CITRATE INHIBITION OF PHOSPHOFRUCTOKINASE AND THE PASTEUR EFFECT*

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A large amount of recent evidence clearly indicates that the regulation of phosphofructokinase (PFK) activity is fundamental in the control of glycolysis (Lowry and Passonneau, 1964) through a series of feedback mechanisms (Sols et al., 1963). Endproduct inhibition of PFK by ATP (Viñuela et al., 1963) may account for the Pasteur effect in certain tissues (Wu, 1964). Nevertheless, it has been reported that in glucose utilizing yeast, where both the Pasteur effect and an involvement of PFK in it were first observed, the level of ATP is essentially the same in aerobiosis and anaerobiosis (Lynen et al., 1959). Evidence reported here indicates that an additional feedback inhibition involving citrate may account for the aerobic regulation of PFK activity in yeast.

EXPERIMENTAL

A considerable increase in the level of citrate and isocitrate by aerobiosis has been found in yeast utilizing glucose, as shown in Fig.1. The changes in the levels of hexosemono- and diphosphate

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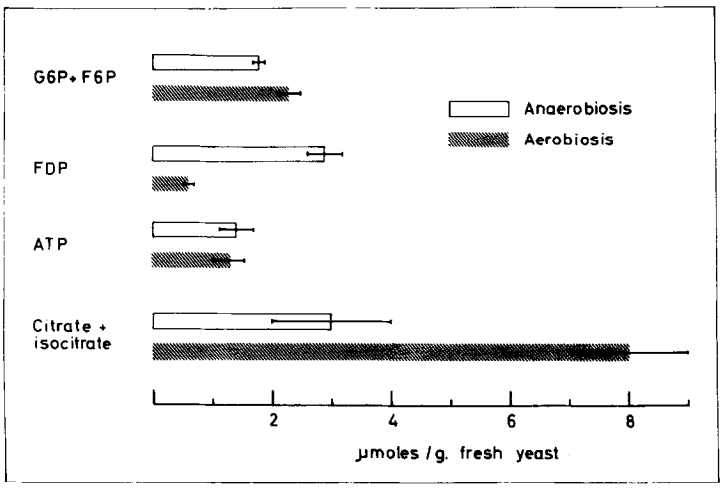

Fig. 1. Levels of hexose phosphates, ATP, and citrate + isocitrate in fermenting yeast.

Baker's yeast (Danubio), suspended in 0.02 M Mg-Cl_2-potassium phosphate (pH 3.5), was pretreated by aeration at 30° for 3 hours with 0.8 M glucose, washed, and suspended in phosphate buffer. Anaerobic and aerobic glucose (0.05 M) fermentation was followed by conventional manometric methods (95% N_2 - 5% CO_2 and 95% O_2 + 5% CO_2 respectively) using 0.25 g. yeast in a volume of 3 ml in large (35 ml) Warburg vessels, at 20°. A very marked aerobic inhibition of fermentation was observed in all cases. After 15 min., each vessel was opened and its content quickly thrown into liquid nitrogen. The deep frozen cakes were pulverized in a stainless steel cylindrical mortar precooled with liquid nitrogen, and extracted with 3 ml of 3 M perchloric acid precooled at -20. The homogenates were centrifuged and the supernatants neutralized with KOH and recentrifuged. Metabolites were estimated in the extracts by conventional spectrophotometric methods, with enzymes and cofactors as follows. G6P + F6P with G6P dehydrogenase, glucosephosphate isomerase and NADP; after completion of this reaction, ATP was estimated by the addition of hexokinase, glucose, and $MgCl_2$. FDP was estimated with aldolase after removal of triose phosphates with triosephosphate isomerase, glycerol-3-phosphate dehydrogenase and NADH. Citrate + isocitrate was estimated with aconitase, isocitrate dehydrogenase and NADH. Aconitase was purified from pig heart (Anfinsen, 1954). All other enzymes were purchased from Boehringer. Averages of at least 6 experiments, with the standard deviations are plotted in the figure.

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and the essentially constant level of ATP in these conditions confirm the early findings of Lynen et al. (1959). A crossover point at the

level of PFK in yeast has also been reported by others (Ghosh and Chance, 1964; Hommes, 1964).

Citrate, first reported to be an inhibitor of PFK from animal tissues (Parmeggiani and Bowman, 1963), is also an inhibitor of yeast PFK b (Viñuela et al., 1964). The inhibition of yeast PFK by citrate within the physiological range of concentrations is shown in Fig. 2. Isocitrate is similarly inhibitory, while α-keto-

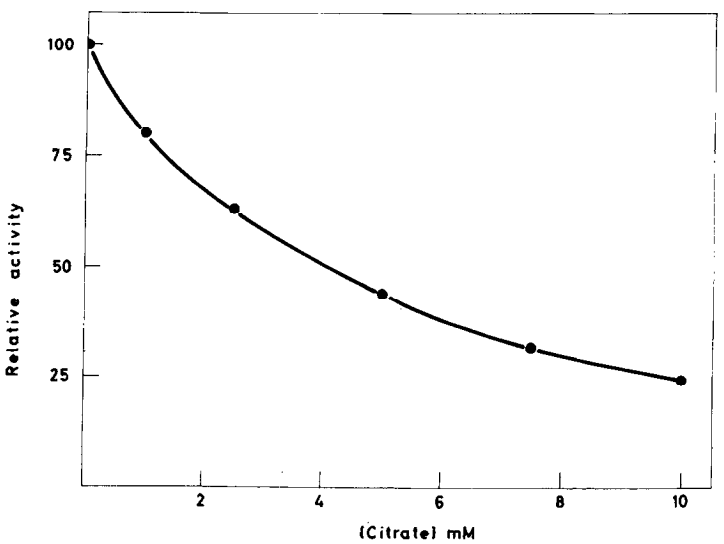

Fig. 2. Effect of citrate concentration on the activity of yeast PFK with 1 mM ATP and 0.25 mM F6P.

The activity of a 20 fold purified preparation of yeast PFK b (Viñuela et al., 1964) was assayed in the presence of 0.2 units each of aldolase, triosephosphate isomerase, glycerol-3-phosphate dehydrogenase, 0.1 mM NADH, 5 mM ethanethiol, 10 mM $MgCl_2$, 1 mM ATP, 0.25 mM F6P, 0.05 M imidazol, pH 7.5, and citrate (neutralized sodium salt) as indicated in the figure, in a total volume of 2 ml. The reaction was started by the addition of the PFK preparation and followed at room temperature by the decrease in optical density at 340 mμ.

glutarate is not. The results in Fig. 3 suggest that citrate may increase the sensitivity of PFK to inhibition by physiological concentrations of ATP.

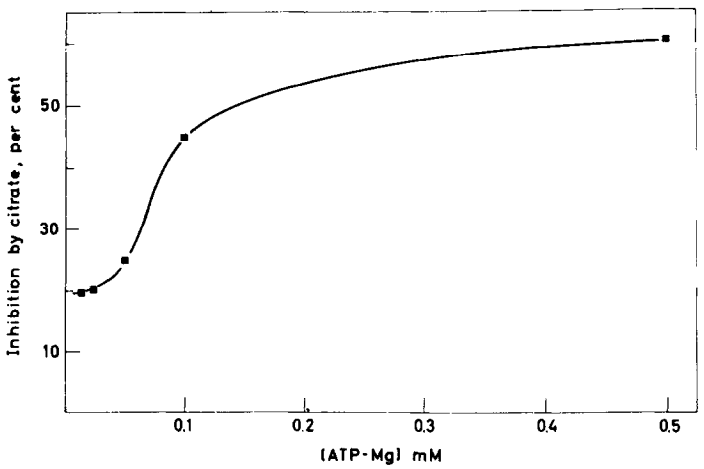

Fig. 3. Inhibition of yeast PFK by citrate at different concentrations of ATP.

The activity of a 150 fold purified preparation of yeast PFK b (Salas and Viñuela, 1965) was assayed in the presence of aldolase, triosephosphate isomerase, glycerol-3-phosphate dehydrogenase, NADH, $MgCl_2$, and ethanethiol as indicated in Fig. 2, with 25 mM potassium phosphate, pH 6.5, 0.5 mM F6P, and ATP-Mg as indicated in the figure, in the absence and in the presence of 10 mM citrate. Per cent inhibition by citrate for each of the concentrations of ATP tested are plotted in the figure.

DISCUSSION

It appears that anaerobic glycolysis in resting yeast can maintain a maximal ATP level, which the more efficient respiration cannot substantially increase. Because of this fact, ATP inhibition of PFK, by itself, could not account for the aerobic decrease in PFK activity involved in the Pasteur effect.

The carbon products of anaerobic glycolysis, largely ethanol and CO_2 in yeast, are merely byproducts. When oxygen availability makes possible the oxidation of mitochondrial NADH, pyruvate is preferentially oxidized to acetyl-CoA because pyruvate oxidase has a much lower Km than pyruvate decarboxylase (Holzer, 1961). After pyruvate oxidation follows the formation of citrate, which is a major metabolic crossroad. Citrate can be oxidized through the energy yielding Krebs cycle, a pathway that can be feedback con-

trolled at the level of its first irreversible step, since the NAD--dependent isocitrate dehydrogenase can adjust citrate oxidation to the energetic requirements of the cell by its allosteric dependance on either AMP (yeast) or ADP (animal tissues) (Hathaway and Atkinson, 1963; Chen and Plaut, 1963; Goebell and Klingenberg, 1964). Citrate can also leave the mitochondria and be used as initial substrate for several biosynthetic pathways, including the fatty acid storage pathway (Kornacker and Lowenstein, 1965). Sensitivity of PFK to inhibition by citrate offers then a mechanism of feedback control of glycolysis by what can be regarded as its aerobic carbon endproduct.

The conclusion that pyruvate oxidation up to citrate is sufficient for the aerobic inhibition of glycolysis in resting yeast is supported by the fact that the Pasteur effect can occur in aconitaseless mutants (Ogur et al., 1964) *. Related to this observation is the recent finding that in aconitase inhibition by fluoroacetate poisoning in isolated heart there is inhibition of glycolysis at the level of PFK when large amounts of citrate accumulate (Bowman, 1964; Williamson et al., 1964).

The sensitivity of PFK to the two endproducts of aerobic glycolysis affords a highly flexible regulation of glycolysis in resting cells. Of these products, ATP is the strongest inhibitor of PFK, but citrate is the main factor in the Pasteur effect in yeast. Aerobic changes in ATP (and the accompanying changes in AMP, a very efficient activator of PFK as competitive antagonist of ATP inhibition) may be the main factor in the Pasteur effect in certain tissues, but some involvement of citrate in the Pasteur effect is likely to be of wide occurrence. Preliminary observations indicate

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* We have confirmed this observation in one of the aconitaseless, citrate accumulating mutants kindly made available to us by Dr. M. Ogur. What was initially puzzling to us was the finding that the Pasteur effect, as measured by glucose utilization, in this mutant was not greater than in normal yeast. This may be due to the fact that citrate inhibition of PFK can be minimized by a decrease in the concentration of ATP.

an aerobic increase of citrate in isolated rat diaphragm utilizing glucose.

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