THE ROLE OF LACTATE IN REGULATION OF THE ENZYMATIC SYSTEM SYNTHESIS PARTICIPATING IN THE ACETATE OXIDATION IN STAPHYLOCOCCUS AUREUS

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Received August 1, 1963

As it is known Staphylococcus aureus grown in a medium containing glucose has a high aerobic and anaerobic glycolysis and lacks the system of acetate oxidation. The synthesis of a functionally active system of the three carboxylic acid cycle (TCA) was demonstrated after the elimination of glucose from the growth medium (Gershanovitch et. al., 1962, 1963; Collins & Lascelles, 1962). Hence the system of acetate oxidation may be characterized as repressible (Jacob & Monod, 1961), and the phenomenon of repression may be regarded as the expression of the glucose effect (Magasanik, 1957, 1961).

Simultaneously with the idea about repression of the synthesis of enzymes by the feed-back control regulation by the products of glucose metabolism (Neidhardt & Magasanik, 1956, 1957; Magasanik, 1961), there are data demonstrating the possibility of the inhibition of the production of catalytically active proteins by metabolites, not related to the end products of this reaction (Gorini et al., 1961; Freundlich & Lichstein, 1963). It must be mentioned that the theoretical possibility of regulation of one process by metabolites produced in other metabolic pathways was postulated by Monod and Jacob (1961). In the present paper we demonstrate the possibility of repression of enzymes of the TCA cycle by the terminal products of the glycolytic process - lactic acid.

We were working with the wild type of Staphylococcus aureus 209 (St.-209) and its mutant St.-UV-3 (Gause et al., 1957). The cells were grown in a synthetic medium with and without glucose (Gardner & Lascelles, 1962). The microorga-

nisms were harvested by centrifugation, washed with Ringer-phosphate, pH 7,3 and suspended in a small amount of this solution. The culture used in this experiment without preliminary aeration will be further refered to as "original culture".

In two equal aliquots of the synthetic medium we transfered equal samples of the original culture and added the substrates (glucose, lactate & others) to one of the flasks. The samples were aerated at 37° by shaking. After two hours the cells were harvested by centrifugation, washed and suspended in Ringer-phosphate buffer, pH 7,3. In one combined experiment in the Warburg apparatus the original culture and the aerated culture were used. The turbidity of all the samples used in the combined experiment were adjusted to the same level which was of definite significance for the comparison of results of these investigations. The TCA activity was measured by the rate of acetate oxidation. The intensity of the aerobic glycolysis was estimated by the rate of CO_2 evolution (bicarbonate buffer pH 7,3) and by lactate accumulation. Lactic acid was determined by Barker & Summerson's method. The activity of hexokinase, lactate, isocitrate, succinate, α-ketoglutarate and malate dehydrogenases in bacterial lysates was investigated by conventional methods (Colowick & Kaplan, 1955). Protein was determined by the Lowry technique. All the data are computed per mg of protein.

St.-209 grown in medium containing glucose had an active hexokinase and lactate dehydrogenase, but no isocitrate dehydrogenase and α-ketoglutorate dehydrogenase activity. The activity of succinate and malate dehydrogenases of this microorganism was low. St.-UV-3 had no hexokinase and lactate dehydrogenase activity. When growing in a medium with glucose this strain exerted all the activity of the dehydrogenases involved in the TCA cycle.

The cells of St.-209 grown in the presence of glucose were transfered to a fresh nutrient medium and aerated for two hours. Elimination of glucose in aerobic conditions resulted in the appearance of the system of acetate oxidation. The activity of the glycolytic system did not change. Glucose prevented the emergence of functionally active TCA and did

not provoke the further induction of the glycolytic system of St.-209 grown in a medium with glucose (fig. 1).

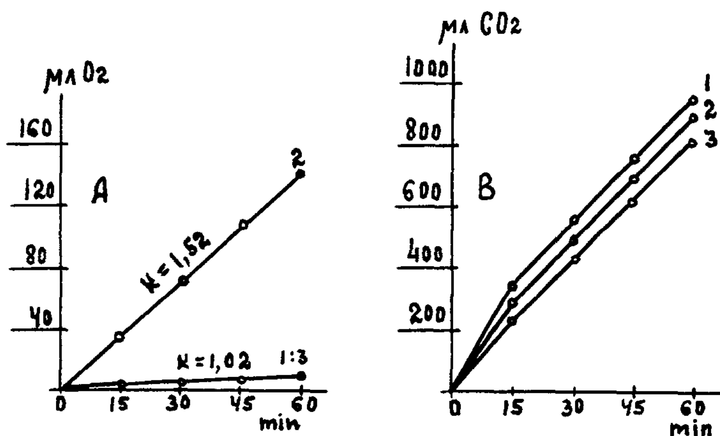

Fig. 1. Derepression of the system of TCA and glycolytic activity of St.-209 grown in a medium with glucose. An 11-hour culture of St.-209 grown in a medium containing glucose. A - intensity of acetate oxidation; B - the intensity of glycolysis. 1 - the original culture; 2 - the culture after aeration in a medium without glucose; 3 - the culture after aeration in a medium containing glucose. The data are corrected for endogenous values. The vessel of the Warburg apparatus contains: 1,9 ml of Ringer-phosphate, pH 7,3 (in aerobiosis) or 1,9 ml of Ringer-bicarbonate (in anaerobiosis), 0,5 ml of Na-acetate (terminal concentration M/30) or glucose (terminal concentration M/50), 0,5 ml of the bacterial suspension. In the central vessel in the aerobic samples - 0,1 ml 10% NaOH. The gas phase in the aerobic vessel-air, in the anaerobic - N_2. The time of incubation - 1 hour. Temperature - 37° . K - the ratio Q_{O2} in the presence of acetate/endogenous Q_{O2}. Lactate accumulation in samples 1,2 and 3 respectively 4,27; 4,7 and 4,9 mg.

The cells were grown in a medium without glucose and aerated. Two hours aeration of the culture in a medium without glucose enhanced the acetate oxidation activity of TCA and did not effect the activity of the glycolytic reactions. Glucose in the conditions of aeration prevented further increasing of acetate oxidations and markedly induced the glycolytic system in cells grown without glucose (fig. 2).

So it seemed that the activation of the system of glycolysis and repression of the system of TCA in St.-209 are related phenomena. Then the question arose whether products formed during the glycolytic process of St.-209 are

able to repress the system of TCA. To get an answer to this question the repressing activity of pyruvate, lactate and acetate were studied paralelly with the repressing activity of glucose. The results are summarized in table 1.

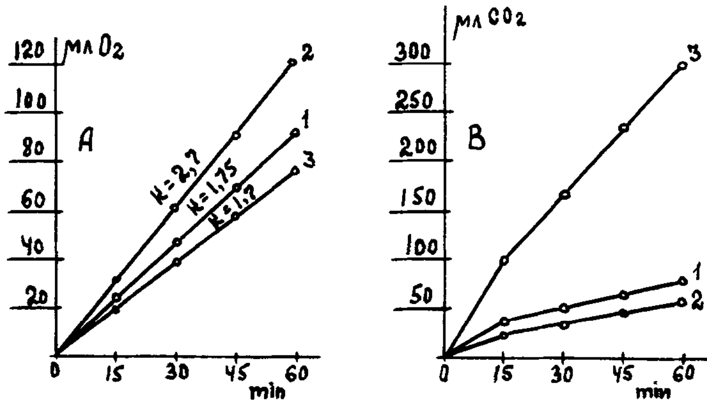

Figure 2. Derepression of the system of TCA and the glycolytic activity in St.-209 grown in a medium without glucose. A 18-hour culture of St.-209. Conditions of the experiments and symbols - see fig.1. Lactate accumulation in anaerobic conditions in samples 1,2 and 3 respectively - 0,35; 0,29 and 1,18 mg.

TABLE 1

INFLUENCE OF THE PRODUCTS OF GLYCOLYTIC METABOLISM ON THE DEREPRESSION OF THE SYSTEM OF ACETATE OXIDATION IN St.-209

An 11-hour culture grown in the presence of glucose. The concentration of substrates in the aerated medium 0,09 M. E - endogenous Q_{O_2}; A - Q_{O_2} in the presence of acetate; K - the ratio A/E. The other experimental conditions are described in fig. 1.

Original culture			Aeration without substrate			Aeration in the presence of substrate		
E	A	K	E	A	K	E	A	K
						Na-lactate		
*143	159	1,07	275	695	2,22	233	316	1,34
						Na-pyruvate		
**159	159	1,0	171	382	2,15	185	400	2,17
						Na-acetate		
**103	100	0,98	207	485	2,34	178	348	1,94
						Glucose		
**139	134	0,96	275	501	1,79	238	283	1,18

*average of 5 experiments; **average of 2 experiments

It was established that during the aeration lactate (0,09 M) inhibited by 60-80 % the synthesis of the system of acetate oxidation. The cells grown in the presence of lactate weakly oxidized acetate (fig. 3). The repressing activity of lactate was demonstrated at pH 7,4 and also at pH 5,9. Pyruvate did not influence the derepression of the enzymes of the TCA cycle. When acetate was added to the aerated medium the rate of TCA derepression decreased approximately by 30%.

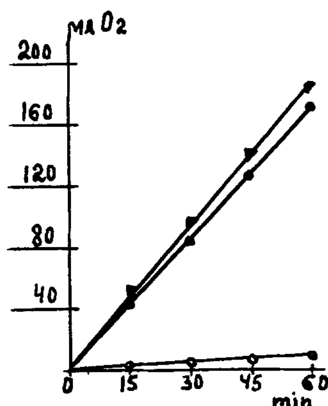

Fig. 3. Oxidation of acetate by St.-209 grown in a medium containing lactate and acetate.
In the growth medium - 0,09 M lactate or acetate. A 18-hour culture. The data are corrected for endogeneous values. -o-o-o- the growth medium with lactate; -•-•-•- the growth medium containing acetate ; -▾-▾-▾- the basal medium. The conditions of the experiments in the Warburg apparatus see in fig. 1.

Acetate did not influence the synthesis of the TCA system if used as a complementary substrate for the growth of the culture under stationary conditions (fig. 3). It may be supposed that in the aerated culture intensively utilizing acetate through the derepressed TCA system, a great quantity of intermediate products are accumulated preventing the subsequent synthesis of some enzymes of the Krebs cycle by feedback control mechanism. The absence of the repressing action of pyruvate on the synthesis of TCA enzymes and repressing action in the system containing glucose could be explained by the continuous generation of $NADH_2$ in the oxidation stage of the glyceraldehyde-3-phosphate in the process of glyco-

lysis. This DPNH generation shifts the lactate dehydrogenase reaction to the side of lactic acid accumulation.

Really, when the Warburg vessel contains M/30 glucose or pyruvate the production of lactic acid per 1 mg of protein is respectively 2.100 and 400 μg. Hence the absence of the repressing activity of pyruvate on the TCA system may be explained by the fact that this substrate utilization is not related with the accumulation of sufficient quantities of lactic acid. On the other hand these data demonstrate that the repressing action of lactate is not related with the transformation of lactate into acetate (or pyruvate) and involvement of lactate in the TCA cycle. We are in right in concluding this if we consider that the lactate in Staphylococcus aureus may be transformed into acetate only through the stage of pyruvic acid (Gershanovitch et al., 1962; Gardner & Lascelles, 1962).

Experiments with the mutant St.-UV-3 have also demonstrated that lactate but not the products of its metabolism possibly block the TCA system synthesis. The above mentioned mutant practically does not utilize lactate (the lack of the lactate dehydrogenase and terminal flavoprotein oxidases). The TCA cycle is the sole system of substrate utilization of St.-UV-3 coupled with energy accumulation. Therefore the blockage of the TCA system synthesis of the mutant had to arrest its multiplication.

The mutant utilized a very small amount of glucose by the oxidative pathway. As the mutant grew slowly without glucose we studied the influence of different substrates on the multiplication of St.-UV-3 in the medium containing glucose. It revealed that pyruvate and acetate had no influence on the growth of the mutant. 0,135 M lactate completely blocked the multiplication of the microorganisms (fig. 4).

All these data allow us to suppose that lactate accumulating in the cells in sufficient concentrations repressed the synthesis of the TCA enzymes.

The result of the investigations of St.-209 demonstrates that preferential utilization by the cells of a definite source of energy may be determined not only by the

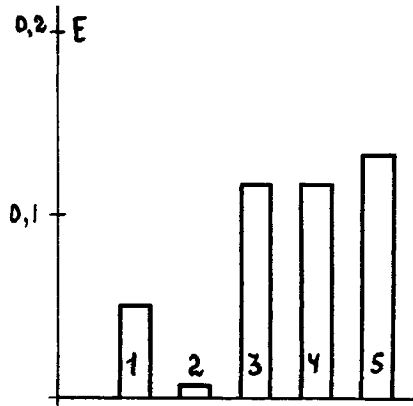

Fig. 4. The influence of different substrates on the multiplication of St.-UV-3. The culture was grown for 42 hours. The concentration of the substrates - 0,135 M. E - optical density (the cell - 0,5 cm) . 1 - basal medium; 2 - basal medium + glucose; 3 - basal medium + glucose + lactate; 4 - basal medium + + glucose + pyruvate; 5 - basal medium + glucose + + acetate.

Pasteur and Crabtree effects that control the activity of given systems but also by the rate of induction of glycolysis and the repression of the enzymes of the biological oxidation system by the terminal products of the glycolytic pathway. Possibly these trends also effect the tissues of higher organisms and particularly the cells in which the highly intensive aerobic glycolysis is coupled with the damage of the chain of biological oxidation.

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