Inhibition of rat liver acetyl CoA carboxylase by chloride

John B. Allred and Karla L. Roehrig

Department of Food Science and Nutrition, The Ohio State University, Columbus, OH 43210

Summary

The activity of acetyl CoA carboxylase in both crude and purified rat liver preparations was reduced in the presence of sodium or potassium chloride and increased in the presence of potassium acetate. The chloride inhibition was not competitive with bicarbonate. The use of Tris-chloride buffer did not alter the apparent pH optimum of the enzyme when compared with Tris-acetate buffer.


Supplementary key words: ion and buffer effects on acetyl CoA carboxylase activity

Chloride ion has been widely used as the anion in assay buffers (1-8) and in the study of properties (9-20) of acetyl CoA carboxylase [EC 6.4.1.2. Acetyl CoA: carbon dioxide ligase (ADP-forming)]. During the course of the development of a rapid assay for this enzyme (21), we discovered that chloride ion was a potent inhibitor of its activity. A study of the effect of chloride and other ions on the activity of acetyl CoA carboxylase was initiated and is reported here. The effect of these ions on the activity of the enzyme is sufficient to warrant care in the interpretation of results obtained in their presence.

METHODS AND MATERIALS

Heat-activated acetyl CoA carboxylase

Liver from fed rats was homogenized in 1.5 volumes of cold (0-4°C) 0.3 M mannitol and centrifuged at 27,000 g for 20 min. The supernatant was incubated at 37°C for 30 min to activate the enzyme as previously described (21), then cooled in ice for an additional 30 min. An aliquot of the heat-activated enzyme was assayed by fixation of radioactive bicarbonate in acid-stable form in one minute (except Fig. 3) in the presence of various concentrations of ions as indicated in the Figures and Tables. The assay reaction mixture, previously described (21) was modified by omitting potassium acetate and, in some cases, substituting other buffers for Tris-acetate as indicated.

Citrate-activated acetyl CoA carboxylase

Acetyl CoA carboxylase was purified by the polyethylene glycol (PEG) precipitation method of Hardie and Cohen (22) except that PEG was increased to 5% as suggested by A. Tausif and P. Srere. This preparation involves a six-hour dialysis at room temperature in the presence of 25 mM potassium citrate and yielded an active enzyme which showed only one detectable peak on Sepharose 6B which was eluted with the void volume indicating a molecular weight of at least 4 x 10^6. Electrophoresis in the presence of SDS on 5% polyacrylamide gel showed only one major band when stained with Coomassie Brilliant Blue. This preparation was assayed spectrophotometrically using the reaction mixture previously reported (21) modified by omitting the radioactive bicarbonate and including 0.3 mM phosphoenol pyruvate, 0.1 mM NADH, 20 units of pyruvate kinase, and 30 units of lactate dehydrogenase (6). The reaction was started by the addition of acetyl CoA and the acetyl CoA-dependent disappearance of NADH was measured at 340 nm was used as a measure of enzyme activity. With this preparation, no decrease in the concentration of NADH was observed in the absence of acetyl CoA.

RESULTS AND DISCUSSION

The effect of including the sodium and potassium salts of chloride and acetate in the assay reaction mixture on the activity of heat-activated acetyl CoA carboxylase is shown in Fig. 1a. Both sodium and chloride ion were inhibitory while acetate ion increased activity. There was a linear decrease in the activity as the concentration of sodium chloride increased and the data in the inset show that this linear effect was observed even with very low concentrations of the salt. This suggested that even the small amount of chloride usually added as the anion of magnesium

1 Personal communication.
The indicated concentration of potassium acetate (● —), sodium acetate (○ —), potassium chloride (—— x —), or sodium chloride (—— ■ —) was included in the assay reaction mixture. The reaction was initiated by the addition of enzyme representing 8 mg of liver and activity was measured by fixation of radioactive bicarbonate in acid stable form in 1 min. Specific activity of the control was 0.89 pmol product/min/g liver.

The indicated concentration of potassium acetate (● —), sodium acetate (○ —), potassium chloride (—— x —), and sodium chloride (—— ■ —) was included in the assay reaction mixture. Purified enzyme (2.4 µg protein) was added and the reaction was initiated by the addition of acetyl CoA. The specific activity of the control was 1.61 units/mg protein.

(16 mM) may decrease activity. We found in similar but separate experiments that substitution of magnesium acetate for magnesium chloride increased activity about ten percent.

The inhibition of acetyl CoA carboxylase by chloride ion has been observed with a variety of preparations. For example, the crude homogenate as used in the experiment shown in Fig. 1a is inhibited by chloride ion before it is heated. Enzyme in Sephadex G-25-treated high speed supernatant (21) was similarly inhibited by chloride ion. The data in Fig. 1b show that the ion inhibition persists in citrate-activated, purified enzyme in that essentially the same effects of the sodium and potassium salts of chloride and acetate were observed as were observed with the crude preparation. The similarity of the salt effects on the crude preparation (Fig. 1a) and the purified enzyme (Fig. 1b), as well as preparations of intermediate purity (data not shown), indicate that these ion effects are likely inherent properties of the enzyme. It should also be noted that the similar ion effects were observed with two different assay methods which indicates they are not a function of particular assay conditions.

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The assay of acetyl CoA carboxylase by the fixation of radioactive carbon from bicarbonate in acid stable form is initiated by the addition of enzyme and is linear for more than one minute (21). The data in Fig. 2 show that sodium chloride did not affect the linearity of product formation with time. This indicates that the interaction between the enzyme and the salt was rapid relative to the rate of product formation.

The effect of pH on activity of heat-activated enzyme. The standard bicarbonate fixation assay was used except that the Tris acetate-potassium acetate buffer was replaced with 0.06 M Tris acetate (● —), Tris chloride (○ —), or potassium phosphate (—— x —) buffer. Specific activity was measured by incubating heat-activated enzyme representing 8 mg liver for 1 min.
formation and that the presence of sodium chloride does not invalidate the assay method.

Although the mechanism of inhibition by chloride ion has not been established, one obvious possibility is that chloride competes for the bicarbonate binding site on the enzyme. If this were the case, chloride inhibition would be a function of the bicarbonate concentration in the assay reaction mixture. This was shown not to be the case by experiments showing that sodium chloride did not affect the apparent $K_m$ for bicarbonate but rather altered the $V_{max}$ (data not shown). Chloride inhibition could not be overcome by increasing the bicarbonate concentration (data not shown).

Inhibition of acetyl CoA carboxylase by chloride could clearly affect the apparent pH optimum of the enzyme if chloride were the buffer anion because, at a lower pH, the chloride ion concentration would be increased. Fig. 3 shows a comparison of the apparent pH optimum of acetyl CoA carboxylase when chloride and acetate were used as anions. In both cases, the maximum activity was observed at pH 7.8. As the pH was increased above the optimum, there was less difference in activity between the two anions. This could be attributed to a lower concentration of chloride at higher pH values. With potassium phosphate buffer, the pH optimum was clearly lower (Fig. 3), in agreement with earlier observations (23), although activity was also lower.

Several buffers were evaluated relative to 0.06 M Tris acetate (Table 1). In general, increasing the concentration of the buffer to 0.27 M decreased activity. Substitution of chloride or sulfate for acetate resulted in less activity. Of the several buffers tested, none were found to give higher activity than Tris acetate although triethanolamine acetate was as good.

Much of the current research on acetyl CoA carboxylase is related to the short-term control of the enzyme and particularly the relationship between enzyme activity and rates of lipogenesis in in vivo systems (18, 19). Such studies must clearly take into account the ion effects on acetyl CoA carboxylase activity reported here.

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