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Mechanistic Investigations of Anaerobic Sulfatase-Maturating Enzyme: Direct C_β H-Atom Abstraction Catalyzed by a Radical AdoMet Enzyme

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Sulfatases belong to at least three mechanistically distinct groups, namely the Fe(II) α-ketoglutarate-dependent dioxygenases,[1] the recently identified group of Zn-dependent alkylsulfatases,[2] and the broad family of arylsulfatases.[3] Arylsulfatases are widely spread from prokaryotes to eukaryotes and are commonly designated as "sulfatases". Sulfatases are unique in requiring an essential post-translational modification of a critical active-site cysteinyl or seryl residue to 3-oxoalanine usually called C_α-formylglycine (FGly).[4] In sulfatases, it has been proposed that the resulting modified amino acid is hydrated as a geminal diol to perform a nucleophilic attack on the sulfur atom of the substrate. This leads to the release of the desulfated product and the formation of a covalent sulfate-enzyme intermediate.[5,6] The second hydroxyl group on the gem-diol is essential for the release of the inorganic sulfate as proven by the inactivation of sulfatase bearing a seryl residue instead of the FGly residue.[7]

In prokaryotes, the 3-oxoalanine formation is catalyzed by at least three enzymatic systems, but only two are currently identified.[8] The first one, called FGE (formylglycine-generating enzyme), uses molecular oxygen and an unidentified reducing agent to catalyze the aerobic oxidation of a cysteinyl residue into FGly.[9] The second one, called anSME (anaerobic Sulfatase Maturating Enzyme), has been recently demonstrated to possess a typical radical AdoMet cluster and thus belongs to the *S*-adenosyl-L-methionine (AdoMet)-dependent superfamily of radical enzymes.[10,11] We have established that anSMEs are dual substrate enzymes able to catalyze the oxidation of both cysteinyl or seryl residues and, in contrast to FGE,[9] are able to activate sulfatases under anaerobic conditions.[8,11] Nevertheless, the mechanism by which these enzymes catalyze the anaerobic oxidation of cysteinyl or seryl residues is still obscure.

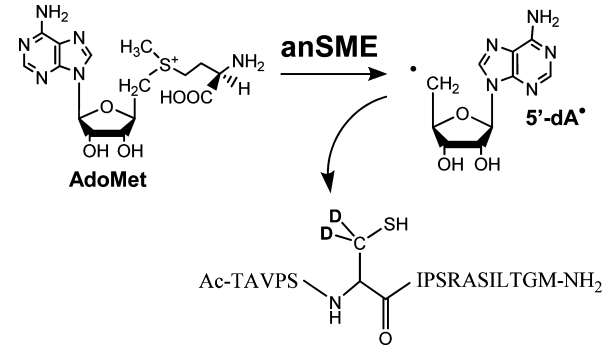
Belonging to the radical AdoMet enzyme family, anSMEs are likely to use AdoMet to generate a 5′-deoxyadenosyl radical.[12,13] Herein, using a new labeled substrate, we demonstrate that anSME uses a 5′-deoxyadenosyl radical to catalyze direct H-atom abstraction from the substrate. Furthermore, our data also established that anSMEs are the first radical AdoMet enzymes catalyzing a post-translational modification involving C_β H-atom abstraction.

The anSME enzyme from *C. perfringens* (anSMEcpe) was purified and reconstituted as previously described[11] (see Supporting Information). AnSMEcpe is able to oxidize a 23-mer peptide containing the critical cysteinyl residue target of the post-translational modification;[10] nevertheless, this peptide proved to be unstable and not suitable to obtain quantitative data. We thus designed a shorter peptide of 17 residues (17C peptide) encompassing the sulfatase consensus motif and the critical cysteinyl residue.[3,14]

This peptide was incubated under strict anaerobic conditions with AdoMet and reconstituted anSMEcpe. Mass spectrometry analysis clearly showed its conversion into a new peptide with the expected mass loss of 18 Da. This shift, consistent with the oxidation of the cysteinyl residue into FGly, was further ascertained by a labeling experiment with dinitrophenyl hydrazine (DNPH) (Supporting Information Figure S1). A similar peptide with a cysteinyl to alanyl substitution (17A peptide) was in contrast not an enzyme substrate demonstrating that the cysteinyl residue is the target of the modification (Supporting Information Figure S2).

We hypothesized that, to oxidize the cysteinyl residue, the 5′-deoxyadenosyl radical produced by anSME directly abstracted a hydrogen atom present on the C_β. To probe this hypothesis, we synthesized a deuterated substrate (termed 17D peptide), which had the same sequence as the 17C peptide but contained a [β,β-^{2}H]-cysteinyl residue (Scheme 1).

Scheme 1. Oxidation of Cysteinyl-Containing Peptide by the 5′-dA• Produced by anSME



The 17D peptide proved to be a substrate for anSMEcpe as shown by MALDI-TOF mass spectrometry (Figure 1 and Supporting Information Figure S3). Indeed, when the 17D peptide was used as a substrate, a new peptide with a mass shift of 19 Da (instead of 18 Da) was produced corresponding to the loss of HDS and the formation of the aldehyde function as confirmed by DNPH labeling (Supporting Information Figure S3).

Nevertheless, HPLC analysis demonstrated that oxidation of the 17D peptide was slower than that of its undeuterated counterpart (17C). Indeed, we determinate an apparent kinetic isotope effect of 5.6, demonstrating that C_β-H/D bond cleavage from the cysteinyl residue constitutes the rate-determining step of the catalytic reaction (Figure 2).

However, in contrast to near stoichiometric FGly-peptide and 5′-deoxyadenosine (5′-dA) formation with 17C peptide, 4.5 times more 5′-dA than FGly-peptide was produced using 17D peptide (Supporting Information Figure S4). This result indicated an uncoupled production of 5′-dA.

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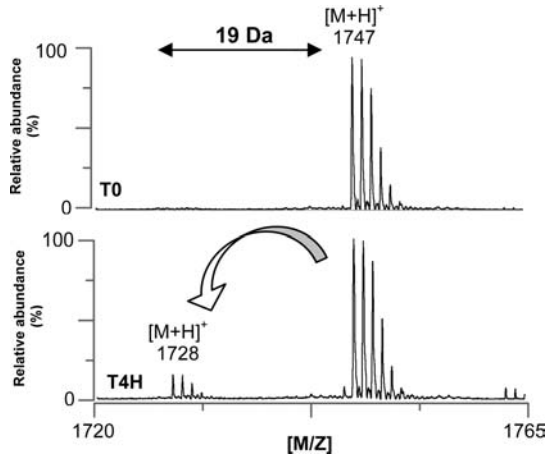

Figure 1. MALDI-TOF mass spectrometry analysis of 17D peptide (500 μM) incubated with reconstituted anSMEcpe (30 μM) under reducing conditions in the presence of AdoMet (1 mM).

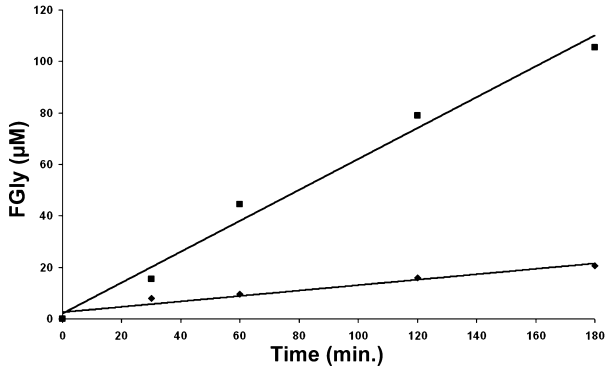

Figure 2. Time-dependent formation of FGly-containing peptides during incubation of 17C (■) or 17D (◆) peptides (500 μM) with anSMEcpe (30 μM) and AdoMet (1 mM) under reducing conditions.

The 5′-deoxyadenosine enzymatically produced during incubation with the 17D peptide was purified by HPLC and analyzed by mass spectrometry and NMR spectroscopy (Figure 3 and Supporting Information Figures S5 and S6). This compound had a different isotopic distribution compared to the standard, indicating the possible formation of monodeuterated 5′-deoxyadenosine. ^{1}H NMR analysis clearly showed a signal upfield-shifted by 0.017 ppm characteristic of the nuclear shielding effect of the geminal ^{2}H isotope and originating from the CH_2D protons (Figure 3).

HPLC analysis, mass spectrometry, and NMR spectroscopy established that substrate deuterium was incorporated into 20−30% of the 5′-dA produced by anSMEcpe. This result was in agreement with the amount of FGly-containing peptide produced and further confirmed that anSME catalyzes direct abstraction of a C_β H-atom on the cysteinyl residue.

Altogether our results support that anSMEcpe generates a 5′-deoxyadenosyl radical by reductive cleavage of AdoMet; this radical species then abstracts one of the C_β H-atoms from the cysteinate residue resulting in the formation of a radical intermediate. This intermediate is probably further oxidized to yield a thioaldehyde intermediate. The existence of thioaldehyde intermediates have been previously proposed for other enzymes but never experimentally evidenced, consistent with their extreme reactivity.[15,16] We suggest that the thioaldehyde intermediate formed by anSME is further hydrolyzed by a water molecule either activated by the enzyme or from the bulk solvent.

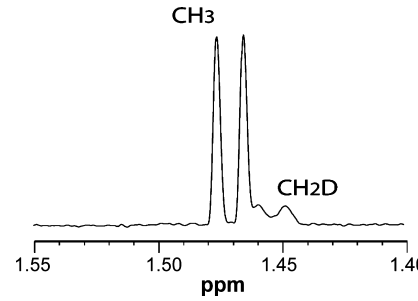

Figure 3. ^{1}H NMR spectroscopy analysis of the 5′-deoxyadenosine produced during incubation of reconstituted anSMEcpe (30 μM) with AdoMet (1 mM) and 17D peptide (500 μM) under reducing conditions (see Supporting Information).

Although several radical AdoMet enzymes have been reported to abstract C_α H-atom in post-translational modification reactions (e.g., pyruvate formate lyase activase and ribonucleotide reductase activase[12,13]), anSMEs are the first radical AdoMet enzymes described to date catalyzing C_β H-atom abstraction on a peptidyl substrate.

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Supporting Information Available: Experimental procedures and additional data (mass spectrometry, HPLC, and NMR analysis). This material is available free of charge via the Internet at http://pubs.acs.org.

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