ALKALINE PHOSPHATASE DEREPRESSION IN VEGETATIVE CELLS OF BACILLUS SUBTILIS BY GLUCOSE AND ITS REVERSAL BY LACTATE

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SUMMARY: Alkaline phosphatase synthesis in the vegetative cells of Bacillus subtilis is strongly stimulated by glucose and glycerol in the low phosphate derepressive media. The glucose stimulation is totally removed by 2% lactate. This reversal of glucose stimulation by lactate is clearly demonstrated when the enzyme formation is progressing at a maximal linear rate. Abolition of glucose derepression by lactate is characterized by initial total halt followed by a diminished rate of synthesis.

INTRODUCTION

In bacteria, the inhibition by one carbon source of the formation of an enzyme system required for the metabolism of another carbon source is known as catabolic repression (1). In addition to this, the selection of a preferred source of constituents required for cell growth also causes repression. Thus, inorganic phosphate represses alkaline phosphatase synthesis in bacteria (2). Substances other than inorganic phosphate have not been reported to repress this enzyme, although it has been suggested that the regulation mechanism of alkaline phosphatase might be activated by something other than phosphate starvation in sporulating cells of B. subtilis (3). In this communication we report that alkaline phosphatase formation in vegetative cells of B. subtilis is derepressed by glucose and glycerol, whereas lactate, pyruvate and succinate abolish this glucose effect.

METHODS

Organism and culture condition: B. subtilis strain SB15 kindly supplied by Dr. A. Tsugita, Osaka University, Japan, was used in the experiments. Details of the culture maintenance and growth conditions have been described previously (4). The basal nutritive solution contained 1% Bacto-Peptone (Difco, selected batches containing low inorganic phosphate), 0.6% Tris hydroxymethylaminomethane (Tris), 0.3% sodium chloride, 0.005% manganese sulfate and 0.9×10^{-4} M magnesium sulfate and 1.2×10^{-4} M calcium chloride added separately after sterilization. Carbon sources were added to this basal media as required.

The 1% Bacto-Peptone media contained 0.2 - 0.3 mM inorganic phosphate (P_i); removal of phosphate from this medium by precipitation (4) lowered the concentration of P_i to 0.05 - 0.1 mM. Cell growth is expressed either as total dry weight per ml or as relative increment $\frac{M_t}{M_o}$ where M_t and M_o are the dry weights of the organism in mg/ml at times 't' and 'o', determined from the standard calibration curves of the dry weights against absorbencies of the cell suspensions at 540 nm in a Klett-Summerson colorimeter.

Enzyme assay: Alkaline phosphatase activity was assayed in aliquots of whole culture as described previously (4). The results are expressed as nmoles of p-nitrophenol produced per minute at 37° C (units of enzyme activity) per ml suspension. To facilitate comparison of the repression or derepression curves, the enzyme activities are expressed as suggested by Paigen (5) as $\Delta E = \frac{E_t - E_o}{M_o}$, where E_t and E_o are enzyme activities at times 't' and 'o'.

RESULTS

It is evident from Fig. 1 that formation of alkaline phosphatase shows

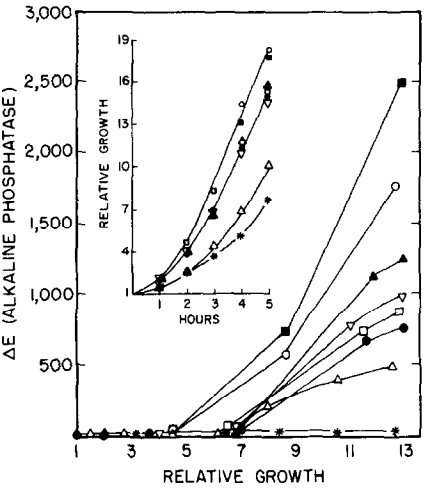

Fig. 1. Alkaline phosphatase synthesis and growth (insert) in peptone medium with and without any carbon sources. The cells after 10 hours of growth in peptone medium containing 0.1% sodium lactate and 2 mM disodium hydrogen phosphate (4) were washed and used to inoculate the required growth medium. An aliquot of the whole culture was used for enzyme estimation; ●, peptone alone; peptone with ▲ , 0.1% lactate; △ , 2% lactate; O, 0.1% glucose; ■ , 0.5% glycerol; □ , 0.5% succinate; ▽ , 0.5% pyruvate; ✱ , 10 mM disodium-hydrogen-phosphate.

a great variation under different growth conditions. The synthesis of this enzyme is increased by the addition of glucose or glycerol in comparison to its basal synthesis in peptone medium alone; whereas, pyruvate, succinate and lactate cause a smaller change. The effect of lactate is notable that low concentrations (e.g., 0.1%) slightly stimulate the enzyme synthesis and higher concentrations (e.g., 2%) cause diminution below the basal level. Under comparative conditions 10 mM inorganic phosphate causes total repression of alkaline phosphatase synthesis. The enzyme synthesis is further characterized by a prolonged lag which is significantly shorter in the presence of glucose or glycerol than in the presence of other substances. Though compared to glucose and glycerol, the growths (Fig. 1, insert) in the presence of 0.1% lactate, succinate, pyruvate and peptone alone do not show significant variation; 2% lactate and 10 mM phosphate cause a slower growth rate. The

maximum amount of alkaline phosphatase formed in the presence of 2% lactate is only about 20 - 25% of the amount synthesized in 0.5% glycerol or 0.1% glucose media.

Alkaline phosphatase synthesis is adequately derepressed in low phosphate peptone medium without removal of P_i; of course, the amount of enzyme synthesized is higher in the medium from which P_i has been removed. The effect of carbon sources could be equally demonstrated in either media whether P_i has been removed from them or not.

Stimulation of alkaline phosphatase synthesis by varying concentrations of glucose and lactate (Fig. 2) reaches a maxima at about 0.2 - 0.3%.

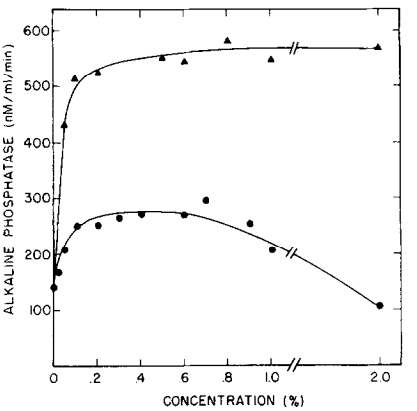

Fig. 2. Effect of different concentrations of glucose (▲), and lactate (●), on the alkaline phosphatase synthesis. The enzyme activity was estimated after 10 hours of growth in the respective medium.

Glucose stimulation is about four fold higher than lactate. The lactate effect contrasts with that of glucose by showing inhibition of synthesis even below the basal level at high concentrations.

It is pertinent to examine the effect of pH alteration induced by the composition of the media (glucose or lactate) on the enzyme formation, and also the action of glucose and lactate on in vitro enzyme activity. The possible

influence of pH alteration was examined by comparing the enzyme formation in glucose and 2% lactate media with and without maintaining the pH at a constant level. The pH of the glucose medium changed from 7.0 to 6.7 and that of lactate medium from 7.0 to 7.3 during an eight-hour period of growth. The growth and alkaline phosphatase synthesis of the pH-adjusted medium compared to the unadjusted one show little difference. The compounds used in the present experiment as the carbon sources of the growth media cause no appreciable alteration of the activity of alkaline phosphatase, purified from B. subtilis, except for a slight stimulation at high concentrations of lactate.

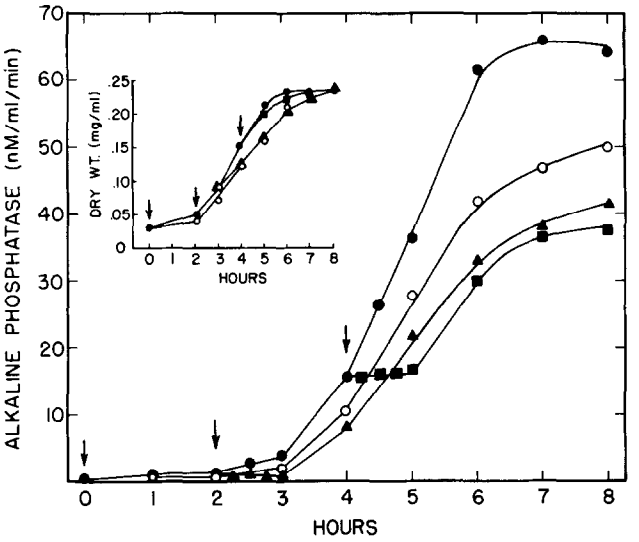

Fig. 3. Effect on the alkaline phosphatase synthesis and growth (insert) of lactate addition (2% final concentration) at various time intervals; ● , glucose alone; glucose with lactate, added at O, 0 hour; ▲ , 2 hours; ■ , 4 hours.

In Fig. 3, results are presented to show the effect of lactate on alkaline phosphatase synthesis of the cells growing in glucose medium. The cells were inoculated in 0.1% glucose medium in separate flasks; sterile solution of sodium lactate was added at a final concentration of 2% in those flasks at various time intervals, namely, immediately after inoculation (0 hr), and after

2 or 4 hours of growth in glucose. Though there is only 10 - 15% inhibition of growth after the addition of the lactate (Fig. 3) the enzyme synthesis shows significant changes. The results are expressed as units of enzyme activity per ml culture at different time intervals, because the experiments were continued well into the stationary phase of growth when the calculation of relative growth is of no value. Effect of lactate addition varied depending on the time of growth (Fig. 3, arrows). Additions at 0 and 2 hours increase the characteristic lag followed by a diminished rate of synthesis. On the other hand, the addition at 4 hours causes total halt of alkaline phosphatase synthesis for about an hour (1/2 - 3/4 generation) followed by recovery of the synthesis at a lower rate than the untreated culture. The greater effect of lactate, when enzyme synthesis is progressing at the linear maximal rate, might be due to a specific action on alkaline phosphatase synthesis, i.e., greater is the synthesis the more obvious will be the inhibition. Specificity of the action is further suggested from the fact that during the one hour period after addition of lactate the increment of cell mass, representing total protein synthesis, remains unaffected (an increase of 0.05 mg dry wt/ml culture), but there is 95 - 100% reduction in the alkaline phosphatase synthesis.

The effect of pre-growth in glucose and subsequent transfer into media containing lactate and vice versa can be seen in Fig. 4. Following the transfer, the washed cells continue to grow immediately, although the rate of growth depends on the composition of the media used. Unlike growth, resumption of alkaline phosphatase synthesis is preceded by a lag which again depended on the composition of growth media in which the cells were transferred, i.e., cells in lactate media have longer (15 - 20%) lag than those in glucose. The enzyme synthesis started after the lag very sharply at a maximal linear rate, which is always the same in glucose medium whether the cells are

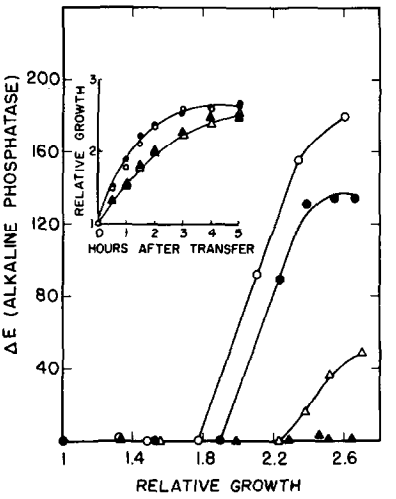

Fig. 4. Effect on alkaline phosphatase synthesis and growth (insert) of cells pregrown in glucose and transferred to lactate medium and vice versa. The cultures were grown for 3 hours and the cells were washed and suspended in the fresh medium. The turbidity of this suspension was adjusted equivalent to that of lactate pregrown cells; pregrown in 0.1% glucose and transferred to, ●, 0.1% glucose; ▲, 2% lactate; pregrown in 2% lactate and transferred to, ○, 0.1% glucose; △, 2% lactate.

pregrown in glucose or lactate. Transfer of the cells into lactate media causes a significant decline in the rate of synthesis and the total enzyme content of the cells. It is interesting that glucose pregrown cells show much more intense repression than lactate pregrown cells on transferrence to the lactate media.

DISCUSSION

Results presented in this paper clearly show that the rate and the amount of alkaline phosphatase synthesized by vegetative cells of Bacillus subtilis vary in a complex manner in the presence of various carbon sources. In the usual low phosphate derepression medium (4) glucose and glycerol cause stimulation; and lactate, pyruvate and succinate cause repression of alkaline phosphatase synthesis. The formation of alkaline phosphatase in the

vegetative stage of growth is characterized by a prolonged lag for about 2 generations following which the synthesis started at a linear rate which might show a further small rise. The repression by lactate has the following significant characteristics: (i) the basal level of enzyme synthesis (in peptone medium alone) is not appreciably repressed; (ii) derepressions of alkaline phosphatase formation in glucose or glycerol media are almost totally abolished; (iii) repression is expressed by immediate cessation followed by synthesis at a diminished rate; (iv) transfer of cells from glucose to lactate causes much more intense repression in comparison to the cells continuously growing in lactate medium. The experiments demonstrate a phenomenon not reported earlier, namely, that alkaline phosphatase can undergo repression and derepression under specific metabolic conditions, even though the cells are in the usual low phosphate derepressive medium. Hence, the synthesis of alkaline phosphatase must be regulated by metabolic parameters other than the level of inorganic phosphorus in the growth media. Further, the results suggest a specific role of alkaline phosphatase in carbohydrate metabolism.

Though conclusive interpretation of the results is not possible now, some speculation can be made as a working hypotheses. It seems likely from the present study that alkaline phosphatase might have a role in the glucose catabolism through transphosphorylation reaction or by virtue of increasing the inorganic phosphate pool of the cell. It is possible that the synthesis of this enzyme is repressed by lactate or some other substrates, because their metabolism might lead to the need of glucose (or hexose) conservation. Thus only the gluconeogenic carbon sources so far tested cause elimination of the glucose derepressible enzyme synthesis. Of course, fluctuation of intracellular inorganic phosphate pool under varying metabolic conditions might be the true regulatory factor. It is not unlikely that depletion of this pool

will be greater under glycolytic than in gluconeogenic conditions. Though this repression has the characteristics of catabolite repression, attempts to reverse this effect by cyclic AMP were unsuccessful. Any effect of cyclic AMP would be masked because the compound is rapidly degraded with the consequent liberation of inorganic phosphate which would lead to intense repression of alkaline phosphatase synthesis.

ACKNOWLEDGMENTS

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