Anion Effects on Pyruvate Kinase

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Rabbit muscle pyruvate kinase is shown not to be activated by several anions (acetate, chloride, lactate, nitrate and sulphate) when tested as their tetramethylammonium salts. This contradicts the conclusions of Focant & Watts (1973).

Although anions are known to be effectors of creatine kinase (Watts, 1973), cations (univalent and bivalent) are well established as effectors of pyruvate kinase (Kayne, 1973). Focant & Watts (1973) have concluded that anions also activate rabbit muscle pyruvate kinase. These authors seem to have been unaware that Na^+ serves as activating univalent cation for pyruvate kinase and will replace the commonly used K^+ , albeit less efficiently (Kayne, 1973). Accordingly, when Focant & Watts (1973) report the activation of pyruvate kinase by acetate, chloride, lactate, nitrate, chlorate, sulphate and phosphate, it is not in the absence of activating cation, because Na^+ was used as the counter-ion in each case. To determine if these anions activate, one must use a truly inactive cation such as tetramethylammonium ion as the counter-ion. We report that, as their tetramethylammonium salts, acetate, chloride, lactate, nitrate and sulphate are not activators of rabbit muscle pyruvate kinase, and suggest that the other anions reported by Focant & Watts (1973) would not activate either, if tested as their tetramethylammonium salts.

Experimental

Tris-ADP was obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.), and cyclohexylammonium phosphoenolpyruvate, tetramethylammonium hydroxide and Na_2NADH were from Calbiochem (Los Angeles, Calif., U.S.A.). Tetramethylammonium chloride was from Eastman (Rochester, N.Y., U.S.A.), cysteine was from Nutritional Biochemicals (Chagrin Falls, Ohio, U.S.A.) and lactic acid was from Mallinckrodt (St. Louis, Mo., U.S.A.). Rabbit muscle pyruvate kinase and rabbit muscle lactate dehydrogenase were from Boehringer-Mannheim (New York, N.Y., U.S.A.). All other chemicals were reagent grade. Double-distilled water was used for all solutions. Except for tetramethylammonium chloride, solutions of tetramethylammonium salts were prepared by neutralizing the appropriate acid with tetramethylammonium hydroxide. Except for use of tetramethylammonium salts, conditions similar to those of Focant & Watts (1973) were used. The en-

zymes were dialysed against 25 mM-Tris-acetate buffer, pH 8.6, 10 mM-magnesium acetate and 1 mM-mercaptoethanol for 3 days with six buffer changes. Nevertheless, in the absence of added salt, dialysed pyruvate kinase exhibited 0.4% of its activity with added KCl. All enzymic assays were performed at 26°C on a Gilford recording spectrophotometer. A less-than-saturating concentration of phosphoenolpyruvate was used in view of the competition reported between anions and phosphoenolpyruvate (Focant & Watts, 1973).

Results and discussion

Table 1 presents the results obtained at 40 mM concentration of the various salts. The tetramethylammonium salts of acetate, chloride, lactate, nitrate and sulphate are observed to have an activity not significantly different from no salt addition. The sodium salts give significantly greater activity, as observed by Focant & Watts (1973), and the values

Table 1. *Effects of several salts on the activity of pyruvate kinase*

The reaction mixture contained 100 mM-Tris-acetate buffer, pH 8.3, 3 mM-magnesium acetate, 1 mM-cysteine, 0.2 mM-cyclohexylammonium phosphoenolpyruvate, 1 mM-Tris-ADP, 0.2 mM-NADH, 25 μg of lactate dehydrogenase and 25 μg of pyruvate kinase in a total volume of 1.0 ml.

Salt (40 mM)	Relative activity
None	0.004
Tetramethylammonium acetate	0.004
Tetramethylammonium chloride	<0.001
Tetramethylammonium lactate	0.006
Tetramethylammonium nitrate	0.002
(Tetramethylammonium) ₂ sulphate	0.003
NaCl	0.051
Sodium lactate	0.041
NaNO_3	0.030
KCl	1.00
Potassium acetate	1.00

are close to those reported for Na^+ in the literature (Kayne, 1973). The potassium salts exhibit optimum activity. Similar results were observed at lower concentrations of the various salts and with replacement of the Tris-acetate buffer with tetramethylammonium tris(hydroxymethyl)methylaminopropanesulphonate buffer, pH 8.3. All anions tested as their tetramethylammonium salts did not activate pyruvate kinase.

Focant & Watts (1973) also report protein difference spectra with NaNO_3 and sodium lactate, which they interpret as due to nitrate and lactate complexes with pyruvate kinase. The authors apparently failed to note that Na^+ ions, like K^+ ions, give rise to a protein difference spectrum, as reported by Suelter *et al.* (1966). Focant & Watts (1973) also note that lactate inhibits pyruvate kinase activated with K^+ . With the use of tetramethylammonium lactate, such inhibition has been confirmed; under the conditions used in Table 1, but with 100mM-KCl, a 40% inhibi-

tion is observed at 40mM-tetramethylammonium lactate, and 42% inhibition is observed with 40mM-sodium lactate. Such inhibition is not surprising in view of the stereospecific reduction of pyruvate to lactate which takes place on pyruvate kinase with NaBH_4 (Phillips *et al.*, 1973).

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