maximal capacity to synthesize the enzyme  $\alpha$ -glucosidase also reflects the amount of mRNA coding for this enzyme. Moreover, glucose appears to affect the increase in the content of mRNA coding for  $\alpha$ -glucosidase during induction, which suggests that repressing glucose concentrations affect not only the translation of mRNA but also the synthesis of mRNA coding for  $\alpha$ -glucosidase.

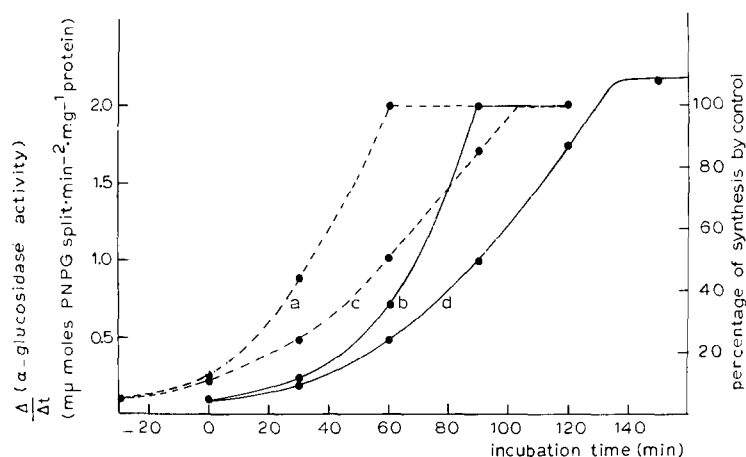


Fig. 15. The changes in the maximal capacity to synthesize  $\alpha$ -glucosidase are compared as determined during and after induction under derepressed (see Fig. 12) and under repressed (see Fig. 14) conditions. Moreover, the data as presented in Table II on the effect of actinomycin D (80  $\mu$ g/ml) on induced  $\alpha$ -glucosidase synthesis under derepressed and repressed conditions are illustrated in this figure. Induction in the presence of 0.1 % glucose and 2 % maltose: a, change in the effect of actinomycin D on  $\alpha$ -glucosidase synthesis when the inhibitor is added at various times (plotted as the percentage of the original or blank synthesis); b, change in the maximal capacity to synthesize  $\alpha$ -glucosidase, as determined from the maximal rate of synthesis after transfer of the protoplasts to an inducer-free medium containing 0.1 % glucose. Induction in the presence of 1 % glucose and 2 % maltose: c, same as a; d, same as b.

## DISCUSSION

From the present experimental results it is seen that maltose as the inducer of  $\alpha$ -glucosidase synthesis stimulates primarily the synthesis of an mRNA. This is concluded from the fact that actinomycin D inhibits  $\alpha$ -glucosidase synthesis in the yeast protoplasts only when present during maltose induction of this synthesis. Moreover under these conditions, a lower rate of enzyme synthesis, induced by sub-optimal maltose concentrations, appeared to result from a lower mRNA level in the protoplasts. Furthermore, the mRNA for  $\alpha$ -glucosidase must be fairly stable because it is synthesized at a significant rate only during a well-defined 90- to 120-min period of induction. This is based on the fact that actinomycin D shows an inhibitory effect on  $\alpha$ -glucosidase synthesis only during the first phase of induction; however, a lasting inhibitory effect is observed on the synthesis of total pulse-labeled mRNA<sup>21,22</sup>. In the later phase, enzyme synthesis continues *via* translation of the mRNA regardless of the presence or absence of the inducer. This was concluded from the experimental results indicating that the removal of the inducer during the actinomycin D-insensitive phase of  $\alpha$ -glucosidase synthesis has no effect on the rate of this synthesis. Moreover, it was shown that both the constant rate of  $\alpha$ -glucosidase synthesis during the later phase of synthesis and the optimal synthetic capacity of the protoplasts to synthesize the enzyme after varying periods of induction directly reflect the amount of mRNA coding for  $\alpha$ -glucosidase. Therefore, the effect of high glucose concentrations on the increase in mRNA content induced by maltose can be tentatively explained as an inhibitory effect exerted at the level of transcription.

In this respect, these results are in accordance with the data published by NAKADA AND MAGASANIK<sup>12</sup>. The experimental results obtained by these authors during a study on the regulatory effects of induction and catabolite repression on the synthesis of the mRNA coding for  $\beta$ -galactosidase in *E. coli* led to the conclusion that the inducer increases and that catabolite repression decreases the rate of synthesis of mRNA specific for  $\beta$ -galactosidase. However, glucose repression of  $\alpha$ -glucosidase synthesis also acts at the translation level. Upon addition of a large amount of glucose during the actinomycin D-insensitive phase of  $\alpha$ -glucosidase synthesis, this synthesis is strongly inhibited. This inhibition proved to be reversible, *i.e.* the capacity to synthesize  $\alpha$ -glucosidase from the mRNA is not diminished by glucose because it is fully restored after the removal of the high glucose concentration.

Thus, it appears that glucose exerts a control at two levels of enzyme synthesis: the first one operates at the transcription level and the second one at the level of translation.

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