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Synthesis and characterization of vitamin B_{12}-hyperbranched polymer

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Abstract—Vitamin B_{12}-hyperbranched polymer was synthesized and characterized by UV–vis and NMR spectroscopy as well as DLS. The shape of hybrid polymer was also directly observed by TEM. Microenvironmental polarity around vitamin B_{12} derivative on the polymer was evaluated by UV–vis absorption peak with α-band of vitamin B_{12} chromophore.

Much attention has been focused in the last decades on the immobilization of metal complexes onto various materials, such as metals, clays, zeolites and polymers, in order to develop good catalytic system.[1] Cobalt complexes, vitamin B_{12} derivatives, are one of the good catalysts for several molecular transformation reactions,[2–6] and effort for immobilization of vitamin B_{12} derivatives on various supports has been reported by several groups. Surface modifications of carbon materials with polymers consisting of epoxy resins and vitamin B_{12} derivative were firstly reported by Scheffold et al.,[7] and some groups used this type of modified electrodes for sensor and electroorganic synthesis.[8,9] Immobilization of vitamin B_{12} derivative onto solid electrode by electropolymerization of vitamin B_{12} derivative containing a pyrrole group in a peripheral alkyl chain was achieved by Keese et al.[10] Rusling et al. also reported preparation of polymer films on graphite carbon electrode, which is constructed by covalently binding poly-L-lysine with vitamin B_{12} derivative.[11,12]

All of these studies used solid-phase support and prepared hybrid materials exhibited both advantages and disadvantages of the support due to the heterogeneous nature of the reaction conditions. For example, heterogeneity of the support reduces the work-up procedures but depresses the access of substrate resulting in poor catalysis. The use of soluble polymer may be applicable for alternative to conventional solid-phase supports to overcome such problem.[13] Dendric polymers, particularly perfect dendrimers and hyperbranched polymers, offer a wide range of new possibilities.[14] Since dendrimers have to be prepared in tedious multi step syntheses, difficult to large-scale syntheses, and show high cost, hyperbranched polymers offer a promising alternative among these soluble polymers.[15–19]

Hyperbranched polymers are rapidly and conveniently prepared in one-pot synthesis and its dendritic topology is prominent scaffolds for the immobilization of various functional compounds. Hyperbranched polymers also provide appropriate functional groups for easy attachment of target molecules.[20] Therefore, vitamin B_{12} derivative-hyperbranched polymer composite will provide desirable hybrid catalyst. In this study, the synthesis and characterization of new hybrid materials composed of vitamin B_{12} derivative and hyperbranched polymer is reported.

Hyperbranched polymer (**1**)[27] having hydroxyl group was synthesized by quasi-living radical co-polymerization of 2-(*N*,*N*-diethyldithiocarbamoyl)-ethyl methacrylate (EMA-DC) and hydroxyethyl methacrylate (HEMA) under irradiation with UV light (100 W high-pressure mercury lamp, Senlights).[21–23] Progress of co-polymerization was monitored by means of NMR and HPLC, and photoreaction was terminated when conversions of substrates were reached to nearly 50% ($M_n = 12{,}600$; $M_w/M_n = 2.6$). Vitamin B_{12} derivative having carboxylic group (**2**)[28] was synthesized by

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Keywords: Vitamin B_{12}; Hyperbranched polymer; Hybrid material; Microenvironmental polarity.

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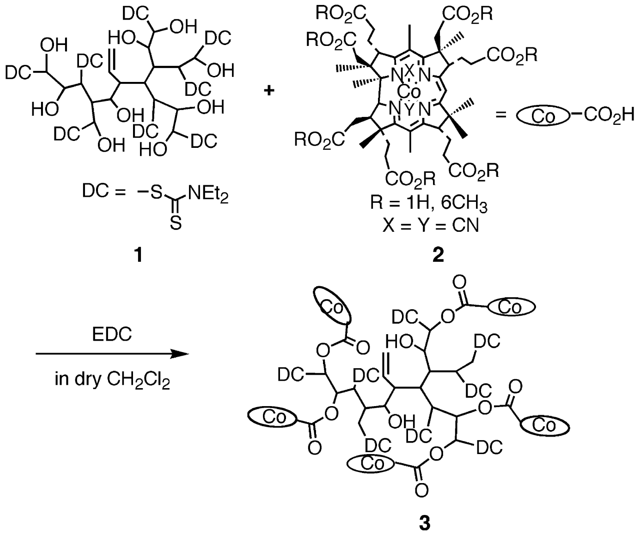

Scheme 1.

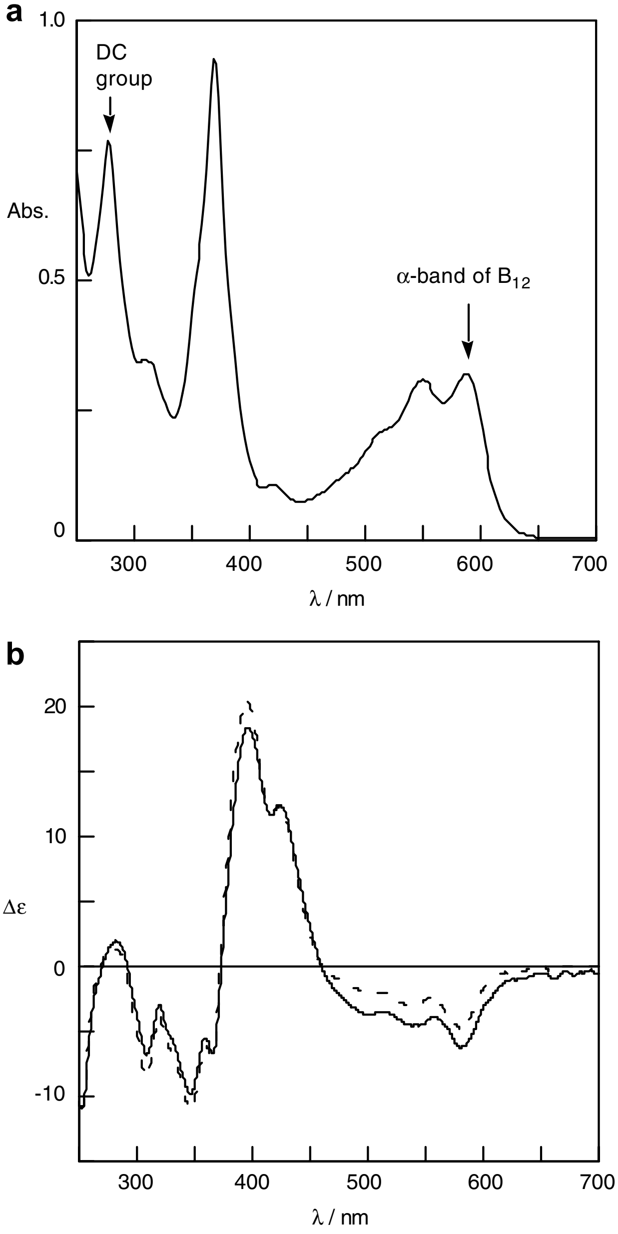

Figure 1. UV–vis absorption spectrum of **3** in CH_2Cl_2 (a), and CD spectra of **3** (solid line) and **2** (broken line) in methanol at 293 K.

hydrolysis of dicyano-cobyrinic acid heptamethyl ester[24] by Na_2CO_3. Synthesis of B_{12}-hyperbranched polymer (**3**) was achieved as shown in Scheme 1. Typical reaction condition is described as follows. To a solution of **1** (100 mg, 0.257 mmol of hydroxyl groups), **2** (556 mg, 0.511 mmol) and 4-dimethylaminopyridine (94 mg, 0.769 mmol) in 2 mL of dry CH_2Cl_2 was added *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) (198 mg, 1.03 mmol) under nitrogen atmosphere at 273 K. The solution was further stirred for 4 h at room temperature. Then the solution was washed with H_2O (15 mL × 2), and organic layer was extracted with CH_2Cl_2 (15 mL × 2). After drying over anhydrous Na_2SO_4, the CH_2Cl_2 extract was concentrated to dryness. The target product (**3**) was separated from excess **2** by gel permeation chromatography (GPC) on columns of JAIGEL-2.5H, 3H and 4H (Japan Analytical Industry Co. Ltd) in this sequence with $CHCl_3$ as elute. Thus obtained B_{12}-hyperbranched polymer (**3**) was reprecipitated from $CHCl_3$ upon addition of hexane to afford a purple powder.

Product **3** was characterized by UV–vis, circular dichroism (CD) and NMR spectroscopy.[29] The ^1H NMR spectrum of **3** reveals broad peaks corresponding to units **1** and **2**. The singlet peak characteristic of the C10 position of B_{12} corrin ring was observed at δ 5.5[25] and the methyl peak of the diethyldithiocarbamoyl (DC) group was observed at δ 1.3. The methylene proton of the DC group was overlapped with those of methyl ester groups for B_{12} (δ 3.5–3.85). The absorption spectrum of **3** in CH_2Cl_2 showed the typical shape for the vitamin B_{12} derivative, λ_{max}/nm 317, 371, 422, 550 and 588, combined with peak for the DC groups, λ_{max}/nm 280, as shown in Figure 1a. The CD spectral patterns of **3** are similar to that of **2** as shown in Figure 1b, so that the substitution of the peripheral position of B_{12} with hyperbranched polymer **1** caused no significant structural change. The size of **3** was estimated by dynamic light scattering analysis (DLS) as 5–7 nm. Molecular images of **3** were directly observed by transmission electron microscopy (TEM). TEM micrographs show dark spots in the range of 5–7 nm without staining procedures as shown in Figure 2. This observation clearly indicates that the shape of hyperbranched polymer is similar to that of dendrimers.

Content of B_{12} in hybrid polymer **3** was determined at 0.61 mmol/g by UV–vis spectroscopy, and modified B_{12} account for 72% of hydroxyl group. Esterification of the hydroxyl groups of **1** with B_{12} with various conditions was carried out by changing equivalents of **2** for each hydroxyl groups of **1**, which resulted in the different B_{12}-modification ratio in **3**. The results are summarized in Table 1. It is noted that all of the prepared B_{12}-hyperbranched polymers show high solubility toward a variety of organic solvents and will show potential advantages for soluble polymer-supported catalyst.

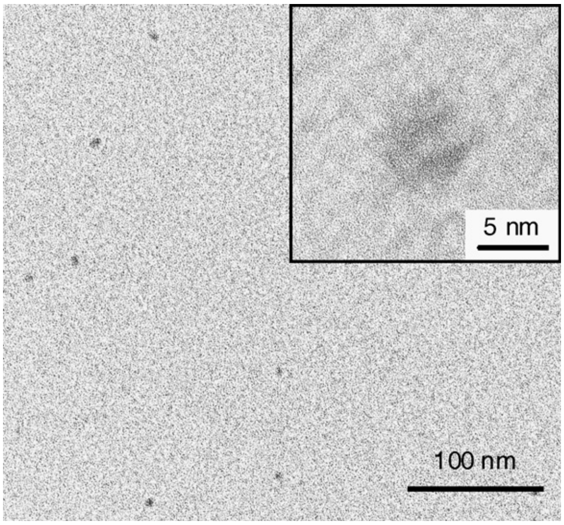

Figure 2. TEM image of **3**.

Table 1. Synthesis of **3** under various conditions[a]

Entry	Molar ratio of **2**/OH-groups of **1**	Contents of B_{12} in **3**[a] (mmol g^{-1})	Modification ratio of OH-groups of **1** with 2 (%)
1	0.1	0.13	6
2	1	0.54	51
3[b]	2	0.61	72

[a] The contents of B_{12} in the hybrid polymer **3** were determined by UV–vis absorption peaks at 588 nm ascribed to B_{12}.

[b] The UV–vis, CD and TEM data shown in Figures 1 and 2 were obtained using this sample.

The microenvironmental polarity for B_{12} on the polymer was evaluated in reference to the α-band of the B_{12} measured in various organic media.[26] The solvent dependence of the UV–vis absorption spectrum of **3** is summarized in Figure 3. The wavelengths of the α-band for **3** varied from 590.0 nm (16,949 cm^{-1}) in non-polar solvent (1,4-dioxane) through 587.4 nm (17,024 cm^{-1}) in a more polar solvent (CH_3CN) to 584.8 nm (17,099 cm^{-1}) in protic solvent (methanol).[30] These values were nearly identical with those of dicyano-cobyrinic acid heptamethyl ester in each organic solvent, and this result indicates that microenvironmental polarities for B_{12} on the polymer are similar to those in organic media.

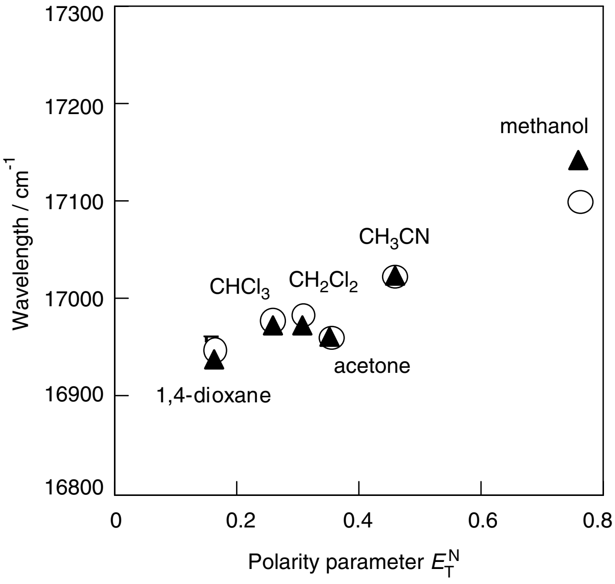

Figure 3. Correlations of α-band of **3** (open circle) and dicyano-cobyrinic acid heptamethyl ester (triangle) with solvent polarity parameters, E_T^N.

In summary, new vitamin B_{12}-hyperbranched polymers were successfully synthesized. Modification of vitamin B_{12} on hyperbranched polymers is controlled by reaction conditions. Application of this hybrid polymer for several molecular transformations is in progress in our laboratory.

Acknowledgement

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27. Compound **1**: UV–vis (in $\mathrm{CH_2Cl_2}$): [$\lambda_{\max}$/nm], 250, 280; IR, ν/cm^{-1}: 1727 (ester C=O), 3437 (O–H); ^{1}H NMR ($\mathrm{CDCl_3}$, 500 MHz): $\delta = 1.25$, 1.29 ($-\mathrm{N(CH_2C}\mathit{H}_3)_2$), 3.57 ($-\mathrm{NC}\mathit{H}_2\mathrm{CH_3}$), 3.76 ($-\mathrm{CH_2C}\mathit{H}_2\mathrm{SC(S)N}-$), 3.81 ($-\mathrm{CH_2C}\mathit{H}_2\mathrm{OH}$), 4.00 ($-\mathrm{C}\mathit{H}_2\mathrm{CH_2SC(S)N}-$), 4.08 ($-\mathrm{C}\mathit{H}_2\mathrm{CH_2OH}$), 4.17 ($-\mathrm{NC}\mathit{H}_2\mathrm{CH_3}$). The methyl protons ($-\mathrm{CH_2C(C}\mathit{H}_3)-$) were overlapped with the signals of the methylene protons in hyperbranched polymer main chains (0.7–2.2 ppm); ^{13}C NMR ($\mathrm{CDCl_3}$, 125 MHz): $\delta = 11.6$, 12.6 ($-\mathrm{N(CH_2}\mathit{C}\mathrm{H_3)_2}$), 17.6 ($-\mathrm{CH_2C(}\mathit{C}\mathrm{H_3)}-$), 34.7 ($-\mathit{C}\mathrm{H_2SC(S)N}-$), 44.8 ($-\mathrm{CH_2}\mathit{C}\mathrm{(CH_3)}-$), 46.9, 49.8 ($-\mathrm{N(}\mathit{C}\mathrm{H_2CH_3)_2}$), 54.0 ($-\mathit{C}\mathrm{H_2C(CH_3)}-$), 60.2 ($-\mathrm{CH_2}\mathit{C}\mathrm{H_2OH}$), 63.4 ($-\mathrm{C(O)O}\mathit{C}\mathrm{H_2}-$), 66.8 ($-\mathit{C}\mathrm{H_2CH_2OH}$), 177.8, 177.8 ($-\mathit{C}\mathrm{(O)OCH_2}-$), 194.1 ($-\mathrm{S}\mathit{C}\mathrm{(S)N}-$); $M_\mathrm{w} = 12{,}600$ by GPC measurement, $M_\mathrm{w}/M_\mathrm{n} = 2.6$, $T_\mathrm{g} = 53$ °C. Content of hydroxyl group in **1** was determined at 2.57 mmol/g by following calculation formula: $1/\{(M_\mathrm{w}$ of EMA-DC$)+(M_\mathrm{w}$ of HEMA$)\}$ since co-polymerization was proceeded with 1:1 ratio of starting EMA-DC and HEMA which was confirmed by NMR.
28. Compound **2**: Although hydrolysis of dicyano-cobyrinic acid heptamethyl ester by solid $\mathrm{Na_2CO_3}$ in methanol afforded a mixture of various hydrolyzed products, mainly mono and di hydrolyzed product, dicyano-cobyrinic acid hexamethyl ester (**2**) was selectively extracted with $\mathrm{CH_2Cl_2}$. Thus obtained **2** was analyzed by HPLC (column, Acquity BEH C18, Waters Co. Ltd) as $\mathrm{CH_3CN}$ and $\mathrm{H_2O}$ elutes. MALDI-TOF-MS (dithranol matrix, *m*/*z*): $[\mathrm{M}-\mathrm{CN}]^+$, 1048.4, $[\mathrm{M}-2\mathrm{CN}]^+$, 1022.3. UV–vis (in $\mathrm{CH_2Cl_2}$): [$\lambda_{\max}$/nm], 279, 316, 371, 423, 550, 589.
29. Compound **3**: Yield, 213 mg. UV–vis (in $\mathrm{CH_2Cl_2}$): [$\lambda_{\max}$/nm], 280, 371, 422, 550, 588; IR, ν/cm^{-1}: 1729 (ester C=O), 3431 (O–H); ^{1}H NMR ($\mathrm{CDCl_3}$, 500 MHz): $\delta = 1.3$ ($-\mathrm{N(CH_2C}\mathit{H}_3)_2$), 3.5–3.85 (br, $-\mathrm{NC}\mathit{H}_2\mathrm{CH_3}$, $-\mathrm{CH_2C}\mathit{H}_2\mathrm{SC(S)N}-$, $-\mathrm{CH_2C}\mathit{H}_2\mathrm{OH}$, $\mathrm{C(O)OC}\mathit{H}_3$ of $\mathrm{B_{12}}$), 4.00 ($-\mathrm{C}\mathit{H}_2\mathrm{CH_2SC(S)N}-$), 4.1–4.5 (br, $-\mathrm{C}\mathit{H}_2\mathrm{CH_2OH}$, $-\mathrm{NC}\mathit{H}_2\mathrm{CH_3}$, $-\mathrm{C(O)OC}\mathit{H}_2\mathrm{C}\mathit{H}_2\mathrm{OC(O)}-$), 5.55 (C(10)–*H* of $\mathrm{B_{12}}$).
30. 1,4-Dioxane 590.0 nm (16,949 cm^{-1}), $\mathrm{CHCl_3}$ 589.0 nm (16,978 cm^{-1}), $\mathrm{CH_2Cl_2}$ 588.4 nm (16,995 cm^{-1}), acetone 589.6 nm (16,961 cm^{-1}), $\mathrm{CH_3CN}$ 587.4 nm (17,024 cm^{-1}), methanol 584.8 nm (17,099 cm^{-1}). The resolution of the spectrometer was 0.2 nm.