1-11-7. Biological Luminescent Probes

Overview

Biological luminescent probes are imaging tools used to visualize the structures of cells and cell organelles, tissues, and organisms, as well as the various biological phenomena that occur within them. These probes include chemically synthesized small-molecule compounds and semiconductor nanoparticles, as well as fluorescent proteins that are expressed within organisms using genetic techniques. Combined with imaging equipment such as fluorescence microscopes, these probes enable non-invasive tracking of living cells and model animals with high spatiotemporal resolution. The use of luminescent probes in the life sciences, medicine, and pharmacology fields is expected to increase in the future.

Current Status and Frontiers

Many organic and inorganic compounds exhibit fluorescence or phosphorescence when excited by light. Luminescence imaging techniques that exploit these properties to visualize cellular structures, the microenvironments of organelles, and the dynamics of proteins and bioactive substances are attracting increased attention in life science, medicine, and pharmacology research. In 1985, Tsien et al. reported the fluorescent probe Fura-2, which selectively binds to Ca^{2+}, and this discovery led to significant advances in research aimed at visualizing the intracellular dynamics of Ca^{2+} and other Ca^{2+}-related biological phenomena. Since then, chemists have developed small-molecule fluorescent probes for visualizing a variety of biomolecules. For example, Urano and Nagano et al. developed numerous small-molecule fluorescent probes for which the fluorescence "on/off" switch can theoretically be controlled by manipulating photoinduced electron transfer reactions, thus enabling visualization of intracellular enzyme activity. Urano and coworkers also developed a rapid fluorescence imaging method for cancer that uses fluorescent probes capable of detecting enzyme activity specific to cancer cells.

Many compounds present in living organisms exhibit luminescence, which is observed as background fluorescence (autofluorescence). To minimize the effects of autofluorescence and enable highly sensitive observation of biomolecules, various probes have been developed that exhibit emission in the near-infrared region of 650–900 nm, which is known as the "biological window". Recent research has also focused on the development of probes that emit light in the 1100–1350 nm range, referred to as the "second biological window", with the aim of applying these probes in studies using small animal models. In addition, time-resolved fluorescence spectroscopy is gaining attention as an alternative method for avoiding the effects of autofluorescence. Because the autofluorescence lifetime is several nanoseconds, the use of rare earth elements and metal complexes with longer emission lifetimes as probes would allow observation of probe emission after the autofluorescence has decayed. Yoshihara and Tobita et al. developed a methodology for measuring intracellular and *in vivo* oxygen levels by observing the

phosphorescence of iridium complexes.

The emission wavelength of semiconductor nanoparticles, also known as quantum dots, can be controlled by exploiting quantum size effects, resulting in sharp emission wavelengths and high-emission quantum yields. Semiconductor nanoparticles also exhibit superior photostability compared with conventional organic compound–based luminescent probes. The applications of previous semiconductor nanoparticles have been limited due to their strong hydrophobicity and risks associated with heavy metal toxicity. To address these shortcomings, considerable research has focused on enhancing biocompatibility by modifying the particle surface with polymers and other materials to impart hydrophilicity. Additionally, quantum dots using low-toxicity metals and carbon quantum dots generated from nano-sized carbon particles are being developed as new biocompatible luminescent probes.

Shimomura et al. first discovered green fluorescent protein (GFP) in 1962 from the jellyfish *Aequorea victoria*. Since the GFP gene was cloned in 1992, its fluorescent properties have been elucidated, and it is widely used as a fluorescent probe. Unlike chemically synthesized probes, GFP can be synthesized directly by an organism of interest. Furthermore, by linking the GFP gene to a target protein gene, expression of the target protein can be visualized by monitoring GFP fluorescence. The fluorophore of GFP consists of three amino acid residues; mutation of these amino acids has enabled the development of fluorescent proteins that exhibit blue or yellow fluorescence. Proteins that exhibit changing fluorescent properties upon light irradiation and proteins that exhibit differing fluorescence wavelengths depending on the stage of the cell cycle have also been developed, thus enabling the real-time visualization of many biological phenomena. These fluorescent proteins can also be used in small animals, and transgenic mice expressing a variety of fluorescent proteins have been generated and now serve as powerful research tools in the life sciences, medicine, and pharmacology fields.

References

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Future Perspectives and Directions

Issues to be resolved and realized within the next 10 years include the following: (i) the development of practical luminescent probes that can be used in the “second biological window”, and (ii) the development of luminescent probes that target specific human diseases.

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