Oxidation of chiral 9-fluorinated substrates by castor stearoyl-ACP Δ^9 desaturase yields novel products

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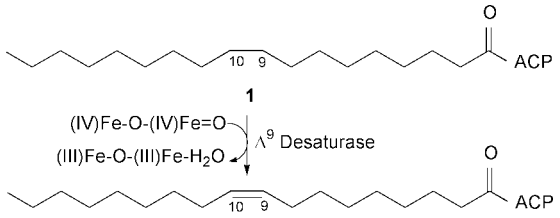
Desaturation of (*S*)-9-fluorostearoyl-ACP using stearoyl-ACP Δ^9 desaturase yielded mainly (*E*)-9-fluoroalk-9-enic product, while desaturation of (*R*)-9-fluorostearoyl-ACP gave the novel (*Z*)-9-fluoroalk-9-ene and (*Z*)-9-fluoroalk-10-ene.

The O_2-dependent conversion of stearoyl acyl carrier protein (ACP) **1** to oleyl-ACP **2** is a remarkable example of a highly



chemo-, regio- and stereoselective enzymatic process.[1] The enzyme responsible for this fascinating reaction, stearoyl-ACP Δ^9 desaturase, has been isolated from castor seed and is the only protein of its type to be thoroughly characterized. The X-ray crystallographic structure of this protein confirmed the presence of a non-heme diiron oxidant which is strategically located at the centre of a narrow, hydrophobic pocket.[2] The amino acid co-ordination of the catalytic diiron centre and the fold of the protein are very similar to that found in methane mono-oxygenase—an enzyme which converts methane and other small alkanes to the corresponding alcohol.[3] It has long been suggested that desaturation and hydroxylation reactions are closely related[4a] and that precise control of substrate conformation and oxidant location may play an important role in determining reaction outcome.[1,4b] We decided to probe the catalytic versatility of the stearoyl-ACP Δ^9 desaturase by challenging this enzyme with a substrate bearing a potentially non-oxidizable, CHF-group at C-9. The availability of chiral 9-hydroxystearic acid derived from natural sources offered a convenient synthetic entry into this project. Here, we report the results of this study.

Each enantiomer† of methyl 9-fluorostearate, (9*S*)-**3** and (9*R*)-**3**, was incubated separately as the ACP derivative (200 nmol) with freshly prepared Δ^9 desaturase (14 nmol) and required cofactors in a total volume of 250 μl for 30 min.[6] The reaction was quenched by addition of THF and the thioester fraction was reduced ($NaBH_4$) to give the corresponding terminal alcohols. These products were analyzed directly by ^{1}H-decoupled ^{19}F NMR and by GC/MS (60 m SP-23 capillary column) as the tetramethylsilyl derivatives.

We were somewhat surprised to observe that very little starting material could be detected in the extracts of both incubations, indicating that both (*S*)- and (*R*)-9-fluorostearates could function as substrates under these conditions (Fig. 1). No obvious inhibitory effects were noted and the product profile did not appear to change as a function of % conversion. The major product formed in the desaturation of (9*S*)-**3** was, after reductive workup, (*E*)-9-fluorooctadec-9-en-1-ol **4** (Scheme 1 and Fig. 1a)‡—identical in all respects (MS, ^{1}H-decoupled ^{19}F NMR, GC rt of TMS derivative) to a sample derived by LAH reduction of previously identified, methyl (*E*)-9-fluorooctadec-9-enoate.[7] Interestingly, this material was accompanied by a small (4–7% of the total products) but reproducible amount of a *threo*-9,10-fluorohydrin **5** (MS: *m/z* 215 $(C_9H_{18}OTMS)^+$, ^{1}H-decoupled ^{19}F NMR: δ −195.25).§ Notably, no products attributable to 9,9-fluorohydrin formation (9-keto- or its reduction product, 9-hydroxystearoyl alcohol) were observed.

Desaturation of the ACP derivative of (9*R*)-**3**, yielded two major novel fluorinated olefinic products, **6** and **7** (Scheme 1 and Fig. 1b).‡ **6** was determined to be the geometric isomer of **4** on the basis of its GC retention time (*ca*. 0.2 min shorter than the corresponding (*E*)-product), similar mass spectrum (*m/z* 358 M^+, 343 $(M - CH_3)^+$, 338 $(M - HF)^+$, 323 $[(M - CH_3) - HF]^+$) and ^{1}H-decoupled ^{19}F NMR chemical shift (δ −110.45 ppm; lit. (*Z*)-7-fluorotetradec-7-ene (δ −110.37 ppm)).[8] The second novel product **7** was identified as (*Z*)-9-fluorooctadec-

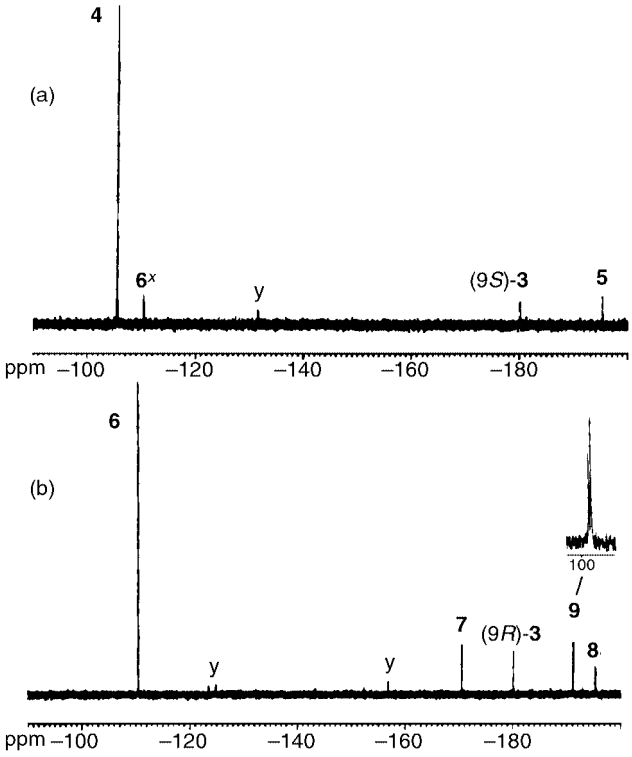


Fig. 1 ^{1}H-decoupled ^{19}F NMR (376.5 MHz) of products obtained from desaturation of (a), (9*S*)-**3**; (b), (9*R*)-**3**. Unidentified, minor peaks of variable intensity are marked with a 'y'. *x* Product derived from a minor amount of (*R*)-9-fluorostearoyl-ACP—the levels of this diastereomer appear to be enhanced relative to the (*S*)-9-fluoro-isomer in the ACP synthase-mediated acylation reaction as was previously noted in preliminary studies using racemic 9-fluorostearate as substrate.

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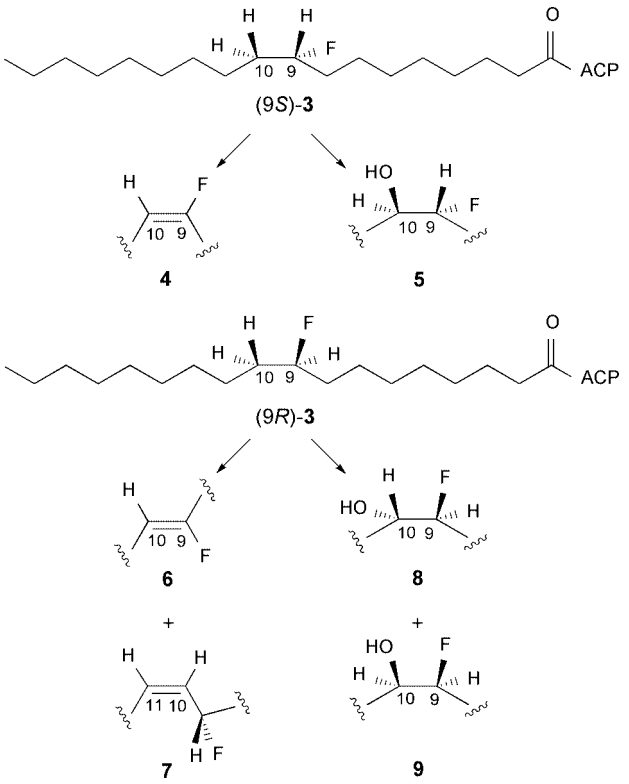
10-en-1-ol by comparison of its GC retention time and ^{1}H-decoupled ^{19}F NMR chemical shift (δ −170.54) with those of (*Z*)-8- and (*Z*)-11-fluorooctadec-9-en-1-ol (δ −170.61 and −170.34, respectively).¶ In addition, a mixture of stereoisomeric hydroxylated products (Scheme 1) was also observed in the product profile: *threo*- **8** (*m*/*z* 215 $(C_9H_{18}OTMS)^+$, ^{1}H-decoupled ^{19}F NMR: δ −195.27) and *erythro*-9,10-fluorohydrin **9** (*m*/*z* 215 $(C_9H_{18}OTMS)^+$; ^{1}H-decoupled ^{19}F NMR: δ −191.18 (.23)‖).

The formation of **4** with 'normal' regio- and stereochemistry *via* desaturation of (9*S*)-**3** indicates that this substrate can adopt the required gauche conformation for what is thought to be a diiron(IV)oxo-mediated, *syn*-dehydrogenation process.[7] However, when removal of the hydrogen occupying the '*pro-R*' position at C-9 is blocked by fluorine substitution as in (9*R*)-**3**, substantial reorientation of substrate occurs and two 'novel' olefinic products (**6**, **7**) are produced as depicted in Scheme 1. There is some precedent for a substrate-induced shift in the regiochemistry of desaturation,[6] but the observation of a switch in stereochemical outcome as displayed by the production of **6**, was completely unexpected. In a previous series of experiments, incubation of racemic 9-fluorostearoyl substrate with a structurally unrelated yeast Δ^9 desaturase produced only the anticipated methyl (*E*)-9-fluorooctadec-9-enoate.[7]

A small amount of hydroxylated materials *is* observed in the product profile of both fluorosubstrates (9*S*)-**3**, (9*R*)-**3**. These compounds do not appear to be formed by hydration of the corresponding fluoroolefins during the workup§ and thus it is tempting to attribute the formation of these products to an MMO-type hydroxylation reaction.[9] It is interesting to note that the stereochemistry of these compounds is dependent on the chirality of the fluorosubstrate: exclusively *threo*-product (**5**) was obtained from (9*S*)-**3**, while a mixture of *threo*- and *erythro*-fluorohydrins (**8**, **9**)‖ was formed from a conformationally distorted (9*R*)-**3** (Scheme 1). Experiments to further investigate the origin of these novel hydroxy products are in progress.

In summary, we have demonstrated a remarkably high preference for the catalysis of dehydrogenation rather than oxygen transfer by stearoyl-ACP Δ^9 desaturase. In addition, valuable new insights into the enantioselectivity of oxidative attack have been obtained.



Scheme 1

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Notes and references

† Methyl (*S*)-9-fluorostearate was synthesized *via* extraction of dimorphecolic ((*R*)-9-hydroxyoctadec-10,12-enoic) acid from *Dimorphoteca* seeds[5a] followed by reduction of double bonds using H_2/Pt and fluorination of the resultant methyl (*R*)-9-hydroxystearate with diethylaminosulfur trifluoride (DAST).[5b] The corresponding (*R*)-9-fluoro-enantiomer was prepared by inverting the stereogenic center of methyl (*R*)-9-hydroxystearate using the Mitsunobu reaction[5c] followed by fluorination using DAST. The absolute configuration of the methyl (*R*)- and (*S*)-9-hydroxystearates was confirmed using standard methods;[5d] the % ee of the alcohols (and hence that of the final fluorinated products[7]) was estimated to be more than 98%.[5d] The ACP derivatives of each enantiomer were prepared according to established enzymatic methods.[5e]

‡ The position of the double bond in **4**, **6** and **7** was confirmed by the results of a labelling experiment in which [10,10-$^2H_2$]-(*R*,*S*)-9-fluorostearoyl-ACP was converted to olefinic products with the concomitant loss of one deuterium: *m*/*z* 359 M^+, 344 $(M - CH_3)^+$, 339 $(M - HF)^+$, 324 $[(M - CH_3) - HF]^+$). The deuterated substrate was prepared from [1,1-$^2H_2$]-bromononane *via* a modification of a published procedure.[7] Since the allylic fluoride **7** is partially hydrolyzed to a pair of corresponding isomeric allylic alcohols (*m*/*z* 329 $[C_{11}H_{19}(OTMS)_2]^+$, 227 $(C_{10}H_{18}OTMS)^+$) during the work-up procedure ($NaBH_4$), the ratio of **6**/**7** is highly variable and ranges from 1 to 3 as measured by GC/MS. The recovery of total products and remaining substrate was estimated to be *ca*. 70–90% of theoretical.

§ The structural assignments were consistent with data obtained using readily available synthetic 9(10),10(9)-fluorohydrins.[7] Control experiments showed that the observed fluorohydrin products did not arise by hydration of fluoroolefins and *vice versa*: (a) no olefinic products were detected when the workup conditions of desaturase assay were simulated using synthetic fluorohydrin; (b) no fluorohydrin was detected as by-products of (*R*,*S*)-8- and (*R*,*S*)-11-fluorostearoyl-ACP desaturation; (c) no fluorohydrin was detected when a typical desaturase assay spiked with (*Z*)-9-fluorooctadec-9-en-1-ol was worked up. Under the same conditions, 9-ketostearoyl alcohol was reduced to the corresponding alcohol. (The detection limits in these control experiments was *ca*. 0.1% of total products.)

¶ Prepared by desaturation of the corresponding (*R*,*S*)-8- and (*R*,*S*)-11-fluorostearoyl-ACP substrates using castor stearoyl-ACP Δ^9 desaturase. The substrates were prepared by standard methods.[7]

‖ The *erythro*-9,10-fluorohydrin fraction appeared to contain some regioisomeric *erythro*-10,9-fluorohydrin as determined by ^{19}F NMR and GC ret. time, the appearance of a diagnostic cleavage ion in the mass spectrum (*m*/*z* 303 $(C_9H_{17}(OTMS)_2)^+$ and use of samples spiked with a synthetic standard of regioisomers (see footnote §). This result does not appear to be an artifact of the workup; fluorine scrambling may be due to a reversible elimination/addition of HF or direct fluorine migration[10] in the unsolvated environment of the active site.

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