19.1 Introduction to Reactive Oxygen Species

Molecular Probes has available several probes that either generate or detect various reactive oxygen species (Table 19.1), including singlet oxygen ($^1O_2$), superoxide ($\cdot O_2^-$), hydroxyl radical ($HO\cdot$) and various peroxides (ROOR′) and hydroperoxides (ROOH). Section 19.2 focuses on these probes and their applications *in vitro* and *in vivo*. Produced during a number of physiological processes, such as Alzheimer's disease,[1] apoptosis (Section 15.5) and phagocytosis (Section 16.1), activated oxygen species react with a large variety of easily oxidizable cellular components, including NADH, NADPH, dopa, ascorbic acid, histidine, tryptophan, tyrosine, cysteine, glutathione, proteins and nucleic acids.[2–6] Reactive oxygen species can also oxidize cholesterol and unsaturated fatty acids, causing membrane lipid peroxidation.[7,8] Using the Amplex Red reagent (Section 19.2), researchers have discovered that antibodies can convert molecular oxygen to H_2O_2, which may be important in understanding a new chemi-

Table 19.1 Reactive oxygen species.

Reactive Oxygen Species	Structure	Detection Reagents	
Hydrogen peroxide	H_2O_2	• Carboxy-H_2DCFDA (C-400) [1–3] • CM-H_2DCFDA (C-6827) [4,5] • Dihydrocalcein AM (D-23805) • Dihydrorhodamine 123 (D-632, D-23806) [6] • Dihydrorhodamine 6G (D-633) [7]	• H_2DCFDA (D-399) [8–11] • Lucigenin (L-6868) [12,13] • Luminol (L-8455) [14] • RedoxSensor Red CC-1 (R-14060) [15]
Hydroxyl radical *	$HO\cdot$	• CM-H_2DCFDA (C-6827) [16] • Proxyl fluorescamine (C-7924) [17]	• TEMPO-9-AC (A-7923)
Hypochlorous acid	HOCl	• Dihydrorhodamine 123 (D-632, D-23806) [18] • Luminol (L-8455) [19–21]	
Nitric oxide	NO	• DAF-FM (D-23841) [22,23] • DAF-FM diacetate (D-23842, D-23844) [22,23]	• DAA (D-23840) [24] • Luminol (L-8455) [25]
Peroxyl radical	$HOO\cdot$	• BODIPY FL EDA (D-2390) [26] • BODIPY 665/676 (B-3932) [27] • H_2DCFDA (D-399) [28–32] • Carboxy-H_2DCFDA (C-400) [33] • CM-H_2DCFDA (C-6827)	• DPPP (D-7894) [34–36] • Luminol (L-8455) [37–39] • *cis*-Parinaric Acid (P-1901) [40,41] • RedoxSensor Red CC-1 (R-14060) [15]
Peroxynitrite anion †	$ONOO^-$	• H_2DCFDA (D-399) [42,43] • Carboxy-H_2DCFDA (C-400) • CM-H_2DCFDA (C-6827) • Coelenterazine (C-2944) [44]	• Dihydrorhodamine 123 (D-632, D-23806) [42,45–47] • Dihydrorhodamine 6G (D-633) • Luminol (L-8455) [42,48,49]
Singlet oxygen ‡	$^1O_2$	• *trans*-1-(2′-methoxyvinyl)pyrene (M-7913) [50,51]	
Superoxide anion	$\cdot O_2^-$	• Coelenterazine (C-2944) [52,53] • Dihydroethidium (D-1168, D-11347, D-23107) [54,55] • Fc OxyBURST Green assay reagent (F-2902) [56,57] • OxyBURST Green H_2DCFDA, SE (D-2935) [58,59] • OxyBURST Green H_2HFF BSA (O-13291) [60] • Lucigenin (L-6868) [61,62] • Luminol (L-8455) [63]	• MCLA (M-23800) [64,65] • MTT (M-6494) [66] • NBT (N-6495) [67] • RedoxSensor Red CC-1 (R-14060) [15] • TEMPO-9-AC (A-7923) • XTT (X-6493) [68]

* Hydroxyl radicals can also be photosensitized by malachite green isothiocyanate (M-689) or generated by a *N*-(1,10-phenanthrolin-5-yl)iodoacetamide (P-6879) metal–ligand complex. **†** 3-Nitrotyrosine, a product of this potent nitrating reagent, can be detected with an anti-nitrotyrosine antibody (A-21285). **‡** Singlet oxygen can also be photosensitized by hypocrellin A (H-7515), hypocrellin B (H-7516), hypericin (H-7476), rose bengal diacetate (R-14000), tetrabromorhodamine 123 (T-7539) and merocyanine 540 (M-24571).

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cal arm of the immune system, as well as the evolution of antibodies and the role they may play in human diseases.[9,10] Several reviews have discussed the chemistry of the different reactive oxygen species and their lipid peroxidation products.[11–16]

The importance of the nitric oxide radical (abbreviated NO) and other reactive oxygen species as biological messengers has been increasingly recognized during the last several years.[17–22] Section 19.3 is devoted to our probes for promoting, inhibiting or detecting nitric oxide production in a variety of experimental systems.

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19.2 Generating and Detecting Reactive Oxygen Species

Molecular Probes prepares an assortment of probes for the generation of reactive oxygen species (ROS) — singlet oxygen, hydroxyl radicals, superoxide, hydroperoxides and peroxides (Table 19.1) — as well as for their fluorometric detection in solution. Although there are no equilibrium sensors that continuously monitor the level of reactive oxygen species, this section discusses a number of probes that trap or otherwise react with singlet oxygen, hydroxyl radicals or superoxide. The optical or electron spin properties of the resulting products are in some way a measure of the presence or quantity of the reactive oxygen species and, in some cases, can measure the kinetics and location of their formation.

Generating Singlet Oxygen

Singlet oxygen is responsible for much of the physiological damage caused by reactive oxygen species, including nucleic acid modification through selective reaction with deoxyguanosine.[1] The lifetime of singlet oxygen is sufficiently long (4.4 microseconds in water[2]) to permit significant diffusion in cells and tissues.[3] In the laboratory, singlet oxygen is usually generated in one of three ways: photochemically from dioxygen ($^3O_2$) using a photosensitizing dye;[4] chemically, either by thermal decomposition of a peroxide or dioxetane; or by microwave discharge through an oxygen stream. Singlet oxygen can be detected by its characteristic weak chemiluminescence at 1268 nm[5] or at 634 and 703 nm.[6]

Hypocrellins and Hypericin

Among the most efficient reagents for generating singlet oxygen are the photosensitizers hypocrellin A (H-7515, Figure 18.10), hypocrellin B (H-7516, Figure 18.11) and hypericin (H-7476, Figure 18.9). These heat-stable dyes exhibit quantum yields for singlet oxygen generation in excess of 0.7, as well as high photostability, making them important agents for both anticancer and antiviral therapy.[7–13] Hypocrellins are also specific and potent inhibitors of protein kinase C[14,15] (Section 18.3), whereas hypericin is an effective inhibitor of both protein kinase C and tyrosine protein kinase[16] with antiretroviral activity.[17]

Because their chemical reactivities are well characterized,[18,19] hypocrellins and hypericin are amenable to conjugation to a variety of primary and secondary detection reagents. Not only do these photosensitizing dyes efficiently oxidize diaminobenzidine (DAB) to form an insoluble, electron-dense DAB oxidation product (see

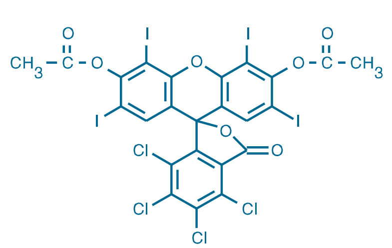

Figure 19.1 R-14000 rose bengal diacetate.

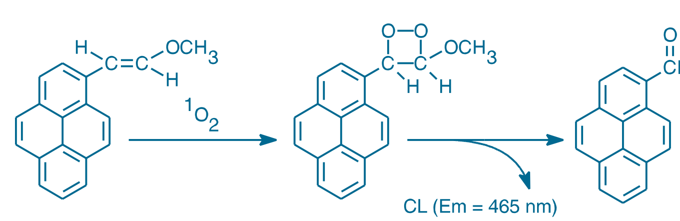

Figure 19.2 Reaction of *trans*-1-(2′-methoxyvinyl)pyrene (M-7913) with singlet oxygen ($^1O_2$), yielding a dioxetane intermediate that generates chemiluminescence (CL) upon decomposition to 1-pyrenecarboxaldehyde.