ACTIVATION OF WHEAT GERM ACETYL CoA CARBOXYLASE BY POTASSIUM AND RUBIDIUM

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SUMMARY

Wheat germ acetyl CoA carboxylase requires certain alkali cations to exhibit maximal activity. Maximal activation results when 60 mM K^+ or Rb^+ are included in the assay mixture, whereas only marginal activation occurs in the presence of similar concentrations of Li^{++} and Na^{++}. Cs^{++} activates, but less effectively than K^+ or Rb^+. Since it is also possible to activate the enzyme maximally using 20 mM potassium isocitrate, but not 20 mM sodium isocitrate, activation of the wheat germ enzyme is due to a cation effect and not to citrate anion.

INTRODUCTION

In higher plants, acetyl CoA carboxylase catalyzes the formation of malonyl CoA from acetyl CoA in a reaction requiring Mg^{++}, ATP and bicarbonate. This enzyme has been studied in embryonic plant tissue (1,2), as well as in chloroplasts (3,4). Like its counterpart in mammalian (5) and procaryotic tissue (6), these plant enzymes contain covalently-bound biotin which initially becomes carboxylated at the expense of Mg-ATP and then serves as the carboxyl donor to the acceptor, acetyl CoA to yield malonyl CoA.

Plant acetyl CoA carboxylase has been purified extensively from both wheat germ (1) and barley embryos (2). In each case, a large protein of approximately 350 kilodaltons was obtained. Once purified, the carboxylase showed a tendency to aggregate with a loss of enzymic activity. Moreover, when the purified enzyme was applied to gel filtration columns, the enzymatic activity recovered from the column invariably was located at the leading edge of the major eluting protein peak (1). Recently Brock and

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Kannangara (2) reported that including 1% NaCl in the elution buffer not only reduced the apparent molecular weight of an aggregated enzyme preparation purified from barley embryos, but also caused the enzymatic activity to be recovered symmetrically throughout the entire protein peak. Despite the apparent reduction in aggregation, their enzyme preparation still rapidly became inactive when stored frozen in 50% ammonium sulfate (2).

During the course of continuing investigations in our laboratory (1,7,8) to purify and characterize wheat germ acetyl CoA carboxylase, as well as its counterpart in photosynthetic tissue, we had occasion to test the effect of cations on the activity of this enzyme. We noticed that while KCl was capable of dissolving an aggregated partially inactivated enzyme preparation, KCl had an additional effect in that it promoted an activation of the enzyme. It is the purpose of this communication to describe briefly the conditions for the activation of wheat germ acetyl CoA carboxylase using various alkali cations.

MATERIALS AND METHODS

Acetyl CoA carboxylase was purified from raw wheat germ (General Mills, Inc., Vallejo, California) using a modification of the procedure worked out by Hatch and Stumpf (7) and Heinstein and Stumpf (1). The enzyme was extracted from an acetone powder using 100 mM potassium phosphate buffer (pH 8.3) containing 1 mM EDTA and 1 mM DTT. The resulting extract was then treated with 0.05 vol of 1 M $MnCl_2$ to remove nucleic acids and the precipitate was discarded. The enzyme was precipitated by bringing the supernatant fraction to 50% saturation with ammonium sulfate and sedimented. The precipitated enzyme was then resuspended in extraction buffer, 20% saturated with ammonium sulfate and the material not dissolved was discarded. The enzyme was again precipitated by bringing the suspension to 50% saturation, and then, after sedimentation, was suspended in 10 mM potassium phosphate (pH 8.3) containing 1 mM EDTA and 1 mM DTT. A precipitate appeared during dialysis to remove residual ammonium sulfate which was centrifuged down and discarded. The super-

natant was employed for all experiments reported here and represents a 10-fold purified preparation.

Acetyl CoA carboxylase activity was determined in a reaction mixture containing in a final volume of 0.5 ml: 10 mM TAPS pH 8.5, 2.5 mM ATP, 5 mM $MgCl_2$, 2.5 mM acetyl CoA, 0.5 mg BSA, 55 µg of enzyme protein, and where indicated the appropriate amount of the specific salt. In performing the reaction, all components except acetyl CoA were incubated at 33°C for 3 minutes, and then acetyl CoA was added to begin the reaction. The reaction was terminated 10 minutes later by the addition of 0.1 ml 17.5M acetic acid, and then the acid stable radioactivity was determined by drying an aliquot on filter paper strips and counted, using Bray's solution in a Beckman Scintillation counter. All activities are expressed as nmoles acid stable radioactivity $minute^{-1}$ mg $protein^{-1}$.

RESULTS AND DISCUSSION

Figure 1 shows that the presence of KCl in the reaction mixture markedly stimulated acetyl CoA carboxylase activity. The preparation used for these measurements yielded between a 3 and 4 fold increase in activity with maximal stimulation taking place between 30 and 60 mM KCl. The actual degree of stimulation varied from one preparation to another, values between

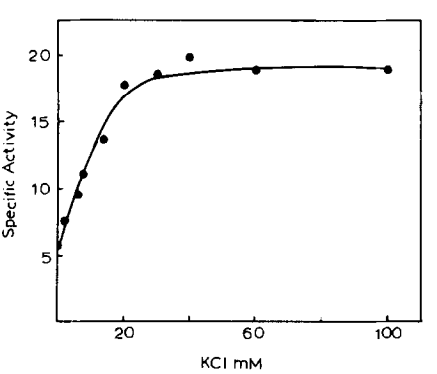

Figure 1. Activation of Acetyl CoA Carboxylase by KCl.

a 2 and a 6-fold increase in activity having been observed. Frequently, the degree of stimulation appeared to become greater as the length of time following the preparation of the enzyme increased. While the data reported here were obtained using an ammonium sulfate precipitated enzyme preparation, a K^+ stimulation of a similar magnitude has also been observed using preparations purified nearly 500-fold from the original extract.

Earlier studies with the wheat germ enzyme indicated that it could be stimulated by incubating in the presence of a sulfhydryl reducing reagent prior to measuring catalytic activity (1), and it seemed possible that the stimulation observed using K^+ might be related to this effect. The data in Table I show that including dithiothreitol increased the acetyl CoA carboxylase activity slightly in the absence of KCl as well as in its presence. However, the degree of stimulation by KCl was similar both in the pre- and absence of dithiothreitol, suggesting that K^+ stimulation was not related to a sulfhydryl site activation.

Table II shows the effect of including various alkali cations in the reaction mixture. Both LiCl and NaCl were nearly without stimulatory effect, whereas both KCl and RbCl stimulated acetyl CoA carboxylase about three-fold. CsCl likewise increased carboxylase activity, but not to the same extent as Rb^+ and K^+. Thus, specificity is exhibited by the enzyme for the activating cation and this specificity may be related to ion size.

Table I. The Effect of Dithiothreitol on Wheat Germ Acetyl Activity

Activator	Specific Activity	Percent of Basal Activity
Basal	8.8	----
60 mM KCl	22.4	255
Basal + 1 mM DTT	10.4	----
1 mM DTT + 60 mM KCl	26.0	250

Table II. Cation Specificity for Acetyl CoA Carboxylase Activation.

Salt Added	Specific Activity	Percent of Basal Activity	Ionic Radius (Å) unhydrated[1]	Ionic Radius (Å) hydrated[2]
Basal	7.35	----	----	----
60 mM LiCl	10.22	139	0.68	3.60
60 mM NaCl	8.56	116	0.97	3.65
60 mM KCl	21.62	295	1.33	4.05
60 mM RbCl	24.42	332	1.47	4.20
60 mM CsCl	15.56	212	1.67	4.40

[1]L. Pauling (1940) Nature of the Chemical Bond, p. 346, Cornell Univ. Press, Ithaca, New York.

[2]L.J. Mullins (1975) Biophysical J. 15, 921.

Table III. The Effect of Isocitrate on Acetyl CoA Carboxylase Activation.

The assays were performed as described in Materials and Methods except that the Mg^{++} concentration was increased to 10 mM to off set the chelating effect of isocitrate.

Activator	Specific Activity	Percent of Basal Activity
Basal	10.19	----
60 mM NaCl	9.84	97
20 mM Na-isocitrate	9.19	90
60 mM KCl	23.22	228
20 mM K-isocitrate	20.05	197

Various tricarboxylic acids including citrate and isocitrate are able to activate acetyl CoA carboxylase from mammalian sources (5). As can be seen in Table III, 20 mM K^+ isocitrate activates the wheat germ enzyme to nearly the same extent as 60 mM KCl. The effect undoubtedly is related to the 60 mM K^+ cations accompanying the isocitrate, however, since 20 mM

sodium citrate is without a stimulatory effect. This result confirms and extends the earlier experiments of Burton and Stumpf (8) who also concluded that tricarboxylic acids were without effect on the plant enzyme. It should also be pointed out that in the case of the wheat germ enzyme, inactivation is accompanied by aggregation, whereas citrate activation of the mammalian enzyme results in polymerization (5).

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