REVIEW

Photo-induced protein modifications: a range of biological consequences and applications

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Abstract

Proteins are the most abundant biomolecules in living organisms and tissues and are also present in many natural and processed foods and beverages, as well as in pharmaceuticals and therapeutics. When exposed to UV–visible light, proteins containing endogenous or exogenous chromophores can undergo direct and indirect photochemical processes, resulting in protein modifications including oxidation of residues, cross-linking, proteolysis, covalent binding to molecules and interfaces, and conformational changes. When these modifications occur in an uncontrolled manner in a physiological context, they can lead to biological dysfunctions that ultimately result in cell death. However, rational design strategies involving light-activated protein modification have proven to be a valuable tool for the modulation of protein function or even for the construction of new biomaterials. This mini-review describes the fundamentals of photochemical processes in proteins and explores some of their emerging biomedical and nanobiotechnological applications, such as photodynamic therapy (PDT), photobonding for wound healing, photobioprinting, photoimmobilization of biosensors and enzymes for sensing, and biocatalysis, among others.

Keywords Protein photochemistry · Photosensitization · Photooxidation · Photocrosslinking · Photobonding
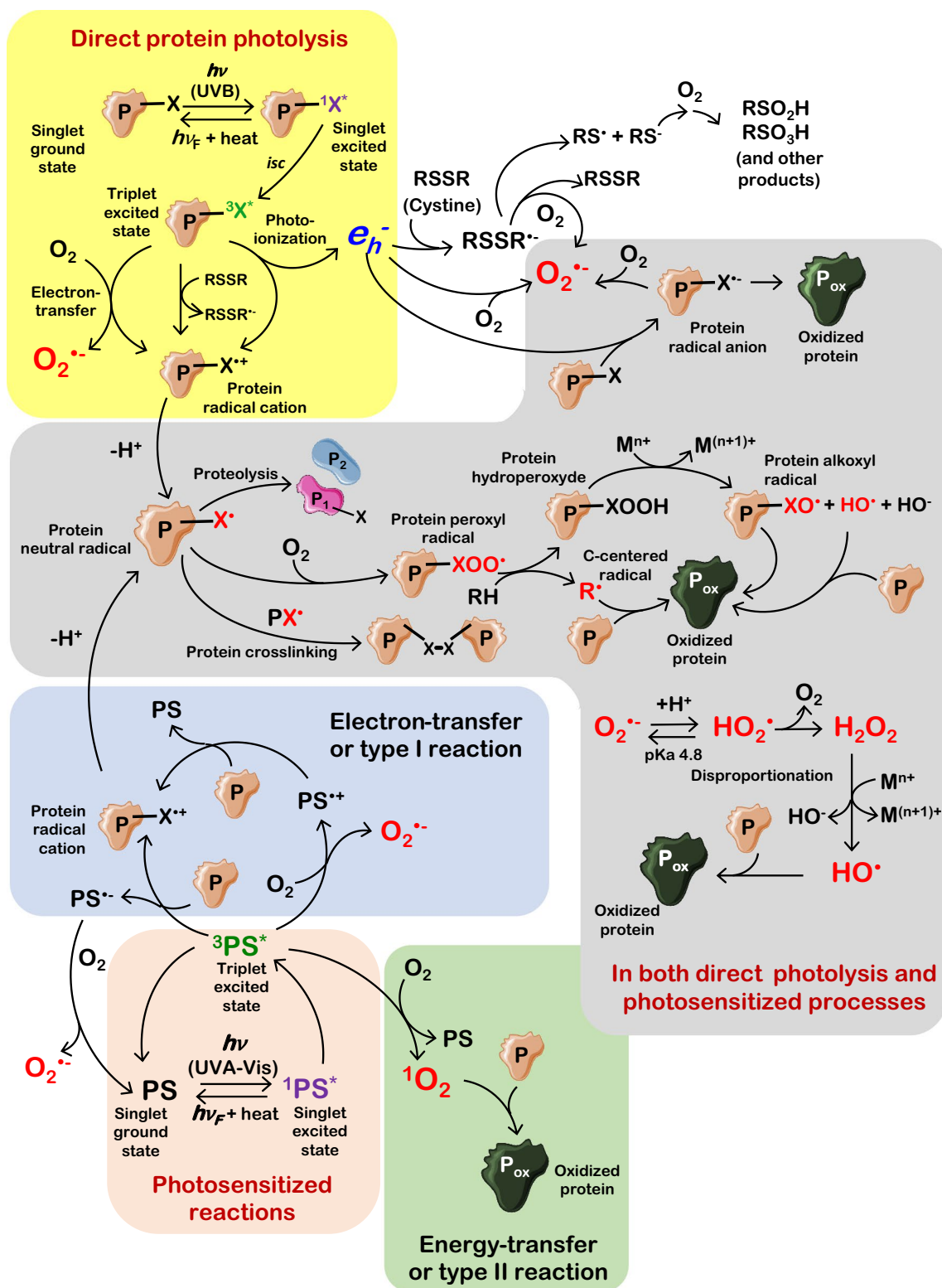
Direct and photosensitized photochemistry of proteins

The light absorption properties of proteins depend on the amino acid sequence, and generally, the lowest-energy UV absorption bands of proteins are between 250 and 320 nm, mainly due to tryptophan (Trp, $\lambda_{max} = 280$ nm and $\varepsilon = 5600$ M^{-1} cm^{-1}), tyrosine (Tyr, $\lambda_{max} = 275$ nm and $\varepsilon = 1400$ M^{-1} cm^{-1}), phenylalanine (Phe, $\lambda_{max} = 257$ nm and $\varepsilon = 200$ M^{-1} cm^{-1}), and, to a lesser extent, disulfide bonds, with a broad absorbance between 250 and 320 nm (Prasad et al. 2017). Therefore, when exposed to ambient light, proteins with aromatic residues (PX, X = Tyr, Trp, and Phe) absorb mainly solar or artificial UVB radiation (280–320 nm), generating the short-lived (ns) singlet excited state (P^1X^*), Fig. 1. This state decays by photophysical

unimolecular pathways to the ground state, emitting fluorescence and heat, and by intersystem crossing (*isc*) to the long-lived triplet state (P^3X^*). The latter excited state can generate the neutral radical (PX$^\bullet$) and the solvated electron e_h^- by unimolecular photoionization reaction, or reacts with dissolved oxygen O_2 and other molecules (e.g., cystine RSSR), generating the protein radical cation (PX$^{\bullet+}$) that rapidly deprotonate to the neutral radical (PX$^\bullet$), and the anion radical superoxide $O_2^{\bullet-}$ and RSSR$^{\bullet-}$, respectively (Kerwin and Remmele Jr. 2006; Davies 2016). Subsequently, the PX$^\bullet$ can add O_2 to form the peroxyl radical PXOO$^\bullet$, which in the presence of a hydrogen donor RH yields the protein hydroperoxide PXOOH and a carbon-centered radical R$^\bullet$. In turn, the e_h^- may react either with O_2 or any C- and N-terminus of other protein residues or RSSR, to produce $O_2^{\bullet-}$ and radical anions (e.g., P$^{\bullet-}$ and RSSR$^{\bullet-}$). Under physiological conditions, $O_2^{\bullet-}$ is the predominant species in equilibrium with HO$_2^\bullet$ (pKa = 4.8), which can be disproportionate to hydrogen peroxide (H_2O_2) (Hayyan et al. 2016). Although these reactive oxygen species (ROS) are relatively damaging to biomolecules, the presence of heavy cations (M^{n+} = Fe^{2+}, Cu^{2+}, etc.) disproportionate H_2O_2 to form the highly reactive hydroxyl radical (HO$^\bullet$) and also catalyzes the degradation

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Fig. 1 Schematic representation of the main photophysical and photochemical pathways occurring by direct UVB excitation of the proteins, and by UVA-vis excitation of a photosensitizer molecule (PS) leading to photosensitized reactions. Adapted from (Kerwin and Remmele Jr. 2006; Pattison et al. 2012; Davies 2016; Baptista et al. 2021; Hipper et al. 2021)

of PXOOH to yield alkoxyl radicals PXO$^\bullet$ and HO$^\bullet$ causing extensive oxidative damage (Davies 2016; Baptista et al. 2021). Besides all the photoinduced oxidative degradation pathways shown in Fig. 1; the generation of crosslinking (PX-XP) and proteolysis products by the radical recombination of two PX$^\bullet$ and radical-mediated break-bond chain reactions are also feasible, respectively (Pattison et al. 2012; Davies 2016; Hipper et al. 2021).

Although proteins are transparent to the light above the UVB region, some endogenous (e.g., pterins, flavins, porphyrins) or exogenous (e.g., organic dyes, drugs, metal complexes) molecules, either unbound or covalently or non-covalently bound to proteins, can absorb UVA-vis light (320–800 nm) and trigger photochemical reactions that modify another molecular entity, in most cases without self-degradation. This process is called *photosensitization*, and the molecule that absorbs the light is the photosensitizer (PS) (Baptista et al. 2021). In biological milieus, photosensitized processes can generate secondary reactive intermediates (e.g., reactive oxygen species, side-chain radicals) that damage or modify the protein structure (Alarcón et al. 2009, 2010, 2017; Zainudin et al. 2019; Savina et al. 2020; Lorente et al. 2021). Photosensitized processes are initiated by the long-lived (μs) triplet excited state of PS (^{3}PS*) formed by intersystem crossing from the singlet excited-state ^{1}PS* (Baptista et al. 2021), Fig. 1. Since the excited states are stronger oxidizing or reducing agents than the ground states, and ^{3}PS* can react with surrounding molecules, such as PX and O$_2$ by an electron-transfer reaction depending on the value of the free energy change as the driving force, i.e., $\Delta G = -nF\Delta E$. Thus, depending on the difference between the excited-state reduction potential value of ^{3}PS*, $^*E_\text{red}^\circ$(PS*/PS$^{\bullet-}$), and oxidation potential of the PX, E_ox° (PX/ PX$^{\bullet+}$), the formation of PS$^{\bullet-}$ and PX$^{\bullet+}$ can be feasible. In aerated neutral aqueous solutions, E_red° (O$_2$/ O$_2^{\bullet-}$) = -0.18 V (*vs.* NHE at 25 °C) (Koppenol et al. 2010), then the electron-transfer reaction from ^{3}PS* to O$_2$ will occur at excited state oxidation potentials of PS $^*E_\text{ox}^\circ$(PS*/PS$^{\bullet+}$) > 0.18 V, producing O$_2^{\bullet-}$ and PX$^{\bullet+}$. Once PX$^{\bullet+}$ is formed, this species follows the cascade of side reactions discussed above to produce proteolysis, cross-linking, and oxidized products. Eventually, ground-state PS is recovered from both ion-radical species PS$^{\bullet+}$ or PS$^{\bullet-}$ by the back electron-transfer reactions with O$_2^{\bullet-}$ and PX, respectively, Fig. 1.

Moreover, ^{3}PS* can react with O$_2$ (a triplet state) by energy transfer to generate the basal state of PS and singlet oxygen ^{1}O$_2$, which is the lowest excited state of O$_2$ with an energy gap $E_S(^1\text{O}_2) = 22.5$ kcal.mol^{-1}. Since the excited triplet-state energy, $E_T(^3\text{PS*})$, of most PS is higher than $E_S(^1\text{O}_2)$, the energy-transfer reaction is very efficient ($k \approx 10^9$ M^{-1} s^{-1}) because it is a downward energy and spin-allowed process (Schweitzer and Schmidt 2003). Both electron- and energy-transfer processes of ^{3}PS* with O$_2$ are termed type I and type II mechanisms, respectively. However, this classification does not mean that only ROS species are involved in oxidative degradation pathways of any biological substrate (BS), since in type I processes the chemical changes can be also produced by radical species formed by oxidation of BS, i.e., BS$^{\bullet+}$/BS$^\bullet$, despite O$_2^{\bullet-}$ is involved in further reactions; while in type II reactions, ^{1}O$_2$ is the only ROS responsible for BS photooxidation (vide infra) (Baptista et al. 2021). Finally, oxygen-independent photosensitized reactions can also occur, such as the formation of photo-adducts by covalent binding of the PS to the protein (P-PS), resulting in the formation of a macromolecular PS (Baptista et al. 2017, 2021).

Protein photooxidation

Direct photolysis or photosensitized reactions with a PS result in photooxidative changes of the proteins that may include the formation of side chain carbonyls and (hydro) peroxides by the addition of oxygen atoms, intra- and inter-molecular crosslinking via radical species, fragmentation of the main chain bond, mainly involving Trp, Tyr, cysteine (Cys), histidine (His), and methionine (Met) residues, as described in several reviews (Kerwin and Remmele Jr. 2006; Grosvenor et al. 2010; Pattison et al. 2012; Hawkins and Davies 2019; Hipper et al. 2021).

Under aerobic conditions, both direct photolysis and photosensitized type I reactions can give rise to similar intermediate and end products, as the key intermediate PX$^\bullet$, which by sequential side reactions with the addition of oxygen atoms and/or scission of bonds produces oxidized side-chain radicals and also the highly reactive HO$^\bullet$. In contrast, in photosensitized type II reactions, ^{1}O$_2$ is the only oxidation intermediate, which is a non-radical, highly reactive, electrophilic species with a lifetime of ≈ 3 μs in aqueous media (Schweitzer and Schmidt 2003), enough to diffuse into protein solutions by reacting with π- and *n*-electrons of Tyr, Trp, Met, Cys, and His, oxidizing them with rate constants between 0.2 and 5×10^7 M^{-1} s^{-1} (Michaeli and Feitelson 1994). Typical reactions of ^{1}O$_2$ with olefinic bonds include (i) [2 + 4] cycloadditions to produce endoperoxides, (ii) [2 + 2] cycloadditions forming dioxetane molecules, and (iii) *ene*-type reactions or phenol oxidations to produce hydroperoxides; while residues with sulfuryl groups are oxidized to sulfoxides (Greer 2006). Figure 2 summarizes the main photooxidation products obtained from the degradation of Tyr, Trp, Phe, Cys, Met, and His residues of proteins, among others, by both direct photolysis and photosensitized reactions (Kerwin and Remmele Jr. 2006; Pattison et al. 2012; Schöneich 2017; Hipper et al. 2021). These oxidative modifications in proteins have biological consequences
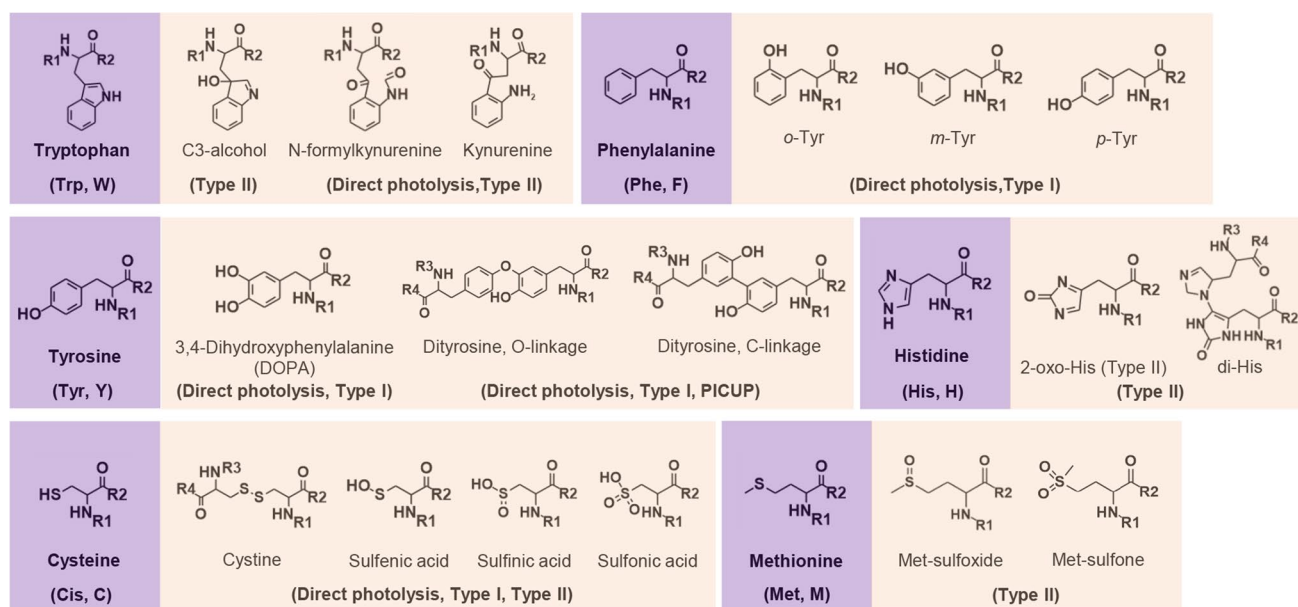
Fig. 2 Representative oxidation derivatives of the reactive residues Trp, Tyr, Phe, His, Cys, and Met of proteins exposed to direct photolysis and photosensitized reactions. Adapted from (Kerwin and Remmele Jr. 2006; Grosvenor et al. 2010; Pattison et al. 2012; Davies 2016; Hipper et al. 2021)

like protein denaturalization, aggregation, malfunctioning, loss of enzymatic activity, changes in cell signaling, redox homeostasis, proteolytic turnover of damaged molecules, and cell survival (Pattison et al. 2012; Fuentes-Lemus and López-Alarcón 2020). Among the deleterious effects of photooxidations can be mentioned the formation of molecular filters and insoluble protein aggregates in cataractogenesis (Davies and Truscott 2001). In particular, oxidation of Trp in proteins leads to the formation of *N*-formyl kynurenine (NFK) and kynurenine (KYN) residues, which are UVA photosensitizers that transform the oxidized protein into a macromolecular PS (Parker et al. 2004; Savina et al. 2020). In addition, Tyr- and Trp-derived radical residues exposed to the solvent are prone to recombine to form diTyr and diTrp crosslinking, as well as Tyr-Trp crossed dimers (Fuentes-Lemus et al. 2022).

Foods and beverages may be affected by photooxidations. For instance, blue-light absorption by riboflavin (vitamin B2) as endogenous PS in milk and beer leads to the $^1O_2$-mediated oxidation of sulfur-containing amino acids in proteins with the formation of off-flavors and off-odors (Hellwig 2019). The photo yellowing of wool fibers is also produced by $^1O_2$-mediated oxidation of Trp to form NFK and KYN and of Tyr in diTyr, and DOPA, among others (Dyer et al. 2006). During the preparation and handling of therapeutic antibodies and protein preparations, photooxidative degradation occurs during exposure to ambient light by impurities acting as PS. Since the impurities can be not destroyed at the end of photosensitization reactions, to ensure the quality of proteins in complex matrices, it is necessary to analyze the photodegradation processes of protein formulations and to protect them from ambient light during manufacturing and storage (Hipper et al. 2021).

Oxidative damage of biological substrates (DNA, lipids, proteins, etc.) produced exclusively by type I and II photosensitized reactions that ultimately lead to cell death is called photodynamic action (PDA) (Kessel 2021). This effect is being used beneficially in medical and clinical applications and is called photodynamic therapy (PDT), which includes the elimination of tumor cells (Benov 2015), pathogenic microbes (Liu et al. 2015; Vera et al. 2021), and the treatment of skin wounds (Nesi-Reis et al. 2018). The advantages of PDT are its near-null invasiveness, high spatial control and target selectivity, low inflammatory effects, no or very low development of microbial resistance, and the absence of toxic effects in the dark (Cieplik et al. 2018).

Photocrosslinking

Protein photocrosslinking refers to the photoinduced formation of intra- or inter-protein covalent bonds, conducting structural changes, dimerization, and/or oligomerization (Mishra et al. 2020). Photocrosslinking mechanisms can include: (i) recombination of intermediate protein radicals PX$^\bullet$ generated by either direct photolysis or photosensitized reactions (Fig. 1) (Wertheimer et al. 2019; Redmond and Kochevar 2019); and (ii) by photoclick chemistry approaches

using specific agents as photoinitiators (e.g., aryl azides, diazirines, and benzophenones) that can undergo various reactions such as 1,3-dipolar cycloadditions, Diels – Alder and inverse electron demand Diels – Alder additions, radical propagation and chain-transfer, and nucleophilic addition (Fairbanks et al. 2021). Upon absorption of light by the photoinitiator, the above reactions proceed by any of these mechanisms: (i) photocleavage with loss of N_2, CO_2, or some protecting group to generate a reactive intermediate; (ii) isomerization of the photoactivated precursor to give rise to a highly unstable intermediate that can react with the molecular partner or revert to the non-activated state; and (iii) by the intervention of a photocatalyst (Kumar and Lin 2021). These "photoclick handles" can be incorporated into proteins by chemical binding, by chemoenzymatic modification, or by site-directed mutagenesis by specific amino acid substitution. (Yamaguchi et al. 2016; Sadiki et al. 2020; Sandland et al. 2021).

As for the crosslinking of native proteins, the so-called induced photocrosslinking of unmodified proteins (PICUP) (Fancy and Kodadek 1999; Kodadek et al. 2005) is a convenient and efficient method, since by means of a brief irradiation (few seconds) with blue light of the tris(2,2′-bipyridyl) ruthenium (II) complex, $Ru(bpy)_3^{2+}$, in the presence of an electron acceptor such as the persulfate anion $S_2O_8^{2-}$, the oxidized cation $Ru(bpy)_3^{3+}$ is produced, a potent oxidant capable of abstracting an electron from a donor amino acid as Tyr, to generate a protein tyrosyl radical (PTyr$^\bullet$) and recover $Ru(bpy)_3^{2+}$, Fig. 3. The recombination reaction between PTyr$^\bullet$ of different neighboring proteins give rise to covalent cross-linking via diTyr bonds. Since diTyr bonds are detectable by UV–vis and fluorescence spectroscopies (for deprotonated diTyr, $pK_a \approx 7$, $\lambda_{ab} = 320$ nm and $\lambda_{em} = 400$ nm) (Malencik and Anderson 2003), and by SDS-PAGE, the PICUP method allows the easy monitoring of the

oligomer populations as a function of light dose (Kodadek et al. 2005; Borsarelli et al. 2012; Rey et al. 2021). It has been recently shown that the oligomerization pattern obtained by PICUP is almost O_2-independent, but under aerobic condition the $^1O_2$-mediated oxidation of protein residues also occurs, increasing the total content of carbonyl groups with the formation of NFK and KYN by oxidation of Trp residues (Rey et al. 2021), Fig. 3. This is the consequence of the efficient generation of $^1O_2$ by $Ru(bpy)_3^{2+}$ in protein solutions (Giménez et al. 2016). Therefore, to avoid or minimize $^1O_2$-mediated modification of proteins by PICUP, anaerobic conditions are recommended.

Protein photocrosslinking is a powerful tool for the study of protein–protein interactions in living cells combined with the identification of the crosslinked proteins by mass spectrometry, enabling the identification of protein–protein complexes and the mapping of protein interaction networks (Müller et al. 2019). The PICUP method has been applied to study the effect of oligomerization on the interactions of several neuronal amyloidogenic proteins (Bitan and Teplow 2004; Piening et al. 2006; Borsarelli et al. 2012). Compared to chemical crosslinking, photocrosslinking offers unique advantages, such as the rapid production of oligomers under mild conditions, with high selectivity and no toxicity due to the low concentration of photocrosslinking agents used (Mishra et al. 2021).

Photobonding

Photopolymerization is a special medical application of photosensitized crosslinking that uses visible light to bond tissues for wound suturing and tissue repair (Tsao et al. 2012; Pupkaite et al. 2016, 2017; Alarcón et al. 2017; Redmond and Kochevar 2019). In photobonding, the main

Fig. 3 Schematic representation of the photocrosslinking reaction of unmodified proteins (PICUP) under both anaerobic and aerobic conditions. The latter condition includes photosensitized generation of singlet oxygen ($^1O_2$) by the $Ru(bpy)_3^{2+}$ coordination complex and subsequent $^1O_2$-mediated oxidation of the protein and/or cross-linked oligomers. Adapted from (Fancy and Kodadek 1999; Rey et al. 2021)
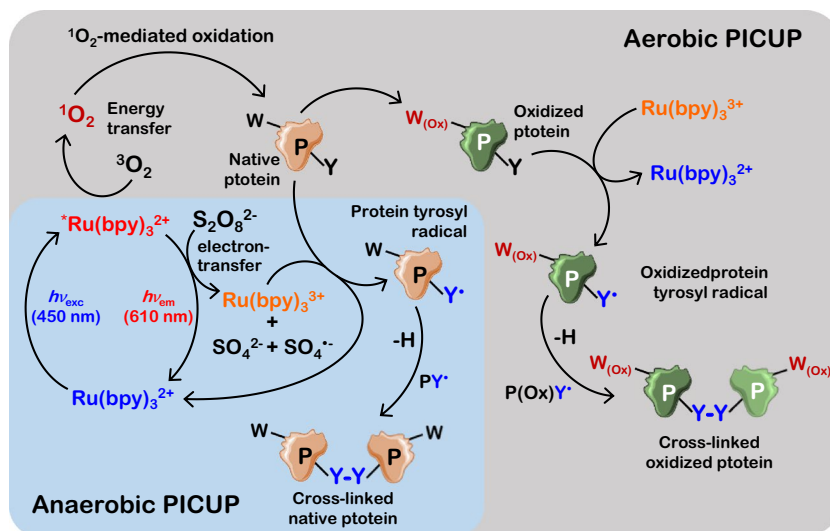
target macromolecule for tissue attachment is collagen, an abundant extracellular protein that provides support. Typically, a PS (e.g., riboflavin or rose Bengal) is irradiated with visible light, triggering by radical chemistry the covalent cross-linking between collagen and target tissue to bind them together. Photobonding applications also include the treatment of accommodative intraocular lenses to reverse presbyopia (Alejandre-Alba et al. 2018) and in tissue bioprinting where photoactivated materials can be used to drive the formation or degradation of chemical bonds with spatiotemporal control (Van Hoorick et al. 2019; Mu et al. 2020). Photobonding offers many advantages over other medical treatments, such as the absence of long-term side effects when properly administered, and is usually performed as an outpatient procedure in a short time. In summary, photobonding is an adhesive- and solvent-free alternative to traditional tissue bonding methods that can be cytotoxic and is also a promising technique in regenerative medicine, such as for wound closure and tissue bioprinting with a resolution and build size ranging from nanometers to centimeters (Mironov et al. 2009; Mu et al. 2021).

Light-induced formation of new biomaterials

Due to the suitable reactivity of various residues, different sizes, and shapes, proteins are adequate building blocks for the preparation of new biomaterials. Bovine serum albumin, lysozyme, collagen, and fibrinogen are examples of proteins used for this purpose, and the design of biomaterials can combine them with synthetic chemical groups, other macromolecules, and/or nanomaterials (Jutz and Böker 2011; Bao et al. 2015). Recent examples are the preparation of biomaterials with specific properties, such as hydrogels, which can be used for tissue engineering and drug delivery (Elvin and Vuocolo 2011; Abbate et al. 2012), allowing further functional modulation by anchoring molecular modules to the sidechains of the backbone proteins (Hardy et al. 2018); and protein/enzyme immobilization onto carbon-based materials, metallic surfaces, or protein fibrils, for many applications including biocatalysis and biosensing (Chaves et al. 2016; Alonso et al. 2018; Thomas et al. 2020).

In some cases, it is possible to take advantage of intrinsic structural features of proteins, such as the accessibility of photoreactive residues that are not compromised in the active/binding site, thus allowing direct or photosensitized crosslinking (Chaves et al. 2016; Della Ventura et al. 2019). Some photoclick reactions were also adapted to be selectively targeted to side chains of Cys or Lys residues (Alonso et al. 2018; Choi et al. 2020; Guo et al. 2020). Thanks to the development of numerous strategies to modify or replace specific amino acid residues, the incorporation of photoclick

handles into protein structure has been greatly improved in the last decade (Fairbanks et al. 2021). As mentioned above, these strategies include chemical modifications, chemoenzymatic modifications, and optogenetic approaches. (Reddington et al. 2013; Thomas et al. 2020).

Summary and perspectives

Proteins are the most abundant biomolecules in living organisms and tissues and are also present in many natural and processed foods and beverages, as well as in pharmaceutical and therapeutic (Hayes 2020; Jiang et al. 2020; Hipper et al. 2021). Whether under direct or photosensitized illumination, proteins can be converted into reactive macromolecules that can result in a plethora of modifications, such as oxidation and reduction of amino acid residues, conformational changes, proteolysis, cross-linking, covalent binding to other molecules, immobilization on surfaces and interfaces, formation of nanoaggregates and nanocomposites, all modifying the functionality of native proteins. Promising new applications related to these photoinduced modifications of proteins are emerging in the fields of biomedicine and nanobiotechnology, such as PDT, photobonding and wound healing, photo-bioprinting, photo-immobilization of biosensors and enzymes for sensing and biocatalysis, among others.

Declarations

Ethical approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

References

Abbate V, Kong X, Bansal SS (2012) Photocrosslinked bovine serum albumin hydrogels with partial retention of esterase activity. Enzyme Microb Technol 50:130–136. https://doi.org/10.1016/j.enzmictec.2011.11.002

Alarcón E, Edwards AM, Garcia AM et al (2009) Photophysics and photochemistry of zinc phthalocyanine/bovine serum albumin

adducts. Photochem Photobiol Sci 8:255–263. https://doi.org/10.1039/b815726j

Alarcón E, Edwards AM, Aspee A et al (2010) Photophysics and photochemistry of dyes bound to human serum albumin are determined by the dye localization. Photochem Photobiol Sci 9:93–102. https://doi.org/10.1039/b9pp00091g

Alarcón EI, Poblete H, Roh H et al (2017) Rose Bengal binding to collagen and tissue photobonding. ACS Omega 2:6646–6657. https://doi.org/10.1021/acsomega.7b00675

Alejandre-Alba N, Gutierrez-Contreras R, Dorronsoro C, Marcos S (2018) Intraocular photobonding to enable accommodating intraocular lens function. Transl vis Sci Technol 7:1–9. https://doi.org/10.1167/tvst.7.5.27

Alonso R, Jiménez-Meneses P, García-Rupérez J et al (2018) Thiol-ene click chemistry towards easy microarraying of half-antibodies. Chem Commun 54:6144–6147. https://doi.org/10.1039/c8cc01369a

Bao C, Zhu L, Lin Q, Tian H (2015) Building biomedical materials using photochemical bond cleavage. Adv Mater 27:1647–1662. https://doi.org/10.1002/adma.201403783

Baptista MS, Cadet J, Di Mascio P et al (2017) Type I and type II photosensitized oxidation reactions: guidelines and mechanistic pathways. Photochem Photobiol 93:912–919. https://doi.org/10.1111/php.12716

Baptista MS, Cadet J, Greer A, Thomas AH (2021) Photosensitization reactions of biomolecules: definition, targets and mechanisms. Photochem Photobiol 97:1456–1483. https://doi.org/10.1111/php.13470

Benov L (2015) Photodynamic therapy: current status and future directions. Med Princ Pract 24:14–28. https://doi.org/10.1159/000362416

Bitan GAL, Teplow DB (2004) Rapid photochemical cross-linkings - a new tool for studies of metastable, amyloidogenic protein assemblies. Acc Chem Res 37:357–364. https://doi.org/10.1021/ar000214l

Borsarelli CD, Falomir-Lockhart LJ, Ostatná V et al (2012) Biophysical properties and cellular toxicity of covalent crosslinked oligomers of α-synuclein formed by photoinduced side-chain tyrosyl radicals. Free Radic Biol Med 53:1004–1015. https://doi.org/10.1016/j.freeradbiomed.2012.06.035

Chaves S, Pera LM, Avila CL et al (2016) Towards efficient biocatalysts: photo-immobilization of a lipase on novel lysozyme amyloid-like nanofibrils. RSC Adv 6:8528–8538. https://doi.org/10.1039/C5RA19590J

Choi H, Kim M, Jang J, Hong S (2020) Visible-light-induced cysteine-specific bioconjugation: biocompatible thiol–ene click chemistry. Angew Chemie - Int Ed 59:22514–22522. https://doi.org/10.1002/anie.202010217

Cieplik F, Deng D, Crielaard W et al (2018) Antimicrobial photodynamic therapy–what we know and what we don't. Crit Rev Microbiol 44:571–589. https://doi.org/10.1080/1040841X.2018.1467876

Davies MJ (2016) Protein oxidation and peroxidation. Biochem J 473:805–825. https://doi.org/10.1042/bj20151227

Davies MJ, Truscott RJW (2001) Photo-oxidation of proteins and its role in cataractogenesis. J Photochem Photobiol B Biol 63:114–125. https://doi.org/10.1016/S1011-1344(01)00208-1

Della Ventura B, Banchelli M, Funari R et al (2019) Biosensor surface functionalization by a simple photochemical immobilization of antibodies: experimental characterization by mass spectrometry and surface enhanced Raman spectroscopy. Analyst 144:6871–6880. https://doi.org/10.1039/c9an00443b

Dyer JM, Bringans SD, Bryson WG (2006) Characterisation of photo-oxidation products within photoyellowed wool proteins: tryptophan and tyrosine derived chromophores. Photochem Photobiol Sci 5:698–706. https://doi.org/10.1039/b603030k

Elvin C, Vuocolo T (2011) Photochemical crosslinking of proteins to make novel biomedical materials. Aust Biochem 42:15–18

Fairbanks BD, Macdougall LJ, Mavila S et al (2021) Photoclick chemistry: a bright idea. Chem Rev 121:6915–6990. https://doi.org/10.1021/acs.chemrev.0c01212

Fancy DA, Kodadek T (1999) Chemistry for the analysis of protein-protein interactions: rapid and efficient cross-linking triggered by long wavelength light. Proc Natl Acad Sci U S A 96:6020–6024. https://doi.org/10.1073/pnas.96.11.6020

Fuentes-Lemus E, López-Alarcón C (2020) Photo-induced protein oxidation: mechanisms, consequences and medical applications. Essays Biochem 64:33–44. https://doi.org/10.1042/EBC20190044

Fuentes-Lemus E, Hägglund P, López-Alarcón C, Davies MJ (2022) Oxidative crosslinking of peptides and proteins: mechanisms of formation, detection, characterization and quantification. Molecules 27:1–31. https://doi.org/10.3390/molecules27010015

Giménez RE, Vargová V, Rey V et al (2016) Interaction of singlet oxygen with bovine serum albumin and the role of the protein nano-compartmentalization. Free Radic Biol Med 94:99–109. https://doi.org/10.1016/j.freeradbiomed.2016.02.014

Greer A (2006) Christopher foote's discovery of oxidation reactions. Acc Chem Res 39:797–804

Grosvenor AJ, Morton JD, Dyer JM (2010) Profiling of residue-level photo-oxidative damage in peptides. Amino Acids 39:285–296. https://doi.org/10.1007/s00726-009-0440-7

Guo AD, Wei D, Nie HJ et al (2020) Light-induced primary amines and o-nitrobenzyl alcohols cyclization as a versatile photoclick reaction for modular conjugation. Nat Commun 11:5472. https://doi.org/10.1038/s41467-020-19274-y

Hardy JG, Bertin A, Torres-Rendon JG et al (2018) Facile photochemical modification of silk protein–based biomaterials. Macromol Biosci 18:1–6. https://doi.org/10.1002/mabi.201800216

Hawkins CL, Davies MJ (2019) Detection, identification and quantification of oxidative protein modifications. J Biol Chem jbc.REV119.006217. https://doi.org/10.1074/jbc.REV119.006217

Hayes M (2020) Measuring protein content in food: an overview of methods. Foods 9. https://doi.org/10.3390/foods9101340

Hayyan M, Hashim MA, Alnashef IM (2016) Superoxide ion: generation and chemical implications. Chem Rev 116:3029–3085. https://doi.org/10.1021/acs.chemrev.5b00407

Hellwig M (2019) The chemistry of protein oxidation in food. Angew Chemie - Int Ed 58:16742–16763. https://doi.org/10.1002/anie.201814144

Hipper E, Blech M, Hinderberger D et al (2021) Photo-oxidation of therapeutic protein formulations: from radical formation to analytical techniques. Pharmaceutics 14:72. https://doi.org/10.3390/pharmaceutics14010072

Jiang L, Wang M, Lin S et al (2020) A quantitative proteome map of the human body. Cell 183:269-283.e19. https://doi.org/10.1016/j.cell.2020.08.036

Jutz G, Böker A (2011) Bionanoparticles as functional macromolecular building blocks - a new class of nanomaterials. Polymer (guildf) 52:211–232. https://doi.org/10.1016/j.polymer.2010.11.047

Kerwin BA, Remmele RL Jr (2006) Protect from light: photodegradation and protein biologics. J Pharm Sci 96:1468–1479. https://doi.org/10.1002/jps

Kessel D (2021) Death pathways associated with photodynamic therapy. Photochem Photobiol 97:1101–1103. https://doi.org/10.1111/php.13436

Kodadek T, Duroux-Richard I, Bonnafous JC (2005) Techniques: oxidative cross-linking as an emergent tool for the analysis of receptor-mediated signalling events. Trends Pharmacol Sci 26:210–217. https://doi.org/10.1016/j.tips.2005.02.010

Koppenol WH, Stanbury DM, Bounds PL (2010) Electrode potentials of partially reduced oxygen species, from dioxygen to water. Free

Radic Biol Med 49:317–322. https://doi.org/10.1016/j.freeradbiomed.2010.04.011

Kumar GS, Lin Q (2021) Light-triggered click chemistry. Chem Rev 121:6991–7031. https://doi.org/10.1021/acs.chemrev.0c00799

Liu Y, Qin R, Zaat SAJ et al (2015) Antibacterial photodynamic therapy: overview of a promising approach to fight antibiotic-resistant bacterial infections. J Clin Transl Res 1:140–167. https://doi.org/10.18053/jctres.201503.002

Lorente C, Serrano MP, Vignoni M et al (2021) A model to understand type I oxidations of biomolecules photosensitized by pterins. J Photochem Photobiol 7:100045. https://doi.org/10.1016/j.jpap.2021.100045

Malencik DA, Anderson SR (2003) Dityrosine as a product of oxidative stress and fluorescent probe. Amino Acids 25:233–247. https://doi.org/10.1007/s00726-003-0014-z

Michaeli A, Feitelson J (1994) Reactivity of singlet oxygen toward amino acids and peptides. Photochem Photobiol 59:284–289. https://doi.org/10.1111/j.1751-1097.1994.tb05035.x

Mironov V, Visconti RP, Kasyanov V et al (2009) Organ printing: tissue spheroids as building blocks. Biomaterials 30:2164–2174. https://doi.org/10.1016/j.biomaterials.2008.12.084

Mishra PK, Yoo CM, Hong E, Rhee HW (2020) Photo-crosslinking: an emerging chemical tool for investigating molecular networks in live cells. ChemBioChem 21:924–932. https://doi.org/10.1002/cbic.201900600

Mishra P, Ahmed T, Singh L (2021) A comparative study of biosynthesized silver-nanoparticles from citrus maxima peel, pulp and seed: a special retrospect for antimicrobial activity. J Pharm Res Int 454–463. https://doi.org/10.9734/jpri/2021/v33i45b32827

Mu X, Sahoo JK, Cebe P, Kaplan DL (2020) Photo-crosslinked silk fibroin for 3D printing. Polymers (basel) 12:1–18. https://doi.org/10.3390/polym12122936

Mu X, Agostinacchio F, Xiang N et al (2021) Recent advances in 3D printing with protein-based inks. Prog Polym Sci 115:101375. https://doi.org/10.1016/j.progpolymsci.2021.101375

Müller F, Graziadei A, Rappsilber J (2019) Quantitative photo-crosslinking mass spectrometry revealing protein structure response to environmental changes. Anal Chem 91:9041–9048. https://doi.org/10.1021/acs.analchem.9b01339

Nesi-Reis V, Lera-Nonose DSSL, Oyama J et al (2018) Contribution of photodynamic therapy in wound healing: a systematic review. Photodiagnosis Photodyn Ther 21:294–305. https://doi.org/10.1016/j.pdpdt.2017.12.015

Parker NR, Jamie JF, Davies MJ, Truscott RJW (2004) Protein-bound kynurenine is a photosensitizer of oxidative damage. Free Radic Biol Med 37:1479–1489. https://doi.org/10.1016/j.freeradbiomed.2004.07.015

Pattison DI, Rahmanto S, Davies MJ et al (2012) Photo-oxidation of proteins. Photochem Photobiol Sci 11:38–53. https://doi.org/10.1039/c1pp05164d

Piening N, Weber P, Högen T et al (2006) Photo-induced crosslinking of prion protein oligomers and prions. Amyloid 13:67–77. https://doi.org/10.1080/13506120600722498

Prasad S, Mandal I, Singh S et al (2017) Near UV-visible electronic absorption originating from charged amino acids in a monomeric protein. Chem Sci 8:5416–5433. https://doi.org/10.1039/c7sc00880e

Pupkaite J, Temkit M, Kochevar I et al (2016) Tissue photo-bonding using biopolymer crosslinked with rose bengal. Eur Cells Mater 31:342107

Pupkaite J, Ahumada M, Mclaughlin S et al (2017) Collagen-based photoactive agent for tissue bonding. ACS Appl Mater Interfaces 9:9265–9270. https://doi.org/10.1021/acsami.7b01984

Reddington S, Watson P, Rizkallah P, et al (2013) Genetically encoding phenyl azide chemistry : new uses and ideas for classical biochemistry. 1177–1182. https://doi.org/10.1042/BST20130094

Redmond RW, Kochevar IE (2019) Medical applications of rose bengal- and riboflavin-photosensitized protein crosslinking. Photochem Photobiol 95:1097–1115. https://doi.org/10.1111/php.13126

Rey V, Abatedaga I, Vera C et al (2021) Photosensitized formation of soluble bionanoparticles of lysozyme. ChemistrySelect 6:13443–13451. https://doi.org/10.1002/slct.202103215

Sadiki A, Kercher EM, Lu H et al (2020) Site-specific bioconjugation and convergent click chemistry enhances antibody–chromophore conjugate binding efficiency. Photochem Photobiol 96:596–603. https://doi.org/10.1111/php.13231

Sandland J, Rimmer SD, Savoie H, Boyle RW (2021) Bio-orthogonal conjugation of a cationic metalloporphyrin to BSA and HSA via "click" chemistry. ChemBioChem 22:2624–2631. https://doi.org/10.1002/cbic.202100176

Savina ED, Tsentalovich YP, Sherin PS (2020) UV-a induced damage to lysozyme via type I photochemical reactions sensitized by kynurenic acid. Free Radic Biol Med 152:482–493. https://doi.org/10.1016/j.freeradbiomed.2019.11.017

Schöneich C (2017) Sulfur radical-induced redox modifications in proteins: analysis and mechanistic aspects. Antioxidants Redox Signal 26:388–405. https://doi.org/10.1089/ars.2016.6779

Schweitzer C, Schmidt R (2003) Physical mechanisms of generation and deactivation of singlet oxygen. Chem Rev 103:1685–1757. https://doi.org/10.1021/cr010371d

Thomas SK, Jamieson WD, Gwyther REA et al (2020) Site-specific protein photochemical covalent attachment to carbon nanotube side walls and its electronic impact on single molecule function. Bioconjug Chem 31:584–594. https://doi.org/10.1021/acs.bioconjchem.9b00719

Tsao S, Yao M, Tsao H et al (2012) Light-activated tissue bonding for excisional wound closure: a split-lesion clinical trial. Br J Dermatol 166:555–563. https://doi.org/10.1111/j.1365-2133.2011.10710.x

Van Hoorick J, Tytgat L, Dobos A et al (2019) (Photo-)crosslinkable gelatin derivatives for biofabrication applications. Acta Biomater 97:46–73. https://doi.org/10.1016/j.actbio.2019.07.035

Vera C, Tulli F, Borsarelli CD (2021) Photosensitization with supramolecular arrays for enhanced antimicrobial photodynamic treatments. Front Bioeng Biotechnol 9:655370. https://doi.org/10.3389/fbioe.2021.655370

Wertheimer CM, Elhardt C, Kaminsky SM et al (2019) Enhancing rose bengal-photosensitized protein crosslinking in the cornea. Investig Ophthalmol vis Sci 60:1845–1852. https://doi.org/10.1167/iovs.19-26604

Yamaguchi A, Matsuda T, Ohtake K et al (2016) Incorporation of a doubly functionalized synthetic amino acid into proteins for creating chemical and light-induced conjugates. Bioconjug Chem 27:198–206. https://doi.org/10.1021/acs.bioconjchem.5b00602

Zainudin MAM, Poojary MM, Jongberg S, Lund MN (2019) Light exposure accelerates oxidative protein polymerization in beef stored in high oxygen atmosphere. Food Chem 299:125132. https://doi.org/10.1016/j.foodchem.2019.125132