ENZYMATIC AND METABOLIC BEHAVIOR OF FLUOROSUCCINIC ACIDS

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Enzymatic hydrogen transfer reactions may be modified if hydrogen is replaced by fluorine at or near a position where hydrogen is a potential leaving group (proton, radical or hydride). For this purpose, fluorosuccinic acids have been used as substrate analogs for the study of several enzymes, utilizing succinate as substrate, which are normally involved in coenzyme-dependent, hydrogen transfer reactions. These include the succinic dehydrogenase complex obtained from beef heart mitochondria, in which a key step is substrate-flavin interaction, and the methyl malonyl CoA mutase and related enzymes obtained from propionibacteria, which involve a substrate-adenosyl-B_{12} interaction.

The effect of substituting monofluorosuccinate (prepared by the method of Dean and Pattison [1963]) for succinate as measured by the succinate dehydrogenase, the succinate-cytochrome c reductase, and the succinate oxidase activities of non-phosphorylating beef heart submitochondrial particles (King, 1967a) is shown in Table I.

As indicated here, the rate of "fumarate" appearance as measured by absorbancy at 230 mμ was equivalent to the

Table I. Activity of monofluorosuccinate as substrate for succinate dehydrogenase, succinate-cytochrome c reductase and succinate oxidase

System	Conditions	Activity (succinate = 100)
a) succinate dehydrogenase:	cyt c reduction with PMS‡	33
b) succinate reductase:	cyt c reduction at 550 mμ (Slater, 1950)	37
c) succinate oxidase:	(1) Product appearance at 230 mμ* (Slater, Bonner, 1952)	35
	(2) O_2 uptake (manometric) (Keilin and Hartree, 1940)	38 (max)**

pH 7.4, 67 mM phosphate, 25°C (except manometric carried out at 30°), 30 μM c present in dehydrogenase and reductase assays, 12 μM c in succinate oxidase.

‡ phenazine methosulfate (0.3 mmolar). Cytochrome c replaces dichlorophenol indophenol as electron acceptor from PMS (King, 1967b).

* fluorofumarate (assumed E_{mM} identical to that of fumarate).

** maximal rate, obtained initially with fresh fluorosuccinate; rapid decline in activity observed during the reaction due to formation of oxaloacetate.

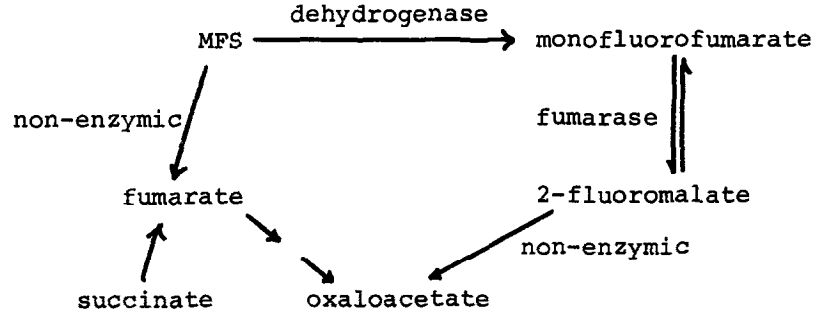


Figure 1

oxidation rate obtained manometrically by O_2 uptake. However, the addition of fumarase in the spectrophotometric assay clearly shows that the product of succinic dehydrogenase and fluorosuccinate is not fumarate (Nicholls & Brodie, in preparation). The oxidation of the natural substrate gives a continued increase in absorbancy at a rate approximately 25% that prior to the addition of fumarase. This corresponds to the equilibrium constant of 4 for the malate-fumarate system under these conditions. Fluorosuccinate oxidation, however, ceases abruptly upon addition of fumarase. Manometrically, fumarase was found to accelerate markedly the decline in the initial oxidation rates occurring with fluorosuccinate. These results are readily explained as due to the formation of oxaloacetate, a potent inhibitor of the dehydrogenase.

This was supported by the observation that treatment of fluorofumarate with fumarase resulted in a rapid decline in absorbancy at 230 mμ, followed by a slower rise. The spectrum of the final product was identical to oxaloacetate. Furthermore, fluoride was released during the reaction as shown by the formation of the yeast peroxidase fluoride complex (Nicholls & Brodie, in preparation). As suggested by Clarke, et al (1968), these results are best explained in terms of the formation of an unstable 2-fluoromalate which spontaneously hydrolyses to give oxaloacetate and fluoride ion.

These reactions are summarized in Fig. 1.

Allen et al (1964) have described a multiple enzyme system from P. shermanii (see Fig. 3) in which propionyl-

CoA is synthesized from succinate, CoA and ATP with concomittant carboxylation of pyruvate to oxaloacetate (assayed with malic dehydrogenase and NAD). We find this system utilizes monofluorosuccinate in place of succinate. The first reaction in the sequence forms monofluorosuccinyl CoA by the action of CoA transferase. This CoA ester is an excellent substrate for the B_{12} coenzyme dependent methyl malonyl CoA mutase, as shown in Fig. 2. The K_m for the reaction using chemically synthesized monofluorosuccinyl CoA is about 5 times and the maximal velocity is 25% that with the natural substrate. The oxidation of NADH on the addition of methyl malonyl CoA mutase is shown in Fig. 2.

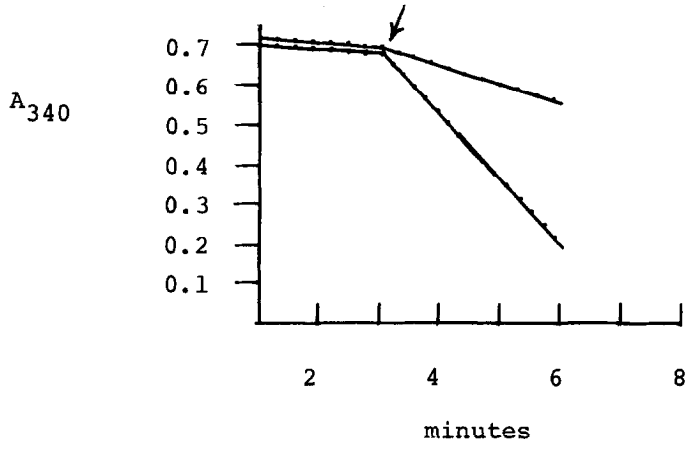
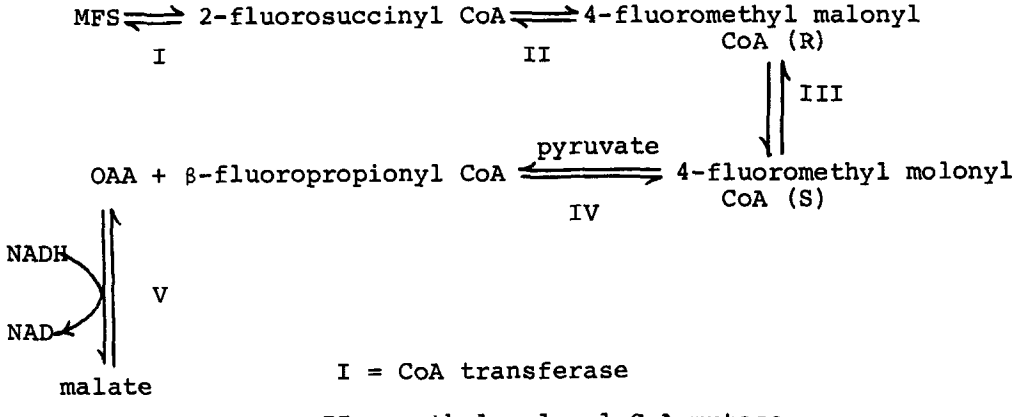


Fig. 2. Spectrophotometric assay of methyl malonyl CoA mutase. The assay mixture contained in micromoles: potassium phosphate buffer, pH 7.4, 4; NADH, .04; sodium pyruvate, 2.0; acetyl CoA, 0.25; adenosyl-B_{12}, .002; fluorosuccinate (upper curve) or succinate (lower curve), 1.0; and in units: malic dehydrogenase, 0.5; oxaloacetate transcarboxylase, 0.1; CoA transferase, 0.1; methyl malonyl CoA racemase, 0.1; and methyl malonyl CoA mutase, specific activity 0.8, added at indicated time in limiting quantities to a total volume of 0.32 ml.

Chemically synthesized monofluorosuccinyl CoA and the product of the CoA transferase system behave similarly although not identically in the methyl malonyl CoA mutase assay. Chemical characterization of the initial enzymatic product as the 2-fluoro succinyl CoA is still largely inferential due to the marked susceptibility of the product both to hydrolysis and elimination of HF. However, the assay also involves the racemization of the fluoromethyl malonyl CoA (step III, Fig. 3); it is improbable that a racemase substrate could have arisen from a 3-fluoro succinyl CoA by the action of methyl malonyl CoA mutase.



I = CoA transferase

II = methyl malonyl CoA mutase

III = methyl malonyl CoA racemase

IV = transcarboxylase

V = malic dehydrogenase

Figure 3

The postulated reaction scheme to account for NADH oxidation is the pathway described by Allen, et al (1964) and shown in Fig. 3.

Unlike monofluorosuccinate, α, α-difluorosuccinate (DFS) had no substrate activity in any of the systems tested. Instead, DFS, exhibited the properties of a competitive inhibitor with a K_i of ∿400 μM for succinic dehydrogenase. Preliminary experiments with the methyl malonyl mutase system indicate that, DFS, presumably as the CoA ester, is a weak, competitive inhibitor.

This work shows that we have the following series of analogs for studying hydrogen transfer reactions with an example shown in parentheses. (1) A fluorine gem to the leaving group (L-monofluorosuccinate and succinic dehydrogenase), (2) a fluorine vicinal to the leaving group (2-fluorosuccinyl CoA and methyl malonyl CoA mutase), and (3) a fluorine in the position of the leaving group (D-monofluorosuccinate and succinic dehydrogenase).

Electronic and steric effects of fluorine replacement in the flavin and adenosyl-B_{12} catalyzed hydrogen migrations are also reflected in the visible spectra of the appropriate enzyme-substrate complexes (unpublished observations).

In addition, environmental NMR spectroscopy by both F^{19} and double resonance techniques, now being undertaken, offers another approach to understanding the structure of these labile enzyme-substrate complexes.

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