Influence of Certain Cations on Activity of Succinyl CoA Synthetase From Tobacco\textsuperscript{1, 2}

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Abstract. Succinyl CoA synthetase from Nicotiana tabacum exhibited a requirement for univalent and divalent cations. Mn\textsuperscript{2+} replaced Mg\textsuperscript{2+} in the assay medium and Co\textsuperscript{2+} and Ca\textsuperscript{2+} partially replaced Mg\textsuperscript{2+}. Addition of Zn\textsuperscript{2+} resulted in no enzyme activity. The enzyme was activated by univalent cations K\textsuperscript{+}, Rb\textsuperscript{+}, NH\textsubscript{4}\textsuperscript{+}, and Na\textsuperscript{+}; Li\textsuperscript{+} showed little or no activation. Maximum enzyme activity varied significantly with potassium salts of different anions. Greatest activation was obtained with K\textsubscript{2}PO\textsubscript{4} and, respectively, KCl, KNO\textsubscript{3}, K\textsubscript{2}SO\textsubscript{4} and KF exhibited steady decreasing enzyme activation.

Materials and Methods

Materials. Potassium ATP, GSH, hydroxylamine HCl, tris buffer and polyvinylpyrrolidone 360 were obtained from Mann Research Laboratories. Succinic acid was obtained from Sigma Chemical Company. All other reagents were commercial preparations of high purity. The tris salt of ATP was made by passing a solution of dipotassium ATP through an ion exchange column containing Dowex 50 resin, 50 to 100 mesh, (tris cycle). The tris salt of succinate was prepared by neutralizing the acid to pH 7.4 with tris.

Methods. Succinyl CoA was obtained from an aceton powder from leaves of tobacco (Nicotiana tabacum L. var. Burley 21) cultured in the greenhouse. The acetone powder was prepared using 0.002 g GSH per g fresh tissue and blended in 10 to 20 volumes of cold acetone. One g acetone powder was extracted with stirring for 30 min at 4° with 15 ml of 16.7 mM potassium phosphate buffer, pH 7.2, containing 1 g polyvinylpyrrolidone 360. The crude extract was clarified by centrifugation at 12,000g for 15 min. Cold acetone was added drop-wise to the supernatant solution to a concentration of 25% by volume and stirred for 2 hr at 4°. The precipitate was removed by centrifugation at 10,000g for 10 min and discarded. The supernatant solution was made to 60% acetone by volume and stirred for 2 hr at 4° and centrifuged. The precipitate was dissolved in 16.7 mM tris-HCl buffer, pH 7.2, and dialyzed overnight against the same buffer. The dialyzed solution was applied to a Sephadex G-75 column, 1.5 × 20 cm. The column was eluted with the tris-HCl buffer. The fraction eluted between 15 and 20 ml was used for the various experiments. These purification steps resulted in approximately 200-fold increase in specific activity of the enzyme with a final specific activity.

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of 10 to 13 μmole succinohydroxamic acid formed per 30 min per mg protein.

**Succinyl CoA Synthetase Assay.** The enzyme was assayed by following the rate of succinohydroxamic acid formation (3). The reaction mixture contained the following constituents in a 1 ml volume: 0.48 mmole NH₄OH; 0.05 mmole tris, pH 7.2; 0.05 mmole potassium succinate, pH 7.2; 10 μmole GSH; 5 μmole MgCl₂; 2.5 μmole ATP; 0.065 μmole CoA; and 0.05 to 0.1 unit of enzyme. After 30 min incubation at 37° the reaction was stopped by addition of 0.5 ml of a 3 x HCl solution containing 0.10 mmole FeCl₃ and 0.09 μmole trichloroacetic acid. The protein precipitate was removed by centrifugation at 10,000 g for 15 min and optical density determined at 540 μg. One unit of activity is defined as the amount of enzyme catalyzing the formation of 1.0 μmole of succinohydroxamic acid in 30 min. Protein concentration of extracts was determined with Poli-Ciocâltean reagent (7). Bovine albumin was used as protein standard.

In experiments designed to determine divalent cation specificity the standard assay solution was adjusted to pH 6.8 and the cation concentration was decreased. These precautions were necessary because some of the tested metals tend to form their hydroxides at a basic pH, and therefore are only very slightly soluble.

Assays for univalent cation requirements were modified by substitution of tris succinate, tris ATP, and tris neutralized NH₄OH for their potassium salt counterparts.

**Results**

**Divalent Cation Activation of Succinyl CoA Synthetase.** Succinyl CoA synthetase from tobacco exhibited an absolute requirement for divalent cation. The effect on enzyme activity by substitution of various divalent cation chlorides is shown in Fig. 1. The assay solution was lowered to pH 6.8 and the concentration of divalent cations was reduced to minimize their precipitation from the assay solution. The enzyme activating capacities of Mg²⁺ and Mn²⁺ were approximately equal at all concentrations tested. The Km for MgCl₂ was 4 mM. Compared to Mg²⁺,

![Graph showing the influence of various concentrations of univalent cations on the activity of succinyl CoA synthetase](image)

**FIG. 2.** The influence of various concentrations of univalent cations on the activity of succinyl CoA synthetase. Tris succinate and tris ATP plus the univalent cation were used in the standard assay.

10 % activation was produced by Ca²⁺ concentrations below 2.5 mM and 38 % activation by concentrations above 2.5 mM. Zn²⁺ was the least effective divalent cation tested and caused no enzyme activation below 1.5 mM and only slight activation at higher concentrations. Co²⁺, above 3.0 mM, was apparently a more effective activator than Mg²⁺. However, accurate comparisons were difficult because of high spectrophotometric readings for the controls and uncertainty as to the Co²⁺ concentration in solution.

**Univalent Cation Activation of Succinyl CoA Synthetase.** The curves in Fig. 2 illustrate the influence of univalent cations, added as the chloride salt, on enzyme activity. In these experiments tris succinate, tris ATP, and tris-neutralized NH₄OH were used in the standard assay. Slight activity was occasionally detected in the absence of added salts. Analysis of the assay solution by flame photometry indicated the presence of 77 μM K⁺.

Maximum enzyme activity with the chloride salts of K⁺, Rb⁺, NH₄⁺, and Na⁺ was observed at concen-

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Fig. 3. The influence of potassium salts of several anions on the activity of succinyl CoA synthetase. Tris succinate and tris ATP plus the potassium salt were used in the standard assay.

Effect of Anions on Enzyme Activity. Univalent cation activation of succinyl CoA synthetase was not independent of the anion (Fig. 3). Optimum concentration varied slightly with potassium salts of different anions; however, maximum enzyme activity varied markedly with the different potassium salts. At maximum enzyme activity the substitution of the PO₄³⁻ salt gave 120% of the activity obtained with Cl⁻, whereas in the presence of the NO₃⁻ and SO₄²⁻ salts enzyme activity was 70% of that obtained with Cl⁻. KF caused slight but constant activation of succinyl CoA synthetase.

Discussion

Succinyl CoA synthetase from tobacco exhibited a requirement for univalent cations. Maximum enzyme activity was produced by K⁺ and Rb⁺ at a concentration of 0.06 M. Enzyme activity was inhibited by Li⁺ above 0.01 M and the addition of Na⁺ and NH₄⁺ resulted in intermediate enzyme activity. The partial activation of succinyl CoA synthetase by Na⁺, compared with K⁺, was the same as that observed with acetic thio kinase (2). These results are in contrast to the observation of Na⁺ being an activator for pyruvate kinase isolated from higher plants (8).

Observed differences in maximum enzyme activity with different anions of K⁺ cannot be explained at the present time. The pH of the assay solutions was not altered appreciably with the addition of the PO₄³⁻; and the pH was within the optimum range previously reported (5). If the data were expressed in K⁺ equivalents, the order of rank among the anions would remain the same with respect to enzyme activity: PO₄³⁻ > Cl⁻ > NO₃⁻ > SO₄²⁻ > F⁻. The lack of enzyme activation by potassium halides other than Cl⁻ has also been observed with pyruvate kinase (8). The physiological importance of the various univalent cation activators of succinyl CoA synthetase is not fully understood. Rb⁺ and NH₄⁺ are relatively efficient activators of pyruvate kinase, phosphotransacetylase, and aldehyde dehydrogenase as well as succinyl CoA synthetase and may substitute for K⁺ as an univalent cation in plant metabolism. Richards (11) has reported that the addition of Rb⁺ to barley decreased the K⁺ requirement for growth. However, the concentrations of Rb⁺ or NH₄⁺ normally present in plant material are insufficient to be physiologically important. Also, because of the toxicity of high concentrations of either Rb⁺ or NH₄⁺ to plants the most probable physiologically active univalent cation is K⁺ (2).

Previous reports (1, 5, 9, 10) have shown succinyl CoA synthetase to require a divalent cation. This requirement was satisfied by Mg²⁺, Mn²⁺, and Co²⁺. The observation that Co²⁺ activated the enzyme significantly more than Mg²⁺ cannot be explained with present data and is in contrast to other data (9). However, at very low concentration Gibson et al. (1) showed that activation by Co²⁺ was equal to or greater than activation with Mg²⁺. Maximum activity with Ca²⁺ was only 38% of Mg²⁺ and substitution of Zn²⁺ resulted in only 10% of the activity obtained with Mg²⁺.

Literature Cited