Alkaline phosphatase activity in Vibrio cholerae strain 569B grown in low-phosphate medium was stimulated if glucose or glycerol was used as the carbon source. No such stimulation was observed, however, if tricarboxylic acid cycle intermediates like succinate or citrate were used. Experiments using specific enzyme inhibitors strongly indicated that the metabolic reactions of the glycolytic pathway from glyceraldehyde 3-phosphate to 2-phosphoglycerate play a key role in the stimulation process.

INTRODUCTION

Alkaline phosphatase (APase) of Vibrio cholerae is different from that found in other bacteria in that it is monomeric (Roy et al., 1982a). However, like the enzymes found in other organisms, its synthesis is repressible by phosphate. Derepression can be achieved by lowering the phosphate content of the growth medium. The presence of glucose in low-phosphate medium (LP) stimulates enzyme synthesis further (Roy et al., 1982b). Similar observations have been reported for the Gram-positive organisms Bacillus subtilis (Ghosh & Ghosh, 1972) and Bacillus licheniformis (Hydren et al., 1977). The mechanism of glucose stimulation, however, has remained obscure. We show here that the metabolic reactions of glycolysis from glyceraldehyde 3-phosphate to 2-phosphoglycerate play a major role.

METHODS

Organism and growth media. Vibrio cholerae Inaba 569B strain used in this study was obtained from the Cholera Research Centre, Calcutta, India. Cultures were stored and maintained under the conditions described by Roy et al. (1982b).

Cultures were grown at 37 °C in LP (phosphate-depleted nutrient broth) as described by Roy et al. (1982b). In some experiments glucose or other intermediates of carbohydrate metabolism were added. Growth was assayed by measuring the OD540 using a Gilford model 250 spectrophotometer. (An OD540 of 1.0 corresponded to 1.2 × 10⁹ cells ml⁻¹.)

Growth conditions. Cells were grown in 40 ml LP for 18 h with shaking (180 r.p.m.) at 37 °C and then used to inoculate 100 ml LP medium in a 500 ml Erlenmeyer flask to give an initial OD540 of 0.2. The suspension was incubated at 37 °C with shaking (180 r.p.m.) and samples were removed at appropriate intervals for the assay of enzyme activity and growth.

APase assay. APase activity in whole or toluene-treated cells was assayed as described by Roy et al. (1982b).

RESULTS AND DISCUSSION

Derepression of APase synthesis in the presence of different carbon sources

In order to see if carbon sources other than glucose would stimulate APase in V. cholerae, we measured APase activity in LP in the presence of different carbon sources. Different degrees of stimulation were obtained with different carbon sources (Fig. 1). APase activity was four times

**Abbreviations:** LP, low-phosphate medium; APase, alkaline phosphatase.

0001-3235 © 1986 SGM
Fig. 1. APase activity in *V. cholerae* strain 569B. Cells were grown in LP (○), LP + 2% (w/v) succinate (△), LP + 2% (w/v) citrate (□), LP + 1% (v/v) glycerol (▲) and LP + 0.1% (w/v) glucose (●).

Fig. 2. Effect of various inhibitors of glycolysis on growth (inset) and APase synthesis in *V. cholerae* strain 569B. Cells were grown in LP + glucose (a) or LP (b) for 3 h and then inhibitors were added (arrows). Samples were withdrawn at different times to monitor growth and APase activity. ○, LP + 0.1% (w/v) glucose; ●, LP alone; △, LP + glucose + 2.5 mM-potassium fluoride or LP alone + 2.5 mM-potassium fluoride; □, LP + glucose + 0.075 mM-iodoacetate or LP alone + 0.075 mM-iodoacetate.

higher in cells grown in LP + glucose compared to those grown in LP alone, and growth in LP + glycerol caused a 2.5-fold enhancement. No stimulation of enzyme activity was obtained when succinate or citrate was the carbon source.

*B* involvement of glycolytic pathway in the glucose derepression of APase

The above experiments indicated that glucose or glycerol, which are metabolized through the glycolytic pathway, stimulated APase under derepression conditions, whereas tricarboxylic acid cycle intermediates did not. Thus it appeared that the metabolic reactions of glycolysis were somehow involved in the observed stimulation. Further, since glycerol enters the glycolytic pathway through the formation of glyceraldehyde 3-phosphate, it can be postulated that
metabolic reactions subsequent to this step were involved in the stimulation. In order to identify the metabolic reactions responsible, specific inhibitors were added to cultures grown in LP or LP + glucose, and APase activity was monitored. The addition of 2.5 mM-potassium fluoride, which inhibits enolase in vivo (Kanapka & Hamilton, 1971) and is also active against V. cholerae enolase in vitro (our unpublished observation), had no effect on the derepression of APase activity by glucose. However, derepression was substantially reduced in the presence of 0.75 mM-iodoacetate (Fig. 2a) an inhibitor of glyceraldehyde-3-phosphate dehydrogenase (Harris & Waters, 1976). Similar observations were made with cells grown in LP + glycerol (data not shown), but the inhibitors had no effect when cells were grown in LP (Fig. 2b). Thus it seemed reasonable to conclude that the metabolic reactions of the glycolytic pathway from glyceraldehyde 3-phosphate to 2-phosphoglyceric acid play, in an as yet undetermined way, a major role in the stimulation of APase activity by glucose in V. cholerae.

One criticism of the above experiments is that iodoacetate, a general -SH inhibitor, could inhibit enzymes other than triose phosphate dehydrogenase. However, the observation that iodoacetate at the concentration used had no effect on cell growth (Fig. 2 inset), or on APase activity when V. cholerae was grown in the absence of glucose, effectively rules out this possibility.

REFERENCES


