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The convenient screening method using albumin for the tumor localizing property of Ga-porphyrin complexes

Yoshinori Nakae[a,b,*], Ei-ichiro Fukusaki[a], Shin-ichiro Kajiyama[a], Akio Kobayashi[a], Susumu Nakajima[c], Isao Sakata[b]

[a] *Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1, Yamadaoka, Suita, Osaka 565-0871, Japan*
[b] *Photochemical Co. Ltd., 5319-1, Haga, Okayama 701-1221, Japan*
[c] *Health Care Administration Center, Obihiro University of Agriculture and Veterinary Medicine, 2-11, Inada-cho, Obihiro, Hokkaido 080-8555, Japan*

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Abstract

Eleven Ga-phorphyrin complexes bearing various lengths of carbon-chain were synthesized and the UV–vis spectra shift patterns in various concentrations of albumin solutions were evaluated. The distributions of these derivatives into the organs were also determined by surface fluorescence using nitrogen-pulsed laser spectrophotometry. The shift patterns between 0 and 0.9% albumin concentrations (albumin test) were correlated to their tumor localizing property. The albumin test was proved to be useful for evaluation of tumor localizing properties of porphyrin derivatives. The tumor localizing ability was increased along with increasing their lipophilicity and the derivative bearing decyl groups as side chains was a maximum. The porphyrin–albumin complex might have key mission for the passive accumulation focusing on the tumor.
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1. Introduction

Photodynamic therapy (PDT) is based on the concept that the photosensitizers can be passively localized (somewhat preferentially) in neoplastic tissue, and subsequently, these photosensitizers can be activated with the appropriate wavelength of light to generate active molecular species, such as free radicals and singlet oxygen ($^1O_2$) that are toxic to cells and tissues [1]. Specific irradiation will reduce the side effect, even if a slight amount of photosensitilizer might be accumulated in normal tissues. However, PDT will be more broadly applied by an enhancement of the selectivity. Selectivity can be further enhanced by binding photosensitizers to molecular delivery systems (i.e. antibody) that have high affinity for target tissue [2–8]. The mechanism of the tumor-localizing property for the photosensitizer was investigated, in which endocytosis mediated by low density lipoprotein (LDL)–LDL receptor would play an important role [9–12]. And porphyrin would be preferentially bound to telomere sequence, which includes more in the chromosome of cancer cells than normal cell [13]. However, no correlation between LDL binding capacity and the tumor localizing ability of the photosensitizers was found [14–16]. Therefore, the mechanism of tumor localizing property of porphyrins has been unclear. Almost of chemical drugs form the complexes with proteins in plasma [17,18] and are often bound to components, e.g. glucuronic acid in organs. The photosensitizers for PDT should be also interact with plasma proteins immediately after intravenous injection. Porphyrin derivatives are thought to interact with albumin, which is the most abundant protein in plasma [16,19–23]. Here we present a simple screening method using albumin for the tumor localizing property.

* Corresponding author. Tel.: +81 862869777; fax: +81 862869778.
E-mail address: pccnakae@opticor.jp (Y. Nakae).

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2. Experimental

2.1. Materials

Protoporphyrin IX dimethyl ester (PP-Me, **1**) was purchased from Sigma–Aldrich, Japan (Toyko) and used without further purification.

Protoporphyrin IX dimethyl ester (50 g, PP-Me, **1**) was dissolved in pyridine (1400 mL). 10% $GaCl_3$ (500 mL) was added and sealed to keep at 150 °C for 90 min. The solution was evaporated to dryness. After the cake was washed with distilled water and dried, it was dissolved in MeOH (200 mL). The solution was added of 2 mol/L KOH in MeOH (400 mL) to hydrolyze at a room temperature for 90 min. The solution was added of saline (1300 mL) and 20% citric acid in water (300 mL) to precipitate. The precipitate was filtered and dried to obtain Ga-PP-H (32 g, **2**, 58%). MS (ESI^+) *m/z*: 629 $[M-35]^+$. IR (KBr) ν: 2917, 1715, 1618, 1380, 1232, 1151, 1124, 1092, 1055, 990, 946, 838, 719 cm^{-1}. UV–vis (MeOH) λ_{max} (ε): 577 (24,600), 538 (20,500), 405 (414,000) nm. Analytically calculated for $C_{34}H_{35}N_4O_6Ga$: C, 61.37; H, 5.30; N, 8.42. Found: C, 61.32; H, 5.28; N, 8.37.

Ga-PP-H (**2**, 1 g, 1.4 mmol) was dissolve in acetic acid (6 mL) and 25% HBr in acetic acid (9 mL) to stand for 48 h at darkness. Acetic acid and HBr were evaporated to remove. The cake was dissolved in various kinds of alcohol (15 mL) to stand at a room temperature for 120 h under darkness. The solution was added of distilled water (200 mL) to precipitate. The precipitation was purified by a ODS column chromatography (water–MeOH) to obtain the intermediates **3**–**13**. Subsequently, compound **3**–**13** (each 0.8 g) was dissolved in *N*,*N*-dimethylacetamide (50 mL) and added of dicyclohexylamine (200 mg, 1.1 mmol), dimethyl-L-aspartate hydrochloride (3 g, 22.6 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (3 g, 15.6 mmol) to stir at a room temperature for 8 h. The solution was added of distilled water (200 mL) to precipitate (**14**–**24**). The precipitation was added of EtOH (16 mL) and 0.7 mol/L sodium hydroxide in water (18 mL) to stir at a room temperature for 90 min. The solution was adjusted to pH 5 with 10% citric acid in water and added of distilled water (200 mL) to precipitate. The precipitation was dried to obtain desired eleven derivatives (**25**–**35**, 0.6–1.1 g, Cn-Ga-DP-Asp) (Fig. 1).

13,17-Bis(1,2-dicarboxyethyl)carbamoylethyl-2,7,12,18-tetramethyl-3,8-bis(1-methyloxy)ethylporphyrin Gallium (III) (C1-Ga-DP-Asp, **25**). MS (ESI^+) *m/z*: 923 $[M-35]^+$. IR (KBr) ν: 2975, 2931, 1722, 1640, 1538, 1448, 1389, 1375, 1232, 1188, 1112, 1084, 981, 943, 842, 727 cm^{-1}. ^{1}H-NMR (MeOH-d_5, 500 MHz) δ: 3.43 (6H, m), 2.32 (4H, m), 2.35 (6H, m), 3.24 (4H, m), 3.80 (3H, s), 3.81 (3H, s), 3.85 (3H, s), 3.88 (3H, s), 4.46 (2H, m), 4.49 (4H, m), 6.26 (2H, m), 10.55 (1H, s), 10.60 (1H, s), 10.92 (1H, s), 10.98 (1H, s) ppm. UV–vis (MeOH) λ_{max} (ε): 570 (24,700), 533 (25,400), 400 (616,300), 380 (83,600) nm. Analytically calculated for $C_{44}H_{53}N_6O_{14}Ga$: C, 55.07; H, 5.57; N, 8.76. Found: C, 55.03; H, 5.49; N, 8.79.

13,17-Bis(1,2-dicarboxyethyl)carbamoylethyl-2,7,12,18-tetramethyl-3,8-bis(1-ethyloxy)ethylporphyrin Gallium (III) (C2-Ga-DP-Asp, **26**). MS (ESI^+) *m/z*: 951 $[M-35]^+$. IR (KBr) ν: 2972, 2929, 1718, 1638, 1390, 1374, 1233, 1137, 1098, 950, 926, 841, 725 cm^{-1}. ^{1}H-NMR (MeOH-d_5, 500 MHz) δ: 1.27 (6H, m), 2.33 (4H, m), 2.38 (6H, m), 3.30 (4H, m), 3.66 (2H, m), 3.80 (3H, s), 3.82 (3H, s), 3.84 (3H, s), 3.86 (3H, s), 3.93 (2H, m), 4.43 (2H, m), 4.55 (4H, m),

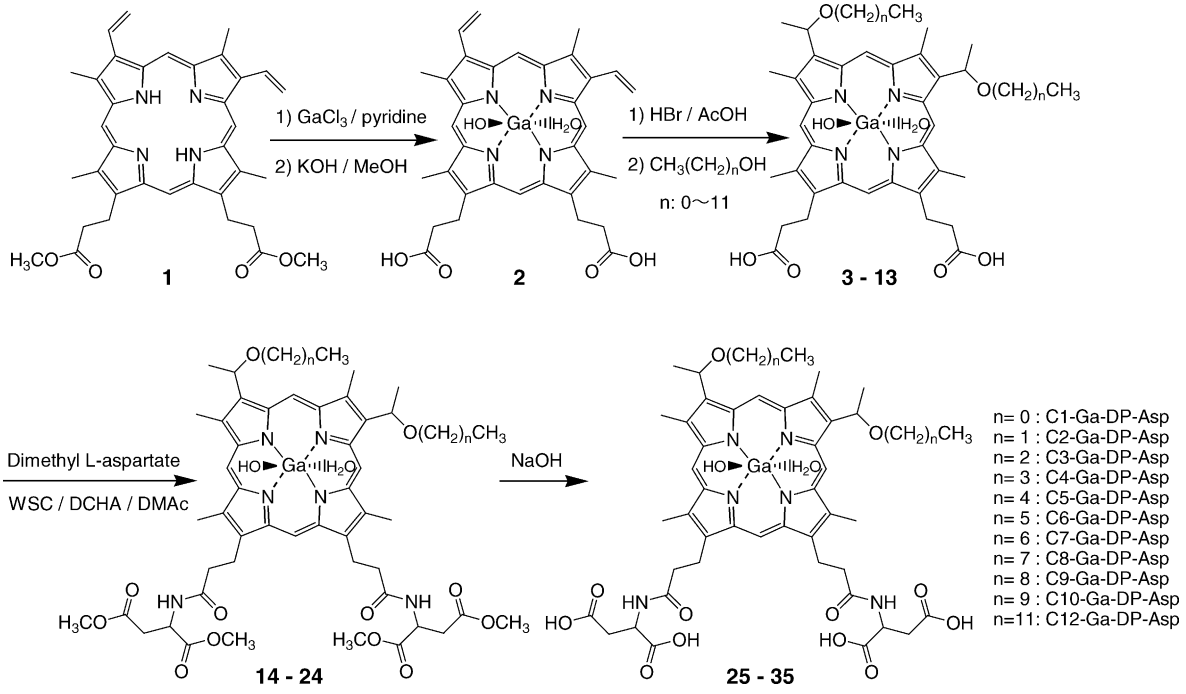

Fig. 1. Synthesis scheme of Ga-porphyrin complex (Cn-Ga-DP-Asp).

6.29 (2H, m), 10.60 (1H, s), 10.62 (1H, s), 11.01 (1H, s), 11.05 (1H, s) ppm. UV–vis (MeOH) λ_{max} (ε): 571 (23,700), 533 (23,700), 400 (613,700), 380 (73,700) nm. Analytically calculated for $C_{46}H_{57}N_6O_{14}Ga$: C, 55.94; H, 5.82; N, 8.51. Found: C, 55.97; H, 5.77; N, 8.56.

13,17-Bis(1,2-dicarboxyethyl)carbamoylethyl-2,7,12,18-tetramethyl-3,8-bis(1-propyloxy)ethylporphyrin Gallium (III) (C3-Ga-DP-Asp, **27**). MS (ESI^+) *m/z*: 979 $[M-35]^+$. IR (KBr) ν: 2933, 2862, 1722, 1638, 1544, 1454, 1389, 1232, 1136, 1092, 974, 943, 840, 730 cm^{-1}. ^{1}H-NMR (MeOH-d_5, 500 MHz) δ: 0.85 (6H, m), 1.74 (4H, m), 2.35 (4H, m), 2.37 (6H, m), 3.29 (4H, m), 3.72 (2H, m), 3.82 (3H, s), 3.83 (3H, s), 3.87 (3H, s), 3.89 (3H, s), 3.92 (2H, m), 4.50 (2H, m), 4.62 (4H, m), 6.30 (2H, m), 10.46 (1H, s), 10.52 (1H, s), 10.91 (1H, s), 11.00 (1H, s) ppm. UV–vis (MeOH) λ_{max} (ε): 570 (23,900), 532 (23,200), 400 (685,700), 380 (72,600) nm. Analytically calculated for $C_{48}H_{61}N_6O_{14}Ga$: C, 56.76; H, 6.05; N, 8.27. Found: C, 56.71; H, 6.13; N, 8.21.

13,17-Bis(1,2-dicarboxyethyl)carbamoylethyl-2,7,12,18-tetramethyl-3,8-bis(1-butyloxy)ethylporphyrin Gallium (III) (C4-Ga-DP-Asp, **28**). MS (ESI^+) *m/z*: 1007 $[M-35]^+$. IR (KBr) ν: 2933, 2871, 1724, 1641, 1542, 1390, 1374, 1232, 1136, 1096, 944, 838, 733 cm^{-1}. ^{1}H-NMR (MeOH-d_5, 500 MHz) δ: 0.82 (6H, m), 1.49 (4H, m), 1.83 (4H, m), 2.33 (4H, m), 2.34 (6H, m), 3.31 (4H, m), 3.69 (2H, m), 3.81 (3H, s), 3.83 (3H, s), 3.85 (3H, s), 3.86 (3H, s), 3.91 (2H, m), 4.47 (2H, m), 4.57 (4H, m), 6.24 (2H, m), 10.50 (1H, s), 10.59 (1H, s), 10.99 (1H, s), 11.09 (1H, s) ppm. UV–vis (MeOH) λ_{max} (ε): 570 (21,600), 533 (20,900), 400 (627,000), 380 (66,100) nm. Analytically calculated for $C_{50}H_{65}N_6O_{14}Ga$: C, 57.53; H, 6.28; N, 8.05. Found: C, 57.48; H, 6.33; N, 8.12.

13,17-Bis(1,2-dicarboxyethyl)carbamoylethyl-2,7,12,18-tetramethyl-3,8-bis(1-pentyloxy)ethylporphyrin Gallium (III) (C5-Ga-DP-Asp, **29**). MS (ESI^+) *m/z*: 1035 $[M-35]^+$. IR (KBr) ν: 2930, 2866, 1723, 1640, 1539, 1391, 1374, 1234, 1136, 1098, 946, 840, 728 cm^{-1}. ^{1}H-NMR (MeOH-d_5, 500 MHz) δ: 0.78 (6H, m), 1.13 (4H, m), 1.46 (4H, m), 1.77 (4H, m), 2.31 (4H, m), 2.38 (6H, m), 3.25 (4H, m), 3.66 (2H, m), 3.74 (3H, s), 3.80 (3H, s), 3.81 (3H, s), 3.84 (3H, s), 3.86 (2H, m), 4.44 (2H, m), 4.61 (4H, m), 6.25 (2H, m), 10.47 (1H, s), 10.66 (1H, s), 10.91 (1H, s), 11.01 (1H, s) ppm. UV–vis (MeOH) λ_{max} (ε): 570 (21,400), 532 (20,800), 400 (629,700), 380 (67,000) nm. Analytically calculated for $C_{52}H_{69}N_6O_{14}Ga$: C, 58.27; H, 6.49; N, 7.84. Found: C, 58.22; H, 6.53; N, 7.81.

13,17-Bis(1,2-dicarboxyethyl)carbamoylethyl-2,7,12,18-tetramethyl-3,8-bis(1-hexyloxy)ethylporphyrin Gallium (III) (C6-Ga-DP-Asp, **30**). MS (ESI^+) *m/z*: 1063 $[M-35]^+$. IR (KBr) ν: 2930, 2859, 1723, 1640, 1543, 1390, 1373, 1232, 1137, 1098, 950, 839, 726 cm^{-1}. ^{1}H-NMR (MeOH-d_5, 500 MHz) δ: 0.79 (6H, m), 1.13 (8H, m), 1.50 (4H, m), 1.76 (4H, m), 2.30 (4H, m), 2.35 (6H, m), 3.27 (4H, m), 3.66 (2H, m), 3.76 (3H, s), 3.79 (3H, s), 3.80 (3H, s), 3.82 (3H, s), 3.85 (2H, m), 4.45 (2H, m), 4.55 (4H, m), 6.26 (2H, m), 10.51 (1H, s), 10.64 (1H, s), 10.99 (1H, s), 11.06 (1H, s) ppm. UV–vis (MeOH) λ_{max} (ε): 570 (21,300), 533 (20,600), 400 (618,700), 380 (65,300) nm. Analytically calculated for $C_{54}H_{73}N_6O_{14}Ga$: C, 58.97; H, 6.69; N, 7.64. Found: C, 58.91; H, 6.73; N, 7.67.

13,17-Bis(1,2-dicarboxyethyl)carbamoylethyl-2,7,12,18-tetramethyl-3,8-bis(1-heptyloxy)ethylporphyrin Gallium (III) (C7-Ga-DP-Asp, **31**). MS (ESI^+) *m/z*: 1091 $[M-35]^+$. IR (KBr) ν: 2929, 2857, 1726, 1643, 1538, 1390, 1374, 1231, 1137, 1100, 944, 839, 725 cm^{-1}. ^{1}H-NMR (MeOH-d_5, 500 MHz) δ: 0.76 (6H, m), 1.15 (12H, m), 1.44 (4H, m), 1.77 (4H, m), 2.32 (4H, m), 2.36 (6H, m), 3.33 (4H, m), 3.67 (2H, m), 3.71 (3H, s), 3.73 (3H, s), 3.78 (3H, s), 3.80 (3H, s), 3.85 (2H, m), 4.39 (2H, m), 4.56 (4H, m), 6.22 (2H, m), 10.48 (1H, s), 10.53 (1H, s), 10.88 (1H, s), 10.96 (1H, s) ppm. UV–vis (MeOH) λ_{max} (ε): 570 (23,000), 533 (22,300), 400 (646,300), 380 (71,100) nm. Analytically calculated for $C_{56}H_{77}N_6O_{14}Ga$: C, 59.63; H, 6.88; N, 7.45. Found: C, 59.60; H, 6.81; N, 7.53.

13,17-Bis(1,2-dicarboxyethyl)carbamoylethyl-2,7,12,18-tetramethyl-3,8-bis(1-octyloxy)ethylporphyrin Gallium (III) (C8-Ga-DP-Asp, **32**). MS (ESI^+) *m/z*: 1119 $[M-35]^+$. IR (KBr) ν: 2928, 2857, 1719, 1638, 1545, 1388, 1234, 1136, 1100, 945, 838, 725 cm^{-1}. ^{1}H-NMR (MeOH-d_5, 500 MHz) δ: 0.77 (6H, m), 1.15 (16H, m), 1.48 (4H, m), 1.76 (4H, m), 2.30 (4H, m), 2.33 (6H, m), 3.30 (4H, m), 3.67 (2H, m), 3.77 (3H, s), 3.79 (3H, s), 3.81 (3H, s), 3.82 (3H, s), 3.87 (2H, m), 4.43 (2H, m), 4.58 (4H, m), 6.28 (2H, m), 10.53 (1H, s), 10.61 (1H, s), 10.94 (1H, s), 11.03 (1H, s) ppm. UV–vis (MeOH) λ_{max} (ε): 570 (23,800), 534 (23,100), 400 (673,900), 380 (74,100) nm. Analytically calculated for $C_{58}H_{81}N_6O_{14}Ga$: C, 60.26; H, 7.06; N, 7.27. Found: C, 60.21; H, 7.00; N, 7.22.

13,17-Bis(1,2-dicarboxyethyl)carbamoylethyl-2,7,12,18-tetramethyl-3,8-bis(1-nonyloxy)ethylporphyrin Gallium (III) (C9-Ga-DP-Asp, **33**). MS (ESI^+) *m/z*: 1147 $[M-35]^+$. IR (KBr) ν: 2927, 2855, 1721, 1639, 1549, 1390, 1232, 1101, 948, 838, 732 cm^{-1}. ^{1}H-NMR (MeOH-d_5, 500 MHz) δ: 0.78 (6H, m), 1.15 (20H, m), 1.50 (4H, m), 1.78 (4H, m), 2.28 (4H, m), 2.35 (6H, m), 3.33 (4H, m), 3.69 (2H, m), 3.74 (3H, s), 3.76 (3H, s), 3.79 (3H, s), 3.81 (3H, s), 3.89 (2H, m), 4.41 (2H, m), 4.56 (4H, m), 6.24 (2H, m), 10.58 (1H, s), 10.62 (1H, s), 10.89 (1H, s), 11.05 (1H, s) ppm. UV–vis (MeOH) λ_{max} (ε): 570 (22,000), 533 (21,300), 400 (677,100), 380 (66,800) nm. Analytically calculated for $C_{60}H_{85}N_6O_{14}Ga$: C, 60.86; H, 7.24; N, 7.10. Found: C, 60.85; H, 7.27; N, 7.02.

13,17-Bis(1,2-dicarboxyethyl)carbamoylethyl-2,7,12,18-tetramethyl-3,8-bis(1-decyloxy)ethylporphyrin Gallium (III) (C10-Ga-DP-Asp, **34**). MS (ESI^+) *m/z*: 1175 $[M-35]^+$. IR (KBr) ν: 2926, 2855, 1725, 1642, 1545, 1391, 1373, 1233, 1100, 949, 839, 725 cm^{-1}. ^{1}H-NMR (MeOH-d_5, 500 MHz) δ: 0.81 (6H, m), 1.14 (24H, m), 1.47 (4H, m), 1.79 (4H, m), 2.31 (4H, m), 2.37 (6H, m), 3.31 (4H, m), 3.64 (2H, m), 3.76 (3H, s), 3.79 (3H, s), 3.82 (3H, s), 3.83 (3H, s), 3.82 (2H, m), 4.46 (2H, m), 4.55 (4H, m), 6.28 (2H, m), 10.46 (1H, s), 10.56 (1H, s), 11.01 (1H, s), 11.09 (1H, s)

ppm. UV–vis (MeOH) λ_{max} (ε): 570 (21,200), 533 (20,500), 400 (642,700), 380 (62,900) nm. Analytically calculated for $C_{62}H_{89}N_6O_{14}Ga$: C, 61.43; H, 7.40; N, 6.93. Found: C, 61.39; H, 7.44; N, 6.99.

13,17-Bis(1,2-dicarboxyethyl)carbamoylethyl-2,7,12,18-tetramethyl-3,8-bis(1-dodecyloxy)ethylporphyrin Gallium (III) (C12-Ga-DP-Asp, **35**). MS (ESI^+) *m*/*z*: 1231 $[M-35]^+$. IR (KBr) ν: 2925, 2855, 1723, 1640, 1549, 1391, 1372, 1233, 1137, 1101, 945, 838, 726 cm^{-1}. ^{1}H-NMR (MeOH-d_5, 500 MHz) δ: 0.80 (6H, m), 1.15 (32H, m), 1.49 (4H, m), 1.76 (4H, m), 2.29 (4H, m), 2.36 (6H, m), 3.24 (4H, m), 3.64 (2H, m), 3.78 (3H, s), 3.80 (3H, s), 3.81 (3H, s), 3.83 (3H, s), 3.90 (2H, m), 4.44 (2H, m), 4.52 (4H, m), 6.23 (2H, m), 10.57 (1H, s), 10.69 (1H, s), 10.93 (1H, s), 11.11 (1H, s) ppm. UV–vis (MeOH) λ_{max} (ε): 570 (22,900), 532 (22,200), 400 (705,000), 380 (69,500) nm. Analytically calculated for $C_{66}H_{97}N_6O_{14}Ga$: C, 62.50; H, 7.71; N, 6.63. Found: C, 62.46; H, 7.77; N, 6.68.

2.2. Instrumentation

Vis spectra were obtained on a spectrometer UV-2400PC (Shimadzu) and infrared spectra on a FTIR-8200 (Shimadzu) using KBr pallete method. 1H NMR spectra were recorded using a XL-500 (Varian) spectrometer for solution in deuteromethanol with tetramethylsilane as internal standard. Mass spectra were obtained on a liquid chromatograph mass spectrometer LCMS QP8000 (Shimadzu) using ESI ion source as an interface. Thin-layer chromatography was carried out with silica gel 60 F_{254} (Merck). Column chromatography was carried out with Chromatorex 100–00 mesh (Fuji Silysia Chemical). Elemental analyses were carried by a vario EL (Elementar).

2.3. Bathochromic, hypsochromic, hyperchromic and hypochromic effects

Human serum albumin was dissolved in and diluted with saline to prepare various concentrations of albumin solution. Three kinds of Ga-porphyrin complexes (**25**, **30**, **35**) ere dissolved in phosphate buffer (pH 8.0) and diluted (5 μg/mL) with saline. These derivatives correspond to the bearing the shortest, half and longest size of carbon side chains. The Ga-porphyrin solution (2 mL) was mixed with various concentrations of albumin solution (2 mL) respectively. UV–vis spectra were measured and the bathochromic, hypsochromic, hyperchromic and hypochromic effects were observed.

2.4. Albumin test

UV–vis spectra of eleven Ga-porphyrin complexes in 0 and 0.9% albumin solution were measured by the above method. The OD_{max} at 0.9% albumin condition was normalized by subtraction with the value in the case with no addition to yield the difference value. The OD_{max} ratio that is OD_{max} (0.9% albumin) per OD_{max} (0% albumin) was calculated. The OD_{max} difference value was multiplied by OD_{max} ratio to yield the albumin test value [16,19–23]. The value expressed numerically the degree of bathochromic, hypsochromic, hyperchromic and hypochromic effects for Ga-porphyrin complexes in albumin solution.

2.5. Biodistribution test

Syrian golden hamsters were implanted with nitrosoamine-induced pancreatic cancer for 4–6 weeks, and porphyrin derivatives (0.025 mmol/kg) which had been diluted with sodium phosphate buffer (pH 7.4) were given by intravenous injection. At 24 h after the injection, the hamsters were exsanguinated to death after blood sampling for serum examination, and dissected to collect the cancer tissues and organs. They were irradiated with nitrogen pulsed laser (wavelength: 337 nm, 2 ns). The excited fluorescent spectrum was measured. Thus, the distributions of the test compounds in the organ were conveniently determined (not so precise as HPLC) by the surface fluorescence intensities using nitrogen pulsed laser spectrofluorometry [24].

2.6. PDT efficacy

Syrian golden hamsters were implanted with nitrosoamine-induced pancreatic cancer. They were injected intravenously porphyrin derivatives (3 mg/kg), which had been diluted with sodium phosphate buffer (pH 7.4). At 3 h after injection, pulsed YAG Laser (λ = 1064 nm, Heraus Lasersonic) with Q switch was irradiated to the cancer surfaces of five hamsters. The energy was 600 J/cm^2. Pulse width was 250 ns and the frequency was 1000/s. At one week after the irradiation, PDT efficacy for the tumors was observed. The long and short diameters (mm) of tumors were measured. The tumor size was calculated as [(long diameter) + (short diameter)]/2.

3. Results

3.1. The bathochromic, hypsochromic, hyperchromic and hypochromic effects

UV–vis spectra of Ga-porphyrin complexes (**25**, **30**, **35**) were shown in Fig. 2. Maximum absorption wavelengths were shifted by addition of albumin for C6-Ga-DP-Asp (**30**) and C12-Ga-DP-Asp (**35**). Absorption wavelengths at nearby 400 nm were markedly red-shifted (ca. 7 nm). A little of bathochromic shift was observed for C1-Ga-DP-Asp (**25**), which is not more than 3 nm. The shift at nearby 400 nm was not more than 1 nm. Absorption wavelengths at nearby 400 nm for the three derivatives were, respectively, almost constant in 0.1–0.9% albumin solutions. The condition of 2.7% albumin was not applicable because it afforded a serious negative effect on the evaluations of the intensities by the variation of baseline. Maximum absorption intensity at nearby

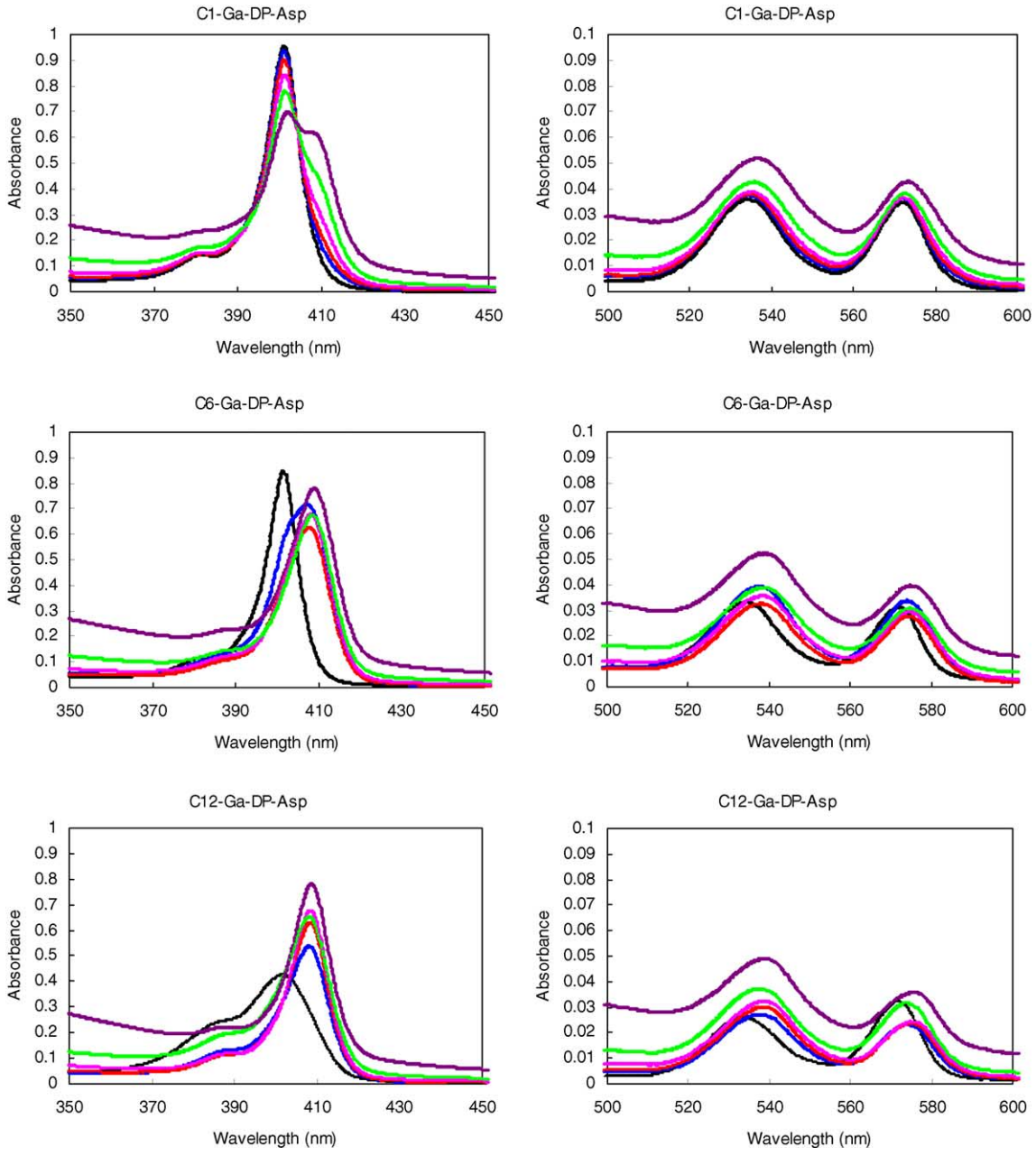

Fig. 2. UV–vis spectra of Ga-porphyrin complexes in various concentrations of albumin (black: 0%, blue: 0.033%, red: 0.1%, pink: 0.3%, green: 0.9%, purple: 2.7%). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

400 nm decreased depending on the increase of albumin concentration and the intensities at nearby 530 and 570 nm increased conversely for C1-Ga-DP-Asp (**25**). The intensities increased or decreased independently on albumin concentrations but the intensities at nearby 400 nm were almost constant in 0.3–0.9% albumin solutions for C6-Ga-DP-Asp (**30**). For C12-Ga-DP-Asp (**35**), all of the maximum absorption intensities increased depending on the albumin concentrations. The intensities at nearby 400 nm were close to each other in 0.1–0.9% albumin solutions. After surveillance the albumin concentration and the absorption wavelength were decided as 0.9% and ca. 400 nm.

3.2. Albumin test

The wavelengths and the intensities ($n = 3$) of Ga-porphyrin complexes (**25**–**35**) were shown in Table 1. The albumin test values were calculated by using them. The albumin test value (Fig. 3) increased gently ranged 1–3, markedly at 4, gently 4–10 of carbon number as side chain. The value at C12-Ga-DP-Asp (**35**) was lower than C10-Ga-DP-Asp (**34**). Therefore, it was suggested that C10-Ga-DP-Asp (**34**) interacted to albumin extremely. And this indicates the balance between hydrophilic and hydrophobic components of Ga-porphyrin complex is important for the binding to albumin.

Table 1
λ_{max} and intensities of Ga-porphyrin complexes

Code	Name	Albumin (0%)		Albumin (0.9%)	
		λ_{max} (nm)	Intensity	λ_{max} (nm)	Intensity
25	C1-Ga-DP-Asp	401.2	0.953	401.4	0.777
26	C2-Ga-DP-Asp	401.2	1.095	401.6	0.886
27	C3-Ga-DP-Asp	401.4	0.965	402.6	0.660
28	C4-Ga-DP-Asp	401.4	1.046	408.0	0.699
29	C5-Ga-DP-Asp	401.6	0.694	408.6	0.566
30	C6-Ga-DP-Asp	401.6	0.843	408.6	0.674
31	C7-Ga-DP-Asp	401.6	0.680	408.2	0.747
32	C8-Ga-DP-Asp	401.8	0.530	409.0	0.600
33	C9-Ga-DP-Asp	401.8	0.518	409.0	0.616
34	C10-Ga-DP-Asp	400.8	0.462	408.8	0.947
35	C12-Ga-DP-Asp	401.6	0.424	408.2	0.651

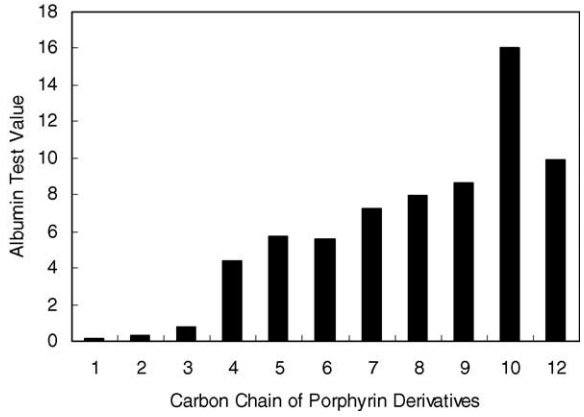

Fig. 3. Albumin test values of Ga-porphyrin complexes bearing various lengths of carbon side chains.

3.3. Tumor localizing property

Porphyrin complexes were injected intravenously into Syrian golden hamsters (three per groups) bearing tumor. At 24 h after administration, the distributions (Fig. 4) of the test compounds in the tumor tissues and other organs were determined by the surface fluorescence intensities using nitrogen pulsed laser spectrofluorometry. C10-Ga-DP-Asp (**34**) was accumulated with the highest concentration in cancer tissue and the concentrations decreased according to decreasing methylene units (carbon number as side chain). C12-Ga-DP-Asp (**35**) was accumulated with lower than C10-Ga-DP-Asp (**34**).

3.4. PDT efficacy of C10-Ga-DP-Asp (34)

YAG Laser was irradiated to five hamsters with no administration as a control. No disappearance and no reduction of tumor were observed one week after administration although an ulcer was observed. Compared with control group, remarkable tumor-reducing effect was found with C10-Ga-DP-Asp (**34**) administered group. The tumor was disappeared in two cases, reduced the size to 1/3 in two cases and to 1/4 in one case.

4. Discussion

Albumin test value increased with increasing of methylene unit as the side chain and reached maximum at decyl group. The correlation between carbon chain length and albumin test value was similar to the correlation between carbon chain length and tumor localizing property. The result suggested that albumin test would be applicable to the convenient evaluation method of tumor localizing property and that the biochemical property of porphyrin–albumin complex is of considerable importance for the tumor localizing property. The localization of many photosensitizing agents has been attributed to distribution of low density lipoprotein (LDL)-bound drug as a function of the relative numbers of LDL receptors in different tissues. While *N*-aspartyl chlorin e6 binds mainly to mouse plasma high density lipoproteins (HDL) and albumin, with only 1% bound to LDL [16]. The distribution mechanism of Ga-porphyrin complex to different tissues cannot be explained on the basis of an LDL-mediated mechanism as same as *N*-aspartyl chlorin e6. A number of

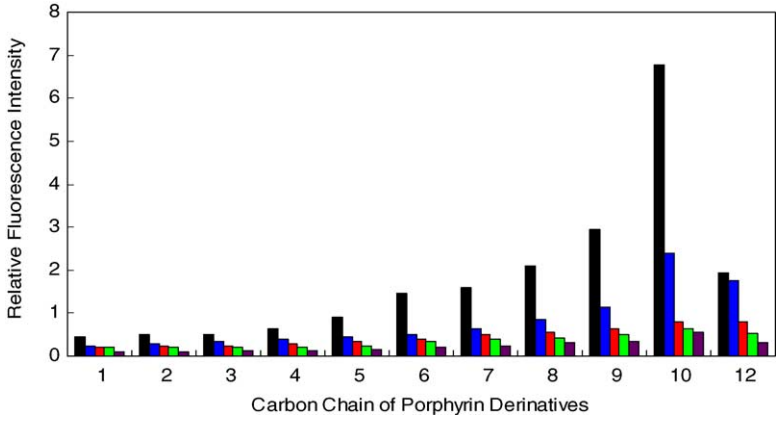

Fig. 4. Tumor localizing properties of Ga-porphyrin complexes bearing various lengths of carbon side chains (black: tumor, blue: plasma, red: liver, green: lung, purple: kidney). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

derivatives with high tumor localizing property will be found and the tumor localization mechanism will be explicated. The explication of the mechanism will be contributed to the creation of further excellent photosensitizers.

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