

Photochemotherapy: Light-Dependent Therapies in Medicine

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Because health care in America impacts everyone, any discussion of the topic captures the attention of students in the chemistry classroom, especially those interested in allied health careers. Photochemotherapy, the use of ultraviolet, visible, or near-infrared light with the administration of a photosensitizer in the treatment of disease (1), provides a method of bridging a discussion of chemistry with one of health care. Topics such as UV-vis spectroscopy, MO theory, polarity, and biochemistry can be introduced during a discussion of photochemotherapy.

The most commonly known form of photochemotherapy is photodynamic therapy (PDT), highlighted in the May 1999 issue of this *Journal* (2). Although PDT is predominately used for the treatment of cancers (3), this paper will focus on the chemical concepts that have led to the less well-known uses of PDT, as well as the photochemotherapeutic method of extracorporeal photophoresis, or ECP. Photochemotherapy can be used to treat people with acne (4), macular degeneration (a leading cause of blindness in those over age 65 years, ref 5, 6), intimal hyperplasia (commonly causing a decrease in the diameter of arteries, ref 7, 8), and a wide range of autoimmune disorders (9–16) from psoriasis to rheumatoid arthritis and AIDS.

What Is Photodynamic Therapy?

Photodynamic therapy is characterized by three basic steps (Figure 1, ref 17). A photosensitizing agent is first chosen for the patient. Next, the photosensitizer must be administered, followed by a waiting period for absorption of the molecule into the cells. The final stage in the therapy is the death of the photosensitized cells, which is induced by visible light (usually 630–800 nm). Photodynamic therapy is a treatment primarily used for cancer (2, 17).

Only two photosensitizing agents are available in the United States for PDT treatment (6, 18), although many other compounds are under investigation (3). These photosensitizing agents are usually derivatives of the porphyrin macrocycle (Figure 2, ref 17). A discussion of the final step in PDT, which follows, will reveal the common thread that enables the use of these various compounds. It is first important to briefly discuss the events leading to the use of these

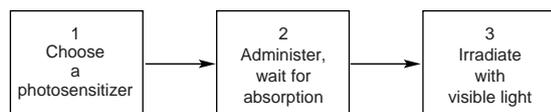


Figure 1. Three basic steps of PDT (17).

second-generation photosensitizers (17), so that a clear understanding of the ongoing nature of this research is established.

Hematoporphyrin derivative (HpD) was the first PDT agent approved by the United States Food and Drug Administration (USFDA). In December 1995, the USFDA granted approval of HpD for the treatment of obstructing esophageal cancer, followed by approval of HpD for the treatment of non-small-cell lung cancer in January 1998 (21). Age-related macular degeneration may now be treated with HpD as well. Approval was granted for this most recent application in April 2000 (6).

Hematoporphyrin derivative is a variable mixture of porphyrin dimers and oligomers with evidence that the component porphyrins are bonded by ether and ester linkages (Figure 3, ref 17). The lack of a single, pure compound is a significant problem when HpD is used clinically. Because any two batches of HpD will have variable concentrations of different compounds, the effectiveness of the samples for use in PDT may be unreliable. Scientists are not even sure which compound in HpD provides the most desirable characteristics. The inability to purify and characterize the components of HpD was an important factor leading to the need for new photosensitizers (17).

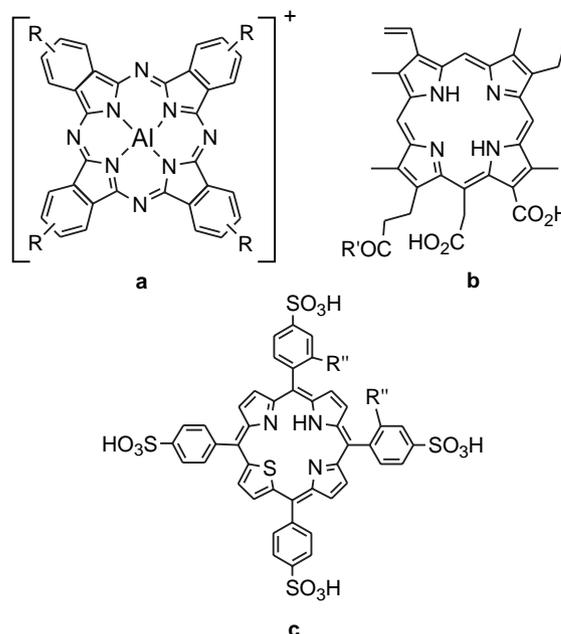


Figure 2. Some common PDT agents based on the porphyrin macrocycle: (a) aluminum phthalocyanine (R = H or SO₃) (19), (b) chlorin e₆ (R' = OH) (17), (c) 21-thiaporphyrin (R'' = OCH₃) (20).

The first and so far only one of these new photosensitizers to be approved by the USFDA is 5-aminolevulinic acid (ALA). Approval was granted for the treatment of precancerous skin lesions with ALA in December 1999 (18). Aminolevulinic acid is the precursor to the body's natural porphyrin (protoporphyrin IX or PPIX) in the heme-biosynthetic pathway (Figure 4). When ALA is applied to epithelial cells, the cells become loaded with ALA and the negative feedback mechanism that controls heme production is bypassed, causing the accumulation of PPIX in tissue (9).

The accumulation of ALA can be optimized with the additional use of iron-chelating agents. These agents, such as desferrioxamine (Figure 5), bind the iron and prevent the completion of heme production. Since the last step, illustrated in Figure 4, is inhibited, there is an augmentation of PPIX accumulation, important since the porphyrin is the photosensitizer (9).

The type of photosensitizer chosen and type of tumor being treated dictates the specifics of the second step of PDT. Most photosensitizers are injected, but oral or topical administration is also possible (3). The waiting period is the time allotted to achieve maximum localization of the drug within the target cells. The duration of this period may range from a few hours to a few days, depending on the type of photosensitizer used and tissue involved (3). Aminolevulinic acid, for example, can be applied directly to the skin. Studies show that PPIX accumulation is at its peak six hours after ALA application in this manner (9).

Localization of agents that are administered systemically is controlled by various factors. Polarity may be an issue, as tumors and acne-causing bacteria absorb lipophilic agents well (3, 4). Many other types of abnormal cells have been found to absorb porphyrin derivatives as well, but the exact mechanisms have not been determined in each case (7, 11).

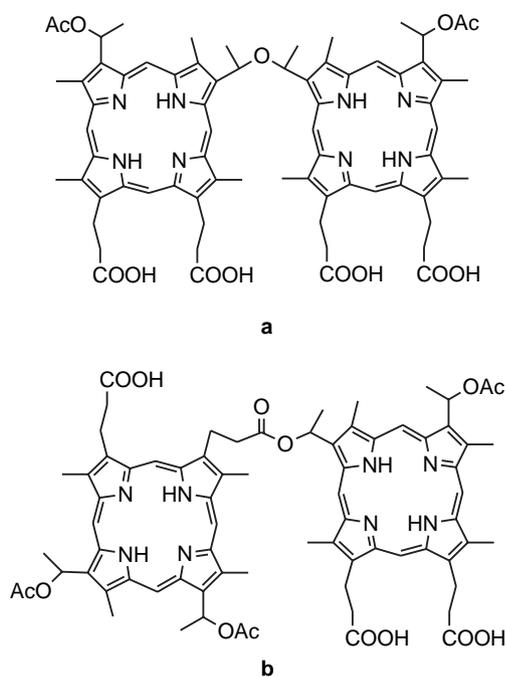


Figure 3. HpD dimers formed by (a) an ether linkage or (b) an ester linkage (17).

The electronic absorption properties of photosensitizing agents become important in the final step of PDT. Absorption of light far into the red region is sought, since increasing the wavelength by 100 nm can increase tissue penetration by up to 0.5 cm (2). Ideally, photosensitizing agents will be able to treat deeper tissues with a less-invasive procedure (17).

The wavelengths at which high values of molar absorptivity (ϵ) occur are specific to the agent and can be observed in the electronic or UV-vis spectra (Figure 6). Photosensitizing agents with larger ϵ in the desired range can reach the required level of excitation more efficiently. Because of the degree of conjugation typically found in porphyrin derivatives, the valence electrons can move about easily and excited-state energy levels may lie relatively close to the ground state.

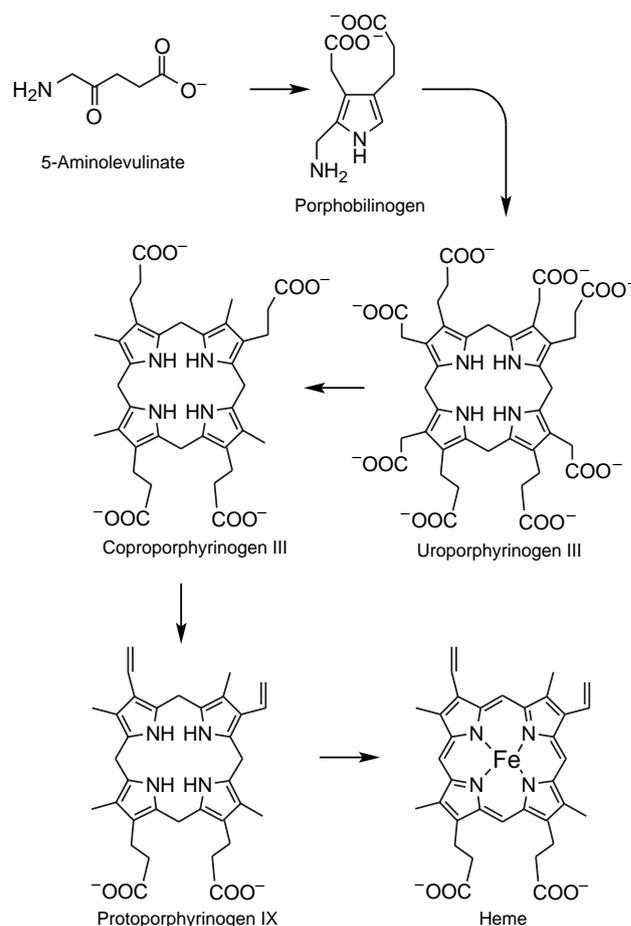


Figure 4. The heme-biosynthetic pathway (22) illustrating the production of heme from 5-ALA. The molecule immediately preceding heme is natural porphyrin (PPIX), not shown.

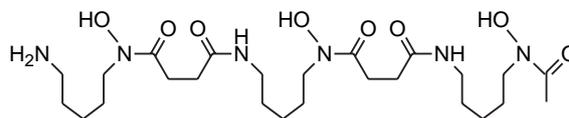


Figure 5. Desferrioxamine (23); an iron chelator that is commonly referenced in general chemistry laboratory manuals.

The frequency of light required to reach an excited-state energy level from the ground state may, therefore, lie within the visible region (24).

Singlet oxygen is the key to PDT; photosensitizing agents are only the carriers of the energy (17). Because singlet oxygen phosphoresces at 1270 nm and is transparent at such wavelengths, the ground-state molecule does not absorb the energy directly from light. The use of a photosensitizer allows the electronic transition to occur. Molecular oxygen can accept the energy readily due to the two unpaired electrons (Figure 7a, ref 26). The singlet species (Figure 7b) is extremely reactive, lasting for less than 0.04 μs and traveling less than 0.02 μm (3) before reacting with nearby biomolecules (17).

Unlike chemotherapy and radiation therapy, the target biomolecules in PDT are not the DNA strands of the cell (19). The cellular target that is currently being studied the most is the mitochondria (19, 27–29). More specifically, it is thought that the singlet oxygen species reacts with molecules in the plasma membrane (19), causing reactions similar to those in Figure 8.

The oxidation of the molecules within the membrane can cause important changes in membrane permeability and physiology (19). For example, the oxidation of long chain fatty acids can disrupt the normal stability of the cell membrane. In the mitochondria, this leads to the release of

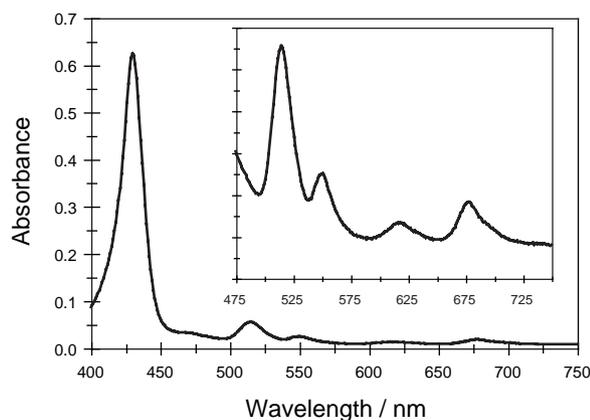


Figure 6. The electronic or UV-vis spectrum of thiaporphyrin (Figure 2c) (25). The absorbance band furthest to the right is located at 679 nm, while that of HpD (not shown) is at 630 nm.

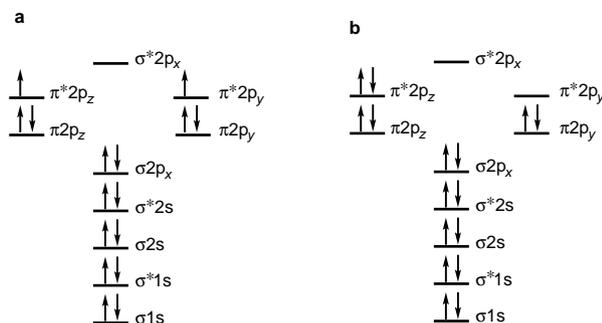


Figure 7. Molecular Orbital diagrams (26) of (a) ground state/triplet O_2 and (b) excited state, singlet O_2 . Notice the paired electrons in the degenerate HOMO of (b).

cytochrome c. Cytochrome c reacts with various other biomolecules in the cytosol, resulting in the activation of caspase-3 (cysteine requiring aspartate protease, ref 28, 30).

The importance of caspase-3 lies in the classification of the protein within the family. The molecule is normally downstream of a long series of caspase family activity. Caspase-3 is considered to be an effector caspase. Effectors have a smaller prodomain region and lack the ability to interact with death adapter molecules in the cell. As an effector, caspase-3 is responsible for the events of active cell death (30), or apoptosis (31). Apoptosis destroys the cell quickly, through DNA fragmentation and protein cleavage (28, 31).

The main drawback to PDT is that many patients experience skin photosensitivity following therapy. This side effect may last for several hours or several days, depending on the photosensitizer used (3). Superficial tissues that contain photosensitizers and are exposed to light are damaged. By adjusting the polarity of the photosensitizer slightly with hydrophilic groups, tissues can metabolize the molecules more quickly and photosensitivity can be minimized (17).

The most obvious benefit of PDT is the minimal damage inflicted upon normal tissue. This type of selectivity is due to the need for photoactivation (21). Some researchers believe that, in the absence of light, photosensitizers may be as harmless as antibiotics when properly used (32). In addition, most PDT agents do not enter the nuclei of affected cells, so there is a low probability of DNA damage and mutations (3).

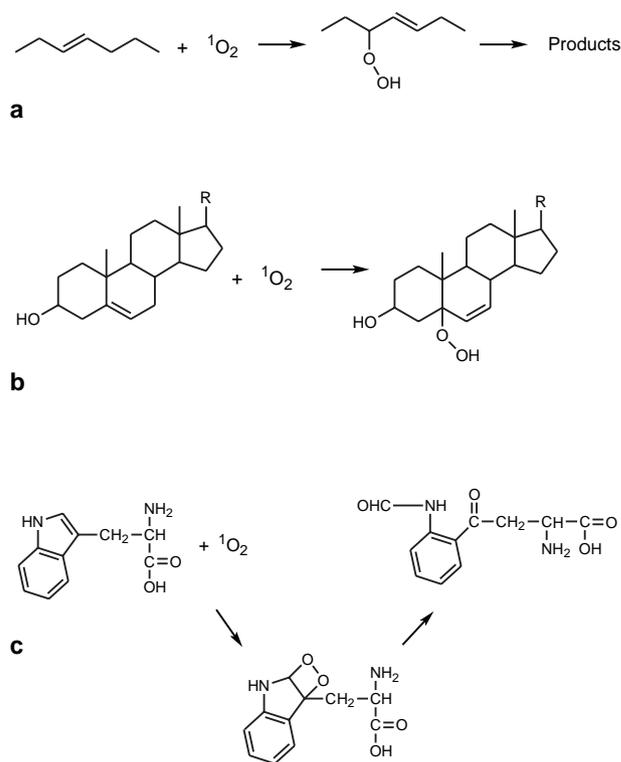


Figure 8. Some typical oxidation reactions that may occur with biomolecules in the cell (17): (a) unsaturated lipid (b) cholesterol (c) tryptophan.

Current cancer therapies include chemotherapy (e.g., antimetabolites, DNA crosslinkers, and DNA binding agents) and radiation. These forms of treatment are administered systemically and are not very selective. Because these treatments affect all of the cells inside the patient, the side effects are greater in number and severity (33).

What Is Extracorporeal Photopheresis?

Extracorporeal photopheresis (ECP) is a form of light-dependent therapy that is approved by the USFDA to treat patients with cutaneous T-cell lymphoma (34). Extracorporeal photopheresis is under study for the treatment of patients with autoimmune disorders as well (35). The procedure is slightly more complicated (Figure 9) by the need to extract the patient's blood and separate the blood into leukocyte-deficient (white blood cell deficient) and leukocyte-rich portions. Since both disorders are mediated by leukocytes, the leukocyte-deficient blood is reinfused into the patient, but the leukocyte-rich blood is treated with the photosensitizer outside the body. The treated portion is then irradiated with light for nearly three hours before reinfusion (16).

The ECP sensitizing agent is 8-methoxypsoralen, or 8-MOP (Figure 10, ref 34, 36). Because 8-MOP is aromatic, hydrophobic, small, and nearly planar, the molecule can fit between the rungs of the double helix, becoming intercalated (37). When exposed to short-wavelength light (334–346 nm), 8-MOP reacts covalently with stacked pyrimidines (Figure 11, ref 16). The resulting cross-link across DNA strands prevents replication and cell division, leading to cell death in the effected leukocytes (37). Methoxypsoralen is known to target T cells, although the exact mechanism has not been determined (39).

While 8-MOP may be used to treat immune disorders, it is usually secondary to steroid therapy with prednisone (40). The reluctance to use ECP is probably due to the fact that 8-MOP is considered to be a mutagen and possible carcinogen (41). The consideration of possible effects of 8-MOP and prednisone is important, since steroid therapy also carries with it a long list of potentially devastating side effects, including immunosuppression, severe weight gain and diabetes, loss of bone and muscle mass, cataract, and psychological disorders (42).

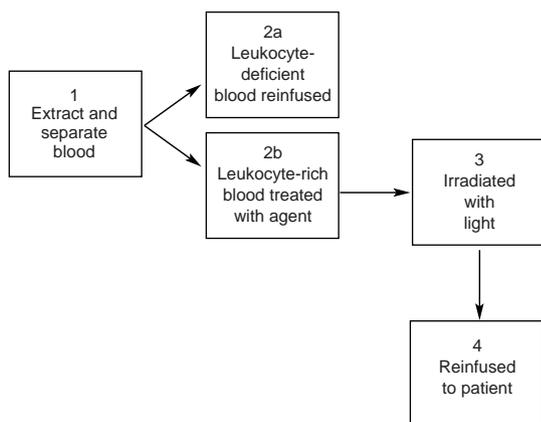


Figure 9. The steps of ECP (16).

Conclusion

Photodynamic therapy and extracorporeal photopheresis are both forms of light-dependent therapy. While they depend on different photosensitizers and different methods, PDT and ECP both have potential applications beyond the treatment of cancer. Although the authors do not intend to prove safety or efficacy in a clinical tone, the topic of photochemotherapy does provide an excellent opportunity to catch the interest and improve the education of students at various levels.

While light-dependent therapy presents new alternatives for a number of medical conditions, a great deal of research remains to be performed, especially in the development of new photosensitizers that are more effective and less toxic. For porphyrin-derived photosensitizers, this may be simply adjusting polarity and light-absorption properties by manipulating core and peripheral groups. In some cases, brand new photosensitizers may need to be developed or engineered.

We have brought attention to one example of the strong impact that chemistry can have on the health care field. As health care professionals seek to improve the methods by which they treat their patients, it is necessary to reinforce to them the importance of chemistry in manipulating their patients' physiology. Students interested in this arena must be made aware of this impact.

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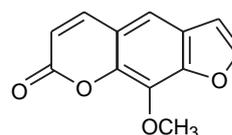


Figure 10. 8-Methoxypsoralen or 8-MOP (36).

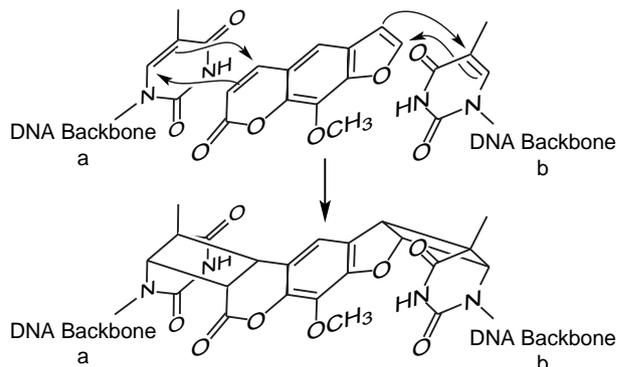


Figure 11. The action of 8-MOP. Notice that (a) is below the plane of 8-MOP and is binding through the furan-side adduct and (b) is above the plane of 8-MOP and is binding through the pyrone-side adduct (37).

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