Palladium Complexes for Peptide and Protein Modification and Cross-linking

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ABSTRACT: Protein and peptide cross-links are common in biological systems that contribute to complicated protein structures and make them function effectively. Apart from natural cross-links, cross-linking reagents are used in a variety of techniques for protein and peptide researches. Hence, many types of cross-linking reagents and methods are discovered and utilized, including palladium complexes. In this review, we will introduce general cross-linking techniques and some common reagents firstly. Further, some applications and methods of cross-linking induced by palladium complexes are presented. Finally, we will illustrate the properties and development of palladium-mediated arylation of protein and peptide.

INTRODUCTION

Protein cross-links can be defined as covalent bonds between two amino acid side chains either within a single polypeptide chain (intramolecular, Figure 1a) or between from separate polypeptides (intermolecular, Figure 1b). One of the most widespread types of cross-links in biological systems are the disulfide bonds linking two thiol groups of cysteine residues. These natural disulfide bonds play an important role in stabilizing protein's secondary or tertiary structures, guaranteeing their basic functions.

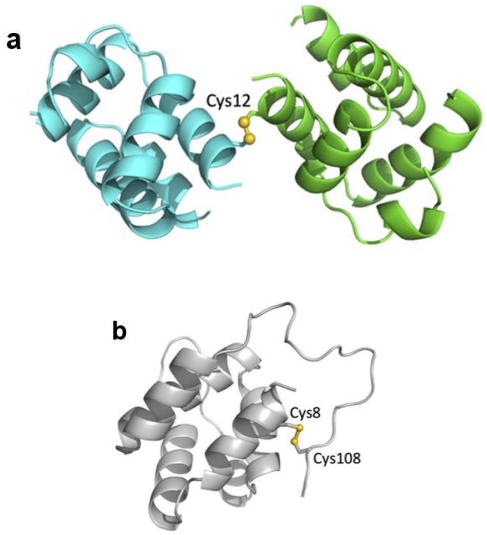

Figure 1. Examples of disulfide bonds in (a) intra-molecular and (b) intermolecular bonds cross-links.

Apart from these natural cross-links, cross-linking reagents (or crosslinker) are used in a variety of techniques to assist in determining partners and domains of protein interactions, three-dimensional structures of proteins, and molecular associations in cell membranes. They are also used to, for instance, immobilize proteins on solid supports for affinity purification, to conjugate haptens to carrier proteins for immunization, and to prepare antibody-enzyme conjugates for detection procedures (Figure 2).

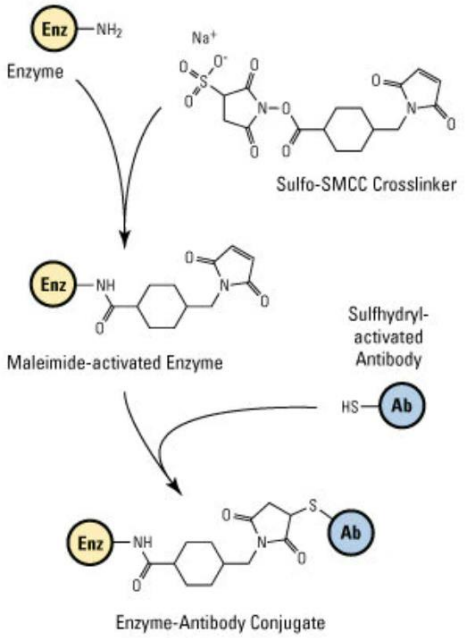

Figure 2. An example of antibody-enzyme conjugate preparation.

Generally, in cross-linking techniques, reagents, such as N-hydroxysuccinimide activated esters (NHS ester), maleimide, hydrazide, and 1-Ethyl-3-[3-dimethylamino-propyl]carbodiimide hydrochloride (EDC), are commonly used (Figure 3). In addition to these organic crosslinkers, metal complexes can also be applied. Here we mainly discuss palladium complexes.[1-2]

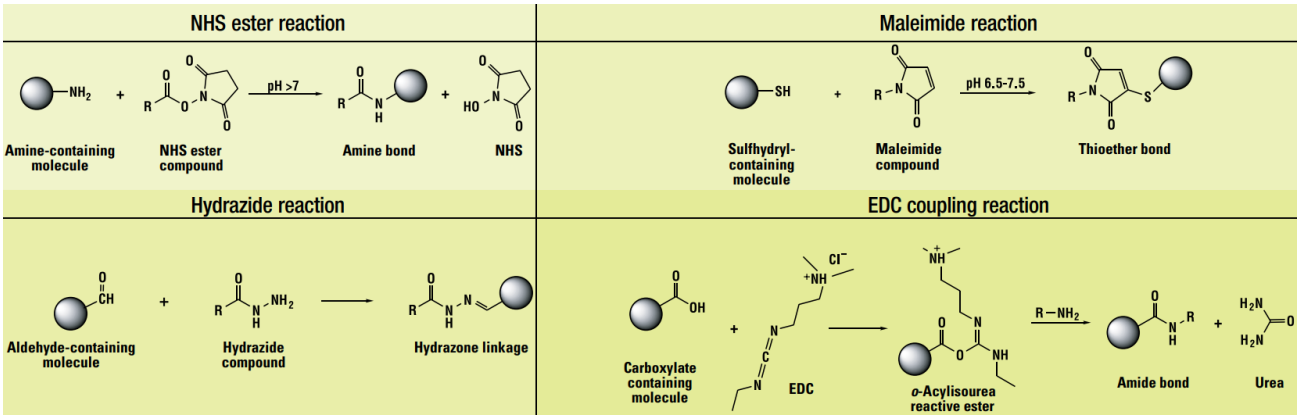

Figure 3. Some common cross-linking reagents and their reactions.

APPLICATIONS OF PALLADIUM COMPLEXES IN EARLY YEARS

There are several different cross-linking mechanisms where radicals are key media. Among these, photolysis produces reactive intermediates capable of insertion into C-H bonds, and aryl azides and benzophenones absorb in the UV region and have low extinction coefficients. Cross-linking reagents that could be activated efficiently with visible light and which would then mediate protein cross-linking very rapidly are needed.[3]

A Pd(II) porphyrin complex is designed to photoinduce protein cross-linking.[4] Glutathione S-transferase (GST), a native homodimer 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. Electron transfer from the excited state of the palladium porphyrin to the APS results in the formation of the porphyrin radical cation and that this species would act as the initiating oxidant. After the radical is induced in benzophenone from the tyrosine residue in protein 1, a series of reactions contributes to its connection with a nucleophile in protein 2 and the cross-linking succeeds (Figure 4).

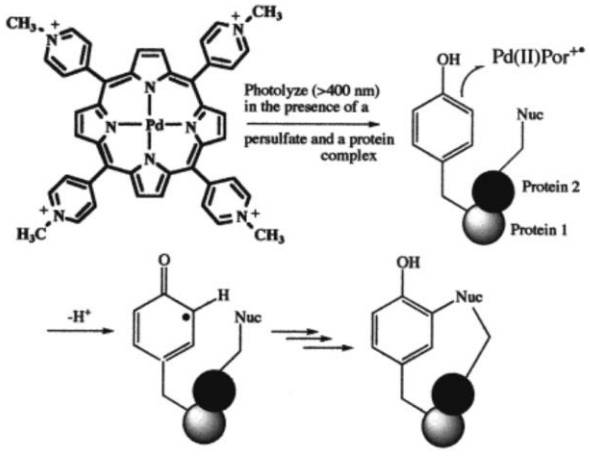

Figure 4. Cross-linking mediated by palladium porphyrin.

Except radical induced cross-linking, the cross-coupling catalyzed by palladium complexes is known for its effective carbon-carbon bonds or some other hetero-carbon bonds formation, and hence can be used to complete cross-linking. In particular, cross-coupling on peptides and proteins has received much attention because of the prospect of selective formation of a nonlabile linkage at a residue functionally orthogonal to natural amino acids.[5] The following figure demonstrates an example of Suzuki-Miyaura cross-coupling on a serine protease, subtilisin Bacillus lentus (SBL) mutant S156C (Figure 5).[6] The process is simple with moderate condition but several steps for preparing reactants are required, such as the arylation at the single cysteine on SBL.

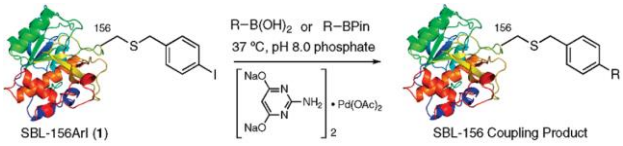

Figure 5. Suzuki-Miyaura cross-coupling on a protein.

PALLADIUM-MEDIATED ARYLATION

Here palladium oxidative addition complexes (OACs) of the type [LPd(Ar)X] (L = biaryl-phosphine, X = Cl, Br, OTf, Nu = S (Cys), NH (Lys)) are introduced, which can undergo a mechanism for mild and site-selective bioconjugation that takes advantage of the reactivity of organometallic reagents (Figure 6).[7] After the halogen is substituted by the nucleophile group, mainly thiol and amine group on the side chain of cysteine and lysine respectively, reductive elimination is completed, managing to finish arylation. By this simple protocol, the arylation biomolecules is completed within minutes and in generally high yields.[8] However, these complexes are more cysteine-selective because the nucleophilicity of amine group is lower and the following reductive elimination needs to be done in the presence of a weak base due to the lower acidity of the palladium–amine complex.

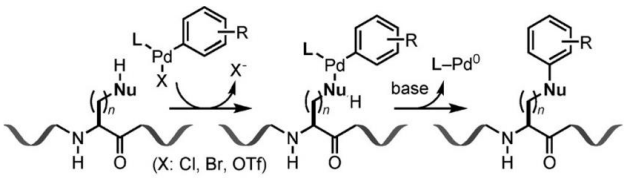

Figure 6. Cross-linking strategy with OAC

This arylation strategy can be used for, for example, peptide macrocyclization, which contains cross-linking of two amino acid side chains.[9] The following figure shows peptide macrocyclization that is demonstrated using a 4,4'-benzophenone-linked bis-palladium reagent along with a few others, resulting in effective crosslinking of two cysteine residues at the i, i + 4 and i, i + 7 positions of two unprotected peptides respectively (Figure 7). Compared to their linear counterparts, macrocyclic peptides often display enhanced binding affinity, resistance to protease degradation, and in certain cases, readily enter cells.[10]

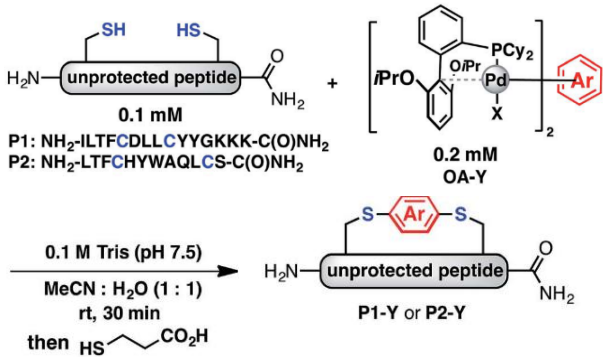

Figure 7. Peptide macrocyclization by palladium-mediated arylation.

Small molecule OACs are mainly discussed previously, which combine with thiol groups effectively. However, new strategies are needed to complete cross-linking between proteins and peptides and protein-palladium complexes should be synthesized preliminarily (Figure 8A).[11] Two methods are discussed through retrosynthesis analysis. Adapting strategies used on small molecules to access electrophilic OACs of protein substrates is challenging and hampered pre-dominantly by three factors (Figure 8B, blue): (1) a two-step process that first necessitates installation of an aryl halide prior to oxidative addition; (2) ineffective oxidative addition due to nonspecific metal chelation, which often requires a large excess of Pd reagents to overcome; and (3) the requirement to use an inert atmosphere and organic solvents. Compared to this, these challenges can be overcome if small molecule OACs are synthesized in advance (Figure 8B, red), which involves the use of a Pd-functionalized NHS (N-hydroxy-

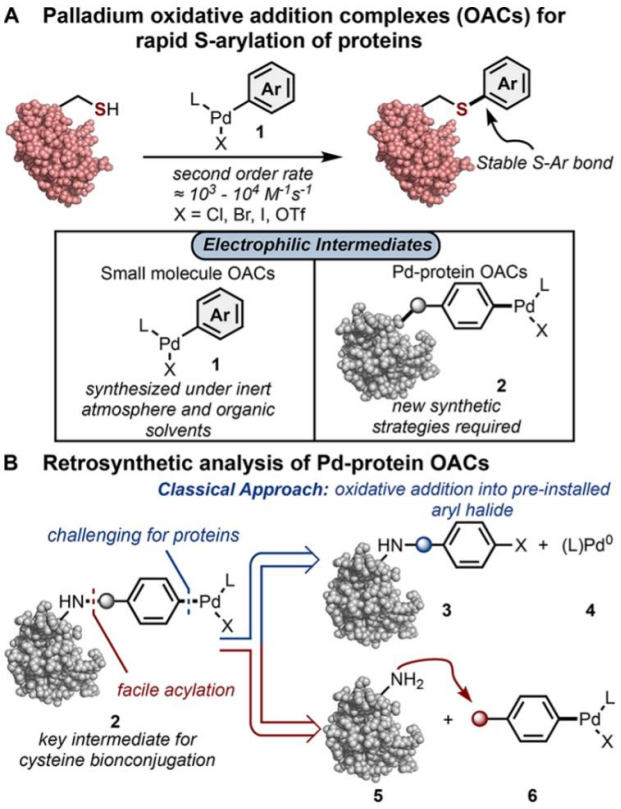

Figure 8. Strategy for formation of OACs of protein substrates.

succinimidyl) ester reagent. NHS esters are electrophilic groups capable of acylating numerous types of small molecules and materials containing exposed nucleophilic residues, including side chains of amino acids. Therefore, combined to Pd-OACs, they are amine-selective, which enables them to react with free lysine amine groups (Figure 9A). After combining with a lysine on ribonuclease A, Pd-RNase A-OAC **12** is formed (Figure 9B).

Pd-RNase A-OAC **12** can undergo efficient conjugation with thiol containing substrates to complete the protein homodimerization (Figure 10A). Antibody-protein conjugates can also be synthesized with trastuzumab **14** (Figure 10B). The protein can be substituted into peptide, for instance, EGP-peptide, to form antibody-peptide conjugates (Figure 10C). These demonstrate that Pd-OACs offer great diversity of cross-linking between proteins and peptides.

In order to develop the lysine-selectivity of OAC, NHS group is changed using phenyl N-(4-bromophenyl)-carbamate and then both intramolecular (Figure 11A) and intermolecular (Figure 11B) cys-lys protein cross-linking can be completed.[12] To verify its lys-selectivity, after the intramolecular cross-linking in peptide **2** is completed first (Figure 12a), the lysine in peptide **2** is mutated to leucine

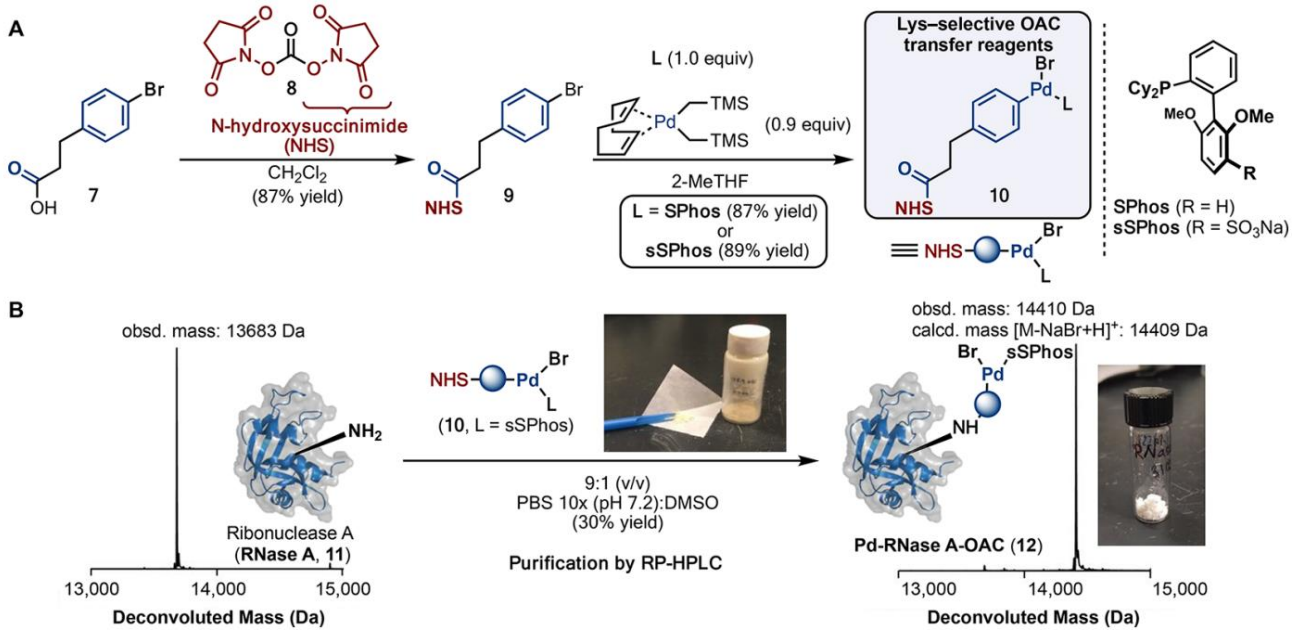

Figure 9. Pd-RNase A-oxidative addition complex (OAC).

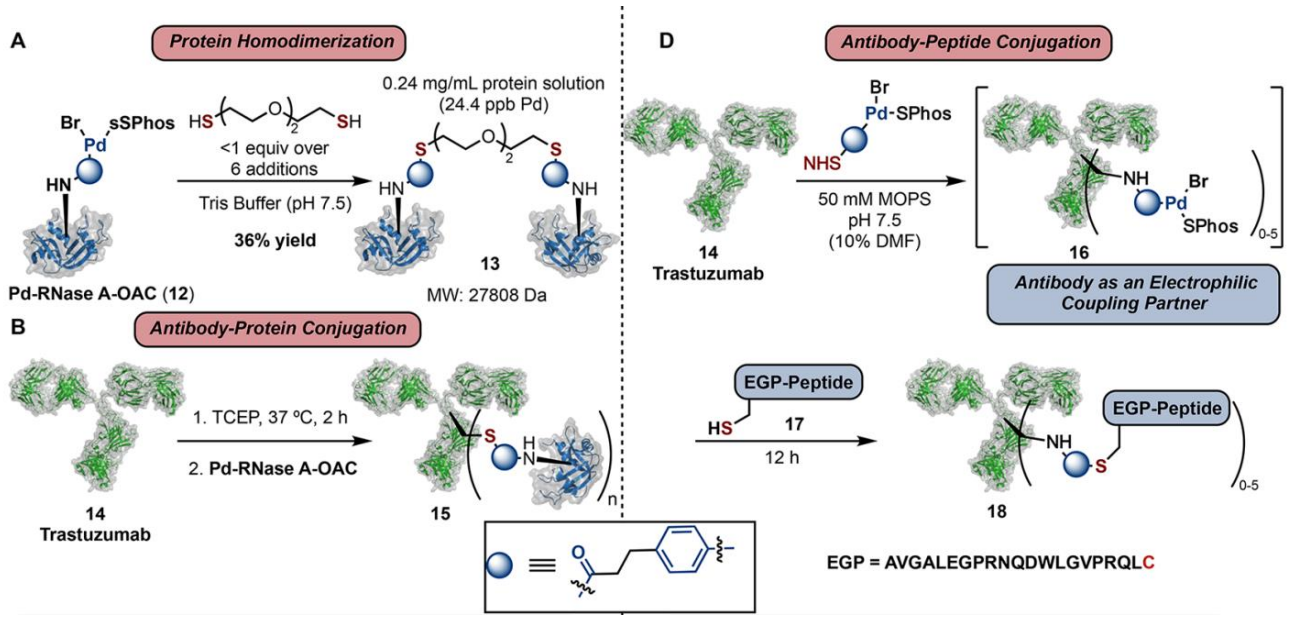

Figure 10. Cross-couplings of Pd-protein OACs enables protein homodimerization, antibody-protein conjugation and antibody-peptide conjugation.

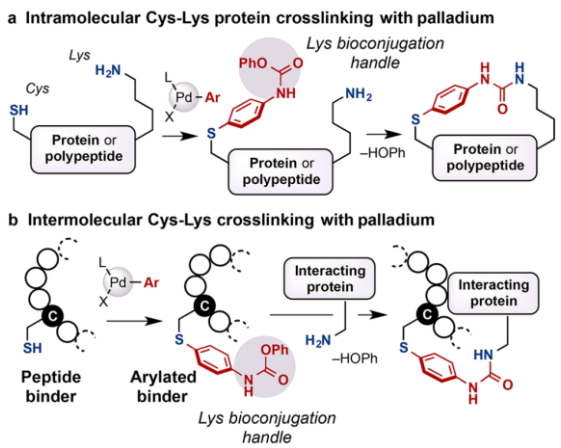

Figure 11. Strategy for palladium-mediated peptide and protein cross-linking.

(Figure 12b). Then MS analysis is introduced and finds that cross-linking fails after the mutation.

CONCLUSION

In summary, palladium complexes, especially palladium oxidative addition complexes (OACs) of the type [LPd(Ar)X], are useful for bioconjugation due to their broad function group tolerance and advent of aqueous cross-coupling after researches for decades. A variety of non-natural amino acids and functional groups can be combined with these complexes with controllable selectivity and low toxicity, showing great application potential in further protein and peptide analysis and modi-

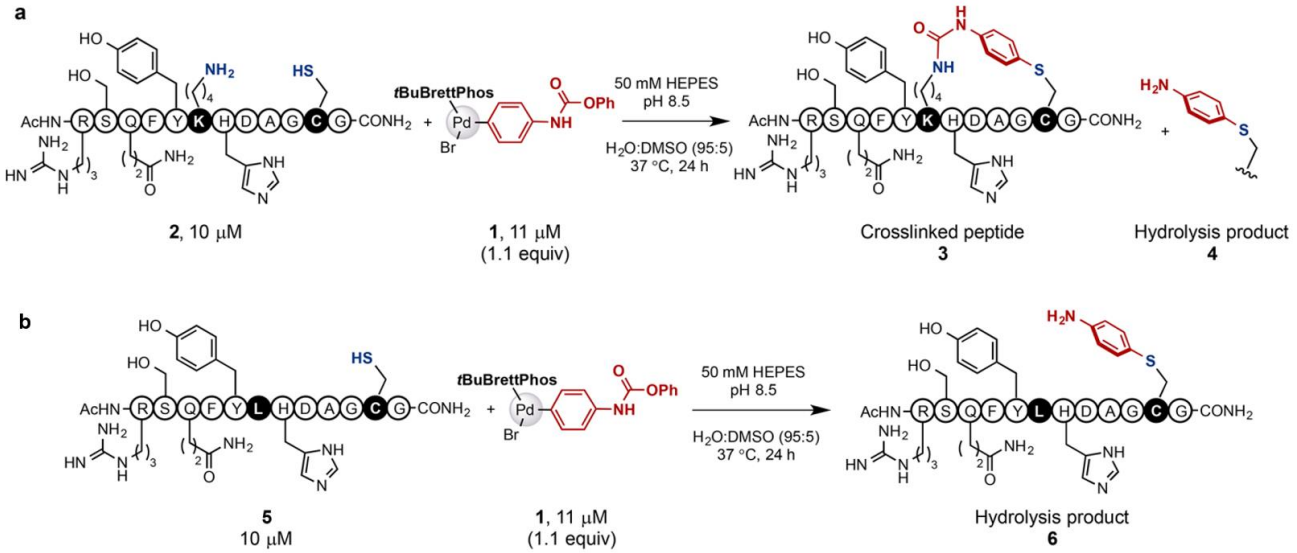

Figure 12. Chemoselectivity of palladium complex 1-mediated peptide cross-linking is confirmed by mutation study.

fications in the fields of biochemistry.

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