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ARTICLE TYPE

Chirality induction and amplification in methylene blue H-aggregates *via* D- and L-phenylalanine pre-adsorbed on the tungsten oxide nanocolloid surface

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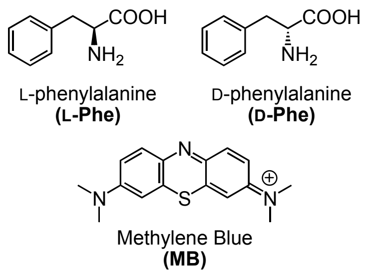
In the methylene blue (MB)/phenylalanine (Phe)/tungsten(VI) oxide (WO_3) colloid ternary aqueous solution, the MB H-aggregates, which could recognize the chirality of D- and L-Phe, were formed and investigated by means of UV-Vis absorption and CD spectroscopy. These results demonstrate a chirality transfer and amplification from only the pre-adsorbed Phe molecules to MB aggregates formed on the WO_3 colloid surface *via* non-covalent interactions.

Amino acids are important bioactive substances, they are widely used in the food, chemical and pharmaceutical industries.[1,2] Enantioselective recognition of amino acids is an important methodology for providing a better understanding of the origin of homochirality and leading to better development of biochemical and pharmaceutical chiral devices.[3-6] Great efforts have been made to recognize amino acid enantiomers, but designing an efficient method to recognize amino acids is still a challenging task.[7] There are great interests concerning the transformation of chiral information from a chiral template to achiral molecules and supramolecular systems.[8] By forming intrinsically chiral assemblies, or by aggregating on a chiral template, the symmetric achiral molecules could also present supramolecular chirality. Construction of a chiral molecular assembly is very important in supramolecular chiral recognition.[9-11] The driving force for the formation of chiral supermolecules is generally constructed on the non-covalent interactions, such as van der Waals interactions, hydrogen bonds, π-π stacking, electrostatic interaction and hydrophobic interactions.[12-14]

Phenothiazine derivatives such as methylene blue (MB) play important roles in spectroscopic studies for their novel and tunable spectroscopic, photophysical and photochemical properties.[15,16] Their unique planar as well as rigid molecular geometry and aromatic electronic feature delocalized over the molecular frame enable them to be well investigated in the field of constructing supramolecular assemblies by non-covalent interactions. In our previously reported work,[17] we found that the cationic MB dyes could form ideal H-type (face-to-face fashion) trimer on the negative charged tungsten(IV) oxide (WO_3) nanocolloid surface *via* the supramolecular self-assembly process in the aqueous solution.

Inspired by this finding, we have introduced the concept of "chirality" into the MB/WO_3 binary system by adding phenylalanine (Phe) which is one of the amino acids that are joined together to form proteins. Once the supramolecular chirality in the MB/Phe/WO_3 ternary system has been achieved, one can expect that the system would show more important and more interesting optical active architectures from the viewpoints of chiral recognition mechanism and organic/inorganic hybrid material. Herein, we describe an investigation of supramolecular chirality in the MB/Phe/WO_3 ternary system.



Scheme 1 Molecular Structures of L-, D-phenylalanine (Phe), and methylene blue (MB).

Preliminary, the adsorptivity of D- and L-Phe on the WO_3

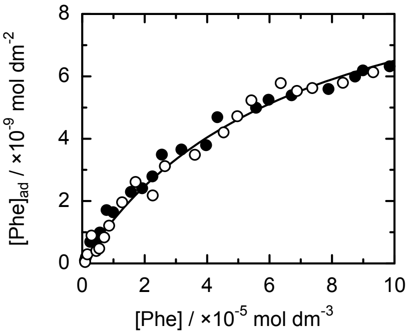


Figure 1 Adsorption isotherm of phenylalanine (open circle [○]: L-Phe, filled circle [●]: D-Phe) measured by HPLC technique onto the WO_3 colloid surface at 25ºC. The solid line is best fit of data to Langmuir equation (see Eq.(2)). Concentration: [Phe] = 1.0×10^{-4} M, [WO_3] = 1.0×10^{-6}~5.0×10^{-2} M, [NaCl] = 1.0×10^{-2} M, and pH 3.0 (HCl).

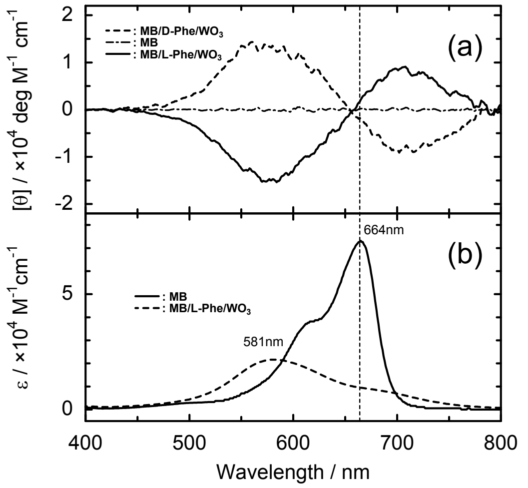


Figure 2 (a) Circular dichroism (CD) spectra of the MB monomer (chain line), the normal addition order MB/L-Phe/WO_3 ternary (solid line), and the normal addition order MB/D-Phe/WO_3 (broken line) ternary aqueous solution. (b) Adsorption spectra of the MB monomer (solid line) and the normal addition order MB/L-Phe/WO_3 ternary (broken line) aqueous solution. Concentration: [MB] = 1.0×10^{-6} M, [Phe] = 1.0×10^{-4} M, [WO_3] = 1.0×10^{-4} M, [NaCl] = 1.0×10^{-2} M, and pH 3.0 (HCl).

collid surface was investigated by HPLC technique. Typically, adsorption equilibrium plots of D- and L-Phe on the WO_3 colloid are shown in Fig. 1. The adsorption isotherm behavior of D- and L-Phe was similar to each other. From the curve-fitting analysis of Langmuir equation (see Eq.(2) in Experimental section), the adsorption constants (K_{ad}) of D- and L-Phe on the WO_3 colloid surface were calculated as $(1.60\pm0.07)\times10^{-4}$ and $(1.57\pm0.06)\times10^{-4}$ dm, respectively. Also, the saturated concentrations (a) were obtained to be $(1.10\pm0.09)\times10^{-8}$ and $(1.08\pm0.09)\times10^{-8}$ mol dm^{-2}, respectively. There was no significant difference between calculated Langmuir adsorption parameter values of D- and L-Phe. Since the isoelectric point of tungsten(VI) oxide is 0.2-0.5,[18] the WO_3 colloid surface is negatively charged in this aqueous condition (pH=3). This isotherm result clearly indicates that both D- and L-Phe, zwitterionic form at pH 3,[19] are adsorbed as monolayer on the WO_3 colloid *via* the electrostatic interaction. Using the observed a values of D- and L-Phe, the area per molecule on the WO_3 colloid surface was calculated to be ca. 150 Å^2/molecule. The projection area of Phe molecule is estimated to be 95-102 Å^2 from CPK model. Therefore, it can be seen that Phe molecules are relatively non-dense-packed on the WO_3 colloid surface, probably due to hydrogen bonds between Phe molecules. This result suggests the possibility that MB molecules would be inserted, adsorbed, and aggregated between Phe molecules pre-adsorbed on the WO_3 surface, as necessary. The Phe/WO_3 binary aqueous solutions were stable without precipitate for a week at room temperature.

The chiral aggregation behaviors of MB dye in the MB/Phe/WO_3 ternary aqueous solution were investigated. Herein, it is noteworthy that cationic MB dyes are favorable to interact electrostatically with negatively charged WO_3 colloid surface.[20] Figure 2(b) shows typical absorption spectra of MB in acidic water (as reference) and the MB/L-Phe/WO_3 ternary aqueous solution. Although MB dye tends to dimerize highly in water, the dimerization of MB dye is almost negligible under this MB concentration (1.0×10^{-6} M).[17] With the addition of the L-Phe/WO_3 binary aqueous solution into the MB aqueous solution, the absorption maximum of MB was blue-shifted to 581 nm from the absorption maximum of monomer (664 nm). The MB/D-Phe/WO_3 ternary system also showed similar UV/Vis spectral changes (not shown). Interestingly, the absorption spectra of MB aggregates in the MB/Phe/WO_3 ternary system were very similar to those of MB H-type (face-to-face fashion) trimer in the MB/WO_3 binary system.[17] Therefore, this intriguing coincidence suggests that MB dye molecules also form the H-type trimers and/or higher aggregates in the MB/Phe/WO_3 ternary system.

Moreover, when the Phe/WO_3 binary aqueous solution was added to the MB aqueous solution, the CD spectrum of the resulting ternary solution displayed the bisignated Cotton effects. The sign of the Cotton effect was reversed by the addition of Phe of opposite chirality (negative and positive CD couplet [20] for D-Phe and L-Phe, respectively), as shown in Fig. 2(a). The comparison of this CD signal peaks to the UV/Vis absorption peaks in the MB/Phe/WO_3 ternary system suggests that the bisignated Cotton effect may result from an exciton coupling within the H-type MB aggregates.[20,21] Meanwhile, MB monomer (chain line in Fig. 2(a)), Phe, WO_3, MB/L-Phe binary, MB/WO_3 binary, and L-Phe/WO_3 binary aqueous solution (see Fig. S1 in ESI) were CD silent in the visible region. Additionally, the order in which MB and Phe are pre-adsorbed on the WO_3 colloid surface has a much greater influence on the supramolecular chirality. The addition in reverse order (*i.e.*, MB/WO_3 binary aqueous solution was added to the Phe aqueous solution) gave no CD signal, even in the MB/Phe/WO_3 ternary system (see Fig. S1 in ESI). It is noted that, in the reverse addition order MB/Phe/WO_3 ternary system, the absorption shoulder peak at the red-flank of MB monomer peak (ca. 700 nm), which was observed in the normal addition order MB/Phe/WO_3 ternary system, disappears. Furthermore, the absorption spectrum in the reverse addition order MB/Phe/WO_3 ternary system is very similar to the one in the MB/WO_3 binary system (in the absence of Phe). These spectral results provide strong evidence that the

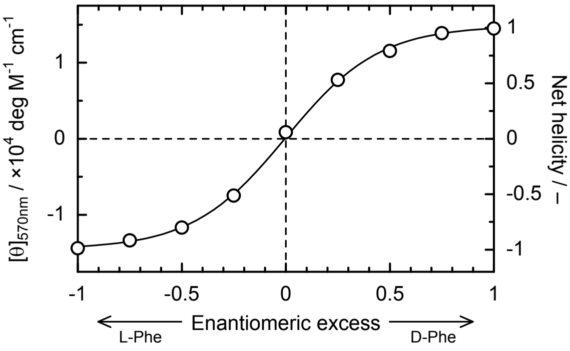


Figure 3 Molar ellipticity ([θ]) and net helicity (= $[\theta]/[\theta]_{max}$) at 570 nm as a function of enantiomeric excess (*ee*) in the MB/Phe/WO_3 ternary system. The solid line indicates the theoretical result that gives the good agreement with experimental data. Concentration: [MB] = 1.0×10^{-6} M, [Phe] = 1.0×10^{-4} M, [WO_3] = 1.0×10^{-4} M, [NaCl] = 1.0×10^{-2} M, and pH 3.0 (HCl).

true interaction between MB and pre-adsorbed Phe molecules occurs during the MB self-assembly on the WO_3 colloid surface. In any case, various findings in this study indicate that there is a "helical" transfer induced from only the chiral Phe molecules pre-adsorbed on the WO_3 colloid surface to MB aggregates formed on the WO_3 colloid surface, *via* non-covalent interactions, though the detail structure and morphology of the supramolecular chiral MB aggregate are not clear at the present stage.

We examined this induced supramolecular chirality of the MB aggregates as a function of the enantiomeric excess (*ee* = ([D-Phe]−[L-Phe])/([D-Phe]+[L-Phe])) of the chiral source of D- and L-Phe mixture in the MB/Phe/WO_3 ternary system. As shown in Fig. 3, the induced CD signal in the MB/Phe/WO_3 ternary system was found to exhibit an "S"-shaped profile when plotted against the *ee*. This finding means a major enantiomer of the chiral source dictates the helical handedness of the supramolecular aggregate, known as the "majority-rules".[22] For example, D-Phe at only 25% *ee* is able to induce a CD signal 54% as large as that observed with pure D-Phe. A chiral amplification in the MB/Phe/WO_3 ternary system is hence shown. Chiral amplification in supramolecular structures has inspired a great variety of research because of its significance in understanding the origin of chirality in nature and the applications in asymmetric synthesis.[23,24] Recently, theoretical models developed by van Gestel et al.[25-27] allow us to quantify the energies that are involved in chiral amplification processes: the mismatch energy (E_M), that is, the penalty that is paid by the minority enantiomer to be present in a helix of its non-preferred sense and the helix reversal penalty (E_R), that is, the penalty that it cost for a helix to change its helical sense. Both energies are derived from experimental data. Following the least-squares-type fitting procedure of van Gestel et al.,[26] it is possible to obtain a good agreement with our experimental data (see Fig. 3) if values of the parameters are E_M = 0.62 kJ mol^{-1} and E_R = 7.7 kJ mol^{-1}. The relatively large value for a helix reversal penalty (E_R) is very informative, because it implies that helix reversals are not likely to occur in MB aggregates formed the WO_3 colloid surface. Basically, this may suggest that, in the presence of racemic phenylalanine in the MB/Phe/WO_3 ternary system, the MB dyes tend to self-assemble into a mixture of right-handed or left-handed aggregates (in adequate proportions), rather than non-helical aggregates. Clearly, further work is necessary to confirm this hypothesis.

In summary, an approach is presented for the first time to induce chirality in H-aggregates of achiral methylene blue dyes by chiral phenylalanine molecules pre-adsorbed on the WO_3 colloid surface. These findings, together with the possibility of fine-tuning both amino acids and organic dyes on the WO_3 colloid surface, open new promising ways towards the design of efficient chiral supramolecular sensors and devices. Furthermore, organic dye aggregates with induced optical activity on the metal oxide should be attractive organic/inorganic hybrid materials in terms of fundamental and application viewpoints. More detailed mechanism of chirality transfer and amplification in the ternary system is under investigation using other chiral amino acids and organic dyes.

Experimental section

Unless specified in particular, all measurements in this study were carried out under the condition that the pH value and ionic strength were fixed at pH 3.0 with hydrochloric acid and 0.1 M (1 M = 1 mol dm^{-3}) with sodium chloride, respectively, in a thermostated room at 25±2 °C.

Materials: Sodium tungstate(VI) dihydrate ($Na_2WO_4{\cdot}2H_2O$), concentrated hydrochloric acid (Analytical Reagent, Wako Pure Chemical, Osaka, Japan), and dialytic membranes with 3500 dialytic modulus (Spectrum Laboratories, CA, USA) were used to prepare tungsten(VI) oxide (WO_3) nanocolloids. Methylene blue (MB) was obtained from Aldrich Chemical Company (Milwaukee, USA) and recrystallized from a chloroform/methanol mixture before use. D- and L-Phenylalanine (Phe) were purchased from Tokyo Kasei Kogyo Co., Ltd (>99 %*ee*, Tokyo, Japan). The water used for all sample preparation was first distilled and then passed through a Milli-Q system (Millipore, USA), resulting in the specific resistivity of 18.2 MΩcm. All other chemicals were of reagent grade from Nacalai Tesque Inc. (Kyoto, Japan), and were used as received without further purification.

Preparation of WO_3 Nanocolloid Aqueous Solution

The preparation procedure of WO_3 colloids is similar to that described by Zou et al.[28] $Na_2WO_4{\cdot}2H_2O$ (100 g, 0.3 mol) was dissolved in 100 mL of water. Conc. HCl (7 mL, 0.7 M) was added dropwise into the solution with efficient stirring. Eventually, a colorless transparent aqueous WO_3 colloid solution (pH 3.3, 0.023 M WO_3) was obtained, which was then closed in a dialytic membrane pipe and dialyzed in a 1000 mL glass beaker containing Milli-Q water for a period of 8 hrs. The Milli-Q water was periodically replaced until chloride ions could not be detected by ion chromatography. The concentration of chloride ion in the WO_3 colloid solution was determined by using portable-type IC analyzer (PIA-1000, Shimadzu, Japan) equipped with an anion-exchange Shim-pack IC-A3 column (Shimadzu, Japan) at 30°C. The eluent used in this study was 4-hydroxybenzoic acid (8.0×10^{-3} M)/bis(2-hydroxyethyl)iminotris-(hydroxymethyl)methane (Bis-Tris; 3.2×10^{-3} M), and the flow rate was 300 μL/min. From TEM measurement, the resulting products are composed of almost spherical particles with diameters over the range 8-24 nm (average diameter: 17.6 nm).[17]

Adsorption Isotherm of D- and L-Phe on the WO_3 Colloid Surface

A typical experiment would consist of first mixing the WO_3 colloid aqueous solution, water, and hydrochloric acid. Then, the D- or L-Phe aqueous solution would be added and stirred for 10 min at room temperature. It was preliminarily established that this time is sufficient for the establishment of adsorption equilibrium. The pH value in the Phe/WO_3 binary system was periodically checked with a F-14 pH meter (Horiba, Japan) equipped with a 6366-10D glass electrode (Horiba, Japan). After filtration using 0.2 micron membrane filter (Ekikuro Disk, Shimadzu, Japan), the concentration of D- or L-Phe was determined by high-performance liquid chromatography (HPLC) on a CTO-20A (Shimadzu, Japan) system equipped with a guard column and a

MIGHTYSIL RP-18 GP AQUA (250 mm L×4.6 mm I.D., 5 μm) column (Kanto Reagents, Japan) with UV (λ=254 nm) detection, using water (100%) adjusted to ion strength (0.1 M) with sodium chloride and pH 3.0 with hydrochloric acid as an eluent at 1 mL/min and 25ºC. We chose optimal conditions for each Phe and plotted the calibration curves. The accuracy of the determination of Phe within the studied concentration range was 3%. The values of adsorption of D- or L-Phe, Δ[Phe] (mol dm^{-3}), could be determined from the difference between the initial and equilibrium concentrations. In this Phe/WO_3 binary system, Δ[Phe] value can be written as

$$\Delta[\mathrm{Phe}] = [\mathrm{Phe}]_{\mathrm{ad}} \frac{S}{V} \qquad (1)$$

where $[\mathrm{Phe}]_{\mathrm{ad}}$, S, and V denote the concentration of D- or L-Phe adsorbed on the WO_3 colloid surface (mol dm^{-2}), the total surface area of WO_3 colloid (calculated from BET specific area value (11.8 m^2 g^{-1})), and the volume of the aqueous phase, respectively. The adsorption isotherms of D- and L-Phe on the WO_3 colloid surface were constructed using these HPLC data. Langmuir isotherm was given by

$$[\mathrm{Phe}]_{\mathrm{ad}} = \frac{aK_{\mathrm{ad}}[\mathrm{Phe}]}{a + K_{\mathrm{ad}}[\mathrm{Phe}]} \qquad (2)$$

where [Phe] denotes the equilibrium concentration of D- or L-Phe in the Phe/WO_3 binary system (mol dm^{-3}). a is the saturated concentration on the WO_3 colloid surface (mol dm^{-2}), and K_{ad} is the adsorption constant onto the WO_3 colloid surface (dm) and defined as the following:

$$K_{\mathrm{ad}} = \frac{[\mathrm{Phe}]_{\mathrm{ad}}}{[\mathrm{Phe}]} \qquad (3)$$

UV-Vis Absorption and Circular Dichroism Spectra Measurement in the MB/Phe/WO_3 Ternary System

The formation of the optically active aggregate of MB on the WO_3 colloid surface in the presence of D- or L-Phe was performed by batch method. UV-Vis absorption and CD spectra measurements in the MB/Phe/WO_3 ternary system were performed by the following procedures; (i) at first, the aqueous phase (4.0 mL) containing WO_3 colloid was introduced into a vial bottle by a calibrated Eppendorf pipette, (ii) then, the aqueous phase (1.0 mL) containing D- or L-Phe was added, (iii) it was stirred for 10 min (as in HPLC adsorption experiment described above), until the adsorption equilibrium of Phe onto the WO_3 colloid surface was achieved, (iv) finally, the Phe/WO_3 binary aqueous solution (2.0 mL) was put into a 1 cm path length optical quartz cell with PTFE stopper, into which the aqueous phase (2.0 mL) containing MB was added in advance., (v) static UV-Vis and CD spectra in the MB/Phe/WO_3 ternary system were obtained on a UV-1600 spectrometer (Shimadzu, Japan) and J-810 spectropolarimeter (Jasco, Japan), respectively.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Supporting UV/Vis and CD spectra in the Phe, WO_3, MB/L-Phe binary, MB/WO_3 binary, L-Phe/WO_3 binary, reverse addition order MB/Phe/WO_3 ternary system. See DOI: 10.1039/b000000x/

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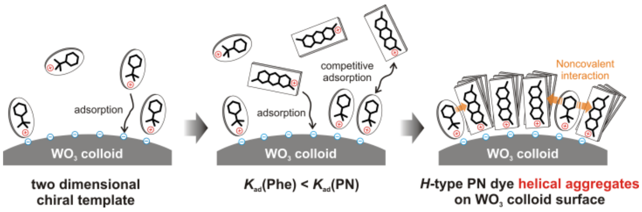

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