

Photodynamic Therapy (PDT)

1. Basic Principles

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Photodynamic Therapy (PDT) is a newly emerging modality against cancer in which a photosensitizing molecule acts against malignant tumors under the influence of light. An overview of the chemical, photochemical and biochemical basis of PDT is presented in this series of articles. In doing so, an attempt has been made to illustrate how a topic such as PDT – which is related to human health – can generate interest amongst students in interdisciplinary areas bordering between chemistry and biology. Besides providing a general introduction to PDT, details of the physical and biochemical mechanisms of photodynamic action are provided in this article.

Introduction

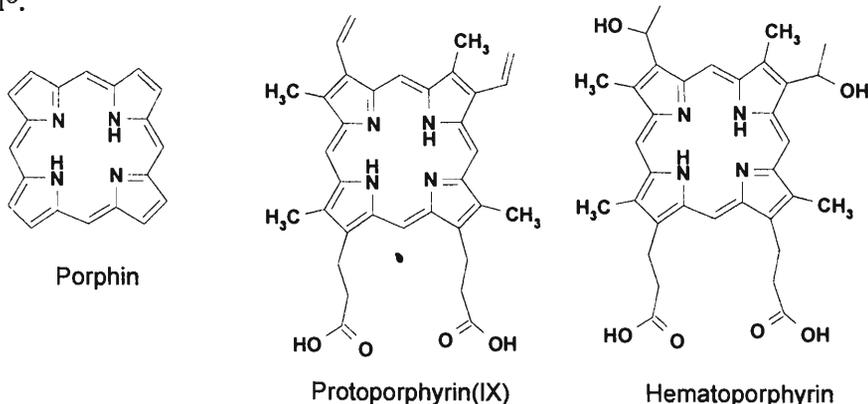
It all began centuries ago; ancient Egyptians knew well that ‘chemicals’ from orally ingested plants can accumulate in skin and, when activated by sunlight, bring about re-pigmentation of the skin. This 4000 year-old-treatment is still used today with psoralins (the ‘chemicals’ which Egyptians did not know) and UV-light exposure, representing the best known treatment for vitiligo – a widespread skin disease. Indeed, a number of skin diseases (acne, eczema, etc.) and others (neonatal jaundice) are being treated by photomedicine – a medical modality in which light in combination with a light-activatable molecule is the key therapeutic ingredient. However, not until the beginning of the previous century was a scientific inquiry into this light-related aspect of medicine undertaken. Raab, in 1900, showed that acridine dyes and light effectively killed *paramecia*. In 1925, Policard examined the ability of porphyrins – a class of tetrapyrrolic pigments which form the core of active sites of hemoglobin, chloroplast, cytochromes and related biomolecules



(Box 1) – to produce phototoxic effects. Mayer-Betz, in 1933, injected himself with around 200 mg of hematoporphyrin (see Box 1 for structure) and suffered no ill effects until he exposed himself to sunlight, whereupon he suffered extreme swelling and remained photosensitive for several months! In spite of

Box 1. Porphyrins – The Colors of Life

Porphyrins are a ubiquitous class of naturally occurring heterocyclic compounds with many important biological representatives including chlorophyll, prosthetic groups of various metalloenzymes/proteins such as hemoglobin, myoglobin, cytochromes, Vitamin B₁₂, etc. There are additionally a multitude of synthetic porphyrinoid molecules that have been prepared for purposes ranging from basic research to real-life applications. All of these molecules share in common the porphyrin macrocyclic substructure comprising four pyrrolic subunits linked by four methine bridges (see structure of unsubstituted porphyrin, i.e. porphin, shown below). Porphyrins are aromatic and they obey Hückel's rule for aromaticity in that they possess $4n+2\pi$ electrons which are delocalized over the macrocycle. While the peripheral positions of the basic porphyrin macrocyclic structure can be substituted by various organic functionalities, its central cavity is capable of binding a variety of metal/metalloid ions. Thus, a great variety of coordination complexes of substituted porphyrin ligands (natural/synthetic) can be envisaged. The iron complex of protoporphyrin IX (see structure shown below) is the prosthetic group of hemoglobin – the red pigment of blood. A variant structure with magnesium inside the macrocyclic cavity forms chlorophyll – the green pigment of plants. Thus porphyrin, the 'nature's ligand of choice', by virtue of its robust but yet substitution-labile structure and ability to bind metal ions in different oxidation states performs central roles in all spheres of life: as co-factors for oxygen or electron transport, for collecting light and transforming energy and as catalysts in biosynthesis. These bioinorganic aspects of porphyrinoid molecules have previously been discussed in *Resonance* (see: V Krishnan, 12, 77, 1997; K H Reddy, 6, 67, 1999). Needless to say, porphyrins are biocompatible molecules and exhibit rich photochemistry and redox chemistry. They are ideal candidates as photosensitizers in photodynamic therapy. Hematoporphyrin (see structure shown below) is the precursor for the currently marketed porphyrin-based drug – Photofrin II®.



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these early discoveries, the use of photomedicine in cancer treatment had to wait for three more decades until Lipson showed, in 1964, that hematoporphyrin preferentially accumulated in cancerous tissue. Even then the progress in this field was very slow for nearly 20 more years. Interest in photomedicine and particularly photodynamic therapy (PDT) was rekindled by Dougherty who showed during the late 1980's that the combination of a photosensitizer and light can indeed be used for cancer treatment. First developed for cancer treatment, PDT is now branching out to many other applications and experts are of the opinion that "in the current millennium, PDT is going to be one of the important modalities in medicine". In this series of articles, we discuss the chemical, photochemical and biochemical basis of PDT in light of its usefulness as an effective modality in the management of human cancers and allied diseases.

Basic Principles of PDT

Cancer is characterized by the uncontrolled growth and spread of abnormal cells and is often detected by the formation of a malignant tumor. The most important step in preventing the spread of cancer is to kill malignant cells, the unlimited growth of which expands locally by invading tissue and spreads to the rest of the body. Current major modes of cancer treatment are surgery, radiation therapy and chemotherapy.

Photodynamic Therapy (PDT) is a newly introduced modality for treating cancer in which a photosensitizing agent acts against malignant tumors under the influence of light. A typical PDT session, as it would be carried out at the clinical level, is illustrated in *Figure 1*.

In the first step, the patient receives an intravenous injection of a photosensitizer, a dye molecule that sensitizes chemical/biochemical reactions upon absorption of light. A period of incubation is needed during which the normal cells get rid of the photosensitizer and the malignant cells accumulate it through mechanisms not yet fully understood. The period of incubation



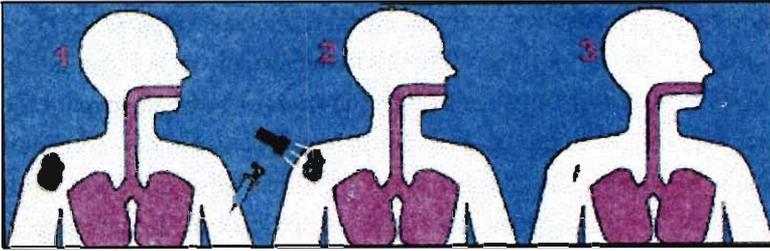


Figure 1. A typical PDT session.

optimizes the ratio of the concentration of the drug in malignant cells to that in normal cells such that the malignant cells have considerably higher concentrations of the photosensitizer. The incubation time can range from a couple of hours to several days depending on the characteristics of the particular photosensitizer. In the second step, the patient returns to the clinic after the incubation period and the clinician exposes *only* the tumor tissue, and *not* the normal tissue, to light of appropriate wavelength and power for absorption by the photosensitizer. Initially, the light sources for clinical PDT were argon-pumped dye lasers. But, these days, solid state diode lasers (so called medical lasers) coupled to optical fibers are increasingly being employed to irradiate the photosensitizers located in any desired part of the body and at any desired wavelength in the visible region of the electromagnetic spectrum.

The generally accepted mechanism of action of PDT is as follows. When sensitizers are illuminated by light of the correct wavelength, they absorb energy and, in turn, transfer it to molecular oxygen. Oxygen in its ground electronic state is a triplet. The energy transferred by the photosensitizer converts triplet oxygen to singlet oxygen, an extremely reactive species that ultimately leads to the destruction of the target cells.

A primary goal of all therapies against cancer is to affect targeted cells while sparing normal cells and tissue; the challenge is to maximize this selectivity. ‘Selectivity’ is a hallmark of PDT that is lacking in traditional anti-cancer therapies. PDT provides a means for achieving high selectivity by its two components: light and photosensitizer. An important thing to remember here is that the photosensitizer administered into the body is not

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active in the absence of light. In fact, the real drug in PDT is 'photosensitizer + light'! The photosensitizer, in the absence of light is thus a 'pro-drug'. The tissues meant to be destroyed are selectively illuminated and the damage is generally confined only to those areas. Other sites are minimally affected, both because the photosensitizers have no toxicity in the absence of light and because they tend to accumulate in the target tissues. In practice, the amount of selectivity depends on both the degree of preferential accumulation of the photosensitizer in the targeted cells and the extent to which illumination is spatially localized to the target.

The prototype, first-generation photosensitizer is hematoporphyrin derivative (HpD) – a mixture of monomeric, dimeric and oligomeric species derived from hematoporphyrin. The partially purified fraction of HpD called Photofrin II® (Porfimer sodium) has been recently approved by regulatory boards in countries like Canada, Japan, Netherlands and United States as a tumor-photosensitizing agent for the PDT of lung, oesophageal, bladder and other cancers. Photofrin II® was originally developed at Roswell Park Cancer Institute, Buffalo, NY (USA) in 1988 and later bought by QLT PhotoTherapeutics, Vancouver, British Columbia (Canada). Like HpD, Photofrin is also an oligomeric hematoporphyrin mixture, formed by ester and ether linkages; the actual chain length of the oligomer is not known (*Figure 2*). Currently there are many porphyrin, modified porphyrin and phthalocyanine based sensitizers, which are under phase I/II/III clinical trials for treatment of a variety of human cancers and also other clinical applications. We shall revert to this topic in a later part of this series.

There is, however, one side effect of PDT. Photofrin II® stays in the skin for approximately 30 days after treatment making the patient sensitive to strong light (recall Mayer-Betz's 1933 experiment!). On a good sunny day, the patient who is administered with this photosensitizer will either have to stay indoors or will need to wear 'hat and gloves' if he/she prefers to be out in the open. Nonetheless, compared with the severe side effects that



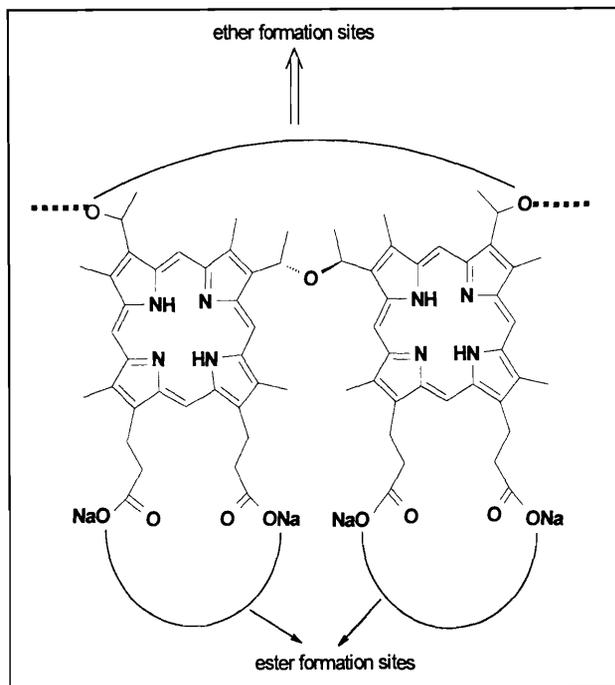


Figure 2. Structure of HpD mixture. It contains dimeric and oligomeric species formed via ether or ester bonds between the monomers, as shown. The commercially available Photofrin II® is a purified, active component of this mixture whose complete structural identity is still unknown.

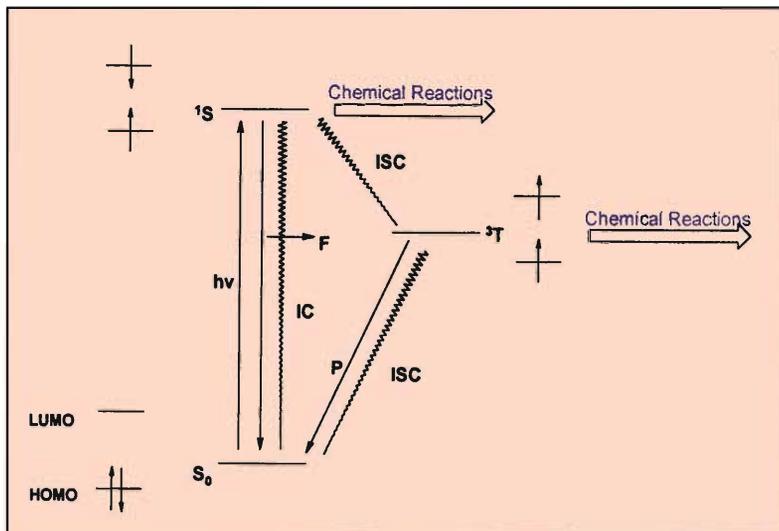
often accompany chemo- and radiation therapy, the problems associated with PDT seems really benign.

Mechanism of PDT Action

The therapeutic efficacy of any anti-cancer drug depends on its mechanism of action. Obviously, tumor eradication is a biochemical/molecular biological, and eventually, a clinical event. But, as far as PDT is concerned, it is intimately connected with the interaction of light with a photosensitizer – a purely physical process. Therefore, we discuss the physical chemistry aspects of photodynamic action first and the biological consequences next.

Physical Chemistry: It all begins with the photosensitizer absorbing a photon of energy from the light source of the correct wavelength. The photosensitizer is activated, with an electron typically being excited from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). A modified Jablonski diagram in *Figure 3* illustrates the excitation process and the fate of such an excited state (1S) of

Figure 3. A modified Jablonski diagram illustrating the various photophysical processes that occur upon irradiation of a PDT photosensitizer.



the photosensitizer. The 1S state can be dissipated by one of many pathways listed below:

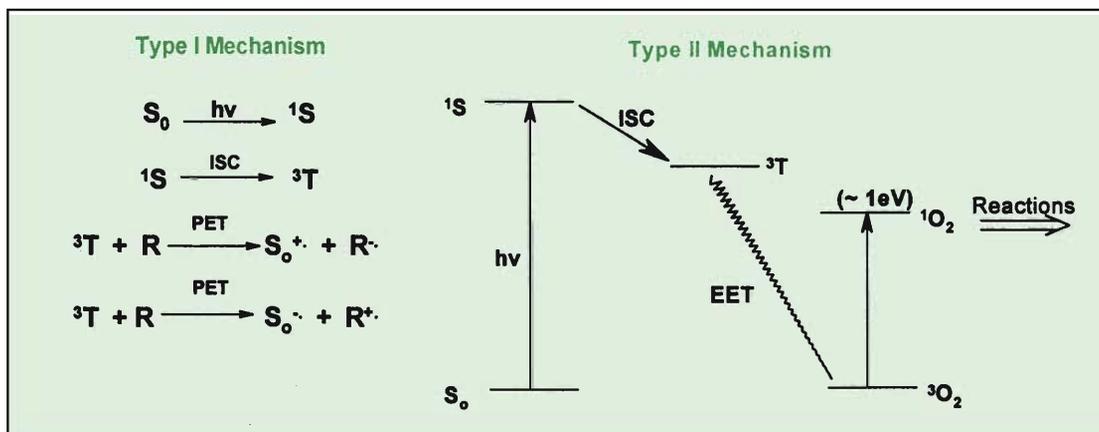
- (i) Radiative decay – fluorescence (F).
- (ii) Intersystem crossing (ISC) to generate the triplet state (3T).
- (iii) Non-radiative decay (internal conversion, IC) with dissipation of heat to generate the ground state (S_0).
- (iv) Chemical/physical reaction with the surrounding substrates.

Among these pathways, intersystem crossing is quite important for PDT because this process generates the triplet state that plays a major role in the photodynamic action. Like the singlet, the reactive, paramagnetic triplet species can dissipate its excess energy by both radiative and non-radiative processes:

- (v) Radiative decay – phosphorescence (P).
- (vi) ISC (non-radiative) to generate the ground state (S_0).
- (vii) Chemical/physical reaction with the surrounding substrates including oxygen.

Of the seven processes listed above, process (vii) is central to the biochemical mechanism of PDT. In order to understand this better, let us examine the underlying photochemical principles.

Steps (i)-(iii), (v) and (vi) are all photophysical processes. But



(iv) and (vii) involve chemical changes. *Two major types of chemical reactions can occur from the excited states of a photosensitizer. These are commonly referred to as type I and type II reactions (Figure 4).* In general, those reactions which involve a photoinduced electron transfer (PET) between the excited state and the substrate (R) are termed as Type I reactions while those involving the electronic excitation energy transfer (EET) are the so called Type II reactions.

Chances of occurrence of both Type I and Type II reactions from the singlet state are rather remote due to the fact that this state is usually short-lived and any reaction involving it as a partner should occur within its lifetime (t) which is typically between nanoseconds ($ns = 10^{-9}$ s) and pico seconds ($ps = 10^{-12}$ s). On the other hand, triplet states, which are generated from the singlet precursor, have a better chance of reacting with other substrates because they are long-lived (t ranges from microseconds, $\mu s = 10^{-6}$ s to milliseconds, $ms = 10^{-3}$ s) and there is enough time for the reaction to occur subsequent to mutual diffusion of the reaction partners. The triplets can undergo either electron transfer or energy transfer reactions with the available substrates. The primary photochemical steps may or may not be involved in bond-making/bond-breaking, but the subsequent steps can involve pure chemical reactions giving rise to new products. Most available data suggest the involvement of an energy transfer reaction between the triplet PDT photosensi-

Figure 4. The Type I and Type II reactions of a photosensitizer. Initial events of Type I and II reactions involve photoinduced electron transfer (PET) and excitation energy transfer (EET), respectively. While S_0 , 1S and 3T represent the ground, singlet and triplet states of the photosensitizer, R represents a biomolecule including oxygen.

Most available data suggests the involvement of an energy transfer reaction between the triplet PDT photosensitizer and molecular oxygen resulting in the formation of singlet oxygen ($^1\text{O}_2$)

tizer and molecular oxygen resulting in the formation of singlet oxygen ($^1\text{O}_2$) *via* the Type II mechanism.

Singlet oxygen, $^1\text{O}_2$, is unstable with a life time of ~ 6 ms in water and a little longer in lipid and cell environments. Thus, it cannot diffuse more than a single cell length during its lifetime. However, this reactive oxygen species is a fairly indiscriminant oxidant that reacts with a variety of biological molecules and assemblies. The actual biochemical mechanism of cell death mediated by $^1\text{O}_2$ will be addressed in the next section.

Although the $^1\text{O}_2$ – mediated mechanism seems to be responsible for the photodynamic action of Photofrin II[®] and several other porphyrin-based PDT agents, participation of the Type I photoprocess from a given triplet sensitizer cannot be altogether ruled out. Indeed, a large body of evidence exists in the PDT literature wherein radical products like superoxide radical anion, peroxy radical, hydroxyl radical, cation radicals, etc. formed *via* Type I mechanism have been cited to be responsible for the observed cytotoxic action. In some other cases, participation of both Type I and Type II mechanisms have been invoked and their relative contributions depend on the photosensitizer.

Biochemical Mechanism: Three events are important here – (i) drug delivery and retention, (ii) molecular mechanisms, and (iii) tumor destruction pathways. These are briefly discussed below.

Sensitizer transport in the body and subsequent specific localization depend on its hydrophobicity, aggregation state and specific ionic state. The main carriers in the blood stream are lipoproteins and serum albumin. Two important observations can be made from the various scenarios proposed for sensitizer accumulation and retention in a tumor. These are:

- (a) high levels of low density lipoprotein (LDL) receptors in proliferating cells associated with cholesterol accumulation;
- (b) lower pH often found in the interstitial fluid of neoplastic cells.

It has been demonstrated that hydrophobic porphyrin-based sensitizers are mainly carried in blood by lipoproteins (which also do not 'like' water), particularly LDLs. LDLs are of particular interest because they are recognized by a specific receptor called the apo B/E receptor, which results in rapid internalization and delivery of the LDL particle to the lysosomal compartment. The number of LDL receptors is generally higher in tumor cells compared to their normal counterparts. This is because the hyperproliferating (rapidly multiplying) cells require an extra supply of cholesterol and phospholipids, which is satisfied through the formation of receptors specifically controlling the metabolism of extracellular LDLs. Consequently, it is thought that hydrophobic sensitizer accumulation by a tumor results from its delivery to cells via the very efficient LDL receptor pathway. However, it has also been demonstrated that some porphyrins are bound to high-density lipoproteins (HDLs) but with a low turnover rate in plasma. On the other hand, hydrophilic photosensitizers, such as water-soluble porphyrins and phthalocyanines are largely carried by albumin and other serum proteins. The retention of such porphyrins and also other charged photosensitizers in tumor cells can sometimes be considerably increased by binding them covalently to serum albumin.

In tumor tissue, the oxygen supply to rapidly growing tumor cells is generally insufficient (but not to the extent that PDT is severely impeded). Glucose is thus partly used *via* the anaerobic pathway producing large quantities of lactic acid. The excess acid produced within the cells is transported to the outer medium. The pH of interstitial fluid in various tumors has been consistently found to be lower than that of normal tissues. Thus, there is a possible involvement of acid-base equilibria in the increased incorporation and retention of sensitizers having ionizable groups by tumors. The distribution patterns of lipophilic or cationic photosensitizers are primarily governed by their electrochemical features. They follow potential gradients in accordance with the Nernst equation and concentrate across the membrane.

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Lipid peroxidation leads to structural and functional damage of cell membranes and of their surroundings.

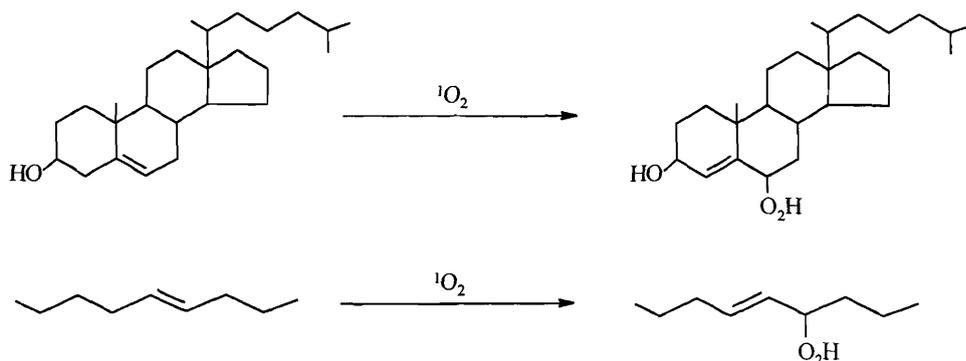
The active species that are generated upon irradiation of the localized photosensitizers has been reported to induce various deleterious reactions in the target tissue components. As stated earlier, $^1\text{O}_2$ is the most prevalent and often quoted reactive oxygen species in PDT. Evidence for the production of $^1\text{O}_2$ during the photodynamic action of Photofrin II[®] is supported by the oxygen-dependency of PDT: in the absence of molecular oxygen ($^3\text{O}_2$), there is no tumor damage, and in the presence of molecular oxygen and a $^1\text{O}_2$ quencher (a compound that eliminates the energy of singlet oxygen), cells that have incorporated Photofrin II[®] are not destroyed.

A few important chemical reactions of $^1\text{O}_2$ are illustrated in *Figure 5*. As shown, oxygen atom transfer can result in the oxidation of both carbon and sulfur centers and in the formation of hydroperoxides from a variety of substrates. The biochemical reactions of $^1\text{O}_2$ include: lipid peroxidation; oxidation of unsaturated fatty acids, proteins with oxidizable amino acid units and bases in DNA. The biological membranes, and in particular mitochondrial membranes are considered as the critical targets for cell killing by photosensitization. For example, Hp photosensitization of cholesterol, a determinant of eukaryotic membrane mechanical stability, gives its 5- α -hydroperoxide, this reaction being specific to $^1\text{O}_2$ mechanism. A similar 'ene' type reaction is observed in the Hp photosensitized oxidation of unsaturated fatty acids. Lipid peroxidation leads to structural and functional damage of cell membranes and of their surroundings including crosslinking for proteins with aminolipids, inactivation of intrinsic enzymes, and increased permeability.

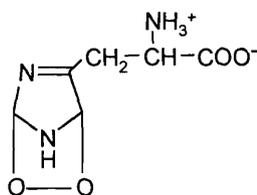
Although PDT acts mainly by effecting membrane damage, photodynamic processes have been reported *via* photosensitized damage of DNA. In cells, DNA-protein crosslinks and DNA single strand breaks have been observed, all of them *via* the $^1\text{O}_2$ pathway. In addition, photo-oxidation of histidine, tryptophan, tyrosine, methionine and cysteine residues can produce reactions with amino groups and intramolecular crosslinking in proteins.



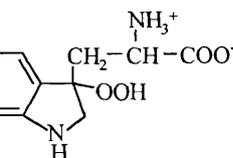
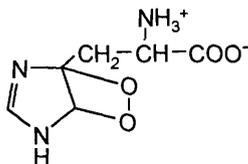
Two typical reactions of 1O_2



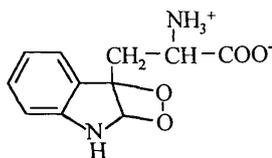
Products with other biological substrates



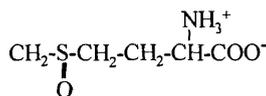
Histidine endoperoxides



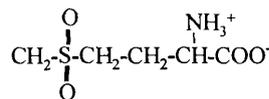
Tryptophan hydroperoxide



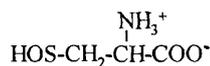
Tryptophan endoperoxide



Methionine sulfoxide



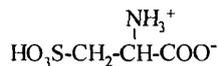
Methionine sulfone



Cysteine sulfenate



Cysteine sulfinic acid



Cysteine sulfonate

Figure 5. Typical examples of reactions of 1O_2 with biomolecules.

Suggested Reading

- [1] W M Sharman, C M Allen, J E van Lier, *Drug Discovery Today*, 4, 507, 1999.
- [2] J M Miller, *J. Chem. Ed.*, 76, 592, 1999.
- [3] L Milgrom, S MacRobert, *Chem. Britain*, 45, May, 1998.
- [4] R Bonnett, *Chem. Soc. Rev.*, 24, 19, 1995.

At the cellular level, the primary mechanism of tumor destruction by PDT was originally thought to be the selective accumulation of HpD in neoplastic cells and their subsequent direct killing *via* photoinactivation of the intracellular photosensitizer. However, several observations show that the effectiveness of PDT may not be related to directly destroying tumor cells. Recent data indicate that initial vascular damage and subsequent tumor cell anoxia may well be an alternative indirect mechanism leading to tumor necrosis. The ultimate mechanism of PDT with HpD (or Photofrin II®) may thus involve killing of tumor cells as mainly a secondary step following damage to the microvasculature and perturbation of the tumor microcirculation. The vascular endothelium may be the main target of tumor photosensitization by HpD. As far as the other photosensitizers are concerned, one or more among the following effects, viz: reduced fluidity of the bilayer, increased permeability and lysis, inactivation of intrinsic enzymes, transporters and receptors, covalent crosslinking of proteins and aminolipids, polypeptide strand scission, DNA damage and mutagenesis have been thought to be operative upon photosensitization. More recently, apoptosis – the so called ‘programmed cell death’ – is also being advocated as a cause of tumor eradication.

Concluding Remarks

This article provides an overview of PDT. Basic physical, chemical and biological principles involved in this new anti-cancer therapeutic modality have been discussed in some detail. Throughout, while trying to focus on the general attributes of PDT, a special effort has been made to drive home the point that this therapy involves an interdisciplinary approach involving chemists, laser physicists, molecular oncologists, clinicians, etc. In the next article of this series, we shall discuss the strategies involved in PDT drug design and development and also on the ‘light-related’ issues of PDT. New directions in the PDT of cancer and newer applications of PDT in managing diseases other than cancer will form the subject matter of the concluding part of this series.

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