EVIDENCE FOR REGULATION OF AEROBACTER AEROGENES pH 6 ACETOLACTATE-FORMING ENZYME BY ACETATE ION

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Summary: Experiments have been carried out with acetic acid and monodeuteroacetic acid (CH_3COOD) at pH 5.5 in H_2O and D_2O respectively, and with acetate analogues at pH 6.5 with substitution in the methyl group. The results indicate that it is the CH_3COO^- which is the activator for the pH 6 acetolactate-forming enzyme.

Introduction: In A. aerogenes (1,2) acetate plays an important role in the regulation of the first enzyme in the fermentative pathway where pyruvate leads to 2,3-butanediol, with acetolactate and acetoin as intermediates. The pH 6 acetolactate-forming enzyme, which is the first enzyme, catalyzes the following reaction:

$$2\ CH_3COCOOH \longrightarrow CH_3COCOHCH_3COOH + CO_2$$

Apparant Michaelis Menten kinetics is only obtained in acetate buffer. It has further been shown that the carboxyl group is essential for this effect. The other buffers tested gave sigmoidal kinetics and a lower V_{max} (1).

In order to study whether the activating effect of acetate upon the pH 6 acetolactate-forming enzyme is due to CH_3COO^-, CH_3COOH, or both, two types of experiments were carried out. First the enzyme activity obtained with CH_3COOH in H_2O was compared to that obtained with CH_3COOD in D_2O at pH 5.5 and at

Table I. Enzyme activity as a function of acetate ion concentration at pH 5.5 in the presence of D_2O or H_2O.

I	II	III	IV	V
mM acetate or monodeuteroacetate added	mM CH_3COO^- present		Activity	
	D_2O	H_2O	D_2O	H_2O
0	-	-	333	266
1.25	0.8	1.1	366	400
2.5	1.6	2.1	400	533
5.0	3.2	4.2	433	533
10	6.4	8.5	533	670
20	12.8	17.0	900	935
40	25.6	33.9	1070	1200
80	51.2	67.8	1800	2000

The incubation mixture contained in 1.0 ml: 5 μmoles sodium pyruvate, 50 μmoles 2-(N-morpholino)ethane sulfonic acid (MES) pH or pD 5.5, 0.05 μmoles $MnCl_2$, 40 μg cocarboxylase, 1.8 μg enzyme protein, and acetate or monodeuteroacetate pH 5.5 as indicated. In the experiments with deuteroacetate, H_2O was replaced by D_2O, MES adjusted to pD 5.5 with NaOD, and acetate pD 5.5 by mixing monodeuteroacetic acid with sodium acetate. $MnCl_2$ and cocarboxylase were dissolved in D_2O. The experiments were started by the addidition of 5 μl of the enzyme dissolved in H_2O. In columns II, and III are mM CH_3COO^- present in the incubation mixtures calculated from the pK^3 values at 25°.

equal buffer concentrations. Under these conditions the proportion of CH_3COO^- is different, due to the differences in the pK values. The other group of experiments were carried out in the presence of acetate analogues with substitutions in the methyl group at pH 6.5. At this pH, most of the compounds were almost dissociated.

Materials and Methods: Crystalline preparations of the pH 6 acetolactate-forming enzyme was purified from A. aerogenes (3) and were used in this work. Enzyme activity was assayed by measuring the production of acetolactate (3) and was defined as the amount of acetolactate formed per mg of enzyme per hour at 37°. The deutero-compounds were obtatined from Fluka AG, Switzerland.

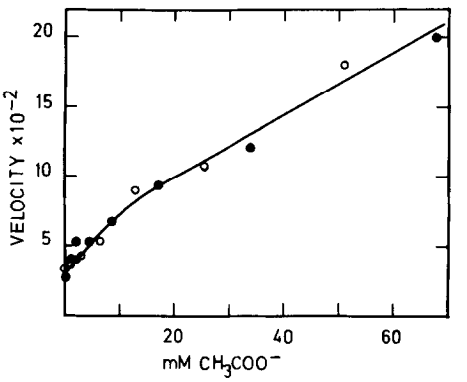

Fig. 1. Enzyme activity as a function of acetate ion concentration at pH 5.5.
The data are replotted from Table I, and show the enzyme activities from columns IV (O) and V (●). Plotted against the concentration of CH_3COO^- from the columns II, and III.

Results: Monodeuteroacetate and acetate have pK values at 25° of 5.25 and 4.75 respectively. Accordingly, at pH 5.5, 63.6% of the monodeuteroacetate and 84.6% of the acetate are present as CH_3COO^-. If the acetate ion is the activator for the enzyme, at the same pH one would expect higher enzyme activity in the presence of a given concentration of acetate than with the same concentration of monodeuteroacetate. The results presented in Table I and Fig. 1, show that with both buffers the activity increases for increasing concentration of CH_3COO^-. In the presence of H_2O and acetate, the enzyme activity is higher than in the corresponding concentration of D_2O and deuteroacetate. The amount of dissociated acetate required for a 100% increase in activity was approximately 6.5 mM.

Little enzyme stimulation was observed in the presence of the sodium salt of n-butyric acid. On the other hand, the salts of n-propionic, monochloroacetic, and trifluoroacetic acids were able to stimulate the enzyme activity at pH 6.5, Table II.

Table II. The effect of acetate analogues upon the activity of the pH 6 acetolactate-forming enzyme from A. aerogenes

Analogue added	% Activity
None	30
Propionate	80
Trifluoroacetate	87
Monochloroacetate	115
Acetate	100

The incubation mixture contained substrate, coenzymes, and enzyme as described in Table I, 50 μmoles MES pH 6.5, and 50 μmoles of the analogues pH 6.5 were indicated. 100% activity corresponds to 1100 umoles acetolactate formed per mg protein per hour.

Discussion: The results show that the enzyme activity was higher in the presence of acetate than at the same concentration of monodeuteroacetate at pH 5.5. This, plus the fact that the enzyme activity can be stimulated by acetate and its analogues with substitution in the methyl group, indicates that it is the acetate ion which activates the enzyme.

It has been pointed out that it is not surprising that the enzyme is not very sensitive to the effect of acetate (1). If the pH 6 acetolactate-forming enzyme demonstrated a high affinity for the acetate ion in vivo, this could cause a drain of pyruvate into this fermentative pathway where pyruvate is required for other metabolic processes. At a slow rate of aeration, the pH in the cultures, which have glucose as the carbon source, drops from 7.0 to 5.6, and then goes up to 6.2. In addition, acetoin and 2,3-butanediol are excreted into the medium when the pH starts to increase (4). This indicates that the pH acetolactate-forming enzyme in vivo is operating in the pH-range 5.5-6.5. In

this pH-range acetic acid is predominantly in the dissociated state.

Since acetate ions have a stimulating effect upon the enzyme activity, the enzyme is more easily subject to regulation in the pH-range where the enzyme is most active, than if the acetic acid has been the effector.

A. aerogenes excretes acetate when grown on glucose. The production of 50 mmoles acetate per 100 mmoles of glucose, has been reported (5,6). This indicates that the intracellular concentration could be in the range where it has a regulatory effect in vivo. Acetate has also been reported to stimulate the pH 6 acetolactate-forming enzyme purified from Serratia marcescens(7), and a similar mechanism may be involved.

I would suggest, from the results described herein that similar experiments with deuteroacids could be performed with other enzymes having weak acids as substrates or effectors.

REFERENCES

1. Størmer,F.C. (1968) J.Biol.Chem. 243, 3735-3739.
2. Størmer,F.C. (1975) Methods in Enzymology (S.P. Colowick and N.O. Kaplan,eds.), Acad.Press.Inc. New York 41, p. B,518-533.
3. Størmer,F.C. (1967) J.Biol.Chem. 242, 1756-1759.
4. Johansen,L., Bryn,K., and Størmer,F.C. (1975) J.Bacteriol. 123, 1124-1130.
5. Neish, A.C., and Ledingham,G.A. (1949) Can.J.Res.B. 27, 694-704.
6. Altermatt,H.A., Simpson,F.J., and Neish,A.C. (1955) Can.J. Microbiol. 1, 473-478.
7. Malthe-Sørenssen,D., and Størmer,F.C. (1970) European J. Biochem. 14, 127-132.