Quercetin-induced Conformational Change of Human Serum Transferrin

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Conformational change of Tf induced by a natural compound, quercetin, is found. A possible interaction mechanism has been proposed based on the pH-dependent release of quercetin from quercetin–Tf complex and molecular modelling. These results present a potential method to use Tf for target-oriented delivery of drug molecules.

Proteins of the transferrin family, which contains serum transferrin and lactoferrin, control iron levels in higher animals through their very tight ($K_{app} \approx 10^{22}\,M^{-1}$) but reversible binding of iron.[1,2] These bilobate molecules[3,4] have two binding sites, one per lobe, each housing one Fe^{3+} and the synergistic CO_3^{2-} ion.[5] Under physiological circumstances, human serum transferrin (Tf) is approximately 30% iron-saturated in the plasma. More than 40 metal ions could bind to Tf besides Fe^{3+}. The binding strength correlates with the acidity of the metal.[6–8] Binding and release of metal ions with Tf, accompanied by substantial conformational change, might act as a delivery system of metal ions.[9,10] In addition, Tf has been proposed as a potential drug transport and delivery system targeting for tumor using covalent conjugation of molecules based on the observation that the expression of the Tf receptor is enhanced in cancer cells compared to normal cells.[11] However, non-conjugated binding of drug molecules with Tf through intermolecular interaction is less reported although this is the major way in the interaction between protein and drug molecules. In this communication, we present such a case that direct binding of a natural flavonoid compound, quercetin (structural formula is inserted in Figure 1), which possesses wide range of biological activities, with Tf.

Quercetin, a member of naturally occurring and widely distributed compounds, the flavonoids, which are ubiquitous phenolic secondary metabolites in plants, fruits, flowers, and plant derived food, generally could interact with many biomolecules including nucleic acids, enzymes, and other proteins.[12–14] In our case, under neutral condition, addition of Tf to the aqueous solution of achiral quercetin results in three negative–positive–negative CD bands ranging from 290 to 600 nm (Figure 1), while no signal is observed for transferrin alone with the same concentration in this region.[23] The relative intensities increase with increasing [Tf]. Judging from the peak shape and band position, the induced signals could not be ascribed to the quercetin because they are quite different from those obtained in the system of the quercetin/human serum albumin.[15] Additionally, in Figure 1, a strong positive peak and a negative peak appeared at 325 and 450 nm, respectively, which is similar to those of Tf with closed-state.[16] Therefore, one may expect these being due to the conformational transition of Tf from open- to closed-state induced by quercetin. This result is a little surprising, to the best of our knowledge, because such conformational transition is only observed when Tf is bound with metal ions. One question arises that how quercetin could induce conformational transition of Tf since, obviously, quercetin could not form the same strong coordinate bonds with Tf compared with metal ions through electrostatic interaction.

In order to elucidate the mechanism of conformational transition of Tf bound with quercetin, the molecular modelling calculations have been carried out by binding site analysis and docking modules of Insight II 2005. The crystal structure of Tf taken from the Protein Data Bank (PDB entry code 1D3K) is used to find the binding site of quercetin. The best energy ranked result is shown in Figure 2. As can be seen, quercetin coordinates with distorted octahedral geometry to Glu-15, Asp-63, Glu-83, Gln-206, and Lys-296 in the N-lobe of Tf via six hydrogen

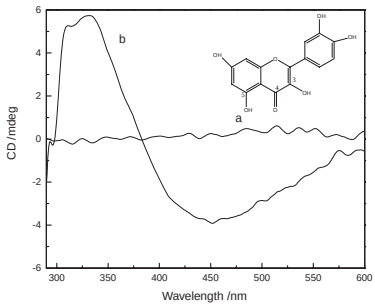

Figure 1. The CD spectra for 40 μM quercetin without (a) and with (b) 10 μM Tf in pH 7.4 PBS buffer at room temperature.

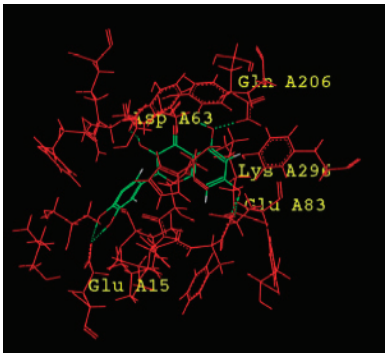

Figure 2. Structural details of the interaction of quercetin with the N-lobe of Tf obtained by molecular modelling method under neutral condition. Quercetin molecule is shown with green lines. The red lines represent the binding pocket of the N-lobe around the quercetin. The hydrogen bonds are denoted with dotted green lines.

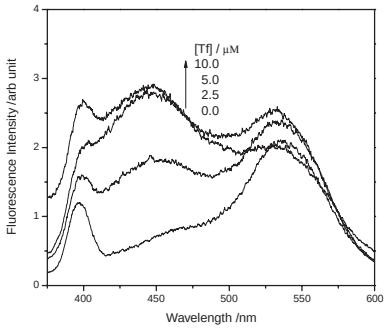

Figure 3. Effect of Tf on the fluorescence spectra of 40 μM quercetin at pH 7.4. The concentration of added Tf is 0, 2.5, 5.0, and 10.0 μM, respectively.

bonds. There is one identical amino acid residue, Asp-63, compared with the binding mode of iron in the N-lobe.[17] The same closure of two domains of N-lobe or C-lobe in Tf is probably due to quercetin binding with Tf via non-covalent bonds since Asp63 may serve as a trigger for the closure of the two domains like in Fe^{3+}-transferrin binding.[18] The hydrogen-bond lengths between Glu-15, Asp-63, Glu-83, Gln-206, and Lys-296 and corresponding positions of quercetin are 1.30 (1.38), 1.45, 1.33, 2.16, and 2.30 Å, respectively, indicating the strong binding between quercetin and Tf, which is consistent with the calculated binding energy (minus 210.68 kcal/mol including minus 47.583 (van der Waals force) and minus 163.097 (electrostatic force) kcal/mol). Additionally, the relative small associate constant ($2.48 \times 10^5\ M^{-1}$), calculated from the CD spectra,[23] is much smaller than that of iron ($10^{22}\ M^{-1}$) under the neutral condition, indicating that the interaction between quercetin and Tf is weaker than that between Fe^{3+} and Tf through electrostatic interaction.

Further fluorescence measurements at λ_{ex} 360 nm provide another support in binding of quercetin to Tf in Figure 3. Under neutral condition, free quercetin exhibits broad fluorescence with two bands, assigned to the fluorescence from normal and intramolecular excited-state proton-transfer (ESPT) tautomer, respectively. Addition of Tf greatly changes the fluorescence spectra of quercetin. A new peak at around 454 nm appears (Figure 3), which can be rationalized in terms of interference with the internal hydrogen bonds of quercetin, i.e., with C(4)=O···HO=C(5) (which facilitates non-radiative deactivation[19]) and C(4)=O··· HO=C(3) (which permits ESPT process[20,21]) occurring at the binding site in Tf.

The binding between antitumor drug and carrier proteins such as monoclonal antibodies, transferrin, or epidermal growth factor are taken up by the cell through receptor-mediated endocytosis. During internalization, the pH value is reduced from 7.4 to 5.5–4.5 within endosomes, and this pH change could be exploited through acid-labile hydrogen bond to the carrier so that the drug can be released inside the tumor cell.[22] Our pH-dependent CD spectra in the quercetin/Tf system[23] have shown that the induced CD signals' intensities of closed-state of Tf decrease upon decreasing the solution pH, and finally disappear when the pH is lower than 5.8, indicating the release of quercetin from quercetin–Tf complex. Molecular modelling calculation on acidic condition pH 4.8 reveals no closure of Tf conformation even though quercetin is bound,[23] consistent with those for Fe^{3+}. Based on these results, one may expect that Tf could also act as a "targeting delivery system" for quercetin, which may be potentially applied in cancer therapy.

In summary, Tf undergoes conformational changes during quercetin uptake and release, suggesting the future usage in targeted drug delivery system. To our knowledge, quercetin is the first known drug molecule to induce the conformational change of transferrin via non-covalent bonds. Further biological experiments are absolutedly necessary in order that Tf is used for the potential targeted delivery system of organic drug molecules for tumor cell.

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23 Supporting Information is also available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

Published on the web (Advance View) November 29, 2006; doi:10.1246/cl.2007.84