Tetrahedron Letters 56 (2015) 884–888

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Digest Paper

Biomolecule-compatible chemical bond-formation and bond-cleavage reactions induced by visible light

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ARTICLE INFO

Article history:
Received 3 November 2014
Revised 5 December 2014
Accepted 6 December 2014
Available online 15 December 2014

Keywords:
Biomolecule-compatible reaction
Visible-light-induced reaction
Aqueous reaction
Chemoselectivity
Functional group compatibility

ABSTRACT

Biomolecule-compatible reactions are useful for biomolecule labeling and manipulations. Light-induced chemical reactions provide high spatial and temporal precision, and visible light imposes less damage to biomolecules compared to UV light. The visible-light-induced biomolecule-compatible reactions reported in the literatures are categorized by reaction mechanisms to oxidative-quench, reductive-quench, and energy-transfer pathways. Their inventions and applications are discussed with the focus on biomolecule-compatibility.

Contents

Introduction

Light is a useful tool to study the microscopic world in biological systems. The biological observation with light using fluorescent proteins[1] and super-resolution microscopy[2] has reached molecular precision. In contrast, the use of light for molecule perturbation by biologists has been lagged behind until the recent development of optogenetics.[3] With optogenetics, the light-responsive protein function can be modulated with visible light in vitro and in vivo. Its powerful impact has revolutionized the research on neurosciences, signal transductions and many other studies in life sciences. With photoaffinity labeling[4] and photodecaging,[5] chemists have been using light to manipulate macromolecules and small molecules. However, many of these photochemical tools use ultraviolet (UV) light, which limits their acceptances by biologists due to the fear of UV light phototoxicity.[6]

In this digest, we review biomolecule-compatible photochemical tools using visible light for chemical bond-formation and bond-cleavage reactions. The recently developed visible-light-induced biomolecule-compatible reactions have found many applications in biomolecule studies. With their further developments, they will be more useful to solve biological questions that are difficult or unimaginable with current methods.

Visible-light-induced biomolecule-compatible reactions

Biomolecule-compatible reactions are chemical reactions that are performed at ambient temperature in aqueous conditions, pref-

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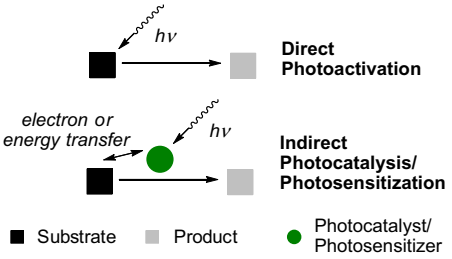
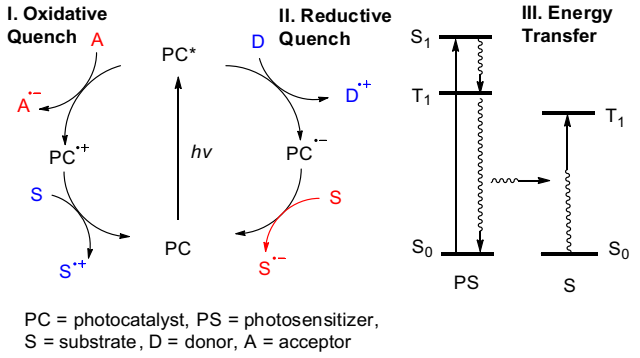
http://dx.doi.org/10.1016/j.tetlet.2014.12.034
0040-4039/



Scheme 1. The direct and indirect photoactivation.



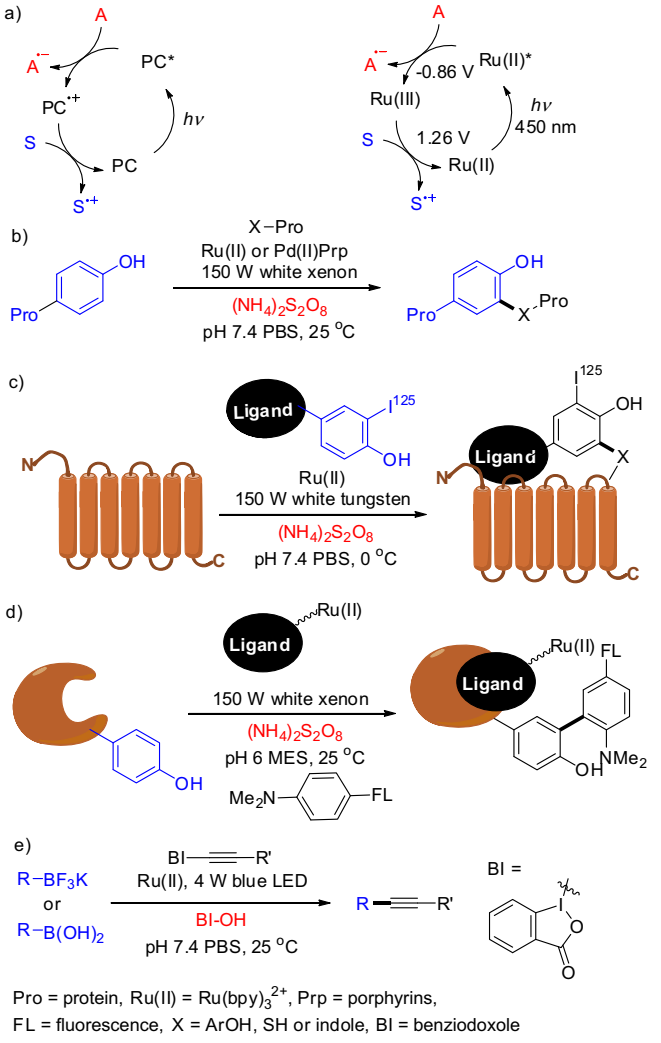
Scheme 2. The reaction mechanisms of photocatalysis and photosensitization.

erably neutral pH, insensitive to the air atmosphere, chemoselective, and compatible with various reactive functional groups on biomolecules. Light-induced chemical reactions provide advantages with stable substrates that are activated only upon light irradiation, which can be precisely controlled with light's high temporal and spatial resolution. The reaction mechanisms can be categorized to direct photoactivation[7] and indirect photocatalysis/photosensitization,[8–10] depending on how the light interacts with the substrate molecule (Scheme 1). The classical direct photoactivation requires UV light. Recently, the two-photon technology enables the longer wavelength activation using strong-energy light sources, which has been extensively reviewed and will not be discussed in this digest.[5] The indirect photocatalysis/photosensitization induced by visible light is the focus of this digest, which will be discussed with the focus on biomolecule-compatibility.

Based on the role of photocatalyst/photosensitizer (PC/PS), the reaction mechanisms are categorized to oxidative-quench, reductive-quench, and energy-transfer pathways (Scheme 2). If the photoexcited photocatalyst PC* loses an electron to the acceptor molecule (A), the oxidized photocatalyst $PC^{\bullet+}$ is generated and constitutes the oxidative-quench pathway I. Similarly, when the photoexcited photocatalyst PC* accepts an electron from the donor molecule (D), the reduced photocatalyst $PC^{\bullet-}$ is generated and makes the reductive-quench pathway II. If the photoexcited photosensitizer PS* does energy-transfer instead of electron-transfer to the substrate (S), the energy-transfer pathway III is named.

Oxidative-quench pathway

The $Ru(bpy)_3^{2+}$ complex is the most-studied and widely-used photocatalyst, which is used as the model for illustration (Scheme 3a).[11,12] After absorbing visible light at the blue light region (450 nm), the ground state photocatalyst $Ru(bpy)_3^{2+}$ is photoexcited to $Ru(bpy)_3^{2+*}$. Its oxidation potential is only −0.86 V and can easily be oxidized by weak oxidants to generate the transient strong oxidant $Ru(bpy)_3^{3+}$ with oxidation potential



Scheme 3. The visible-light-induced biomolecule-compatible reactions with oxidative-quench pathway. (a) The oxidative-quench mechanism and the presentative $Ru(bpy)_3^{2+}$ example. (b) The oxidative cross-linking reaction of phenols. (c) The oxidative cross-linking reaction of phenols on G-protein-coupled receptors. (d) The ligand-directed oxidative cross-linking reaction of phenols on carbonic anhydrase. (e) The visible-light-induced deboronative alkynylation.

at 1.26 V. Some electron-rich substrates can be oxidized by the $Ru(bpy)_3^{3+}$ to yield the radical cation intermediate for further bond formations.

The Kodadek group pioneered the visible-light-induced oxidative cross-linking reaction of phenols in 1999, in which ammonium persulfate ($(NH_4)_2S_2O_8$) was used as the electron acceptor (A) to oxidize the excited photocatalyst (Scheme 3b).[13,14] The oxidized photocatalysts $Ru(bpy)_3^{3+}$ or Pd(III) porphyrin complex can oxidize the tyrosine residue on the protein to produce a tyrosine radical cation. This unstable intermediate can be trapped by the neighboring nucleophilic group phenols, thiols, or indoles to form a covalent bond. This reaction has a high cross-linking efficiency (70–80%) and occurs across a wide pH range in a very short time period (≤1 s).

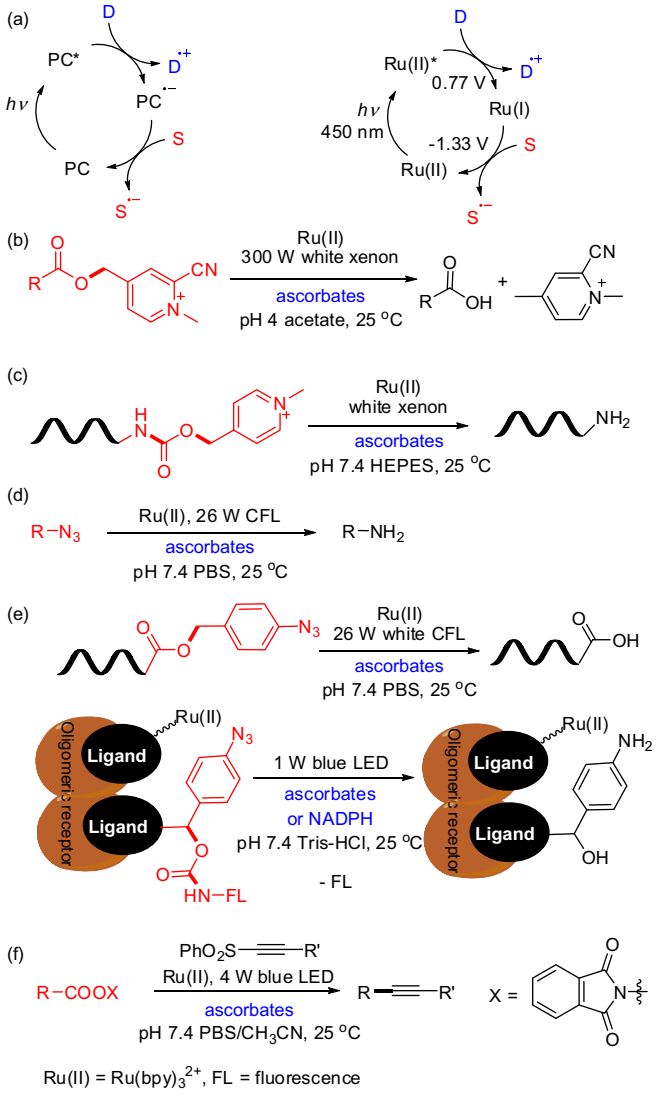
The oxidative cross-linking of phenols is useful for studying interactions of protein–protein and protein–peptide complexes due to the proximity-enabled reactivity. Bonnafous and co-workers covalently labeled the B_2 bradykinlin receptor using ligands with a radio-iodinated phenol moiety, which can be readily monitored by radioactivity (Scheme 3c).[15] Using oxidative crosslinking with agonist or antagonist ligands, the recognition and signaling

mechanisms associated to G-protein-coupled receptors were studied. This visible-light-induced photolabeling reaction was efficient and the B_1 bradykinin, AT_1 angiotensin II, V_{1a} vasopressin and oxytocin receptors were investigated using similar strategies.

Using the ligand-conjugated $Ru(bpy)_3^{2+}$, Nakamura and co-workers selectively labeled the tyrosine residue on carbonic anhydrase closed to the ligand-binding site with a dimethylaniline moiety (Scheme 3d).[16] Compared to the previously used ligand-tethered nucleophilic catalysis strategy,[17] this ligand-directed local single-electron-transfer strategy is more selective and bearing higher reactivity. This visible-light-induced oxidative cross-linking reaction has been applied to other biomolecule studies including metastable amyloidogenic protein assemblies, mammalian cell patterning adhesions, and target protein visualizations in living systems.[18,19]

However, the use of strong oxidant persulfates comes with a price. Slow oxidation of methionine and cysteine is observed with extended incubation time, which suggests $(NH_4)_2S_2O_8$ may oxidize some biomolecules directly.[14] Co(III) complexes sometimes were used as the $(NH_4)_2S_2O_8$ surrogate for electron acceptors, however, much lower yields of cross-linked products were yielded.[14] Therefore, it is desirable to find milder and more selective electron acceptors.

Our group recently discovered that the mild oxidant hydroxylbenziodoxole (BI-OH) can effectively oxidize the excited $Ru(bpy)_3^{2+*}$ (Scheme 3e).[20] This new oxidative-quench system enables the oxidation of stable alkyl trifluoroborates or boronic acids to the alkyl radicals via oxidative deboronation, which can be further trapped by BI-alkyne for alkynylation. This reaction can be carried out in aqueous conditions across different pH ranges including the neutral pH condition. The presence of amino acids (including tyrosine, methionine, and cysteine), nucleosides, oligosaccharides, nucleic acids, proteins, and cell lysates does not affect the reaction, which highlights its mild reaction condition and excellent chemoselectivity.



Scheme 4. The visible-light-induced biomolecule-compatible reactions with reductive-quench pathway. (a) The reductive-quench mechanism and the presentative $Ru(bpy)_3^{2+}$ example. (b) The reductive cleavage reaction of *N*-alkylpicolinium esters. (c) The reductive cleavage reaction of *N*-alkylpicolinium carbamates on DNA. (d) The visible-light-induced azide reduction. (e) The visible-light-induced azide reduction on DNA and protein receptors. (f) The visible-light-induced reductive decarboxylative alkynylation.

Reductive-quench pathway

Similarly, the $Ru(bpy)_3^{2+}$ complex is used as the model of illustration for reductive-quench pathway (Scheme 4a).[11,12] After absorbing visible light at the blue light region (450 nm), the ground state photocatalyst $Ru(bpy)_3^{2+}$ is photoexcited to $Ru(bpy)_3^{2+*}$. Its reduction potential is 0.77 V and can easily be reduced by weak reductants to generate the transient strong reductant $Ru(bpy)_3^{+}$ with oxidation potential at −1.33 V. Some electron-deficient substrates can be reduced by the $Ru(bpy)_3^{+}$ to yield the radical anion intermediate for further bond cleavages and formations.

The Falvey group discovered the visible-light-induced reductive bond cleavage reaction of *N*-alkylpicolinium ester in 2009, in which sodium ascorbates were used as the electron donor (D) to reduce the excited photocatalyst (Scheme 4b).[21] The reduced photocatalyst $Ru(bpy)_3^{+}$ can reduce the *N*-alkylpicolinium to the radical anion. This unstable intermediate releases the carboxylic acid and *N*-alkylpicolinium in mild pH 4 acetate aqueous buffers. However, its biomolecule-compatibility was not demonstrated. Boncella and co-workers later used *N*-alkylpicolinium carbamates to release aliphatic and aromatic amines after CO_2 elimination.[22] In 2012, this reaction was used for the preparation of primary amine linked DNA using the standard phosporamidate chemistry via photo-controlled deprotection (Scheme 4c).[23]

A general biomolecule-compatible visible-light-induced azide reduction was developed by Liu and co-workers in 2011 (Scheme 4d).[24] Using a DNA-encoded reaction discovery system, this chemoselective azide reduction was discovered with serendipity. The azide is a widely used functional group for biocompatible chemistry such as copper-catalyzed click reactions[25] and strain-promoted cyclization reactions.[26] However, its reduction to the amine was not demonstrated on biomolecules. This visible-light-induced azide reduction can be performed on oligosaccharide and oligonucleotide substrates containing azides. The activity of a protein enzyme is not affected after the presence in the azide reduction conditions.

The visible-light-induced azide reduction can be used to release carboxylic acids on a oligonucleotide substrate via 1,6-elemination, which provides an external photocontrol of the deprotection (Scheme 4e).[24] Winssinger and co-workers later demonstrated the visible-light-induced azide reduction to release amines or alcohols on oligonucleotides for nucleic acid sensing and miRNA visualization.[27,28] This azide reduction is superior to widely-used Staudinger reduction, which reagent phosphine is prone to air oxidation side reactions.[29] This azide reduction was also used to identify the acetyl coenzyme A carboxylase and the estrogen receptor on protein oligomeric receptors in cellulo.[30] The ligand-receptor

interaction brings the Ru(II) photocatalyst to close proximity with the azide-based immolative linker, which can promote the visible-light-induced photodecage of the fluorophore rhodamine. The templated reaction rate was found to be at least 30-fold faster than the untemplated reactions.

The visible-light-induced bond-formation reaction via reductive-quench pathway is recently developed by our group (Scheme 4f).[31] The reductive coupling of azides and nitriles was demonstrated previously only in the DNA-linked system, however, its demonstration on small molecules and other biomolecules was not realized.[24] We found the ascorbates readily reduced the photo-excited $Ru(bpy)_3^{2+*}$ to $Ru(bpy)_3^{+}$, which then reduced *N*-acyloxyphthalimide to the radical anion. This unstable intermediate underwent decarboxylation readily to yield the alkyl radical, and was further trapped by alkynyl sulfones for alkynylation. This reaction is applicable in aqueous/organic mixed solvents across different pH ranges including the neutral pH condition. The presence of aliphatic amines, amino acids, nucleosides, oligosaccharides, nucleic acids, proteins, and cell lysates does not affect the reaction.

Energy-transfer pathway

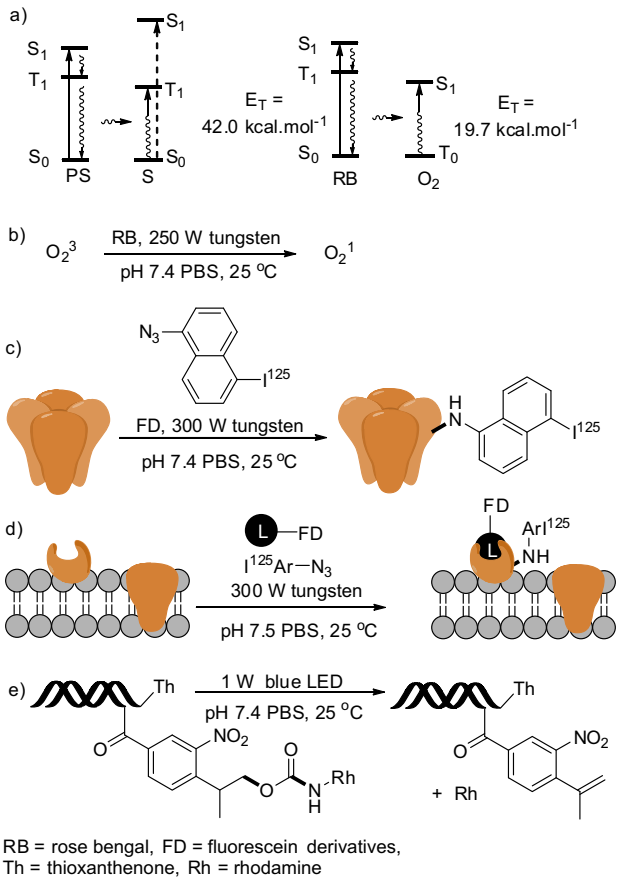
Many photosensitizers have been used for biomolecule studies, including photosensitizers that are clinically used for photo dynamic therapy (PDT). The application of photosensitized singlet oxygen in PDT has been extensively reviewed and will not be discussed in this digest.[32] A typical photosensitizer rose bengal (RB) is used as the model for illustration (Scheme 5a).[33] After absorbing visible light, the ground state photosensitizer RB is excited to RB* S_1 state. After intersystem crossing, a T_1 state of RB* is obtained with excitation energy at 42.0 kcal/mol. Some substrate molecules with the matching lower triplet excitation energy can be sensitized, such as the dioxygen (19.7 kcal/mol), to yield the excited intermediate for further bond formations.

The dioxygen has a low excitation energy and can be easily sensitized (Scheme 5b).[33] The electronic ground state of the dioxygen is a triplet and the singlet is 1.0 eV higher in energy. Singlet oxygen is highly electrophilic and capable of oxidizing phenols, sulfides, and amines.

Organic azides are widely used photoaffinity labeling reagents. After photo activation and nitrogen gas elimination, the resulting nitrene intermediate is highly reactive and useful for protein labeling. In 1987, the Salomon group discovered 5-iodonaphthalene-1-azide can be photosensitized at 480 nm with undecylamine fluorescein for photoaffinity labeling.[34] Comparing to direct photolysis with 314 nm UV light, this sensitization method is more biomolecule-compatible in complex biological systems (Scheme 5c).

As the photosensitization of azides is distance-dependent and confined to the vicinity of the chromophore photosensitizer, this method allowed selective labeling of chromophore-bearing proteins. For example, when fluorescently tagged ligands were applied to rod outer segment and lymphocyte plasma membrane, the photosensitization of 5-iodonaphthalene-1-azide occurred selectively at its nearest neighbors (Scheme 5d).[35] In contrast, random labeling was observed when non-conjugated fluorescein or direct UV photolysis was applied. This sensitization strategy was also used to selectively label functional multidrug transporters in living drug-resistant tumor cells.[36] When the cells exhibit resistance to drugs which are also effective sensitizers, such as doxorubicin, it will be selectivity labeled. When the calcium channel blocker verapamil was added to reverse the resistance to doxorubicin, the doxorubicin-induced selective labeling was abolished.

The visible-light-induced photosensitization also enables photodecaging via bond-cleavage reactions. Steiner and co-workers reported the thioxanthenone absorbing at 405 nm to sensitize the *ortho*-nitro group by intramolecular energy transfer.[37] After hydrogen-transfer, β-elimination and fragmentation, this reaction releases the free hydroxyl group. Winssinger and co-workers later used this photosensitized decaging reaction for DNA sensing (Scheme 5e).[38] They prepared two complementary DNA strands with one carrying the sensitizer thioxanthenone and the other carrying the *ortho*-nitro group. When they were incubated together, the hybridized DNA strands with close proximity facilitated energy-transfer to release a fluorescent rhodamine.



Scheme 5. The visible-light-induced biomolecule-compatible reactions with energy-transfer pathway. (a) The energy-transfer mechanism and the presentative rose bengal example. (b) The photosensitized reaction of dioxygen. (c) The photosensitized reaction of organic azides on proteins. (d) The ligand-directed photosensitized reaction of organic azides on membrane proteins. (e) The photosensitized reaction of visible-light-induced photodecaging.

Conclusion

In conclusion, the visible-light-induced biomolecule-compatible reactions provide high spatial and temporal precision with external light modulation. They are insensitive to visible light without photocatalyst/photonsensitizer and lack the general phototoxicity of UV light. Based on the reaction mechanisms, they are categorized to oxidative-quench, reductive-quench, and energy-transfer pathways. The oxidative-quench type reactions are currently widely used for endogenous protein cross-linking, and new electron acceptors are developed for more selective oxidations. The reductive-quench type reactions are mostly used for visible-light-induced biomolecule photodecaging, and the new bond-formation reactions are emerging. The energy-transfer type reactions are used to generate photoexcited intermediates with visible light rather than UV light irradiation.

Outlook

There are two aspects for the future improvements of visible-light-induced biomolecule-compatible reactions: (i) biomolecule-compatibility: transition metal-free catalysts, stable substrates, and fast reaction kinetics; (ii) visible-light-induction: the quantum efficiency, the selective activation of substrates by the photocatalyst/photosensitizer, and the longer biocompatible wavelength. With their further developments, the visible-light-induced biomolecule-compatible reactions will be more useful and applicable for solving biological questions that are difficult or unimaginable with current methods.

Acknowledgments

Financial support was provided by National Basic Research Program of China 2014CB910304, National Natural Science Foundation of China 21272260, 21472230, 'Thousand Talents Program' Young Investigator Award, and start up fund from State Key Laboratory of Bioorganic and Natural Products Chemistry, and Chinese Academy of Sciences.

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