Reversal by Citrate of the Iodoacetate and Fluoride Inhibition of Glutamic Acid Production by Corynebacterium glutamicum

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C. glutamicum MB-1645 was grown at 28 C in the following medium: MgSO4·7H2O, 0.5 g; MnSO4·H2O, 0.03 g; FeSO4·7H2O, 0.01 g; K2SO4, 1.5 g; (NH4)H2PO4, 1.0 g; (NH4)2HPO4, 1.0 g; (NH4)2SO4, 4.0 g; urea, 1.0 g; d-biotin, 30 µg; p-aminobenzoic acid, 0.05 g; glucose, 10 g; Difco Yeast Extract, 5 g; Difco Vitamin Free Casamino Acids, 5 g; and distilled water, 1,000 ml. The glucose was added separately from a sterile concentrated solution after autoclaving of the medium. After 16 hr, penicillin G was added (10 units/ml). The cells were rapidly chilled in an ice bath, centrifuged in the cold, and suspended at 5 g/liter (dry weight) in the following resting cell medium: KH2PO4, 1 g; MgSO4·7H2O, 0.25 g; urea 6.1 g; (NH4)2SO4, 13.8 g; glucose, 60 or 80 g (added aseptically from a sterile concentrated solution); CaCO3, 30 g; and distilled water, 1,000 ml. The pH was 7.0 to 7.1. Penicillin G was added (10 units/ml) to maintain the excretion of glutamate at a high level for several hours and to insure that the cells were maintained in a non-proliferating state. Incubation was at 32 C on a gyrotrary shaker. At intervals, samples were removed and were centrifuged to sediment the cells. The supernatant fluid was used with no further treatment for the assay of residual glucose by the Glucostat method (Worthington Biochemical Corp., Freehold, N.J.). For assay of glutamic acid, the clarified broth was acidified, heated for 20 min at 60 C, and neutralized to pH 5.0 to 5.5. Glutamic acid was then assayed manometrically by using glutamic acid decarboxylase from Escherichia coli. The glutamate assay was automated with a Technicon autoanalyzer.

In preliminary experiments it was found that 10⁻⁴ to 10⁻³ M concentration of methylene blue, phenazine methosulfate, and sodium azide, inhibitors of terminal electron transfer, was highly inhibitory to the glutamate excretion. Dinitrophenol, an inhibitor of oxidative phosphorylation, was less active, requiring 10⁻² M for complete inhibition. Iodoacetate and NaF, which affect glycolysis, caused, respectively, a 90 and 65% inhibition of glutamate production (Fig. 1A and 1B). The addition of 1% citrate to the resting cell medium completely prevented the effect of either inhibitor. Although some of the citrate was converted directly to glutamate, assays of residual glucose revealed that the major effect of citrate was to reverse the inhibition of sugar utilization. Inhibition of glutamate production caused by azide or dinitrophenol was not prevented by 1% citrate. This failure suggested that the citrate effect was specific for the glycolysis inhibitors, iodoacetate and fluoride. When citrate was added to the medium as late as 6 hr after sodium fluoride, reversal of inhibition was still observed. However, the addition of citrate as early as 45 min after the addition of iodoacetate did not reverse the inhibition. In fact, the only time that citrate pre-
vented iodoacetate inhibition was when it was sterilized with the medium. These results show that the mechanism by which citrate prevents NaF and iodoacetate inhibition is different for each inhibitor. Subsequently, it was found that the only conditions which permitted glutamate production in the presence of iodoacetate were those in which citrate and the inhibitor were autoclaved together. Apparently, citrate directly inactivates iodoacetate during heating of the resting cell medium. Citrate reversal of fluoride inhibition, however, is a true metabolic effect. It is known that fluoride inhibition of enolase is dependent upon the formation of a magnesium-fluorophosphate complex, which inactivates the enzyme (1). A similar complex is responsible for inhibition of phosphoglucomutase (3). Fluoride inhibition of glutamate formation increases with increasing magnesium concentrations, and citrate reversal of fluoride inhibition is antagonized by increasing the magnesium concentration (Fig. 2). Apparently, citrate acts by chelating magnesium and preventing the formation of the inhibitory complex.

The inhibitory effects of methylene blue, phenazine methosulfate, azide, fluoride, and iodoacetate demonstrate the importance of terminal respiration, oxidative phosphorylation, and the EMP pathway for glutamate formation from glucose. The results also point out some dangers involved in interpretation of experiments with inhibitors, i.e., the necessity of sterilizing iodoacetate separately if aseptic conditions are desired and the need for high magnesium concentrations when studying the effect of fluoride on glycolysis.

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LITERATURE CITED


