Organometallic complexes: Catalysis and application in protein modification

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Organometallic catalysis has witnessed phenomenal growth with time and methodologies have been developed to facilitate a variety of chemo-, regio- and stereoselective reactions. The potential of these transformations has been examined to differentiate the reactivity of amino acid residues in a peptide or protein in recent years. However, these biomacromolecules extend stringent chemoselectivity challenge to the classical methodologies. The efforts en route to develop new catalytic approaches in this area has been delineated in this review.

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Metals offer Lewis acidic sites to coordinate with amino acid polymers owing to the abundance of Lewis base centers. Meaningful complexation can be achieved by engineering partners that show bioorthogonal interaction. Such coordination complexes have been found to stabilize secondary structures such as α-helix, β-sheets and various loops. Serving as an inspiration for design of ion sensors, these complexes have also enabled rapid detection of tagged proteins and fluorescent markers. A chemoselective metal coordination with poly-histidine peptide fused to the protein of interest has been instrumental in purification of recombinant proteins. The potential of metal centers for selective coordination in a pool of functionalities make them a probable candidate for their application in post-translational modification (PTM) of proteins. Apart from controlling several intra- and extracellular events, these modifications can regulate the structure and function of proteins. Chemical reactivity of amino acid side chains offer a range of PTM's that includes transfer of phosphate, farnesyl, acyl, ubiquitin, formyl, glycosyl and several other fragments to the protein backbone. This approach is driven by the establishment of unique side chain functionality that can react orthogonally with respect to the residues in natural amino acids. One of the established route to answer this chemoselectivity challenge is enabled by enzyme catalysis. Another classical approach involves incorporation of unnatural amino acid into the protein sequences assisted by engineered bacterial stains. Such mutation would depend on the modified tRNA that suppresses the stop codon. These stop codons introduced into mRNA can in turn define the analogs. However, access to modified suppressor tRNA’s encounters a whole set of new challenges.

In a conceptually infant approach, efforts have been directed towards transition metal catalyzed chemoselective bioconjugation for labeling unmodified natural amino acid polymers. The selective catalytic system involves metallopeptide complexes inspired by metalloenzymes. Here, the first coordination sphere involves side chain amino acid residues that bind to the metal. This regulates the electronic properties of metal and in turn defines the activity of metal-peptide complex. The catalytic fine tuning and selectivity is governed by the second coordination sphere that involves hydrogen bonds, electrostatic, hydrophobic and steric interactions. The peptide sequence constituting the second coordination sphere does not bind with the metal directly but provides a channel for substrates/products and an appropriate microenvironment in the cavity around the active site. In this review, we have highlighted various constraints and methodologies that has directly or indirectly enabled the evolution of this approach.
Organometallic Complexes in Protein Modification

Organometallic catalysis has a profound effect on the C-C and C-X bond formation. Metal center, ligand, additive and solvent are crucial parameters for emergence of an efficient catalytic method. Recent reports have established the approach in differentiating functional groups of amino acid side chain (1a-m, Fig. 1) by exploiting subtle differences in their reactivity. Functional group modification on the surface of proteins is dictated by a new set of parameters defined by the protein itself. This exponentially drops the number of metal catalyzed methodologies that would qualify for their application in this area. A focused approach with collective optimization of parameters is desired to understand the behavior and mode of activation of these catalysts. Efforts for expanding native parameters for organometallic catalysis, i.e., organic solvents, high substrate concentrations (100 mM to 1 M) and high temperature have led to a few successful realization of organometallic milieu that shows high functional group tolerance in the conditions desired for reaction of proteins. The protein modification should be realized in aqueous buffer, low concentration, ambient temperature, and physiological pH. Adhering to these conditions and achieving high rate of reaction is a considerable challenge.

A unique opportunity for bio-orthogonal transformation is offered by unnatural amino acids utilizing “tag and modify” technology. Some of the representative examples include genetic incorporation of azide, unsaturated bonds, and coupling conjugates into proteins that offer the possibility of Cu catalyzed click chemistry, Ru catalyzed olefin metathesis and Pd catalyzed cross coupling reactions. Continuing efforts in this area have established the proof of principle with successful validations utilizing S-allyl cysteine (2), p-iodophenyl alanine (4) and homopropargylglycine (6) outlined in Scheme 1.

Allylation

Electrophilic π-allyl complexes are suitable candidates for labeling of nucleophilic residues on proteins. The required allylic acetate (8), carbonate or carbamate precursors are biologically inert and become reactive only after activation by palladium catalyst. It’s utility has been established in allylation of phenolic groups, for chemoselective functionalization of tyrosine (Scheme 2). The excess allylic acetate (8) forms diene (10) that can be

Fig. 1 – Pool of natural amino acids (1a-m) with reactive side chains.
easily purified due to considerable difference in mass and polarity.

These catalytic systems have the capacity to modify proteins in aqueous medium with high functional group tolerance preserving the protein structure.\textsuperscript{21} Palladium-catalyzed cross-coupling can also modify aryl halides\textsuperscript{22} and boronic acids\textsuperscript{23} within peptide fragments. Difference in access to solvent and microenvironment of amino acid residue can also assist in achieving selective modifications. Solvent-accessible tyrosine residue on chymotrypsinogen A can undergo Mannich-type alkylation\textsuperscript{24} with rhodamine-labeled allylic acetate (8), Pd(OAc)$_2$, and triphenylphosphine tris-sulfonate (TPPTS).\textsuperscript{25} The transformation results in double alkylation where Y171 in T14-15 fragment gets modified predominantly. The catalyst was found to be inert in presence of 19 lysine residues of horse heart myoglobin and cysteine thiolate of H-Ras.

**Reductive amination**

An iridium hydride complex has been reported to reduce imines chemoselectively over their aldehyde precursors. The water-stable [Cp*Ir-(bipy)(H$_2$O)]SO$_4$\textsuperscript{26} complex and its derivatives (13a-c) achieved reductive alkylation of lysine residues of lysozyme at room temperature and neutral pH (Scheme 3). The reaction has been shown to be very successful with structurally diverse proteins including cytochrome c (19 lysines), R-chymotrypsinogen A (14 lysines), myoglobin (19 lysines), ribonuclease A (10 lysines), and an intact virus (bacteriophage MS2, 6 lysines per monomer). Reactivity is dependent on the structure of these biomacromolecules and solvent accessibility of lysine residues. The reductive alkylation adducts are amenable to further modification with alkoxyamines to form oximes.\textsuperscript{21c,27}
Oxidative cross coupling

Oxidative cross-coupling is one of the important techniques for protein modification that utilizes transition metal catalysis. The methodology relies on the formation of tyrosyl radical (19) by oxidation of tyrosine, donating an electron to high-valent transition metal (Ni, Mn, Fe). The tyrosyl radical (19) can couple with another tyrosine linking the two proteins (21) (Scheme 4A). However, tryptophan and cysteine side chain oxidation or their coupling with tyrosyl radical are major side reactions. High reactivity of tyrosyl radical poses enormous challenge and makes it vulnerable for reaction with various residues of nearby proteins. To control the reactivity, a number of catalytic systems have been developed. Ni-GGH (22a), Ni-His8 (22b), and...
Ni-ribonuclease A\textsuperscript{35} complex along with an oxidant were reported to promote protein cross-linking (Scheme 4B). Ni\textsuperscript{2+}-peptide-salen complex offers oxidative cross-linking of DNA to the peptide fragment of metallopeptide complex.\textsuperscript{36}

**Metallocarbenoid mediated conjugation**

Metallocarbenoids\textsuperscript{37} react with 3-methylindole (26)\textsuperscript{38} in presence of water\textsuperscript{39} and a large number of polar residues (Scheme 5A). In this transformation, both N-alkylation and C-alkylation products [(27):(28), 1:1.4, 51% yield] are formed. The residual diazo compound (25) leads to formation of two other side products, alcohol (29) and pyrazole (30). In absence of water soluble substrates, an organic co-solvent was considered necessary to improve the reactivity. Concentration below 100 µM is critical to have control over selectivity. Solvent exposed tryptophan residues (W7 and W14) in myoglobin (10-100 µM) lead to a mixture of mono- and di-labeled products with 60% conversion.\textsuperscript{21d,40} The starting materials were found to be inert in the absence of rhodium catalyst. Single tryptophan residue in subtilisin Carlsberg required lower pH (1.5) for conversion to the monolabeled W113 product.

**Metallopeptides in Catalysis**

Metal-peptide complexes have gained attention for their success in asymmetric C-C bond formation.\textsuperscript{41} Peptides constitute an attractive set of modular chiral pool due to well established synthetic protocols. However, understanding the secondary structure of catalyst is not trivial. Dipeptides [(35-37), Fig. 2] have served as efficient ligands to generate chiral Schiff base for enantioselective catalysis.\textsuperscript{42} The phenol-based Schiff bases (e.g. 35) are better partners for early transition metals (Ti, Zr), whereas, P-containing ligands (e.g. 36) are apt for the late transition metals (Cu, Zn). Some of the successful metallopeptide catalyzed transformations include

\textbf{Scheme 5}

![Scheme 5](image)

\textbf{Fig. 2 – Structures of dipeptide ligands.}
Ti-catalyzed addition of TMSCN to epoxides\textsuperscript{43} and imines (Scheme 6),\textsuperscript{44} copper catalysed conjugate addition (Scheme 7),\textsuperscript{45} Zr-catalyzed addition of dialkylzinc to imines (Scheme 8)\textsuperscript{46} and Al catalysed addition of cyanide to ketones (Scheme 9).\textsuperscript{47}

Proper spatial alignment of Schiff base and amide carbonyls is critical to achieve high selectivity. The chirality of the second amino acid also plays an important role in achieving stereodifferentiation. For example, bifunctional Ti-Schiff base catalyst coordinates with the substrate (38) and carbonyl of peptide (39) to direct the cyanide to activated imine (Scheme 6). Dipeptide serves an ideal length while additional amino acids were found to be detrimental to the selectivity.\textsuperscript{48} A common approach for screening peptide based ligands is hinged on combinatorial screening of library.\textsuperscript{49} However, similar results can also be achieved by an alternative approach where rational screening leads to identification of reactive substrate and metal, Schiff base, ligand-metal combination and peptide. Utilization of this approach led to an efficient Cu catalyzed asymmetric allylic substitution utilizing pyridinyl peptides (44) or (45) (Scheme 7).

(A) Titanium catalysed asymmetric addition of cyanide to imines

(B) Metallopeptide coordination with imine and cyanide

Scheme 6

Scheme 7
In another report, a combinatorial library of peptides was used for enantioselective addition of alkylmetals to C=N bonds \((49)\) (Scheme 8).\(^{46}\) Commercially available \(\text{Et}_2\text{Zn}\) was used as the alkylating reagent. Both early and late transition metals were screened with phenol \((50),\) \((54)\) and phosphine-based Schiff bases \((36)\) and \([\text{Zr}(\text{OiPr})_4]_{\text{HOiPr}}\) was identified as the most effective catalyst. Al-peptide complex was also found to be an efficient catalyst to render asymmetric cyanation of ketones \((56)\) and \((59)\) (Scheme 9). A systematic screening supported by modular peptide-based chiral ligands led to the identification of suitable catalyst.\(^{47,50}\) Acetophenone \((56)\) and TMSCN react in presence of chiral peptide ligand \((57)\) and \(\text{Al(OiPr)}_3\). Stereochemistry of amino acid at C-terminus was found to be critical for enantio-differentiation.

Phosphine, a well-established ligand for the transition metal catalysis, along with peptide ligands have been explored with palladium, ruthenium and rhodium.\(^{51}\) Secondary structure of the peptide plays a key role for bidentate metal chelation. For example, a \(\text{C}_2\)-symmetric cyclic peptide gramicidin S, \((\text{Pro-Val-Orn-Leu-dPhe})_2\), adopts a rigid cyclic \(\beta\)-hairpin structure and predisposes the Orn residues for facile binding with transition metals. Phosphine-gramicidin S conjugate \((63)\) complexed with Rh can serve as an efficient catalyst for
asymmetric hydrogenation (Scheme 10).52 When complexed with Pd, this conjugate renders efficient allylic substitution, albeit with low stereoselectivity.

The contributions to foldamers53 and small peptide helices54 offer another secondary structure where two binding sites are positioned at i and i+4 position for metal binding. With the hypothesis that such structurally defined peptide sequences can coordinate with metal, the nonapeptide KADAALDAK with two carboxylate side chains positioned at i and i+4 was screened.55 In a stereoselective carbenoid insertion to Si-H bond, Rh2(OAc)2-monopeptide complex (69) was found to be modest with respect to bis-peptide complex Rh2(70a)2 or Rh2(70b)2 (Scheme 11). It is important to note that the bis-peptide complexes are formed as separable mixture of parallel (70a) and antiparallel isomers (70b).56 These two isomers of bis-peptide complexes follow independent pathways for enantiomeric differentiation. Other crucial parameter includes nature and spacing of the carboxylates. Increase in spacing between metal and peptide by one methylene (Asp to Glu) compromises the catalysis.

Metallopeptides in Protein Modification

The dirhodium monopeptide complex (72) has been successfully utilized in modification of peptides.57 The key to selectivity was unique interaction between two peptide strands inspired by the role of coiled-coils in protein-protein interaction.58 This proximity-driven approach can gain the rate acceleration up to >103. Coiled-coil sequences with heptad repeats [(75), abcdefg, Scheme 12] position
Scheme 12

Selective modification by Rh complex driven by peptide-peptide recognition

Scheme 13

Chemoselective modification of E3\(_2\)Q (77) in presence of W\(_{\text{random}}\) (78)
peptides, secondary structure plays a crucial role for rendering reactivity and selectivity. β-turns and helices have been shown to predispose binding sites for the coordination with metal. However, multidisciplinary investigation for the role of secondary coordination sphere to regulate the microenvironment of the substrate and thus efficiency of the catalyst is necessary.

References


