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Mitochondria-specific Oxygen Probe Based on Iridium Complexes Bearing Triphenylphosphonium Cation

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Organelle-selective oxygen probe BTP-Mito was designed and synthesized to selectively target mitochondria. BTP-Mito, which is an iridium complex bearing a triphenylphosphonium cation, exhibited selective mitochondria localization in HeLa cells. The phosphorescence of BTP-Mito was significantly quenched by molecular oxygen in living cells, demonstrating that BTP-Mito can be used as mitochondria-specific oxygen sensor.

Oxygen is one of the key metabolites in aerobic systems.[1] In the normal physiological state, the majority of cellular oxygen consumption occurs in mitochondria where oxygen functions as the terminal electron acceptor for the electron-transport chain during aerobic respiration. Oxygen deprivation (hypoxia) is connected with various diseases and occurs in tumor microenvironments.[2] The oxygen level in mitochondria is therefore one of the central parameters in many physiological, pathological, and therapeutic processes.

We recently demonstrated that a phosphorescent iridium(III) complex acetylacetonatobis[2-(2′-benzothienyl)pyridinato-$\kappa N,\kappa C^{3'}$]iridium(III) (BTP, Figure 1) can be used as an optical probe for visualizing the oxygen levels in biological cells and tissues.[3] Under deaerated conditions BTP dissolved in *n*-hexane exhibited red phosphorescence with a moderately long emission lifetime (6.3 μs) and a high quantum yield (0.31). The phosphorescence of BTP was significantly quenched by dissolved oxygen in solution. Similar quenching by oxygen has also been observed for living cells, where BTP showed efficient access to the endoplasmic reticulum (ER) in the cytoplasm.

A great advantage of the iridium complexes lies in their facility in chemical modifications of the ligands. The ligand molecules of BTP consist of two benzothienylpyridinato groups and an acetylacetone as an ancillary ligand. The spectral properties of BTP are determined almost exclusively by the benzothienylpyridinato groups. On the other hand, the acetylacetone moiety scarcely influences the spectral properties of the complex. We can, therefore, improve the physicochemical properties of BTP by introducing an appropriate substituent into the ancillary ligand.

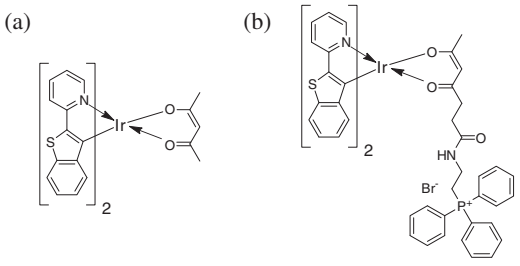

Figure 1. Molecular structures of (a) BTP and (b) BTP-Mito.

Herein, we report a newly developed mitochondria-specific oxygen probe (BTP-Mito).[8] As shown in Figure 1, BTP-Mito has a structure in which BTP is conjugated to a delocalized lipophilic cation (triphenylphosphonium ion; TPP^+). In biological cells the delocalized lipophilic cations are known to preferentially accumulate into mitochondria in response to negative inside transmembrane potentials.[4]

Figure 2 illustrates the absorption and phosphorescence spectra of BTP and BTP-Mito in deaerated tetrahydrofuran (THF). BTP and BTP-Mito exhibit the $S_1 \leftarrow S_0$ absorption bands with their maxima at 486 and 480 nm, respectively, and show intense phosphorescence from a ligand-centered $\pi\pi^*$ excited state with significant admixtures of metal-to-ligand charge-transfer (MLCT) character.[5] On the longer-wavelength tail of the intense $S_1 \leftarrow S_0$ absorption band, a weak absorption band due to the electronic transition to the lowest excited triplet state from the ground state is superimposed. It is noted from Figure 2 that the shape and position of the phosphorescence spectra of BTP are only slightly affected by introducing TPP^+ into the ancillary ligand.

The phosphorescence quantum yield (Φ_p) and lifetime (τ_p) of BTP and BTP-Mito were measured in aerated and deaeraed THF solutions (Table 1).[6] From Stern–Volmer analyses of the phosphorescene quenching due to oxygen, the quenching rate constants (k_q) for BTP and BTP-Mito were determined to be 6.3×10^4 and 5.3×10^4 $mmHg^{-1}\,s^{-1}$, respectively.[8] It is confirmed from Table 1 that BTP-Mito retains the phosphorescence

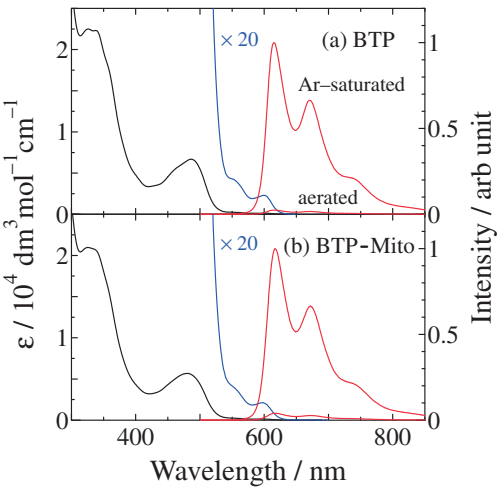

Figure 2. Absorption (black and blue lines) and phosphorescence (red lines) spectra of (a) BTP and (b) BTP-Mito in THF at room temperature. The phosphorescence spectra were taken under Ar-saturated and aerated conditions.

Table 1. Photophysical parameters of BTP and BTP-Mito in THF at room temperature

Compound	ε /dm^3 mol^{-1} cm^{-1}	Φ_p		τ_p/μs		k_q /10^4 mmHg^{-1} s^{-1}
		Aerated	Deaerated	Aerated	Deaerated	
BTP	6700[a]	0.0047	0.30	0.093	5.7	6.3
BTP-Mito	6200[b]	0.0056	0.29	0.110	5.5	5.3

[a]Molar absorption coefficient at 486 nm. [b]At 480 nm.

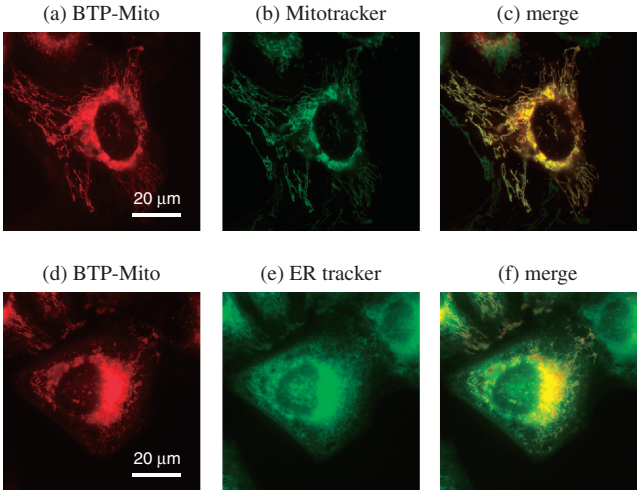

Figure 3. Subcellular localization of BTP-Mito in HeLa cells. BTP-Mito was added to the medium at a final concentration of 500 nM, and HeLa cells were incubated for 5 h at 2.5% O_2. Organelle markers used were Mitotracker and ER tracker which selectively stain mitochondria and ER.

characteristics of BTP, and both compounds show significant response to the variation of the oxygen concentration in solution.

We then verified the intracellular localization of BTP-Mito in living cells by morphologic examination using HeLa cells.[8] Cellular BTP-Mito localization seemed to be limited to the cytoplasm and not to the nucleus (Figure 3). We examined color merging using green-colored mitochondria-specific probe Mitotracker and ER-specific probe ER tracker. We observed a merged yellow color between BTP-Mito and Mitotracker (Figure 3c). However, no green-colored ER tracker merged with BTP-Mito (Figure 3f). These observations suggest that BTP-Mito exhibits selective mitochondria localization in the cytoplasm. This is in remarkable contrast to efficient ER localization of BTP in HeLa cells.[3]

Since we could confirm selective mitochondria localization of BTP-Mito in HeLa cells, we examined oxygen response of the BTP-Mito phosphorescence in living cells. HeLa cells were cultured under 20% or 2.5% O_2 concentrations for 24 h at 37 °C. BTP-Mito was added to the medium at a final concentration of 500 nM and cells were incubated for 4 h, and then the luminescence images were taken under 20% or 2.5% O_2 concentrations (Figure 4). In normoxia (20% O_2), the cell line did not exhibit notable phosphorescence of BTP-Mito. In contrast, it emitted bright phosphorescence in a 2.5% O_2 culture.

The optical properties of BTP-Mito in solutions and living cells demonstrate that BTP-Mito can be applied to real-time monitoring of oxygen levels of mitochondria in living cells. Recently, quantification of cellular oxygen levels based on

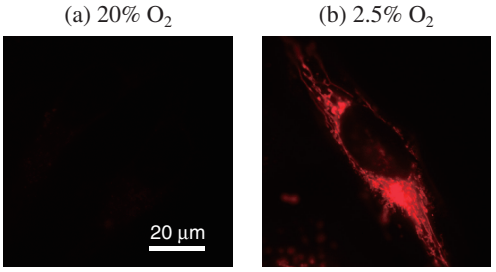

Figure 4. BTP-Mito imaging of HeLa cell line. The HeLa cells were placed at (a) 20% O_2 and (b) 2.5% O_2.

lifetime measurements have been reported by using Pt (or Pd) porphyrins or Ru complexes as phosphorescent oxygen probes.[7] BTP is the first iridium complex-based oxygen sensor for biological cells,[3] and BTP-Mito will become the first mitochondria-specific iridium complex sensor which can be used for lifetime-based oxygen measurements.

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8 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

