REGULATION OF CITRATE LYASE ACTIVITY IN RHODOPSEUDOMONAS GELATINOSA

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Received September 5,1972

Summary: After exhaustion of citrate in the growth medium, the citrate lyase of Rhodopseudomonas gelatinosa is inactivated by deacetylation. Upon addition of citrate to a suspension of cells containing inactive enzyme the lyase is reactivated. The discovery of Buckel, Buschmeier and Eggerer (1), that deacetylation causes loss of activity of purified citrate lyase from Klebsiella aerogenes, therefore, seems to be the basis of a regulatory mechanism of biological significance.

Rps. gelatinosa is one of the few phototrophic bacteria which grow readily on citrate (2). We observed that anaerobically in the light citrate is degraded by this microorganism in a rather uncontrolled manner; degradation products are formed much faster than they can be used for growth. They are excreted into the medium and utilized after exhaustion of citrate (3). The transition from growth on citrate to growth on excretion products (mainly acetate) is indicated by a temporary halt of the growth curve.

Citrate breakdown is initiated by the citrate lyase reaction. After exhaustion of citrate and during growth on acetate, citrate has to be synthesized by the cells since it is a precursor of glutamate. Here we report on experiments concerned with the regulation of citrate lyase activity under conditions which require citrate synthesis.

METHODS

Rps. gelatinosa strain SMG 149 was grown in the following medium:

KH_2PO_4, 1 g; NH_4Cl, 0.4 g; NaCl, 0.4 g; $MgSO_4 \cdot 7\ H_2O$, 0.4 g; $CaCl_2 \cdot 2\ H_2O$, 0.05 g; trace element solution, 1 ml; yeast extract, 0.5 g; sodium citrate or sodium malate, 10 mM; water to 1 000 ml. The pH was 6.7. Cells were grown in screw-cap bottles (500 ml) or in 5-l flasks at approx. 4 000 Lux.

Protein was determined according to Beisenherz et al. (4) after extraction of the pigments with acetone; citrate was determined as described by Dagley (5).

Cell-free extracts were prepared as follows: Approx. 1 g of cells (wet weight) were suspended in 5 ml of 0.1 M triethanolamin-HCl buffer, pH 7.6. The suspension was quickly passed through a French press at a pressure of 8 kg/cm^2. The extract was collected in a tube filled with nitrogen gas and precooled to 0 C. It was centrifuged at 10 000 xg for 15 min in screw-cap tubes.

Citrate synthase activity was determined according to Srere et al. (6) and citrate lyase according to Dagley (7).

Activation by acetic anhydride was carried out as described by Buckel et al. (1). The final concentration of acetic anhydride in the assay mixture was 1.75 mM, and the reaction was started after 1 min by the addition of citrate.

RESULTS

Tab. 1 gives the specific activities of citrate lyase and citrate synthase after growth of Rps. gelatinosa on malate and on citrate for various periods of time. It can be seen that citrate synthase activity did not vary significantly. Citrate lyase could not be detected in malate-grown cells and in cells grown on 10 mM citrate for 18 or 25 hrs. This enzyme, how-

Table 1 Citrate synthase and citrate lyase activity in extracts of Rps. gelatinosa grown on malate and citrate

Substrate and time of incubation (hrs)	Citrate in culture medium (mM)	Specific activity citrate synthase (U/mg)	Specific activity citrate lyase (U/mg)
malate 25	-	0.185	0
citrate 12	0.70	0.192	0.250
citrate 14	1.95	0.183	0.320
citrate 18	0	0.180	0.004
citrate 25	0	0.176	0

5-l cultures inoculated with 500 ml of a 24 to 30 hrs old culture were grown for the time indicated and harvested. The citrate content of the supernatant was determined enzymatically. Cell-free extracts were prepared and analyzed for citrate synthase and citrate lyase activities. The substrate concentration was 10 mM.

ever, could be detected when the bacteria were harvested while decomposing citrate.

Experiments on the mechanism underlying the disappearance of citrate lyase were made possible by the discovery of Buckel, Buschmeier and Eggerer who found that active citrate lyase is an S-acetyl protein and that deacetylated, inactive enzyme can be activated by treatment with acetic anhydride (1). This offered the possibility to distinguish between active and inactive forms of the enzyme.

In the experiment depicted in Fig. 1 cells precultured on malate were inoculated into a citrate-medium, and growth and citrate breakdown were recorded. In addition citrate lyase activity was determined in cell-free extracts before and after treatment with acetic anhydride. After consumption

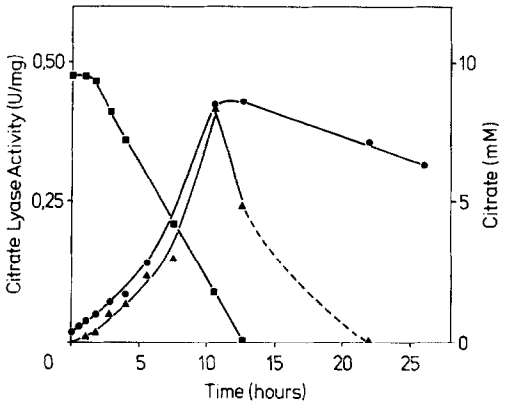

Fig. 1.

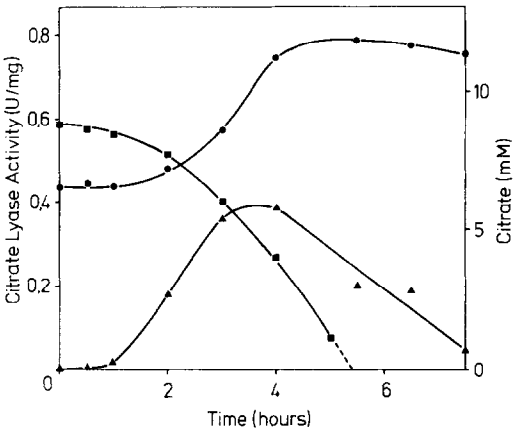

Fig. 2.

Fig. 1. Induction of citrate lyase during growth of Rps. gelatinosa on citrate and inactivation after exhaustion of citrate.

Cells of two 5-l cultures grown on malate were harvested, washed and suspended in 10 l growth medium without a carbon source. After incubation for 2 hrs in the light citrate was added to give a final concentration of approx. 10 mM. 500-ml samples were withdrawn anaerobically. Enzyme activities were determined in the cells as described in Methods.

■ concentration of citrate; ▲ citrate lyase activity; ● citrate lyase activity after treatment with acetic anhydride

Fig. 2. Activation of citrate lyase in cells containing high levels of inactive enzyme.

Cells of two 5-l cultures grown on citrate (10 mM) for 24 hrs were treated in the same manner as described for malate-grown cells in Fig. 1.

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of citrate the enzyme was rapidly inactivated. That this inactivation proceeded by deacetylation is indicated by the high level of lyase activity detectable upon treatment of cell-free extracts with acetic anhydride.

In another experiment it was tried to obtain evidence for the ability of Rps. gelatinosa to convert inactive enzyme into active lyase. Cells grown on citrate medium for 20 hours contained only inactive enzyme; they were harvested, washed and resuspended in fresh citrate medium. Fig. 2 re-

veals that during the first hours of growth on citrate, inactive enzyme was activated.

DISCUSSION

Rps. gelatinosa employs citrate lyase for the degradation of citrate (3). When growing on other substrates, citrate as the precursor of glutamate is formed by the citrate synthase reaction. In the transition of growth from citrate to acetate or other acids a futile cycle with the participation of citrate lyase and citrate synthase is prevented in Rps. gelatinosa by a rapid inactivation of the lyase. The inactivation is caused by deacetylation - a process which was discovered by Buckel et al. (1) with purified citrate lyase from Klebsiella aerogenes. Unlike the citrate lyase from K. aerogenes (8) and Escherichia coli (9) the enzyme from Rps. gelatinosa does not undergo inactivation when incubated with citrate and Mg^{2+} (unpublished results). It, therefore, is conceivable that the observed inactivation reaction is enzyme-catalyzed.

Since inactivated enzyme can be activated again when the cells are suspended in citrate medium, the citrate lyase of Rps. gelatinosa may be added to the group of enzymes which are regulated by chemical modification.

This work was supported by a grant of the "Deutsche Forschungsgemeinschaft".

REFERENCES

1. Buckel, W., Buschmeier, V., and Eggerer, H., Hoppe-Seyler's Z. Physiol. Chem. 352, 1195 (1971)

2. Weckesser, J., Drews, G., and Tauschel, H. D., Arch. Mikrobiol. 65, 346 (1968)

3. Schaab, Ch., Giffhorn, F., Schoberth, S., Pfennig, N., and Gottschalk, G., Z. Naturforsch. 27b (1972) in press

4. Beisenherz, G., Bolze, H. J., Bücher, Th., Czok, R., Garbade, H.K., Meyer-Ahrendt, E., and Pfleiderer, G., Z. Naturforsch. 8b, 555 (1953)

5. Dagley, S., in Bergmeyer, H. U. (editor), Methoden der enzymatischen Analyse, Verlag Chemie, Weinheim/Bergstr., p. 1520 (1970)

6. Srere, P. A., Brazil, H., and Gonen, L., Acta Chem. Scand. 17, S 129 (1963)

7. Dagley, S., in Lowenstein, J. M. (editor), Methods in Enzymology, Vol. XIII, Academic Press, Inc., New York, p. 160 (1969)

8. Dagley, S., and Dawes, E. A., Biochim. Biophys. Acta 17, 177 (1955)

9. Wheat, R. W., and Ajl, S. J., J. Biol. Chem. 217, 909 (1955)