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A diiron protein auto-generates a valine-phenylalanine crosslink

Richard B. Cooley^a, Timothy W. Rhoads^a, Daniel J. Arp^{b,*}, and P. Andrew Karplus^{a,*}

^aDepartment of Biochemistry and Biophysics, 2011 Ag & Life Sciences Bldg, Oregon State University, Corvallis, OR 97331

^bDepartment of Botany and Plant Pathology, 2082 Cordley Hall, Oregon State University, Corvallis OR 97331

Abstract

All known internal covalent crosslinks in proteins involve functionalized groups having oxygen, nitrogen or sulfur atoms present to facilitate their formation. Here, we report a carbon-carbon crosslink between two unfunctionalized sides chains. This valine-phenylalanine crosslink, produced in an oxygen-dependent reaction, is generated by its own carboxylate-bridged diiron center and serves to stabilize the metallocenter. This finding opens the door to new types of post-translational modifications and it demonstrates new catalytic potential of diiron centers.

Many proteins generate covalent crosslinks between two or more of their own amino acids to create a cofactor near their active site (1). Although the mechanisms of crosslink formation and the function of the crosslinked group are not always understood, all characterized crosslinks to date involve side-chains with nitrogen, oxygen or sulfur functional groups to facilitate the making and breaking of the covalent bond(s).

Following up our earlier work on π -helices in proteins (2), we have solved the structure of a rubrerythrin-like protein that appears to conserve certain ancestral features of the ferritin-like superfamily. We call this protein symerythrin to reflect its high level of internal symmetry. The 1.20 Å resolution structure of symerythrin from the photosynthetic eukaryote *Cyanophora paradoxa* ($R/R_{\text{free}} = 10.1/12.5\%$, Figs. 1A and S1, Table S1) reveals an unusual covalent crosslink connecting the aliphatic $C_{\gamma 1}$ -atom of Val127 to the aromatic C_{δ} -atom of Phe17 (Fig. 1B) adjacent to its carboxylate-bridged diiron metallocenter (details to be described elsewhere). LC-MS/MS analysis of in-solution proteolytic digests confirmed the location of the crosslink and its presence in the protein before crystallization (Fig. 1C). This crosslink is notable because it occurs between unfunctionalized side chains.

From our symerythrin expression system, crosslinked (~10%) and non-crosslinked (~90%) forms could be purified separately, allowing us to prove that the crosslink was generated by symerythrin's own metallocenter. Indeed, aerobic incubation of non-crosslinked, iron-reconstituted protein with an electron donor led to complete conversion to the crosslinked form (Fig. 1D). The dependency of crosslink formation on reductant, O_2 , and an intact

*corresponding authors: P. Andrew Karplus, Department of Biochemistry and Biophysics, 2011 Ag & Life Sciences Bldg, Oregon State University, Corvallis, OR 97331, Phone: (541) 737-3200; Fax (541) 737-0481, karplusp@science.oregonstate.edu. Daniel J. Arp, Department of Botany and Plant Pathology, 2082 Cordley, Oregon State University, Corvallis OR 97331, Phone: (541) 737-1294, Fax: (541) 737-5310, Dan.J.Arp@oregonstate.edu.

Supporting Online Material

Materials and Methods

Fig. S1

Table S1

References

diiron center implies that symerythrin's metallocenter forms high-valent diiron-oxygen intermediates resembling those in soluble methane monooxygenase (3) and also proposed to be involved in formation of a recently discovered Tyr-Val ether crosslink in a ribonucleotide reductase R2-like protein (4). By analogy with these systems (5), we hypothesize that as a Q-type (i.e. Fe(IV)-Fe(IV)) intermediate forms, Val127 rotates so its C_{γ1}-atom contacts the activated metal-bound oxygen, which abstracts a hydrogen atom to generate a primary alkyl radical. Val127 then rotates back and adds to the Phe17 phenyl ring to create a cyclohexadienyl radical. Transfer of a single electron to the diiron center, coupled with deprotonation, then generates the crosslink.

While the physiological function of symerythrin remains unknown, evidence shows that the crosslink is crucial for the stabilization of the diiron center (Fig. 1E) by anchoring the N-terminal tail to the core of protein. In doing so, a putative substrate binding pocket is created near the diiron center (Fig. 1B). Given the nature of the crosslink and the significant effort that has been placed in characterizing such hydrocarbon chemistry for its relevance in biological, bioenergetic and environmental applications (6), further study will be of great interest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

The Val-Phe crosslink. (A) The core four helices of symerythrin (green ribbon with helices α A, α C and α D labeled), the N-terminal tail (red coil), the putative substrate binding cavity (olive surface), the diiron center (brown spheres) and the ligating residues (C, N and O atoms are gray, blue and red) are shown. Electron density (blue mesh contoured at $2.2\rho_{\text{rms}}$) shows the covalent bond linking Val127 and Phe17 (yellow atoms). (B) Close up of the crosslink and diiron center (having one additional Glu ligand compared to rubrerythrins) with the same coloring as panel A. Val127 $C_{\gamma 2}$ is 4.5 Å from the bridging oxygen. (C) The MS/MS fragmentation pattern from the triply-charged parent ion at m/z 791.07 identified in proteolytic digests of crosslinked symerythrin. The parent ion mass (2370.181 Da) matches that of residues 120-134 linked to residues 14-18 (calculated MW 2370.182). (D) Purified non-crosslinked symerythrin (N, lane 1) was reconstituted with iron and exposed to O₂ (lane 2) prior to a 40 h incubation with an electron source (e⁻) during which all symerythrin is converted to the crosslinked form (XL, lane 3). Crosslink formation required O₂ (lane 4) and iron (lane 5). (E) Incubation of a chromogenic iron chelator to diferrous non-crosslinked symerythrin (blue line) shows rapid iron removal compared to that of diferrous crosslinked symerythrin (red line).