Reversible inhibition of aldolase by ferricyanide

The inhibitory action of ferricyanide on the aerobic and anaerobic glycolysis of some types of tumour cells[1-3] has now been found to be due to an inactivation of aldolase*.

Homogenates of the Walker 256 rat carcinoma, Crocker mouse-ascites sarcoma 180, Ehrlich mouse-ascites carcinoma, rat-brain cortex and rat-kidney medulla were prepared in 0.125 *M* KCl, with the use of a Potter-Elvehjem homogenizer, and centrifuged at 3000 rev./min for 5 min. The supernatant (S_1) was used as such or after centrifugation at 0–4° at 100,000 × *g* for 60 min (S_2). The glycolytic activity of these supernatants was measured at 25° under air in a modified medium of LEPAGE[4] containing, in a final volume of 1.5 ml, 25 m*M* potassium phosphate buffer (pH 7.4), 1.4 m*M* ATP, 0.4 m*M* DPN, 40 m*M* nicotinamide, 4 m*M* $MgCl_2$ and 5 m*M* fructose 6-phosphate or fructose 1,6-diphosphate. Fluoride and pyruvate were omitted. Final osmolarity was adjusted to 0.25 with KCl. Lactic acid formation was determined colorimetrically[5].

Aldolase, glyceraldehyde 3-phosphate dehydrogenase and a mixture of glycerophosphate dehydrogenase and triosephosphate isomerase were obtained from Boehringer. Aldolase and glyceraldehyde 3-phosphate dehydrogenase were assayed spectrophotometrically by the methods used by WU AND RACKER[6], in the case of aldolase with excess glycerophosphate dehydrogenase and triosephosphate isomerase. Fructose 1,6-diphosphate was estimated spectrophotometrically[7].

Two tumours (Walker and Crocker) with a ferricyanide-sensitive glycolysis were used. Glycolysis in both the low- and high-speed supernatants (S_1 and S_2) from these tumours was inhibited completely by 2–5 m*M* $K_3Fe(CN)_6$. This was the case with either fructose 6-phosphate or fructose 1,6-diphosphate as substrate. A small excess

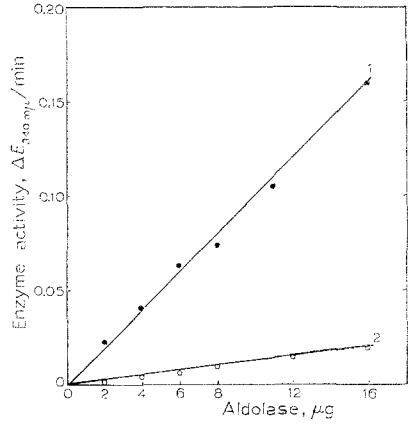

Fig. 1. Effect of ferricyanide on muscle aldolase activity, measured spectrophotometrically[6]. The activities were calculated from the linear decrease of the absorbancy at 340 mμ, measured in a medium containing 0.05 *M* Tris buffer (pH 7.4), 2 m*M* fructose 1,6-diphosphate, 0.1 m*M* DPNH, with excess glycerophosphate dehydrogenase and triose phosphate isomerase, and the indicated amounts of aldolase. Curve 1, control; curve 2, with 0.5 m*M* $K_3Fe(CN)_6$.

Abbreviations: ATP, adenosine triphosphate; DPN, DPNH, oxidized and reduced diphosphopyridine nucleotide; Tris, tris(hydroxymethyl)aminomethane.

* In connection with the stimulation of the hexose monophosphate oxidation by ferricyanide previously reported[3], attention is called to the fact that a correction should be made to the values given for μ-atoms glucose carbon oxidized. All values should be divided by a factor 2.75. This correction does not affect the relative rates of oxidation of C-1 and C-6.

of cysteine, which reduced the $K_3Fe(CN)_6$, partially reversed this inhibition (cf. the disappearance of the ferricyanide effect in intact cells after washing. Fructose 1,6-diphosphatase activity was negligible in the preparations used and the phosphorylation of fructose 6-phosphate to fructose 1,6-diphosphate with ATP, studied in the presence of 1 m*M* iodoacetate, was not affected by 5 m*M* $K_3Fe(CN)_6$.

Concentrations of ferricyanide as low as 0.5 m*M* suppressed the activity of commercial crystalline muscle aldolase almost completely (Fig. 1). The inhibition was completely reversed by 5 m*M* cysteine.

Commercial purified glyceraldehyde 3-phosphate dehydrogenase was practically unaffected by 1 m*M* $K_3Fe(CN)_6$.

Addition of aldolase to a cell-free tumour preparation, in which glycolysis was abolished by ferricyanide, restored the glycolytic activity (Fig. 2). This is not due to removal of ferricyanide, since the ferricyanide concentration at the end of the incubation was still sufficient to inhibit glycolysis in the absence of added aldolase. Glyceraldehyde 3-phosphate dehydrogenase added to the inhibited system did not relieve the inhibition.

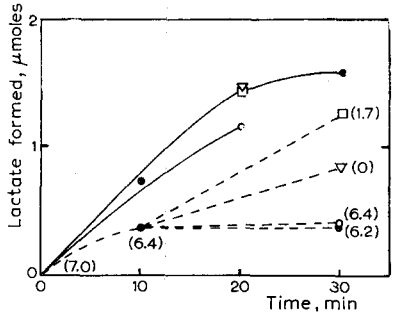

Fig. 2. Effect of ferricyanide on the production of lactic acid from fructose 1,6-diphosphate by a cell-free preparation (S_1) of the Crocker sarcoma 180. See text for reaction conditions. Broken lines represent experiments with 5 m*M* $K_3Fe(CN)_6$. 0.8 mg aldolase (□), 0.8 mg glyceraldehyde 3-phosphate dehydrogenase (○), or 15 μmoles cysteine (▽), respectively, added at zero time in the control experiments and after 10 min in those with ferricyanide. ●, no additions. The values in brackets are the amounts of ferricyanide (μmoles) found at the times indicated.

Although intact normal tissue characterized by high glycolysis[2] and Ehrlich ascites cells[3] are not sensitive to $K_3Fe(CN)_6$, the aldolase activity of cell-free preparations from Ehrlich tumour, rat-brain cortex and rat-kidney medulla was sensitive to ferricyanide. The difference between ferricyanide-susceptible and non-susceptible cells must probably be ascribed to different permeabilities of the cell wall to ferricyanide.

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