statically by these anionic amphipathic activators. Such a factor was removed by partial purification on Sephadex column.

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Department of Medical Chemistry,
Kansai Medical School,
Moriguchi, Osaka (Japan)

KUNIHIKO SAITO
KIYOMI SATO

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Fluoroacetyl-CoA as a substrate for malate synthase

The toxicity of fluoroacetate to warm-blooded animals has been traced to a lethal synthesis of fluorocitrate, a potent inhibitor of aconitase[1]. The K_m for fluoroacetyl-CoA in the reaction with oxaloacetate catalyzed by citrate synthase was found[2] to be $2.5 \cdot 10^{-5}$ M, the same as that for acetyl-CoA in this reaction.

Acetyl-CoA is a substrate for an analogous reaction catalyzed by malate synthase (L-malate glyoxylate-lyase (CoA-acetylating), EC 4.1.3.2), in which glyoxylate is the acetyl acceptor. DIXON, KORNBERG AND LUND[3] in examining the substrate specificity of this enzyme, observed that fluoroacetyl-CoA was cleaved at a rate "approximately one-quarter of the rate observed with similar concentrations of acetyl-CoA".

We report here the results of experiments in which the kinetics of the reaction with fluoroacetyl-CoA are compared to those with acetyl-CoA as substrate. The malate synthase employed was that present in glyoxysomes prepared from the endosperm tissue of germinating castor beans[4], with a specific activity of 0.6–1.4 μmoles/min per mg protein.

Enzyme activity was measured by following the rate of appearance of SH resulting from the cleavage of acetyl-CoA in the presence of glyoxylate[5], with correction, as necessary, for the rate observed before addition of glyoxylate. In the ab-

sence of glyoxylate, acetyl-CoA was not cleaved by the enzyme at a measurable rate, and, as shown in Table I the rate observed when glyoxylate was present was proportional to the amount of malate synthase provided. When fluoroacetyl-CoA alone was added to the enzyme there was a slow production of SH, but the addition of glyoxylate resulted in a 3–4-fold stimulation. The corrected rate of reaction with

TABLE I

RATES OF REACTION OF ACETYL-CoA AND FLUOROACETYL-CoA IN PRESENCE OF MALATE SYNTHASE AND GLYOXYLATE

The reaction mixtures contained, in a final volume of 1.0 ml: 50 μmoles potassium phosphate (pH 8.0), 7.5 μmoles $MgCl_2$, 1 μmole 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) (Aldrich Chemical Co.), 0.2 μmole acetyl-CoA (ref. 6) or fluoroacetyl-CoA (refs. 7, 8) and enzyme as indicated. The change in $A_{412\ m\mu}$ was recorded for 3 min before adding 1 μmole sodium glyoxylate, and from the subsequent trace, initial rates of reaction were calculated. Temp.: 30°.

Amount of enzyme added (μg)	*Sulfhydryl produced (μmoles/min × 100) in presence of*		
	Acetyl-CoA + glyoxylate	*Fluoro-acetyl-CoA*	*Fluoro-acetyl-CoA + glyoxylate**
5	0.6	—	—
14	1.7	—	—
24	2.4	—	0.35
38	4.0	0.15	0.45
48	5.8	0.20	0.75
96	—	0.35	1.35
144	—	0.55	2.10

* Corrected for fluoroacetyl-CoA.

fluoroacetyl-CoA increased with increasing enzyme concentration (Table I) and remained at roughly one-eighth of that observed with acetyl-CoA.

Fig. 1 shows the results obtained when the concentration of fluoroacetyl-CoA was varied in the presence of saturating glyoxylate. The K_m derived from these data is $1.1 \cdot 10^{-5}$ M. K_m values determined similarly for glyoxylate in the presence of an excess of fluoroacetyl-CoA and for the substrates of the normal malate synthase reaction

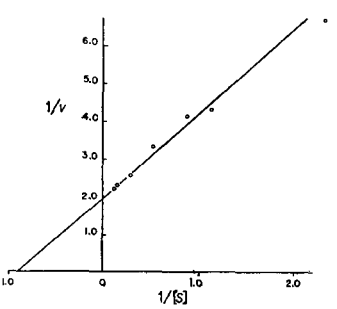

Fig. 1. Reciprocal plot of the effect of fluoroacetyl-CoA concentration S, (10^{-5} M) on reaction velocity v (mμmoles consumed per min). Assay conditions as in Table I, with 60 μg protein/ml and 3.3 μmoles glyoxylate/ml.

TABLE II

K_m VALUES FOR REACTION OF ACETYL-CoA AND FLUOROACETYL-CoA WITH MALATE SYNTHASE

Reaction	*K_m values (M) for*		
	Acetyl-CoA	*Fluoro-acetyl-CoA*	*Glyoxylate*
Acetyl-CoA + glyoxylate	$1.7 \cdot 10^{-5}$	—	$5.8 \cdot 10^{-5}$
Fluoroacetyl-CoA + glyoxylate	—	$1.1 \cdot 10^{-5}$	$5.5 \cdot 10^{-5}$

are given in Table II. The values obtained for this latter reaction are close to those observed previously for the enzyme from castor bean endosperm[9] and from other cells[3]. The K_m for glyoxylate when fluoroacetyl-CoA was the co-substrate is virtually the same as that observed in the presence of acetyl-CoA; that for fluoroacetyl-CoA is slightly lower than that for acetyl-CoA.

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Departments of Chemistry and Biological Sciences, Purdue University, Lafayette, Ind. (U.S.A.)

GARY L. POWELL*
HARRY BEEVERS

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* Present address: Department of Biological Chemistry, School of Medicine, Washington University, St. Louis, Miss. 63110, U.S.A.