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Heme degradation by Staphylococcus aureus IsdG and IsdI liberates formaldehyde rather than carbon monoxide

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Abstract

IsdG and IsdI from *Staphylococcus aureus* are novel heme degrading enzymes containing unusually non-planar (ruffled) heme. While canonical heme degrading enzymes, heme oxygenases, catalyze heme degradation coupled with the release of CO, in this study we demonstrate that the primary C1 product of the *S. aureus* enzymes is formaldehyde. This finding clearly reveals that both IsdG and IsdI degrade heme by an unusual mechanism distinct from the well-characterized heme oxygenase mechanism as recently proposed for MhuD from *Mycobacterium tuberculosis*. We conclude that heme ruffling is critical for the drastic mechanistic change for these novel bacterial enzymes.

Heme degradation in pathogenic bacteria is crucial to acquire iron, an essential nutrient required for its survival and infection, from host heme molecules.[1-3] An enzyme family termed heme oxygenase (HO) is known to degrade heme into iron and biliverdin with the release of a *meso* carbon atom CO (Scheme 1).[4-7] The HO catalysis proceeds through three successive mono-oxygenation reactions where the substrate heme activates oxygen molecules for a series of self-oxidations. Until recently, the HO family was thought to be the only heme degrading enzymes, however, a novel family of heme degrading enzymes has been identified in *Staphylococcus aureus*.[8] IsdG and its paralogue, IsdI, are heme degrading enzymes of *S. aureus* composing of a heme-uptake system called the Isd system.[8] The IsdG-type proteins have distinct structures compared to the HO-family enzymes.[9,10] Especially, heme bound to the IsdG-type proteins show unusual non-planarity, best described as ruffled. This heme ruffling is expected to significantly modulate the O_2 activation chemistry on the heme molecule, and in fact, a novel tetrapyrrole, staphylobilin, having an additional oxidation at a *meso* carbon is produced (Scheme 1).[10] Nevertheless, the unique reaction mechanism of the IsdG-type enzymes remains unclear.

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Supporting Information. Experimental procedures, Figure S1, and Scheme S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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We have recently found that MhuD, an IsdG-type enzyme from *Mycobacterium tuberculosis*, catalyzes the heme degradation without generating CO (Scheme 1).[11] The carbon atom at the site of ring cleavage is not released but retained as an aldehyde group, resulting in formation of a novel chromophore termed mycobilin. This unprecedented observation indicates a unique reaction mechanism of MhuD which is distinct from the well-understood mechanism of canonical HO. We have attributed the drastic mechanism change to the heme ruffling as observed in the *S. aureus* IsdG and IsdI heme bound structures. The *S. aureus* enzymes, however, have been assumed to generate CO despite lack of any experimenl evidence.[10] This assumption is based on the structural similarities of staphylobilin and biliverdin at their ring cleavage site and removal of the *meso* carbon (Scheme 1). To this end, we have experimentally determined the C1 products of *S. aureus* IsdG and IsdI for the first time.

Single-turnover heme degradation reactions by IsdG and IsdI as well as MhuD and rat HO-1 were conducted using ascorbate as an electron donor. HPLC and MS analyses confirm that the four enzymes afford their characteristic tetrapyrrole products (Figure S1). The amount of CO produced was determined by trapping CO by a H64L variant of myoglobin (Mb) which has an extremely high CO affinity.[12] Figure 1 shows the Soret region difference spectra of the ferrous H64L Mb induced by CO generated by the single turnover heme degradation. As reported earlier,[11] while the reaction solution of rHO-1 exhibited a significant difference spectrum, essentially no spectral change was observed for MhuD. The CO yields summarized in Table 1 show almost stoichiometric formation of CO in the HO-1 reaction and its absence in the MhuD reaction. IsdG and IsdI exhibited the CO-induced difference spectra, but their low intensities indicate low yields of CO (~14%, Figure 1 and Table 1). This finding clearly demonstrates that CO is not the major C1 product of the *S. aureus* enzymes.

Since staphylobilin actually lacks one *meso* carbon atom, IsdG and IsdI should afford alternative C1 products. The aldehyde group retained in mycobilin compared to staphylobilin (Scheme 1) suggests formation of formaldehyde (HCHO) and/or its oxidized form, formic acid (HCOOH), by the *S. aureus* enzymes. Formaldehyde in the reaction solution was converted by an acetylacetone method to 3,5-diacetyl-1,4-dihydrolutidine,[13] which was quantitated by HPLC analysis (Figure 2A and Table 1). While rHO-1 and MhuD afford negligible amount of formaldehyde, the prominent signals observed for IsdG and IsdI reveal high yields of formaldehyde in their reactions (approximately 80 %). Quantitation of formic acid was based on its enzymatic oxidation.[14] Formic acid dehydrogenase consumes formic acid to generate an equimolar amount of NADH. HPLC quantitation of NADH (Figure 2B and Table 1) indicates negligible (rHO-1 and MhuD) or very low yields (IsdG and IsdI) of formic acid. These observations firmly identify formaldehyde as the primary C1 product of both IsdG and IsdI.

This study shows that *S. aureus* IsdG and IsdI degrade heme by an unusual mechanism where one *meso* carbon atom of the porphyrin ring is liberated primarily as formaldehyde (Table 1). While canonical HO releases the *meso* carbon as CO,[6,7] only small amounts of CO are produced by the *S. aureus* enzymes. The absence of CO production is first reported for heme degradation by MhuD which retains the *meso* carbon at the cleavage site as the aldehyde group (Scheme 1).[11] The similar *aldehyde* formation by IsdG and IsdI are suggestive of their mechanistic similarity to MhuD catalysis. The low CO yields indicate that the three IsdG-type enzyme reactions do not involve verdoheme, a key intermediate of ring cleavage by HO (Scheme S1). Among three successive oxygenations in canonical HO catalysis, CO is generated at the second step, *meso*-hydroxyheme to verdoheme.[6,7] Hydroxyheme is highly reactive with O_2 to release CO due to its radical character,[6,15] and this rapid auto-oxidation does not require the assistance by the HO enzyme. Thus, the

unique mechanisms of the three IsdG-type enzymes appear to be induced by drastic changes in the intrinsic reactivity of hydroxyheme (or its equivalent). Since the most remarkable structural feature common to hemes in MhuD and IsdG/IsdI is their severe non-planarity,[9-11] we propose that the heme ruffling is critical to suppress CO production and to promote aldehyde formation. The ruffling can modulate reactivity of hydroxyheme through the large steric distortion and/or by changing its electronic configuration.[16] The major difference between the MhuD and IsdG/IsdI reactions is the fate of the aldehyde group; retention in the tetrapyrrole by MhuD or release as formaldehyde by the *S. aureus* enzymes (Scheme S1). While deformylation is known to be mediated by a peroxoheme complex, such a nucleophilic deformylation normally liberates formic acid,[17] which is a minor product of IsdG and IsdI (Table 1).

Great attention should also be paid with regards to the biological significance of the unusual products of the IsdG-type enzymes. Considering the common absence of CO in the *M. tuberculosis* and *S. aureus* enzymes, the most probable scenario is that microbes employing the IsdG-type enzymes do not favor endogenous production of this toxic gas. Exogenous CO is shown to activate the dormancy regulon of *M. tuberculosis* at biologically relevant concentrations[18] and to have a significant bactericidal effect on *S. aureus* at higher concentrations.[19] Moreover, the unique linear tetrapyrroles and formaldehyde may also have some biological functions.

In conclusion, this study shows the unusual formaldehyde formation in heme degradation by *S. aureus* IsdG and IsdI probably due to heme ruffling. It has been postulated that heme ruffling in the IsdG-type enzymes enhances its reactivity while controlling the regioselectivity.[10,16] Our results suggest that heme ruffling opens a new path for its degradation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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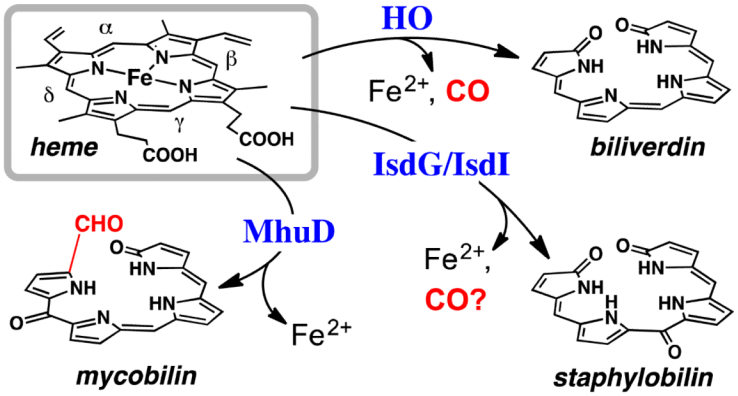

*Substituents of the products are omitted for clarity.

Scheme 1.
Heme degradation products of the HO- and IsdG-type enzymes*

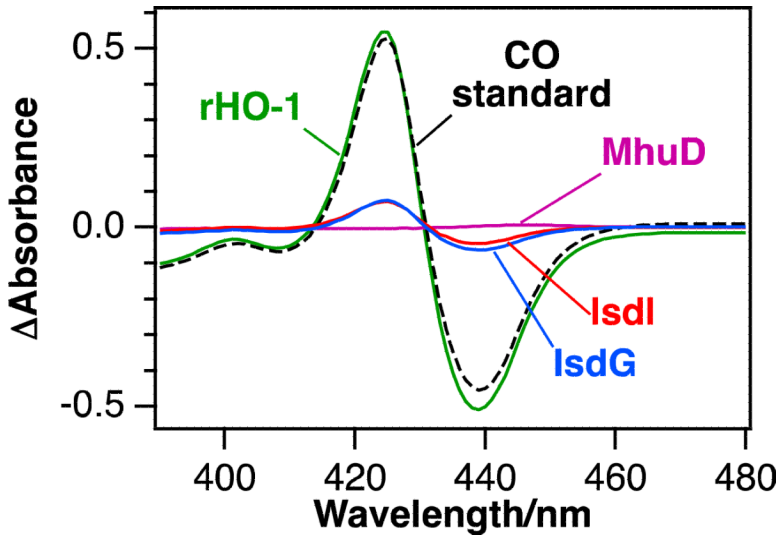

Figure 1.

CO quantification as difference spectra of H64L Mb upon formation of its ferrous CO form. Spectral changes of ferrous H64L Mb were calculated from the spectra taken before and after the heme degradation by 5 μM heme complexes of rat HO-1 (*green*), MhuD (*purple*), IsdG (*blue*) and IsdI (*red*), respectively. The *black broken* line represents difference spectrum of 5 μM ferrous H64L Mb upon saturation with CO.

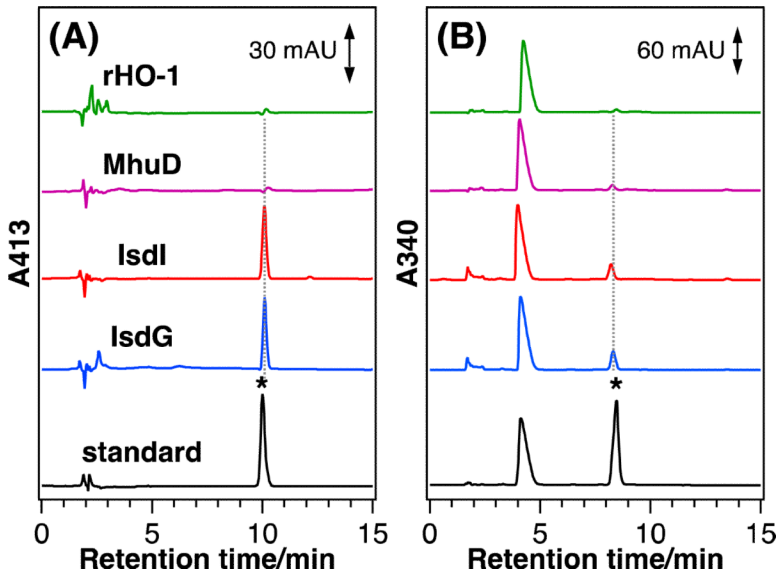

Figure 2.

HPLC chromatograms for quantitation of C1 products. (A) Formaldehyde as 3,5-diacetyl-1,4-dihydrolutidine and (B) formic acid as NADH produced by rat HO-1 (*green*), MhuD (*purple*), IsdG (*blue*), and IsdI (*red*). Product signals are marked with asterisks. Concentrations of enzymes and standards were 20 μM (formaldehyde) and 50 μM (formic acid).

Table 1

Yields of C1 products in heme degradation[a]

Enzyme	CO[b]	HCHO[c]	HCOOH[c]
rHO-1	106 ± 4	4 ± 2	1 ± 1
MhuD	1 ± 1	4 ± 1	5 ± 1
IsdG	14 ± 2	82 ± 1	16 ± 1
IsdI	14 ± 1	81 ± 5	15 ± 1

[a] Yields are given in percent (%)

[b] n=2

[c] n=3