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# A Dual Selective Antitumor Agent and Fluorescence Probe: the Binary BMVC–Porphyrin Photosensitizer

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Photodynamic therapy (PDT) is a promising modality for the treatment of localized tumors.[1–4] To cause cell death during PDT, singlet oxygen can be generated by energy transfer from the triplet state of a photosensitizer to molecular oxygen. Among various photosensitizers, porphyrin derivatives have been widely studied.[5,6] However, porphyrin-based sensitizers have drawbacks to their application in PDT that include poor chemical selectivity toward intended tissue targets and a lack of specific light wavelengths that are optimal for tissue penetration and chromophore excitation. To overcome these shortcomings of the traditional porphyrin chromophore, a number of binary compounds have been recently designed and prepared with a component linked to the porphyrin photosensitizer. The resulting conjugates are highly selective for cancer cells and absorb at longer wavelengths more suitable for tissue penetration.[7–11] For example, Drain and co-workers[7] synthesized porphyrin–saccharide conjugates to increase the uptake of the photosensitizer by specific cancer cells. Dichtel et al.[8] synthesized a binary compound to enhance singlet oxygen generation via fluorescence resonance energy transfer (FRET) by two-photon excitation of the donor chromophore to the central porphyrin acceptor.

Selectivity is a key feature in the design of photosensitizers for killing cancer cells without damaging normal cells. We recently synthesized a novel 3,6-bis-(1-methyl-4-vinylpyridinium)-carbazole diiodide (BMVC) compound with selectivity toward cancer cells over normal cells.[12,13] Specifically, the fluorescence of BMVC detected in cancer cells was found to be much stronger than that in normal cells.[14] Moreover, BMVC has a large cross-section for two-photon absorption around 820 nm,[15] a wavelength that is close to optimal for tissue penetration of the radiation needed to excite the photosensitizer. In addition, the windows of transparency for porphyrin derivatives in the range of 450–500 nm allow us to selectively excite BMVC.[16] For the study reported herein, we combined these features into a binary porphyrin compound for selectivity in PDT.

In this compound, two BMVC molecules are covalently linked to a central 5,10-bis-(4-hydroxyphenyl)-15,20-bis-(4-methoxyphenyl)porphyrin (PAP) molecule at the *ortho* positions, and we termed the resulting binary compound *o*-2B-P. The *ortho* position was chosen for covalent attachment, as it is known that compounds containing *ortho*-5,10-disubstituted water-soluble trimethylaminophenyl groups exhibit better PDT efficacy than those with *para*-5,15-disubstitution.[17] The structure of *o*-2B-P is shown in Figure 1A, and details of its synthesis can be found elsewhere (Supporting Information 1).

We examined the efficiency of energy transfer from the excited state of BMVC to the porphyrin in *o*-2B-P, as well as the efficacy of singlet oxygen generation by the porphyrin in the binary compound. The absorption and fluorescence spectra of *o*-2B-P, PAP, BMVC, and a mixture of BMVC and PAP in DMSO are depicted in Figures 1B and C, respectively. The absorption of *o*-2B-P is almost identical to that of a mixture of BMVC and PAP. The absorption is a linear sum of the absorbances of BMVC and PAP individually, indicating that there is no appreciable interaction between the two chromophores in the ground electronic states. In contrast, the fluorescence of BMVC in *o*-2B-P is almost totally quenched, whereas the fluorescence of PAP is enhanced by at least fivefold upon excitation of *o*-2B-P at 470 nm. Evidently, there is efficient energy transfer from the excited state of BMVC to PAP.

To evaluate the effect of photoinduced formation of singlet oxygen by *o*-2B-P, we applied a photochemical method using a singlet oxygen quencher, 1,3-diphenylisobenzofuran (DPBF), to verify the generation of singlet oxygen.[18] Because the cationic *meso*-tetra-(4-*N*-methylpyridyl)porphyrin (TMPyP) molecule is itself a singlet oxygen photosensitizer,[19,20] we compared the yield of singlet oxygen upon irradiation of *o*-2B-P and TMPyP at various wavelengths. Figure 1D shows a comparison of the absorbance of DPBF at 417 nm in the presence of *o*-2B-P and TMPyP in DMSO as a function of irradiation time at $470 \pm 10$ nm as well as $\lambda_{ex} > 580$ nm selectively from a halogen lamp. Although singlet oxygen generation by *o*-2B-P is slightly lower than that of TMPyP at $\lambda_{ex} > 580$ nm, it is the singlet oxygen generated by *o*-2B-P, not by TMPyP, upon excitation at $470 \pm 10$ nm that provides a more useful measure of the selectivity for PDT efficacy. Despite the large number of porphyrin derivatives in blood and tissues, the porphyrin absorbance at $470 \pm 10$ nm is almost negligible. Accordingly, the 450–500 nm transparent windows of porphyrin derivatives

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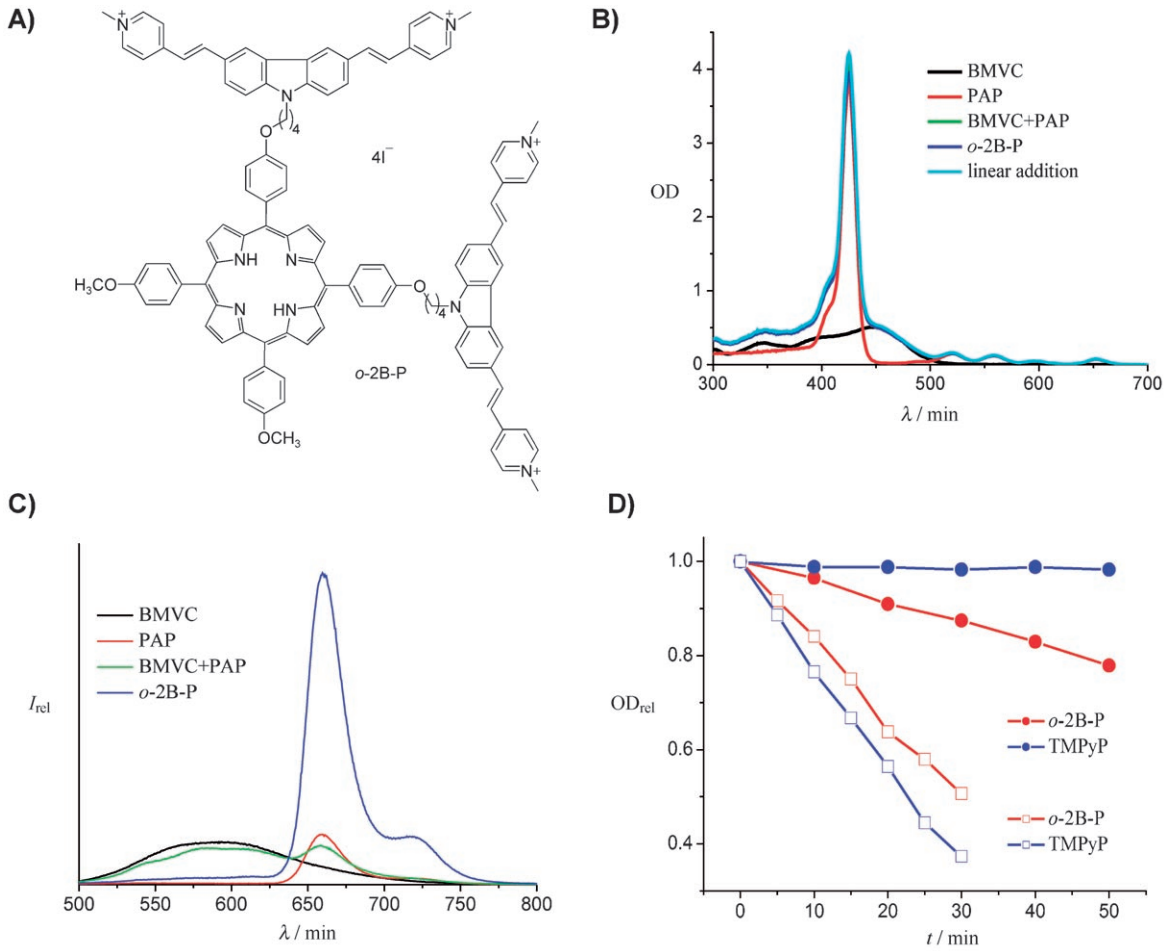


**Figure 1.** A) Structure of *o*-2B-P. B) Absorption spectra of BMVC, PAP, a mixture of BMVC and PAP, *o*-2B-P, and the linear sum of the individual absorbance of BMVC and PAP in DMSO (each at a final concentration of 10 μM). C) Fluorescence spectra of the samples as described in panel B ($\lambda_{ex} = 470 \pm 10$ nm). D) Plots of DPBF absorbance (normalized to $t = 0$ min) in the presence of TMPyP and *o*-2B-P as a function of irradiation time (●: $\lambda_{ex} = 470$ nm, □: $\lambda_{ex} = 580$ nm).

allow us to selectively excite the BMVC moieties in *o*-2B-P. The energy transfer from the excited state of BMVC to the porphyrin together with the cancer cell specificity of BMVC could provide additional selectivity for PDT efficacy without appreciable side effects on normal cells.

We further evaluated the photosensitization effect in human cells using Leica DMIRB fluorescence microscopy in a life-sustaining incubator. Of interest is the photoinduced translocation of *o*-2B-P in normal cells and cancer cells at different rates upon BMVC excitation. Figure 2 shows a bright field and several selected fluorescence images from a real-time video of MCF-7 breast cancer cells and D-551 normal skin cells incubated with 1 μM *o*-2B-P for 6 h after 480 ± 20 nm irradiation from a mercury lamp for ~2 min. These data show that *o*-2B-P is initially localized in the cytoplasm, and the red fluorescence observed here is hardly detected in the nucleus. However, this red emission in the cytoplasm subsequently decreases, and the fluorescence from the cells is dominated by a green–yellow emission in the nucleus, indicating that *o*-2B-P has entered into the nucleus during the time course of irradiation at 480 ± 20 nm. The decrease in red fluorescence may be due to the photobleaching of porphyrin analogues in PDT.[21] Careful examination reveals that *o*-2B-P probably generates holes in the nuclear envelope by singlet oxygen upon BMVC excitation. After damaging the nuclear envelope, *o*-2B-P translocates into the nucleus and shows a bright green–yellow fluorescence. This arises from the interaction between the BMVC moiety of *o*-2B-P and DNA. The fluorescence of BMVC increases by two orders of magnitude upon interaction with DNA in the nucleus and therefore dominates.[12] The marked increase in fluorescence quantum yield of BMVC upon binding to DNA is attributed to the intramolecular twist of the vinyl group limited by the binding interaction with DNA.[16] The excellent contrast in cellular imaging of *o*-2B-P can serve as a marker for the study of PDT.

Of particular interest is the significantly faster photoinduced translocation of *o*-2B-P in MCF-7 cancer cells than in D-551 normal cells, as shown by real-time videos (Supporting Information 2A). Note that the green–yellow color is observed in the nucleus of MCF-7 cells after irradiation for < 20 s, but is barely detected in the nuclei of D-551 cells after irradiation for < 90 s. The fact that the bright fluorescence of BMVC is detected much earlier in the nuclei of cancer cells than in normal cells suggests that BMVC has specificity for the nuclear enve-

  

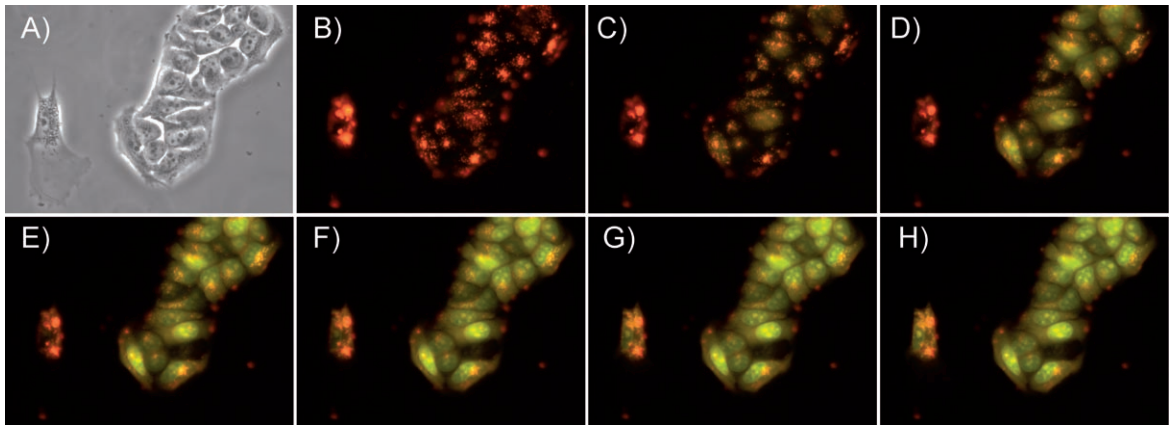


**Figure 2.** Photoinduced translocation of *o*-2B-P in MCF-7 breast cancer cells (clusters at the right of each image) and D-551 normal skin cells (single one at left). A) Bright-field image of the mixture of MCF-7 cancer cells and D-551 normal cells incubated with 1 μM *o*-2B-P for 6 h before irradiation and fluorescence images after irradiation at 480±20 nm for B) 1 s, C) 20 s, D) 40 s, E) 60 s, F) 90 s, G) 120 s, and H) 150 s.

lopes of cancer cells over normal cells, resulting in a more rapid translocation of *o*-2B-P in cancer cells. Similar behavior in photoinduced translocation of *o*-2B-P is also observed in CL1-0 lung cancer cells and MRC-5 normal lung cells; real-time videos are shown in Supporting Information 2B. The key issue is that the time lag between the photoinduced translocation of *o*-2B-P in cancer cells and normal cells may allow us to optimize the conditions for killing cancer cells without appreciable side effects on normal cells.

To clarify the selectivity in PDT on normal versus cancer cells, we compared the bright-field image of a mixture of CL1-0 cancer cells and MRC-5 normal cells incubated with 4 μM *o*-2B-P for 5 h before irradiation (Figure 3 A). The fluorescence images of *o*-2B-P and propidium iodide (PI) after irradiation at 480±20 nm for ~13 s and then storage in the dark for ~78 s are depicted in Figures 3 B and C, respectively. Figure 3 D shows a comparison of the corresponding bright-field image. As a control, the red fluorescence from PI in the nucleus was used as a marker for cell death. Red fluorescence from PI was observed in the nuclei of CL1-0 cancer cells, but was not found in the nuclei of MRC-5 normal cells, suggesting that *o*-2B-P kills cancer cells without damaging normal cells. Note that no dark cytotoxic effect was found after incubation of *o*-2B-P in the medium for 24 h. The rapid cell death induced by photoexcitation of the BMVC moiety in *o*-2B-P is ascribed to necrosis and not to apoptosis. Thus, *o*-2B-P is a novel photosensitizer with both target and irradiation wavelength selectivity for the PDT of cancer cells. Moreover, the excellent contrast in cellular imaging with *o*-2B-P can be applied to monitor the pathway of PDT and serve as a cell death marker. The structure–activity relationship of the cellular response from *o*-2B-P and its derivatives, particularly photoinduced translocation, deserves further study to obtain a better picture of the mechanisms of selectivity as part of the overall development of photosensitizers for PDT in cancer treatment.

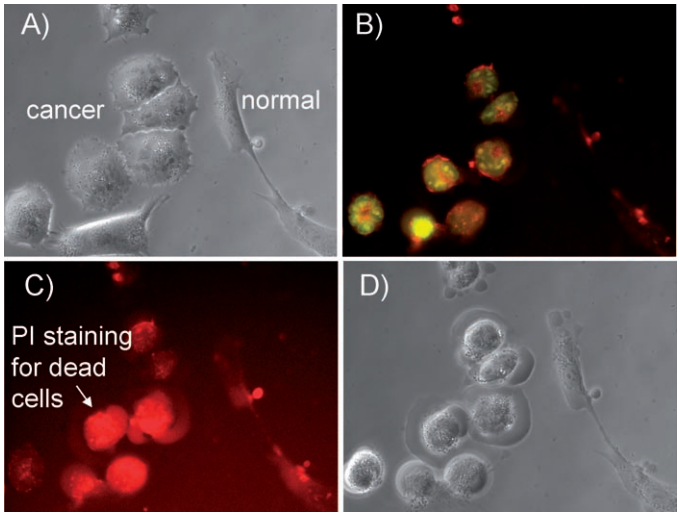


**Figure 3.** PDT effect of *o*-2B-P in CL1-0 lung cancer cells and MRC-5 normal lung cells. A) Bright-field image of a mixture of CL1-0 cancer cells and MRC-5 normal cells incubated with 4 μM *o*-2B-P for 5 h before irradiation. After irradiation at 480±20 nm for ~13 s and then storage in the dark for ~78 s, green fluorescence (B) from the BMVC moiety of *o*-2B-P appeared in the nuclei of cancer cells, and red fluorescence (C) of PI appears in the nuclei of cancer cells. D) The corresponding bright-field images are also shown; it appears that the *o*-2B-P photosensitizer can act as a cell death marker.

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