Synthesis of Protein-Based Photosensitizer for Cancer Therapy
タンパク質からなるがん治療用光増感剤の合成

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This thesis described the synthesis and cytotoxicity of protein-based photosensitizers and antitumor drugs for cancer therapy. In Chapter 1, the theory of photodynamic therapy (PDT) was described as general introduction. PDT induces cell death using reactive oxygen species (ROS), which are generated by light irradiation to photosensitizers accumulated in tumors. The salient advantage of PDT is a non-invasivity, unlike surgery. The partial O_2 pressure in the tumor microenvironment (TME) is less than 2.5 Torr. The hypoxic condition hinders PDT. Three strategies have been proposed for O_2 supply to tumor tissues; (i) direct O_2 transport, (ii) catalytic O_2 generation from hydrogen peroxide (H_2O_2), and (iii) splitting water into O_2 and H_2.

Chapter 2 described the synthesis and PDT activity of hemoglobin–albumin cluster incorporating protoporphyrin IX (PP) [Hb-(HSA-$PP_{0.7}$)$_3$] with O_2 transport capability and metHb-(HSA-$PP_{0.7}$)$_3$ without O_2 transport capability. The PP might bind to a single heme pocket (subdomain IB) in HSA moieties of Hb-HSA_3. Hb-(HSA-$PP_{0.7}$)$_3$ demonstrated higher PDT activity than the PP bound HSA (HSA-PP). The half maximal inhibitory concentrations (IC_{50}) of HSA-PP and Hb-(HSA-$PP_{0.7}$)$_3$ were 2.4 μM and 1.8 μM, respectively. This result implied that O_2 transportation of Hb enhanced photodynamic activity. Surprisingly, metHb-(HSA-$PP_{0.7}$)$_3$ (IC_{50} = 1.8 μM) exhibited much stronger photodynamic activity than that of Hb-(HSA-$PP_{0.7}$)$_3$. Catalytic disproportionation of H_2O_2 to produce O_2 improved hypoxia in TME compared with direct O_2 transport.

Chapter 3 described the synthesis and PDT activity of catalase–albumin cluster incorporating PP [Cat-(HSA-PP)$_5$]. Cat is the highest H_2O_2 disproportionation enzyme. Cat-(HSA-PP)$_5$ showed much greater PDT activity (IC_{50} = 0.7 μM) than metHb-(HSA-$PP_{0.7}$)$_3$ (IC_{50} = 1.8 μM). In addition, the photodynamic activities of Cat-(HSA-PP)$_5$ and HSA-PP under hypoxic conditions ([O_2] = 1%) were evaluated. Although the cell viability was higher than that under normoxic conditions ([O_2] = 20%), Cat-(HSA-PP)$_5$ showed superior photodynamic activity compared to HSA-PP. These results concluded that the enzymatic O_2 formation from H_2O_2 by Cat in tumor cells was valid to promote PDT.

Chapter 4 described the synthesis and PDT activity of zinc-substituted and cross-linked Hb-HSA_3 cluster (ZnXHb-HSA_3). Apohemoproteins can incorporate various metalloporphyrins in their heme pockets. However, an apoHb is easily dissociated to αβ dimer and causes aggregation.

Intramolecularly cross-linking between two Cys-β93 residues of Hb was conducted using 1,6-bis(maleimido)hexane (BMH). XHb-HSA_3 was synthesized according to Chapter 2. The heme of XHb-HSA_3 could be removed by the classical acid–butanone method. Finally, ZnP reconstituted XHb-HSA_3 (ZnXHb-HSA_3) was synthesized, and its PDT activity was evaluated using HeLa cells. Without light irradiation, the ZnXHb-HSA_3 group and the ZnXHb group showed no cytotoxicity, irrespective of the ZnP concentration (0–10 μM). The cell viabilities of the ZnXHb-HSA_3 group and the ZnXHb group decreased markedly by light irradiation, dependent on ZnP concentration. The IC_{50} values of ZnXHb-HSA_3 and ZnXHb were ascertained as 3.4 μM and 7.8 μM, respectively.

Chapter 5 described the synthesis and PDT activity of zinc-substituted myoglobin–albumin fusion protein (ZnMb-HSA). Novel fusion hemoprotein, Mb-HSA, was synthesized using *Pichia pastoris* as a host cell. Mb-HSA was efficiently secreted into the culture medium. The Mb-HSA maintained the individual constitutive protein structures and O_2 binding property of Mb. The heme was removed from Mb-HSA by acid–butanone method, yielding apoMb-HSA. The Mb unit of apoMb-HSA selectively captured ZnP to produce ZnMb-HSA. The PDT activity of ZnMb-HSA (IC_{50} = 5 μM) was almost the same as that of clinically used Photofrin. The ZnMb-HSA showed longer blood persistence than that of ZnMb *in vivo*. It was concluded that ZnMb-HSA is an effective protein photosensitizer for PDT.

Chapter 6 described the synthesis and cytotoxicity of polyoxazoline conjugated L-asparaginase (POx-ASP). L-asparaginase (ASP), a hydrolysis enzyme of L-asparagine to L-aspartic acid and ammonia (NH_3), is used for acute lymphoblastic leukemia (ALL). The administration of ASP from bacteria causes hypersensitivity reactions because of the production of anti-ASP antibodies. POx, a water-soluble polymer, shows biocompatibility and biodegradability similarly to polyethylene glycol (PEG). To reduce immunogenicity, POx-ASP was synthesized (POx binding number per ASP was 10 ± 1). The enzyme activity of POx-ASP (124 ± 10 U/mg) decreased to 60% of ASP (219 ± 10 U/mg). Cytotoxicity of POx-ASP was evaluated using ALL cells (MOLT-4) and chronic myelogenous leukemia (CML) cells (K562). The cytotoxicity of POx-ASP was almost the same as ASP against each cell. Furthermore, the POx-ASP did not produce anti-ASP antibodies from animal experiments using rats. The POx-ASP having a high therapeutic effect and low immunogenicity, is expected to be useful as a new ALL medicine.

Chapter 7 described conclusions and future prospects of this research. All protein-based photosensitizers and antitumor drugs showed superior cytotoxicity in the cell experiments.