Photoinduced Protein Cross-Linking Mediated by Palladium Porphyrins

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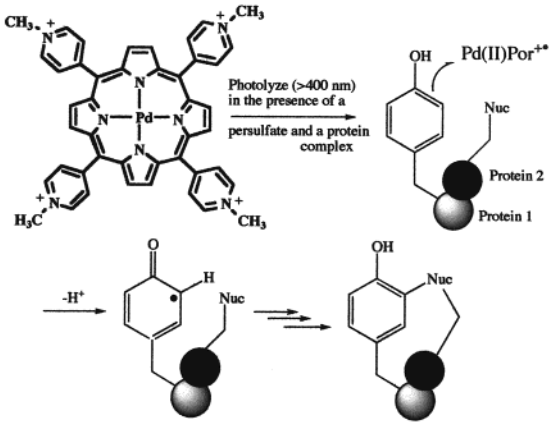
Chemical cross-linking is a useful method to probe protein−protein interactions in solution.[1−3] Of particular utility are cross-linking reagents that are inert until activated. If one has control over the reactivity of the cross-linker, it can be triggered at any point in a catalytic cycle. Furthermore, only activatable reagents can be used in affinity cross-linking experiments[4] in which the cross-linker is appended covalently or noncovalently to a particular protein of interest, which is then reconstituted into the complex under investigation. The most commonly used reagents of this type are aryl azides or benzophenones.[5−10] Photolysis produces reactive intermediates capable of insertion into C−H bonds. While these species have been used to advantage, aryl azides and benzophenones absorb in the UV region and have low extinction coefficients. A significant advance in this area would be to design chemical cross-linking reagents that could be activated efficiently with visible light and which would then mediate protein cross-linking very rapidly.[11]

We and others have shown previously that efficient cross-linking of proteins can be mediated by agents able to oxidize tyrosine or other electron-rich residues at or near the interface of a protein−protein complex.[11−18] It is believed that a tyrosyl radical generated by the oxidation event couples covalently with aromatic or nucleophilic side chains from nearby proteins to cross-link proteins via carbon−carbon or carbon-heteroatom bond formation. Therefore, it was envisioned that a molecule that could be photoexcited to a state capable of oxidizing tyrosine might mediate protein cross-linking with the assistance of a hydrogen atom-abstracting agent (see Scheme 1).

The literature on Pd(II) porphyrin photochemistry suggests that these agents might be useful in this regard.[19−21] To probe this point, glutathione S-transferase (GST), a native homodimer[22] that can also associate into higher-order species, was photolyzed with visible light (>400 nm) in the presence of Pd(II) 5,10,15,20-tetra-(methylpyridinium)porphyrin (Pd(II)TMPyP) and ammonium persulfate (APS), a good electron acceptor.[23] It was hoped that electron transfer from the excited state of the palladium porphyrin to the APS would result in the formation of the porphyrin radical cation and that this species would act as the initiating oxidant (Scheme 1). As shown in Figure 1A (far right lane) cross-linked products corresponding to the GST dimer and higher-order multimers were produced very efficiently under these conditions. An approximately 50% yield of cross-linked products was produced after only a 10 s irradiation using a 150 W xenon lamp and a 400 nm cutoff filter. Little or no cross-linking was observed when either light, APS, or Pd(II) porphyrin was omitted from the solution or when free base porphyrin[24] was substituted for the Pd(II) derivative (Figure 1A and unpublished data of D.A.F.).

Scheme 1



To probe the scope of this reaction, the Pd(II) porphyrin/APS photomediated cross-linking of several proteins was examined. Gal80 protein (Gal80p) is a yeast transcriptional repressor[25] that exists as a mixture of homodimers and tetramers (K. Melcher, T. Kodadek, and S. A. Johnston, unpublished results). Figure 1B shows that cross-linked dimers and tetramers were produced when Gal80 protein was photolyzed for 5 s in the presence of Pd(II)-TMPyP and APS. The fact that little trimer is produced suggests that the cross-linked dimer is even more susceptible to further cross-linking than the unmodified protein, for unknown reasons. Gal80p was also found to cross-link to a radiolabeled 54 residue polypeptide[17] that includes the activation domain of the Gal4 protein, to which Gal80 is known to bind specifically[26,27] (Figure 1C). As will be reported in a full paper, several other proteins known to interact with one another were cross-linked with Pd(II)TMPyP and APS when photolyzed for between 5 and 60 s under these conditions. However, like any cross-linking reaction, the Pd(II)TMPyP/APS protocol is not ubiquitously useful. Avidin,

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(24) Several other metalloporphyrin derivatives were evaluated as well. Fe(III) porphyrins exhibited very weak activity (for a previous report of inefficient heme-based photocross-linking, see: Verweij, H.; Dubbelman, T. M. A. R.; Van Steveninck, J. *Biochem. Biophys. Acta* **1981**, *647*, 87−94) and others tested supported little or no detectable cross-linking.
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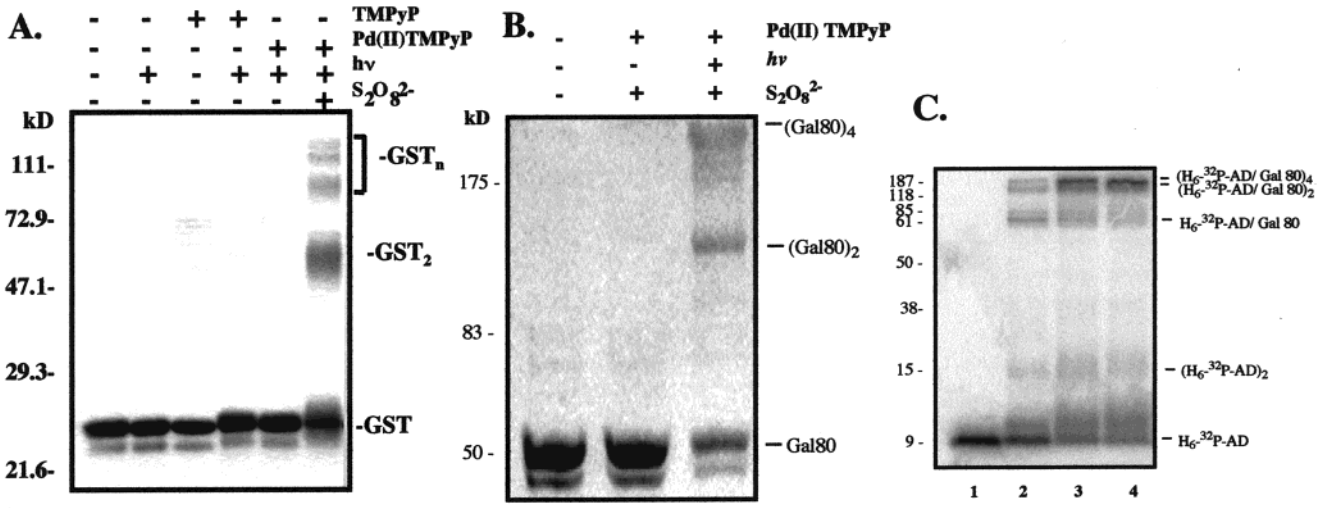

Figure 1. Palladium(II) porphyrin/APS-mediated cross-linking of proteins initiated by visible light. In all cases, when the samples were exposed to light, irradiation was carried out using a 150 W xenon lamp equipped with a <400 nm cutoff filter. When present, the porphyrin concentration was 75 μM, and the APS concentration was 1.25 mM. (A) Cross-linking of GST. In each experiment, the GST concentration was 10 μM. The samples exposed to light were irradiated for 10 s TMPyP represents the free base analogue of Pd(II)TMPyP. Efficient cross-linking is observed only in the presence of the palladium porphyrin, APS, and light. (B) Cross-linking of Gal80 protein. In each experiment the Gal80 concentration was 10 μM. This concentration is much higher than the K_D of the dimer and in the range of the K_D of the tetramer formed through dimer-dimer interactions (K. Melcher, T. Kodadek and S. A. Johnston, unpublished results). The sample in lane 3 was irradiated for 5 s. (C) Cross-linking of a ^{32}P-labeled, His_6-tagged Gal4 activation domain ([^{32}P]H_6AD) (0.5 μM) to Gal80 protein (0.7 μM). A phosphorimage is shown, so that only the bands corresponding to the radioactive AD-containing fusion protein and cross-linked products containing it are observed. Lanes 1−4 differ only in the time of irradiation (0, 10, 20, and 40 s, respectively).

a native tetramer, failed to cross-link under the standard conditions (not shown). This may be due to a lack of electron-rich aromatic residues in the region of the protein−protein interface.[28,29] Finally, several experiments using proteins that do not bind to one another show that this reaction couples only proteins that are stably associated. For example, when ubiquitin, a monomer, was photolyzed in the presence of Pd(II)TMPyP and APS, no cross-linking was observed. Furthermore, addition of excess GST to a solution containing the radiolabeled Gal4 AD and Gal80p did not produce products in which GST was cross-linked to either of the yeast transcription factors (data not shown).

In designing this reaction it was assumed that the protein residues targeted by the photogenerated oxidant would be tyrosine, tryptophan, and perhaps other electron-rich amino acids (see Scheme 1). To test this hypothesis, the radiolabeled Gal4 activation domain/Gal80p complex was subjected to the standard Pd(II)TMPyP/APS-mediated photocross-linking process in the presence of a large excess of various free amino acids (Figure 2). Tryptophan, tyrosine, and cysteine all were potent inhibitors of cross-linking, while threonine and leucine had little effect. Methionine had an intermediate effect. This is the profile that one would expect for a protein-coupling reaction initiated by a photogenerated oxidant.

A limitation of this reaction is the requirement for APS, which is cell-impermeable and can oxidize some cellular components directly. Therefore, it would be useful to find an APS substitute with more favorable properties. Co(III) complexes are sometimes used to accept electrons from excited-state metal complexes. Unfortunately, much lower yields of cross-linked products were produced when $Co(III)(NH_3)_5Cl^{2+}$ was used in place of APS (see Supporting Information). However, we have found recently that $Ru(II)(bpy)_3^{2+}$, like Pd(II) porphyrins, also supports efficient visible light-initiated cross-linking in the presence of APS.[11] $Co(III)(NH_3)_5Cl^{2+}$ supports the $Ru(II)(bpy)_3^{2+}$almost as well as APS (see Supporting Information). More work will be necessary to find APS substitutes that are functional in the Pd(II) porphyrin-mediated reactions.

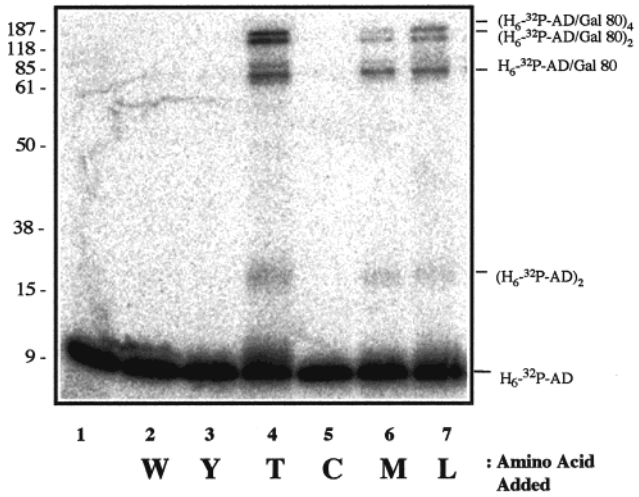

Figure 2. Inhibition of photoinitiated cross-linking reaction by various amino acids suggests that the active reagent is a photogenerated oxidant. Different amino acids (750 μM) were added to a solution containing ^{32}P-labeled Gal4 AD (0.5 μM), Gal80p (0.7 μM), and Pd(II)TMPyP (10.5 μM). APS was added to 150 μM, and the samples were photolyzed for 10 s. The products were analyzed by gel electrophoresis. A phosphorimage is shown, revealing only AD-containing bands. The amino acids added to each reaction are indicated at the bottom (one letter code). The sample in lane 1 was not photolyzed.

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Supporting Information Available: Figure 3, $Co(III)(NH_3)_5Cl^{2+}$ substitutes for APS poorly in the PdTMPyP-mediated reaction but does support tris-bipyridyl ruthenium(II) dication-mediated photocross-linking, although less efficiently than APS. Each solution was photolyzed for 5 s, except that in lane 1, which was not irradiated (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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