Chiral Recognition of Apple Procyanidins by Complexation with Oxo-Titanium Phthalocyanine

Atsuya Muranaka,† Kengo Yoshida,† Toshihiko Shoji,*,‡ Nina Moriichi,‡ Saeko Masumoto,‡ Tomomasa Kanda,‡ Yasuyuki Ohtake,‡ and Nagao Kobayashi*,†

† Department of Chemistry, Graduate School of Science, Tohoku University, Sendai, 980-8578, Japan
‡ Fundamental Research Lab. Asahi Breweries, Ltd. Ibaraki, 302-0106, Japan

1. Materials

Apple procyanidins were purified from a crude apple polyphenol fraction extracted from unripe apples (Malus pumila cv. Fuji) using methods that have been published previously.1

2. General procedures for preparation of TiPc-procyanidin complexes (TiPc)2-2

A mixture of TiOPc (2.77 mg, 3.46 × 10⁻³ mmol) and procyanidin B-2 (2) (1.00 mg, 1.73 × 10⁻³ mmol) was mixed using an agate mortar. After 15 min of grinding, the reaction mixture was dissolved in a small amount of CH₂Cl₂/CH₃OH (50:1 v/v), and size-exclusion chromatography (Bio-beads S-X1, CH₂Cl₂/CH₃OH (50:1 v/v)) was then performed to remove any free TiOPc. An initial dark-green fraction was collected, which yielded the desired TiPc-procyanidin complexes (1.74 mg, 8.11 × 10⁻⁴ mmol, 47%).

3. Spectroscopic measurements

CD spectra were measured on a JASCO J-725 spectrodichrometer using 1 mm quartz cells for procyanidin solutions and 1 cm quartz cells for TiPc-procyanidin solutions. Sample concentrations; [1] = 3.04 × 10⁻⁴ M, [2] = 1.78 × 10⁻⁴ M, [3] = 1.45 × 10⁻⁴ M, [3’] = 1.34 × 10⁻⁴ M, [4] = 1.17 × 10⁻⁴ M. Mass spectra were measured for all complexes using MALDI-TOF/MS (Biflex III, Burker Daltonics). An accelerating voltage of 19.0 kV was used for positive-ion mode spectra in the linear mode. 2,4,6-trihydroxyacetophenone (THAP) was used as a matrix.

4. Computational details

All calculations were performed using the Gaussian 03W software package. The geometry optimization of procyanidin B-2 (2) was carried out using the B3LYP functional with 6-31G* basis sets. A half-chair (quasi-equatorial) conformation based on the heterocyclic ring within the epicatechin unit was used as the initial structure of 2, since this type of structure has been observed
within X-ray crystal data. The structures predicted by DFT are essentially identical to those derived from molecular mechanics calculations using the MM2 force field. Time-dependent Hartree-Fock (TDHF) calculations were used to predict UV-visible absorption and CD spectra based on the ZINDO/S Hamiltonian. Similar positive/negative sign sequences in ascending energy terms, were predicted within both the TDHF calculations and for time-dependent density functional theory (TDDFT) calculations of the CD spectra of the (M)- and (P)-conformers, based on the use of the B3LYP functional with 6-31G* basis sets.

5. Experimental data

![Figure S1](image.png)

**Figure S1.** Separation of TiPc-procyanidin complex (dark-green band) by size-exclusion chromatography (Bio-beads S-X1, CH₂Cl₂/CH₃OH (50:1 v/v)). The blue band corresponds to TiOPc.
Figure S2. FT-IR spectra of a series of procyanidin-phthalocyanine complexes. The dotted line (972 cm\(^{-1}\)) indicates the wavenumber corresponding to the Ti=O stretching observed for TiOPc.
**Figure S3.** MALDI-TOF mass spectra of a series of procyanidine-phthalcyanine complexes. 2,4,6-trihydroxyacetophenone (THAP) was used as a matrix.
Figure S4. Fluorescence spectra of TiOPc (dotted line), and the 1:0.5 (broken line) and 1:1 (solid line) mixtures of TiOPc and epicatechin (1) recorded in CH₂Cl₂/CH₃OH (50:1 v/v) based on an excitation wavelength of 630 nm. An absorbance of 0.10 was obtained at this wavelength prior to measurement.

Figure S5. 400 MHz ¹H NMR spectrum of 1:1 complex formed between TiOPc and epicatechin (TiPc-1) in CDCl₃.
Figure S6. Theoretical UV (a) and CD (b) spectra of two conformers of procyanidin B-2 ((M)-2 and (P)-2) calculated using time-dependent DFT method (B3LYP/6-31G*). Rotational strengths ($R$) are given in cgs ($10^{-40}$ erg esu cm /Gauss).

Figure S1. Comparison of UV-visible and CD spectra of 1:1 (blue) and 2:1 (black) mixtures of TiOPc and dimeric procyanidin (2) in CH$_2$Cl$_2$/CH$_3$OH (50:1 v/v). Each sample concentration is adjusted at the absorbance corresponding to the strongest peak.
6. References

