1990

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Hassan Namdari
University of Rhode Island

Victor J. Cabelli
University of Rhode Island

Citation/Publisher Attribution

Namdari, H., & Cabelli, V. J. (1990). Glucose-mediated catabolite repression of the tricarboxylic acid cycle as an explanation for increased acetic acid production in suicidal *Aeromonas* strains. *J. Bacteriol., 172*(8), 4721-4724. doi: 10.1128/jb.172.8.4721-4724.1990
Available at: http://dx.doi.org/10.1128/jb.172.8.4721-4724.1990

JOURNAL OF BACTERIOLOGY, Aug. 1990, p. 4721–4724
0021-9193/90/084721-04$02.00/0

Vol. 172, No. 8

Glucose-Mediated Catabolite Repression of the Tricarboxylic Acid Cycle as an Explanation for Increased Acetic Acid Production in Suicidal *Aeromonas* Strains

HASSAN NAMDARI† AND VICTOR J. CABELLI*

Department of Microbiology, University of Rhode Island, Kingston, Rhode Island 02881

Received 7 January 1990/Accepted 18 May 1990

Growth in the presence of glucose, even under highly aerobic conditions, significantly reduced the activities of three tricarboxylic acid cycle enzymes, citrate synthetase, α-ketoglutarate dehydrogenase, and malate dehydrogenase, in suicidal but not nonsuicidal *Aeromonas* strains. Pyruvate dehydrogenase activity, however, was significantly increased. The activities of all of the enzymes, as well as the glucose-mediated increase in acetic acid production, were shown to be regulated by catabolite repression. The regulator protein is the same one which regulates the utilization of several sugars.

In an earlier paper (11), we described a phenomenon in which certain strains of *Aeromonas* spp., including all *Aeromonas caviae* and some *A. sobria* isolates, were not recoverable after 24 h when grown in nutrient broth supplemented with glucose. It was shown that death of the cells was due to the accumulation of acetic acid, which is produced in large quantities by these strains even when the cultures are incubated under highly aerobic conditions. The phenomenon was termed "suicide," and the requirements for its manifestation were consistent with the absence of these *Aeromonas* biotypes in acidic lakes in New England and their recovery from alkaline waters in Israel and from sewage at both locations. In this paper, we examine the possibility that the increased production of acetic acid by suicidal aeromonads was achieved by a shutdown of the tricarboxylic acid (TCA) cycle and the diversion of acetyl-coenzyme A produced from pyruvate to the production of acetic acid. We also identify the regulatory mechanism.

Production of acetic acid. Accumulation of acetic acid in nutrient broth-glucose (NBG) (11) shake cultures of the nonsuicidal strains was greatest during the lag period, increased only slightly when the growth and glucose utilization rates were maximal, and then decreased, presumably because acetate was used as an energy source once the glucose was metabolized (Fig. 1A). In the suicidal cultures (Fig. 1B), however, acetic acid continued to be produced until both growth and glucose utilization were prematurely inhibited by the accumulation of acetic acid in its un-ionized form (11). About 43% of the glucose catabolized by the suicidal strains and only 8 to 12% of that used by the nonsuicidal isolates could be accounted for by the acetic acid in the cultures. When the pH of the suicidal cultures was maintained between 6.5 and 7.0, however, the optical density reached a maximum of 1.8, all of the glucose was metabolized, and considerably more acetate was produced, with most of it appearing early in the exponential growth phase (Fig. 2A). *Escherichia coli* resembled the suicidal aeromonads in that high acetate levels also were produced, there was a sharp decrease in pH after 3 h, and about 45% of the glucose metabolized could be accounted for by the acetic acid produced. With *E. coli*, however, most of the acetic acid was produced as the cell population was passing into the stationary phase, and there was little, if any, acetic acid-mediated death of the cells.

Inhibition of TCA cycle enzymes and stimulation of pyruvic dehydrogenase activity. The specific activities of three enzymes in the TCA cycle, malate dehydrogenase (18), α-ketoglutarate dehydrogenase (8), and citrate synthetase (13), in cell-free extracts from Luria broth (LB) cultures were essentially the same whether the nonsuicidal strains were grown in the presence or absence of added glucose or when the suicidal strains were grown in its absence. However, when the suicidal strains were grown in LB supplemented with glucose, the specific activities of all three enzymes were appreciably and significantly decreased (Table 1). LB with glucose, which has a greater buffering capacity and less glucose (0.3%), was used instead of NBG to minimize acetic acid-mediated death of the cells. Pyruvate dehydrogenase activity also was similar with the nonsuicidal strains grown in the presence or absence of glucose and the suicidal strains grown in its absence. When glucose was added to the cultures of the suicidal strains, however, pyruvate dehydrogenase activity was significantly increased rather than decreased (Table 1).

Effect of cAMP on activities of the enzymes and acetic acid production. The addition to LB with glucose of filter-sterilized cyclic AMP (cAMP) at a final concentration of 3×10^{-2} M reversed the glucose-mediated inhibition of the activities of the three TCA cycle enzymes and its stimulation of pyruvate dehydrogenase activity in suicidal aeromonads (Table 1). The activities of the four enzymes in the extracts from the cultures of the nonsuicidal strains were not significantly altered by the addition of cAMP. Moreover, the addition of cAMP to NBG shake cultures of suicidal *Aeromonas* strains reduced acetic acid production, increased the pH, and, because of this, prevented acetic acid-mediated death of the cells (Fig. 2B).

Identity of the catabolite repressor protein. Mutants which simultaneously lost the abilities to utilize lactose, arabinose, and galactose in cAMP-containing media were sought by

* Corresponding author.
† Present address: Mt. Sinai Medical Center, New York, NY 10029.

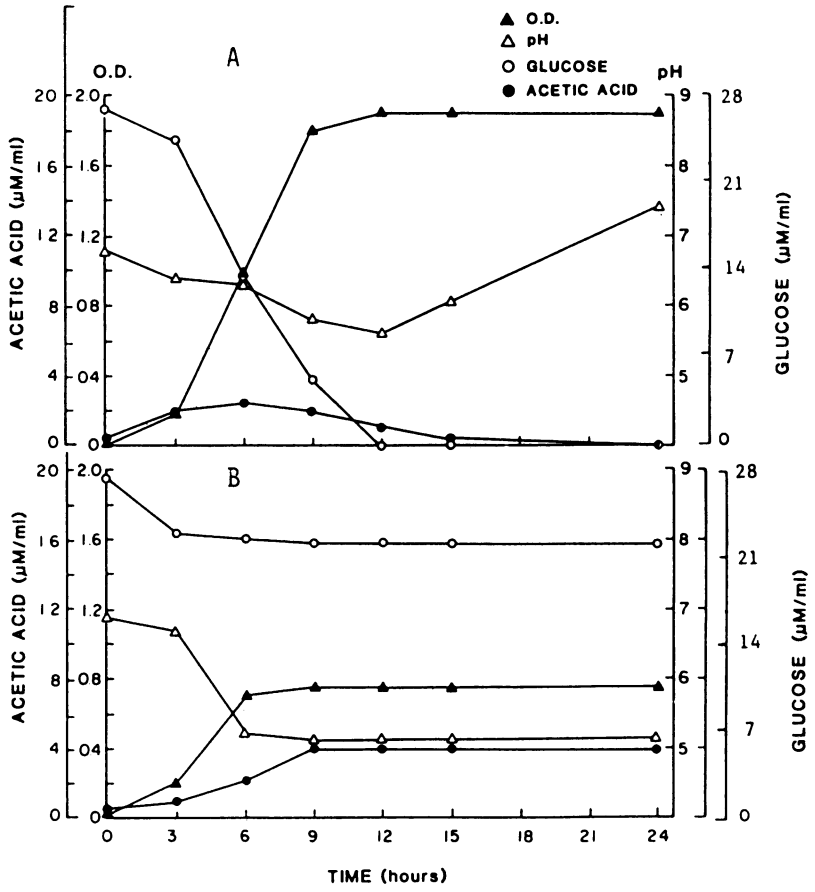

FIG. 1. Acetic acid production relative to glucose utilization, growth as optical density (O.D.), and pH in NBG shake cultures incubated aerobically. (A) Nonsuicidal *Aeromonas* strain (2BT); (B) suicidal *Aeromonas* strain (OP2).

nitrosoguanidine mutagenesis (3). Two types of mutants were obtained (6, 14) (Table 2). Adenylate cyclase (Cya^-) mutants fermented the three sugars only upon the addition of cAMP to the media, while "Crp^-" mutants did not ferment the sugars even when cAMP was added. The presumed Crp^- mutants, in contrast to the wild-type strain, remained suicidal even when cAMP was added to the media. The uptake of cAMP by the presumed Crp^- mutants was examined, since a loss of the ability to transport cAMP would have produced similar findings. The uptake of [^{3}H]cAMP by the CRP mutants was not significantly different from that of the Cya^- mutants or the parental strain, OP2.

Catabolite repression of the TCA cycle enzymes leading to the production of acetic acid under highly aerobic conditions and depression by cAMP also occurs in *E. coli* (1, 7, 9, 10, 18). *E. coli* differs from the suicidal aeromonads, however, in that oxidative phosphorylation and the synthesis of cytochromes also are repressed (2, 5), and the acetic acid is produced late rather than early in the growth cycle.

The increase in pyruvate dehydrogenase activity in *A. caviae* would not only increase the production of acetic acid but also compensate somewhat for the loss in energy due to the glucose-mediated catabolite repression of the TCA cycle. Since neither succinate nor propionate is produced in the shake cultures (11), we conclude that oxidative phosphorylation remains operative and that the increase in pyruvate dehydrogenase activity increases the energy available from the oxidation of the pyruvate to acetyl-coenzyme A. Since the production of pyruvate is regulated by the levels of AMP and ADP in the cells (17) and pyruvate in turn regulates the synthesis of the pyruvate dehydrogenase complex (4), we speculate that the glucose-mediated increase in pyruvate dehydrogenase activity is secondary to the partial shutdown of the TCA cycle.

TABLE 1. Effects of glucose on enzyme activities and reversal by cAMP in suicidal strains of *Aeromonas* spp.

Enzyme[a]	*Aeromonas* type	Sp act in cells grown aerobically with[b]:		
		No glucose	Glucose	Glucose + cAMP
MDH	Suicidal	15.7	3.3*	14.7
	Nonsuicidal	15.1	12.5	15.1
KDH	Suicidal	10.7	2.4*	11.0
	Nonsuicidal	11.5	11.2	11.5
CSN	Suicidal	1,164	209*	1,170
	Nonsuicidal	948	955	1,123
PDH	Suicidal	216	950*	167
	Nonsuicidal	205	264	215

[a] MDH, Malate dehydrogenase; KDH, α-ketoglutarate dehydrogenase; CNS, citrate synthetase; PDH, pyruvate dehydrogenase.

[b] Means from four assays (duplicate assays on extracts from two strains). Specific activity is expressed as micromoles of substrate converted per minute per milligram of protein for MDH, KDH, and PDH and as nanomoles of substrate converted per minute per milligram of protein for CSN. *, Significantly different from nonsuicidal strain, when glucose was omitted or cAMP was added, at $P < 0.01$.

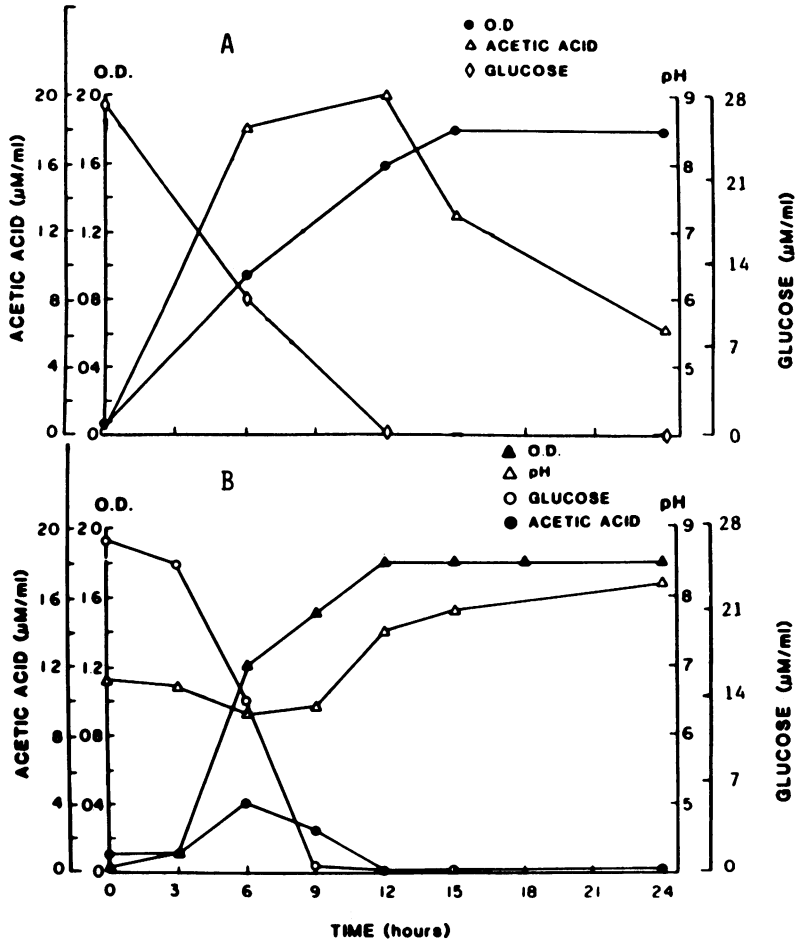

FIG. 2. Acetic acid production relative to glucose utilization, growth as optical density (O.D.), and pH in 30°C NBG shake cultures of the suicidal *Aeromonas* sp. strain OP2. (A) pH maintained between 6.5 and 7.0; (B) cAMP added to medium at a final concentration of 3×10^{-2} M.

The metabolism of *A. caviae* appears to be extremely well adapted to growth in calcium-rich alkaline environments such as those from which most of the strains were isolated. Unlike the nonsuicidal aeromonads, *A. caviae* can grow anaerobically in a glucose-mineral salts medium (11), is anaerogenic, and does not produce 2,3-butanediol as a fermentation end product (15). Also, it utilizes a number of β-glycosides, including cellobiose, esculin, salicin, and lactose; this is due in part to the presence of a β-glycosidase with broad specificity for β-glucosides and β-galactosides (M. Rodgers, personal communication). The most probable explanation for the adaptation towards the production of acetic acid by *A. caviae* is the mobilization of soluble phosphate from insoluble $Ca_3(PO_4)_2$. A second possibility is that the acetic acid acts to liberate nutrients from associated algae. These possibilities need to be examined ecologically.

TABLE 2. Fermentation and suicide reactions of Crp^- and Cya^- mutants in the presence of cAMP

Mutant type	No. of isolates examined	Fermentation of Lac, Ara, Gal[a]	Suicide[a]
Crp^-	3	−[b]	+
Cya^-	5	+[b]	−
Parent		+	−

[a] Mutants and parent ferment glucose with and without cAMP and are $SUIC^+$ without cAMP.

[b] Lactose (Lac), arabinose (Ara), and galactose (Gal) were not fermented in the absence of cAMP.

We gratefully acknowledge suggestions and assistance from Paul S. Cohen.

LITERATURE CITED

1. **Amarasingham, C. R., and B. D. Davis.** 1965. Regulation of α-ketoglutarate dehydrogenase formation in *Escherichia coli*. J. Biol. Chem. **240**:3664–3668.
2. **Broman, R. L., W. J. Dobrogosz, and D. C. White.** 1974. Stimulation of cytochrome synthesis in *Escherichia coli* by cyclic AMP. Arch. Biochem. Biophys. **162**:595–601.
3. **Carlton, B. C., and B. J. Brown.** 1981. Gene mutation, p. 222–242. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
4. **Dietrich, J., and U. Henning.** 1970. Regulation of pyruvate dehydrogenase complex synthesis in *Escherichia coli* K12. Eur. J. Biochem. **14**:258–269.
5. **Dills, S. S., and W. J. Dobrogosz.** 1977. Cyclic adenosine 3′,5′-monophosphate regulation of membrane energetics in *Escherichia coli*. J. Bacteriol. **131**:854–865.

6. **Emmer, M., B. DeCrombrugghe, I. Pastan, and R. Perlman.** 1970. Cyclic AMP receptor protein of *E. coli*: its role in the synthesis of inducible enzymes. Proc. Natl. Acad. Sci. USA **66**:480–487.
7. **Fletchner, V. R., and R. S. Hanson.** 1970. Regulation of the tricarboxylic acid cycle in bacteria. A comparison of the citrate synthetases from different bacteria. Biochim. Biophys. Acta **222**:253–264.
8. **Fortnagel, P., and E. Freese.** 1968. Analysis of sporulation. II. Mutant blocked in the citric acid cycle. J. Bacteriol. **95**:1431–1438.
9. **Gray, C. T., J. W. T. Wimpenny, and M. R. Mossman.** 1966. Regulation of metabolism in facultative bacteria. II. Effects of aerobiosis, anaerobiosis and nutrition on the formation of Krebs cycle enzymes in *Escherichia coli*. Biochim. Biophys. Acta **117**:33–41.
10. **Halpern, Y. S., A. Even-Shoshan, and M. Artman.** 1964. Effect of glucose on the utilization of succinate and the activity of tricarboxylic acid cycle enzymes in *Escherichia coli*. Biochim. Biophys. Acta **93**:228–236.
11. **Namdari, H., and V. J. Cabelli.** 1989. The "suicide" phenomenon in motile aeromonads. Appl. Environ. Microbiol. **55**:543–547.
12. **Ochoa, S.** 1955. Malic dehydrogenase from pig heart. Methods Enzymol. **1**:735–736.
13. **Ohne, M.** 1975. Regulation of the dicarboxylic acid part of the citric acid cycle in *Bacillus subtilis*. J. Bacteriol. **122**:224–234.
14. **Perlman, R. L., and J. Pastan.** 1969. Pleiotropic deficiency of carbohydrate utilization in an adenyl cyclase deficient mutant of *Escherichia coli*. Biochem. Biophys. Res. Commun. **36**:151–157.
15. **Popoff, M.** 1984. Genus III. *Aeromonas* Kluyver and Van Niel 1936, 398^{AL}, p. 545–548. *In* J. G. Holt and N. R. Krieg (ed.), Bergey's manual of systematic bacteriology, 9th ed., vol. 1. The Williams & Wilkins Co., Baltimore.
16. **Requero, A. M., B. E. Corkey, S. Cerdan, E. W. Rode, R. L. Parrilla, and J. R. Williamson.** 1973. Interactions between α-ketoisovalerate metabolism and the pathways of gluconeogenesis and urea synthesis in isolated hepatocytes. J. Biol. Chem. **258**:3673–3681.
17. **Stanier, R. Y., J. L. Ingraham, M. L. Wheelis, and P. R. Painter.** 1986. The microbial world, 5th ed., p. 306. Prentice-Hall, Inc., Englewood Cliffs, N.J.
18. **Takahashi, Y.** 1975. Effect of glucose and cyclic adenosine 3′, 5′-monophosphate on the synthesis of succinic dehydrogenase and isocitrate lyase in *Escherichia coli*. J. Biochem. **78**:1097–1100.