SYNTHESIS OF STEREOSPECIFICALLY DEUTERATED FLUOROACETIC ACID AND ITS BEHAVIOUR IN ENZYMIC ALDOL-TYPE CONDENSATIONS

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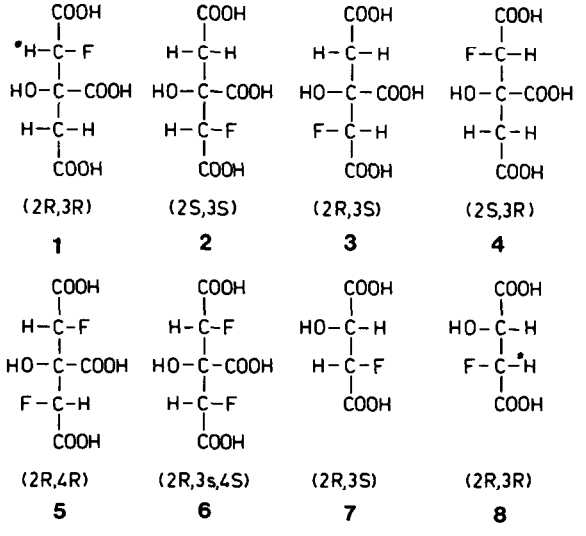
1. Introduction

Fluoroacetyl CoA serves as a substrate analogue both in the citrate synthase and in the malate synthase reactions. It has been established [1] that the former reaction stereospecifically yields (2*R*, 3*R*)-2-fluorocitrate 1. Whereas 1 was found to be a powerful inhibitor of aconitase, the enantiomeric (2*S*, 3*S*)-2-fluorocitrate 2 produced by citrate synthase from racemic 3-fluorooxalacetate and acetyl-CoA as well as the synthetically obtained (2*R*, 3*S*)- and (2*S*, 3*R*)-2-fluorocitrates 3 and 4 were non-inhibitory [2].

By use of unlabeled and of (2*R*)-[2-$^2H_1$]fluoroacetyl CoA we show here that citrate synthase is stereospecific for the H_{Si} atom of the fluoromethyl group, whereas malate synthase is non-stereospecific. Both synthases catalyse the condensations with stereochemical inversion at the fluoromethyl group. Citrate synthase accepts both enantiomers of 3-fluorooxalacetate as substrates with either acetyl CoA or fluoroacetyl CoA as partner. In both cases two diastereomeric products are formed, the configurations of which can be tentatively assigned as shown in 2 and 3 or in 5 and 6, respectively.

Scheme 1



* H = ^2H when starting from (2*R*)-[2-^2H]fluoroacetyl CoA

2. Materials and methods

Citrate synthase (EC 4.1.3.7) and CoA were products of Boehringer (Mannheim), malate synthase (EC 4.1.3.2) was isolated from yeast as in [3].

(2*R*)-2-Fluoroacetic acid was obtained by treatment of (2*R*)-[2-$^2H_1$]glycine [4,5] with sodium nitrite in a hydrogen fluoride/pyridine mixture [6,7]. (Evidence for stereochemical retention in this substitution reaction has been provided by using L-isoleucine and D-*allo*-isoleucine as substrates and comparison of the ^1H NMR spectra of the 2-fluoroacid products with the spectra of the corresponding 2-hydroxy- and 2-chloroacids of known configuration [8].) Fluoroacetyl CoA samples were prepared by the mixed anhydride method [9] and used immediately in the citrate synthase and malate synthase reactions. The conversions were monitored at 232 nm. 2-Fluorocitrate and 3-fluoromalate samples were isolated from the reaction mixtures by chromatography on Dowex 1 (formate form), lyophilization of the fluoroacid containing fractions and extraction of the remaining solid with acetone. Racemic 3-fluorooxalacetic acid [10] was condensed both with acetyl CoA and fluoroacetyl CoA followed by the above workup of the products.

The ^1H NMR spectra of the fluoroacids were recorded in [$^2H_6$]acetone with Bruker WH 500, WH 300 or WH 90 spectrometers.

3. Results

The condensation of fluoroacetyl CoA and oxalacetate on citrate synthase yielded only one diastereomer of 2-fluorocitric acid as verified by its ^1H and ^{19}F NMR spectra (fig.1,2A). When (2*R*)-[2-$^2H_1$]fluoroacetyl CoA was the substrate the doublet at $\delta = 5.28$ ppm ($J_{^1H^{19}F} = 47$ Hz) was lacking in the ^1H NMR spectrum, whereas the doublet at $\delta = -198.6$ ppm ($J_{^1H^{19}F} = 47$ Hz) in the ^{19}F NMR spectrum was replaced by a triplet at higher field ($\delta = -199.2$ ppm, $J_{^2H^{19}F} = 6.5$ Hz, see fig.2B). From the complete retention of deuterium in the reaction of (2*R*)-[2-$^2H_1$]fluoroacetyl CoA and from the stereochemical purity of both the unlabeled and monodeuterated product one can follow that citrate synthase is specific for the H_{Si}-atom of the substrate analogue. Further, stereochemical inversion at the fluoromethyl group can be deduced from the established (2*R*, 3*R*) configuration of the resulting 2-fluorocitrate [1].

When racemic 3-fluorooxalacetate was condensed with acetyl CoA the ^1H NMR spectrum of the product revealed the presence of two diastereomeric 2-fluorocitrates. (In addition to the signals in fig.1 another doublet at $\delta = 5.21$ ppm, $J_{^1H^{19}F} = 47$ Hz, was seen the ratio being 2:1 in favour of the new diastereomer.) If, as is likely, citrate synthase exhibits analogous stereospecificities for the stereoheterotopic faces of the keto group of oxalacetate and 3-fluorooxalacetate,

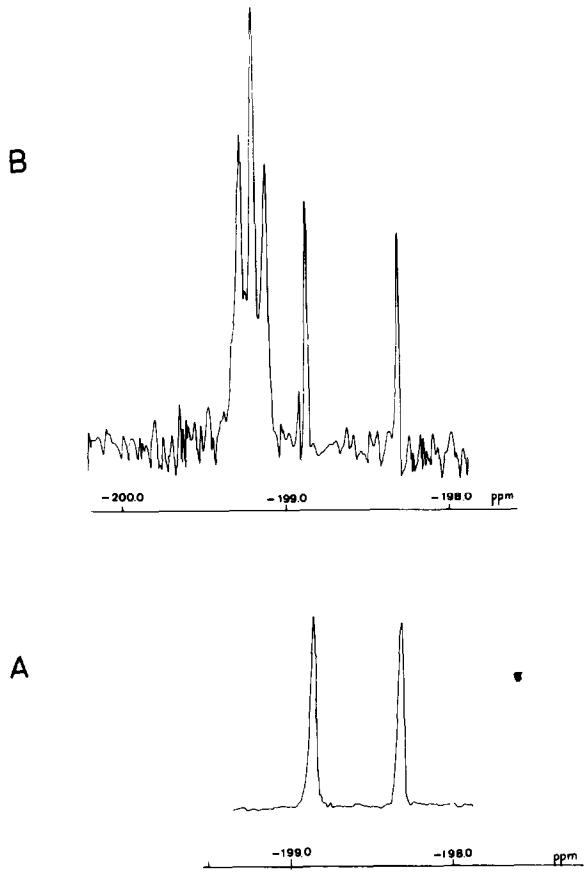


Fig.2. (A) ^{19}F NMR spectrum of the enzymatically produced (2*R*, 3*R*)-2-fluorocitric acid at 84.6 MHz in a [$^2H_6$]acetone and (B) of (2*R*, 3*R*)-2-[2-$^2H_1$]fluorocitric acid obtained by the citrate synthase reaction from (2*R*)-[2-$^2H_1$]fluoroacetyl CoA. (The deuterium content of the starting material was 82% and this is reflected by the presence of the doublet at −198.6 ppm in the spectrum of the product.)

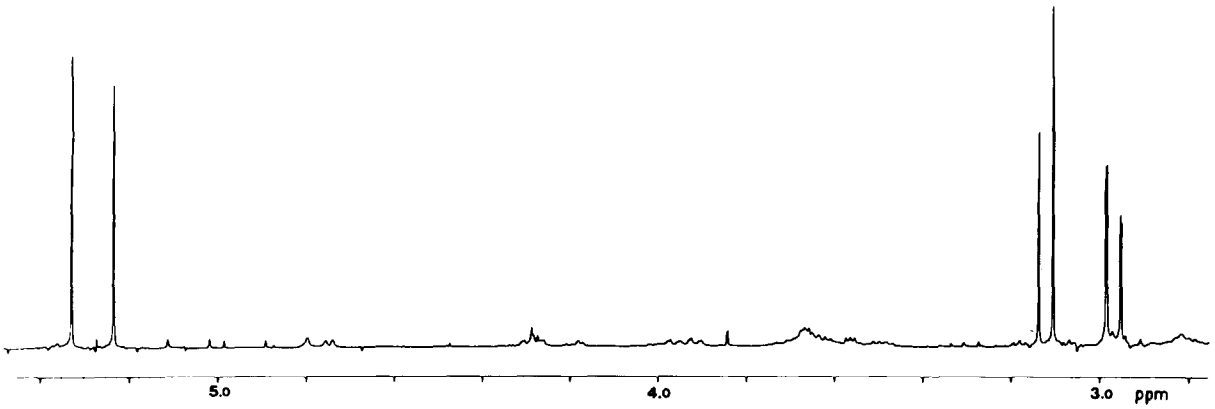


Fig.1. 500 MHz ^1H NMR spectrum of the enzymatically produced (2*R*, 3*R*)-2-fluorocitric acid in [$^2H_6$]acetone (TMS at $\delta = 0.0$ ppm).

then the configurations of the two diastereomeric 2-fluorocitrate products should be (2*R*, 3*S*) and (2*S*, 3*S*) as shown in 3 and 2. It follows therefrom that citrate synthase slightly prefers the (3*R*)-enantiomer of 3-fluorooxalacetate.

In a further experiment we found that citrate synthase catalysed the condensation of fluoroacetyl CoA with racemic 3-fluorooxalacetate quite effectively. Again signals arising from two diastereomeric 2,4-difluorocitrates were seen in the ^{1}H NMR spectrum and, assuming analogous stereospecificity as found in the condensations with only one fluorinated partner, the configurations of these should be (2*R*, 4*R*) and (2*R*, 3*S*, 4*S*) as shown in 5 and 6.

In another set of experiments fluoroacetyl CoA and glyoxylate were condensed on malate synthase. The ^{1}H NMR spectrum revealed that two diastereomeric 3-fluoromalates (7,8) were produced in the ratio of ~4:3.

^{1}H NMR of diastereomer 7: δ = 4.72 ppm, quartet, $J_{H_2H_3}$ = 1.6 Hz, J_{H_2F} = 32.2 Hz, 1H, δ = 5.41 ppm, quartet, $J_{H_2H_3}$ = 1.6 Hz, J_{H_3F} = 47 Hz, 1H;

^{1}H NMR of diastereomer 8: δ = 4.75 ppm, quartet, $J_{H_2H_3}$ = 1.9 Hz, J_{H_2F} = 23.7 Hz, 1H, δ = 5.35 ppm, quartet, $J_{H_2H_3}$ = 1.9 Hz, J_{H_3F} = 47.5 Hz, 1H.

Since malate synthase is stereospecific for the *Si*-face of the glyoxylate carbonyl group, the configurations of these 3-fluoromalates must be (2*R*, 3*S*) and (2*R*, 3*R*), respectively. Further, a crucial coupling constant (J_{H_2F} = 23.7 Hz) identifies 8 as the (–)-*erythro*-isomer in [11], i.e. (2*R*, 3*R*). There are in principle two reasons for the above result:

(i) Strict inversion during the substitution at the fluoromethyl group but only a slight discrimination between the diastereotopic methylene H atoms;

(ii) Inhomogeneous steric course during the substitution with either stereospecific or non-stereospecific removal of one of the diastereotopic H atoms.

Enzymic condensation of (2*R*)-[2-^{2}H]fluoroacetyl CoA with glyoxylate yielded a 3-fluoromalate sample in the ^{1}H NMR spectrum of which the H–CF quartet at δ = 5.41 ppm was lacking. Simultaneously the quartet at δ = 5.35 ppm was considerably diminished so that the ratio of diastereomers 7 and 8 changed to ~3:7 as estimated from the ratio of the quartet at δ = 4.72 ppm and the doublet at δ = 4.75 ppm. This is undoubtedly a consequence of an intramolecular kinetic isotope effect. These results indicate that mechanism (i) is operative, i.e., malate synthase is non-stereospecific for the diastereotopic H atoms of the fluoromethyl group but catalyses the substitution with inversion of configuration.

4. Discussion

These results agree or are compatible with the known stereospecificities of the condensing enzymes towards their normal substrates. Thus, exclusive *Si*-attack is found at the carbonyl groups of oxalacetate and glyoxylate and a stereochemically analogous *Re*-attack at the carbonyl group of 3-fluorooxalacetate. The substitution at the fluoromethyl group of fluoroacetyl CoA takes place in all cases with inversion; a steric course which has already been elaborated by the use of stereospecifically labeled [^{2}H, ^{3}H]acetyl CoA samples [12–15].

In contrast to [2,9] we observed the formation of two diastereomeric 2-fluorocitrates when racemic 3-fluorooxalacetate was condensed with acetyl CoA. The incomplete stereospecificity of citrate synthase for the enantiomeric 3-fluorooxalacetates was also supported by similar results from experiments in which fluoroacetyl CoA was used as the other partner.

The lack of stereospecificity of malate synthase for the diastereotopic 2-H atoms of fluoroacetyl CoA deuterium isotope effect. From the change of the product ratio starting from unlabeled and (2*R*)-[2-^{2}H$_1$]-fluoroacetyl CoA (7:8 ≈ 4:3 → 7:8 ≈ 3:7) a $k_H/k_{^2H}$ value of ~3 can be calculated. This is to be compared with $k_H/k_{^2H}$ value of 4 established with chiral [^{2}H, ^{3}H]acetyl CoA as substrate [16].

Acknowledgements

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Note added

After submission of this communication we learnt from Professor C. Walsh (IMT, Cambridge, MA) that he reached similar conclusions by using stereospecifically tritiated fluoroacetyl CoA (Biochemistry (1980) submitted).

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