INHIBITION OF PHOSPHOGLUCOMUTASE BY CITRATE

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The experiments reported in this paper provide evidence that citrate and, to a lesser extent, isocitrate are the only intermediates of the Kreb's citrate cycle which inhibit the activity of phosphoglucomutase. This effect, like the effect of citrate on phosphofructokinase (Passonneau and Lowry, 1963, Parmeggiani and Bowman, 1963), provides a negative feed-back link from the citrate cycle to a major control point in the Embden-Meyerhof pathway. Evidence will be presented that the inhibitory action is due to the removal of the Mg^{2+} ion necessary for the activity of phosphoglucomutase.

METHODS

Enzyme preparation. About 400 mg. of the thigh muscles of a mouse were homogenised in about 5 ml. of distilled water and the homogenate diluted with water to contain 50 mg. muscle tissue/ml. The homogenate was then transferred to a standard test tube (150 mm. x 16 mm.).

Measurement of enzyme activity. The reaction mixture was made up in a standard test tube and contained 1 ml. glucose-1-phosphate, 10 mM; 1 ml. phosphate buffer, 0.1 M, pH 7.4; and 1 ml. distilled water. 1 ml. distilled water (control) or 1 ml. of the test substance, e.g. citrate, $MgSO_4$ EDTA was added. In some experiments (Table 2) 1 ml. citrate and 1 ml. $MgSO_4$ instead of 2 ml. water were added. In all cases the final volume was 4 ml.

The pH of cis-aconitic, 2-oxoglutaric, DL-malic, maleic and oxaloacetic acids was adjusted to about 7.4 by the addition of N-NaOH. With other test substances the sodium,

or in the case of tartrate the potassium sodium, salts were used.

Three test tubes each containing 4 ml. of the reaction mixture and the test tube containing the muscle homogenate were placed in a water-bath at 37° for 3 min. The reaction was started by adding 1 ml. of muscle homogenate to each of the three reaction mixtures. After incubation for 5 min. the amount of reducing substances, glucose-6-phosphate and fructose-6-phosphate, was determined as follows: 1 ml. Benedict's qualitative reagent was added to each of the three incubation mixtures. The tubes were placed in a beaker of boiling water, a stop watch started and the time in sec. for the first sign of reduction to appear was noted. A fresh homogenate was prepared for each series of three estimations.

Determination of glucose equivalents. The reduction times in sec. were converted to glucose equivalents as follows: Reaction mixtures containing 1 ml. phosphate buffer, 2 ml. distilled water and 1 ml. standard glucose solution (500, 300, 200, 100, 50, 25, and 10 mg./100 ml.) were prepared. The reaction mixtures and a muscle homogenate were equilibrated at 37° for 3 min. 1 ml. Benedict's reagent followed by 1 ml. muscle homogenate were then added to each of three reaction mixtures and the reduction times determined as before.

A graph was drawn relating glucose concentrations (mg/100 ml.) to reduction times (sec.). Glucose equivalents were converted to μmoles/l./sec.

RESULTS AND DISCUSSION

The following are some typical reduction times. Each figure represents the time (sec.) obtained with a different homogenate.

Citrate: 180, 195, 140, 160, 175

Succinate: 32, 33, 36, 31, 38

Tartrate: 39, 35, 36, 35, 32

Control: 36, 32, 39, 34, 31

The citrate figure is the mean of 14 determinations; other figures are the mean of 5 - 7 determinations.

Table 1 shows that citrate and, to a lesser extent, isocitrate are the only intermediates of the citrate cycle which inhibit the activity of phosophoglucomutase. Of the other substances tested, only cis-aconitate and malonate showed significant inhibitory activity. The inhibition by citrate was confirmed by a second method in which fructose-6-phos-

phate was estimated colorimetrically as fructose using a diphenylamine-HCl reagent.

Mg^{2+} is an essential activator of phosphoglucomutase. (Cori and Cori, 1937; Stickland, 1949; Milstein, 1961a). Milstein (1961b) demonstrated that the enzyme was completely

Table 1

Effect of Intermediates of the Citrate Cycle
and Related Substances
on the Activity of Phosphoglucomutase

Addition (1 ml. 50 mM)	Reduction Time (sec.)	Glucose Equivalent (mg/100 ml)	Glucose Equivalent (μmoles/1./sec)
Water (control)	35	195	36
Citrate	171	16	3
DL-isoCitrate	92	30	6
cis-Aconitate	59	70	13
2-Oxoglutarate	35	195	36
Succinate	34	200	37
Fumarate	35	195	36
DL-Malate	33	210	39
Oxaloacetate	34	200	37
Pyruvate	35	195	36
Acetate	35	195	36
Maleate	39	160	30
Malonate	46	120	22
Tartrate	35	195	36

inactive when the last traces of Mg^{2+} ion were complexed with ethylenediaminetetra acetic acid (EDTA). It is probable that citrate and isocitrate also inhibit the enzyme by complexing the Mg^{2+} ion. This suggestion is supported by the results shown in Table 2. The pH of EDTA was adjusted to 6.5 by the addition of N-NaOH.

The results show that 1 ml. 50 mM-citrate (50 μmoles) has the same complexing power as 1 ml. 5 mM-EDTA (5 μmoles).

Table 2

Effect of Complexing Agents and Mg^{2+} ion on the Activity of Phosphoglucomutase

Additions (1 ml.)	Glucose Equivalent μ moles/1./sec.
Citrate (50 mM)	3
Citrate + $MgSO_4$ (0.05 mM)	7
Citrate + $MgSO_4$ (1.0 mM)	11
Citrate + $MgSO_4$ (2.5 mM)	19
Citrate + $MgSO_4$ (5.0 mM)	30
Citrate + $MgSO_4$ (10.0 mM)	37
Citrate + $MgSO_4$ (25.0 mM)	37
$MgSO_4$ (25.0 mM)	35
EDTA (10.0 mM)	2
EDTA (5.0 mM)	3
EDTA (1.0 mM)	9
EDTA (0.5 mM)	39
EDTA (0.1 mM)	39

REFERENCES

Cori, C. F. and Cori, G. T., Proc. Soc. Exp. Biol., N.Y., 34, 702 (1936).
Milstein, C., Biochem. J., 79, 574 (1961a).
Milstein, C., Biochem. J., 79, 584 (1961b).
Parmeggiani, A. and Bowman, R. H., Biochem. Biophys. Res. Commun. 12, 268 (1963).
Passonneau, J. V. and Lowry, O. H., Biochem. Biophys. Res. Commun. 13, 372 (1963).
Stickland, L. H., Biochem. J., 44, 190 (1949).