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A radically different enzyme:

The enzyme co-substrate S-adenosylmethionine is a potential source of two different free radicals, yet only one seemed to occur in nature. The discovery of an unusual enzyme reveals that both radicals can be formed

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One of the ways in which biology extends the functionality and reactivity of the naturally occurring amino acids that make up proteins is by modifying the proteins after their synthesis on the ribosome, a process known as posttranslational modification. Some enzymes catalyse their own modifications, but one or more separate enzymes are sometimes used for this purpose. On page 891 of this issue, Zhang *et al.*¹ report their findings on the posttranslational modification of translation elongation factor 2 (EF2), a factor that is crucial for protein synthesis on the ribosome. This modification generates a structure known as diphthamide, so called because it is the target of the diphtheria toxin. The authors find that the modification is carried out by the Dph2 enzyme in an unprecedented reaction using free radicals, and that a cluster of iron and sulphur atoms in the enzyme is involved.

Diphthamide biosynthesis on EF2 involves a co-substrate known as S-adenosylmethionine (SAM), which can be thought of as a positively charged sulphur atom to which three different groups are attached: a methyl group, a 5'-deoxyadenosyl group and a 3-amino-3-carboxypropyl group (Fig. 1a). The positively charged sulphur makes the adjacent carbons somewhat electrophilic (attracted to electrons), and so the most common reactions of SAM involve the attachment of nucleophiles (electron-donating groups) to these carbons. Such reactions result in 'heterolytic cleavage' of one of SAM's sulphur-carbon (S–C) bonds: the bond breaks so that both of its electrons end up on the sulphur atom. In many biochemical reactions, it is the bond to the methyl group that breaks, a fact that underpins SAM's major role as a methyl donor (Fig. 1a). But biochemical reactions also occur in which one of the other two S–C bonds is broken.

An additional mode of reactivity for SAM operates in the so-called radical SAM enzymes². These enzymes transfer an electron from a chemically reduced cluster of iron and sulphur atoms to SAM, promoting a 'homolytic' bond cleavage, in which the S–C bond to the 5'-deoxyadenosyl group breaks so that one electron ends up on each of the atoms of the bond (Fig. 1b). A radical is generated in this process, which goes on to pluck a hydrogen atom from the enzyme's substrate. This provides a useful way of initiating generally quite difficult, often complex, radical-mediated transformations.

Zhang *et al.*¹ now report that diphthamide synthesis involves a previously unknown reaction of SAM: the homolytic cleavage of the S–C bond to the 3-amino-3-carboxypropyl group (Fig. 1c). They find that this process also requires a reduced iron–sulphur cluster, indicating that the Dph2 enzyme is similar in nature to the radical SAM enzymes, even though it cleaves a different S–C bond of SAM.

Much has been learned about the catalytic mechanism of radical SAM enzymes over the past decade. Spectroscopic techniques have been used to demonstrate that a reduced iron–sulphur cluster donates one electron to SAM to initiate S–C bond cleavage³ and that SAM binds to one of the iron atoms of the cluster through its amino and carboxylate groups⁴. The remaining three iron atoms of the cluster are bound by three cysteine amino-acid residues that occur close together in an evolutionarily conserved motif in the amino-acid sequences of the enzymes. The interaction of SAM with the iron–sulphur cluster has also been shown⁵ to cause electron orbitals on the cluster to overlap with those of the positively charged sulphur atom in SAM, implying that direct overlap of orbitals is necessary for the electron transfer that initiates S–C bond cleavage.

But a puzzling question has remained: why is the S–C bond to the 5'-deoxyadenosyl group of SAM the only one to be cleaved by radical SAM enzymes? This is especially strange given that the S–C bonds to the non-methyl groups in SAM have similar strengths, and that the radicals produced by the homolytic cleavage of these bonds have comparable stability.

Zhang and colleagues' results¹ demonstrate that the S–C bond to the 5'-deoxyadenosyl group is not special in its ability to undergo homolytic cleavage. But it does raise the question of why this bond is exclusively broken by radical SAM enzymes, whereas Dph2 breaks only the S–C bond to the 3-amino-3-carboxypropyl group. The origin of this selectivity probably lies in differences in the way SAM interacts with the iron–sulphur clusters in the enzymes. Electron transfer to SAM is likely to cause the cleavage of a particular bond if the electron enters the 'antibonding' orbital of that bond. In radical SAM enzymes, we would thus expect that the antibonding orbital corresponding to the S–C bond to the 5'-deoxyadenosyl group would overlap with the electron-donating orbital from the iron–sulphur cluster. Consistent with this idea, the crystal structures^{6–10} of radical SAM enzymes in complex with SAM all reveal similar orientations of SAM relative to the iron–sulphur cluster.

But in Dph2, the antibonding orbital of the S–C bond to the 3-amino-3-carboxypropyl group should provide the necessary overlap. Unfortunately, no SAM is bound within the crystal structure of Dph2 reported by Zhang *et al.*¹, and so it remains to be seen whether SAM adopts an orientation in this enzyme that is consistent with this theory.

Intriguingly, Zhang *et al.* report that the structural fold of Dph2 that contains the iron–sulphur cluster is entirely unrelated to the analogous folds of the radical SAM enzymes whose structures have been solved to date. Furthermore, unlike the radical SAM enzymes, the amino-acid sequence of Dph2 does not contain a motif of close-lying cysteines that binds to the cluster. Instead, the three cluster-binding cysteines of Dph2 arise from three

different domains of the protein, and were therefore not previously recognizable as a binding motif.

If other enzymes are identified that homolytically cleave the same S–C bond in SAM as that broken by Dph2, will they also have clusterbinding cysteines in different protein domains, in folds that resemble that of Dph2? Or will their iron–sulphur clusters be bound by the cysteine motif found in radical SAM enzymes? More excitingly, could there be other kinds of fold that also bind to clusters involved in SAM's radical chemistry? Whatever the answers, it seems evident from Zhang and colleagues' work¹ that Dph2 employs analogous reactions to those of the radical SAM enzymes in order to achieve a very different chemical outcome.

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Figure 1. Reaction modes of an enzyme co-substrate

S-Adenosylmethionine (SAM) is a common enzyme co-substrate. It consists of a positively charged sulphur atom to which are bound a methyl group (red), a 5'-deoxyadenosyl group (blue) and a 3-amino-3-carboxypropyl group (green). **a**, SAM often undergoes enzymatic heterolytic cleavage reactions in which the sulphur–carbon (S–C) bond to the methyl group is broken. In these reactions, both of the electrons in the bond end up on the sulphur atom. Curly arrows indicate the movement of pairs of electrons. Nu represents a nucleophile (an electron-pair donor). **b**, SAM also undergoes homolytic cleavage in radical SAM enzymes. In these reactions, SAM accepts an electron (e⁻), whereupon the S–C bond to the 5'-deoxyadenosyl group breaks so that one electron ends up on each of the atoms of the bond. Fish-hook arrows indicate the movement of single electrons. **c**, Zhang *et al.*¹ report that, in the enzyme Dph2, SAM undergoes a previously unknown homolytic cleavage reaction in which the S–C bond to the 3-amino-3-carboxypropyl group is broken.