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Short Communications

The Influence of Univalent Cations on Crystalline Propionyl CoA Carboxylase

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It was previously demonstrated that the propionyl CoA carboxylase activity of acetone powder extracts from rat liver mitochondria can be increased 2–5 times by a preparation obtained from the 105 000 × *g* supernatant [1] and also by potassium ions [2]. The present investigation deals with studies on the influence of potassium and other univalent cations on the enzyme activity of propionyl CoA carboxylase.

Materials and methods. The crystalline propionyl CoA carboxylase was a gift of Dr. Kaziro and Dr. Ochoa of the New York University School of Medicine. The enzyme was a three times recrystallized material with a specific activity of 17 units per mg protein. The unit is defined as the amount of the enzyme which carboxylates one μmole of propionyl CoA* per minute [3]. The material was dissolved in 0.02 M potassium phosphate buffer pH 6.5 containing 0.001 M EDTA and 0.0005 M GSH, a set of conditions under which the enzyme is most stable [4]. In order to minimize the deterioration of the enzyme following repeated freezing and thawing, the solution was divided into portions of 0.1 ml which were immediately frozen again. One such portion was used in each experiment. In order to remove potassium ions the enzyme was diluted with 0.1 M Tris-HCl buffer, pH 7.4, containing 0.001 M EDTA and 0.0005 M GSH, and dialyzed for 4–6 h against 3 × 250 ml of the same buffer. The dialysis was omitted in later experiments. Diluting the original enzyme solution 1:50 with the Tris-HCl buffer mentioned above brought the concentration of potassium ions down to 0.04 μmoles K^+ per incubation mixture — a concentration which does not give any activation effect.

The enzyme assay was based on the $^{14}CO_2$ fixation method and was performed essentially as described by Halenz and Lane [5]. The standard assay mixture contained (in μmoles): GSH, 2.5; ATP, 2; $MgCl_2$, 2; $NaH^{14}CO_3$, 7.5 (1 μC total activity); propionyl CoA, 0.5; Tris-HCl buffer, pH 8.4, 50; solutions of the respective cations (as chloride salts), 0.1 ml; enzyme solution, 0.1 ml (0.025 units). Total volume was 0.85 ml. The reaction was initiated by the addition of propionyl CoA and, after incubation at 37°C for 20 min, was stopped with 0.1 ml 20 % trichloroacetic acid. The mixture was then heated for 5 min at 60°C under a hood to remove the non-reacted $NaH^{14}CO_3$. All incubations were carried out in duplicate. Aliquots (0.05; 0.1 and 0.2 ml) of the clear solutions containing methyl malonate were counted for radioactivity at infinite thinness in a Tracerlab

Table 1. Activation of crystalline propionyl CoA carboxylase by certain univalent cations. 5 μmoles cation added to the reaction mixture for the standard assay with 0.025 units of the enzyme.

Addition	Enzyme activity c.p.m.*	Activation, fold
None	890	1.0
NaCl	1160	1.3
KCl	1510	1.7
NH_4Cl	1420	1.6
RbCl	2050	2.3
CsCl	1780	2.0

* $^{14}CO_2$ fixed, details of the assay in text.

* The following abbreviations will be used throughout this report: propionyl CoA — propionyl coenzyme A, ATP - adenosine-5′-triphosphate disodium salt; GSH-glutathione, EDTA-ethylene diamine tetraacetic acid.

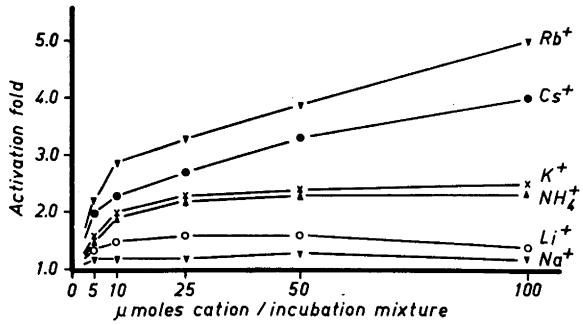

Fig. 1. Influence of univalent cations on the activity of crystalline propionyl CoA carboxylase. Enzyme activity determined by the $^{14}CO_2$ fixation method as described in text. The activity of the enzyme without addition of the different cations (usually 300—800 cpm $^{14}CO_2$ fixed/0.025 enzyme units) is taken as a unit and the activities obtained with the respective cations calculated as multiples thereof.

thin end window counter. The values reported in Tables 1 and 2 refer to the total activity of the solutions. Propionyl CoA was prepared by the method of Simon and Shemin [6]. ATP and GSH were obtained from Sigma Chemical Co. and Fluka, coenzyme A from Sigma.

Results and discussion. Some typical activation values using 5 μmoles of the respective cations per 0.025 units of the enzyme are reported in Table 1. It can be seen that highest activation effects were obtained with Rb^+ and Cs^+, *viz.* 2.3 and 2.0 fold. The effect obtained with K^+ was 1.7 fold which was considerably lower than the 2—5 fold activation effects observed earlier with crude enzyme preparations [2]. When the enzyme solution was dialyzed before assay the activation effects decreased considerably although the base activity of the enzyme, *i.e.* enzyme activity without addition of the respective cations, remained practically unchanged. The reason for this phenomenon could not be understood.

The relation between the concentration of the different salts and their activation effects on propionyl CoA carboxylase is shown in Fig. 1. The activation curves in Fig. 1 are based on 3—4 experiments, each comprising duplicate incubations. The base activity of the enzyme varied from experiment to experiment because of the slow inactivation of the enzyme upon freeze storage: ca 800 c.p.m. $^{14}CO_2$ fixed per standard incubation mixture at the beginning of the investigation and 300 c.p.m. at the end. However, when the base activity was taken as a unit and the activities obtained in the presence of different cations were calculated as multiples thereof, quite constant values were obtained. The activation effects obtained in the different experiments varied in the range ± 0.05—0.15.

The six curves in Fig. 1 can be arranged in three groups according to the order of magnitude of the activation effects obtained. Na^+ and Li^+ (5—100 μmoles) caused the smallest activation effects ranging from 1.2 to 1.6. Since Na^+ was present in the incubation mixture also as $NaH^{14}CO_3$ at a level of 7.5 μmoles and since NaOH had been used for the neutralization of ATP, GSH and propionyl CoA solutions, the activation effects obtained with this cation are difficult to evaluate. Activation effects of an intermediate magnitude were observed with K^+ and NH_4^+. The effects of the latter two cations are very close to each other. The slopes of all six activation curves are greatest in the range 0—10 μmoles. However, even in this respect the two most active cations, Cs^+ and Rb^+, differ markedly from the remaining four (*cf.* Fig. 1).

The activating effects of NH_4^+ and K^+ do not seem to be additive, as demonstrated in Table 2.

The results of this investigation confirm the earlier finding that potassium activates propionyl carboxylase. The activation effects observed with the crystalline enzyme are, however, considerably lower than those obtained in most experiments with the crude enzyme. At the present, no ex-

Table 2. The non-additive character of the activation of crystalline propionyl CoA carboxylase by K^+ and NH_4^+.

Addition to the standard incubation mixture	Enzyme activity c.p.m. $^{14}CO_2$ fixed*
None	950
KCl 5 μmoles	1560
NH_4Cl 5 μmoles	1520
KCl 5 μmoles + NH_4Cl 5 μmoles	1500

* details of the assay in text.

planation can be offered for the observed discrepancy. The observation that the effects of the different univalent ions investigated vary greatly with their size suggests that steric factors may be involved in the metal activation effects.

The amounts of $^{14}CO_2$ fixed in these experiments are considerably smaller than what could be expected from stoichiometric considerations, using 0.025 units of the enzyme per standard incubation mixture. However, the specific activity and the kinetic constants of the enzyme have been determined (by Dr. Kaziro [3]) in a medium containing 150 μmoles K. It is evident from the results reported here that, without any or with only an insignificant concentration of potassium ions, the enzyme activity decreases to levels below the nominal one.

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Di-selena-straight-chain Fatty Acids

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The selena mono carboxylic acids with one selenium atom incorporated in the chain are described in the literature and they have been prepared by syntheses appropriate for selenides. One of the chief methods is the reaction of an alkyl halide with the salt of a selenol acid, and following this procedure many long-chain selena-fatty acids have been made at this Institute [1-3].

During the investigation of the long-chain selena-fatty acids it was decided to investigate the effect of incorporating a second selenium atom in the chain. Such compounds may also be of interest with regard to their biological and pharmacological properties.

Following the usual procedure for the preparation of this type of acid, it was decided to prepare an ω-hydroxy selena acid, halogenate the hydroxy group, prepare the corresponding diselenide and reduce the diselenide; the subsequent reaction with alkyl halide would give the di-selena fatty acid.

In the initial step, ω,ω'-diseleno-diundecanoic acid [4] was prepared and reduced by Rongalite, and by the addition of ethylene bromohydrine, 14-hydroxy-12-selena-tetradecanoic acid was obtained (m.p. 61—62.5°, yield: 61 %). During the second step, the halogenation of the hydroxy group by the hydrogen bromide or hydrogen chloride, either as gas or in solution and with or without heat, caused the appearance of yellow or orange crystals showing that an undesired side reaction had taken place.

In the preparation of a series of 12-selena fatty acids it was found that the reactivity of alkyl bromide with ω-selenol undecanoic acid is substantially greater than that of alkyl chloride and the yield is far better. It was therefore decided to take advantage of this difference in the halide reactivities and use a mixed dihalide. In this case trimethylene chlorobromide was used, which worked successfully according to the following scheme: