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Spectroscopic studies of the interaction between quercetin and G-quadruplex DNA

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Abstract—Quercetin is a kind of flavonoid which has been proved to exhibit anti-tumor activity. The interaction modes of quercetins with monomeric and dimeric G-quadruplexes were studied by absorption, fluorescence, CD, and ^{1}H NMR spectroscopies. The ligands were found to be stacked with terminal tetrads of monomeric G-quadruplexes by intercalation and bound to dimeric G-quadruplexes by groove binding.

In vertebrates, telomeric DNA contains a single-stranded 3′-end overhang with a simple repeat sequence, TTAGGG.[1] In buffer solution containing monovalent cations, this DNA strand could form a stable structure named G-quadruplex (Fig. 1A) composed of stacked tetrads known as G-tetrads.[2,3] Biologically, the G-quadruplex plays an important role in controlling telomerase activity which ties up with cellular immortalization and tumorigenesis.[4] Therefore, molecules, which could stabilize this conformation, are thought to be effective telomerase inhibitors.[5] Most of these ligands have similar feature: extended planar aromatic electron-deficient chromophore with cationic substituents, enabling them intercalate into G-quadruplexes easily and improve its stability, such as anthraquinones,[6] quinoacridines,[7] phenanthrolines,[8] acridines,[9] and carbocyanine dyes.[10] However, these synthetic compounds generally exhibit toxic side effects, such as marrow inhibition, limiting their further application. Looking for non-toxic or less toxic compounds with similar properties is necessary. One may expect that natural compounds having similar structure would be an ideal goal.

As a bioactive plant flavonoid,[11] quercetin (3,3′,4′,5,7-pentahydroxyflavone; Fig. 1B) was reported to exhibit

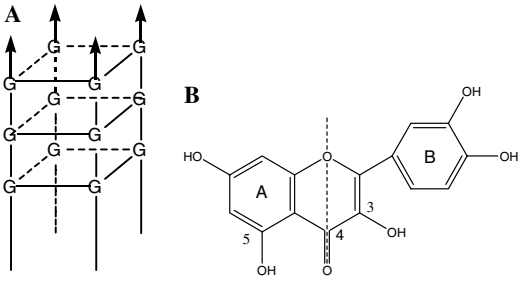

Figure 1. (A) A possible structure of G-quadruplex DNA. (B) The structure of quercetin and the divisions of bands I and II. Ring A, (band II) benzoyl system; ring B, (band I) cinnamoyl system.

anti-cancer, anti-tumor, and other therapeutic activities of significant potency, such as inhibition of the activities of calcium phospholipid-dependent protein kinase, tyrosine protein kinase from rat lung, phosphorylase kinase, phosphatidylinositol 3-kinase, and DNA topoisomerases,[12] and the systemic toxicity is quite low. Compared with the above molecules exhibiting the anti-telomerase activity, quercetin has a planar structure and additional carboxyl group that can be protonated.[13,14] One may expect it could intercalate into G-quadruplexes and exhibit anti-telomerase activity. For this purpose, investigation on the interaction between quercetin and G-quadruplex is necessary.

In this letter, the interaction between G-quadruplex and quercetin[15] has been investigated by using UV–vis

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absorption, fluorescence, ^{1}H NMR, and CD spectroscopies. It was found that quercetin could interact with G-quadruplexes by either groove binding or intercalation. This result encourages us to further explore the anti-telomerase activity of quercetins.

Using ^{1}H NMR and CD spectra, we examined the formation of G-quadruplexes from the DNA samples[16] in PB (10 mM K_2HPO_4/KH_2PO_4, 1 mM EDTA, pH 7.04) without and with KCl (Figs. 4 and 5). In ^{1}H NMR spectra, three and six signals are observed in the chemical shift region of characteristic G-tetrads[2] confirming the formation of parallel monomeric and dimeric G-quadruplexes that formed through end-to-end stacking of 3′-terminal G-tetrads in PB without and with KCl, respectively.[2] Moreover, there are negative and positive peaks at 242 and 264 nm in CD spectra of DNA, also indicating the formation of parallel G-quadruplexes,[17] which is consistent with the NMR results.

Figure 2 shows the absorption spectra of quercetin in the absence and presence of monomeric and dimeric G-quadruplexes. In two buffer solutions, the absorption spectra of free quercetins are very similar to each other exhibiting two absorption peaks at 371 nm (band I) and 260 nm (band II), which could be attributed to cinnamoyl and benzoyl systems, respectively.[11,18,19] However, the absorbance of quercetins with KCl is lower than that without KCl, probably due to the promotion of the aggregation of quercetins by KCl. Adding solution mainly containing monomeric or dimeric form of G-quadruplexes, band I red-shifts to 380 and 376 nm, respectively, and the absorbance of both bands is enhanced, indicating the formation of complex between quercetin and G-quadruplex.

Although quercetins show the similar feature in absorption spectra when they were bound with monomeric and dimeric G-quadruplexes, their fluorescence spectra are greatly different from each other.

Figure 3 shows that free quercetin is weakly fluorescent in aqueous buffer medium with two emission bands

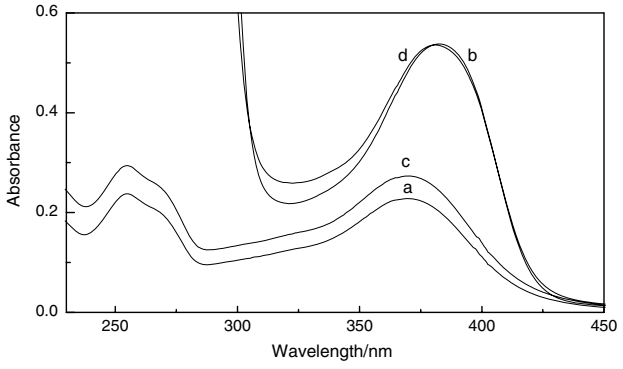

Figure 2. The absorption spectra of 24 μM quercetins (a) without and (b) with 0.2 mM monomeric G-quadruplexes (in strand) in PB (pH 7.04) containing 3.3% (v/v) ethanol, mainly (c) without and (d) with 0.2 mM dimeric G-quadruplexes (in strand) in PB buffer (pH 7.04) containing 100 mM KCl and 3.3% (v/v) ethanol.

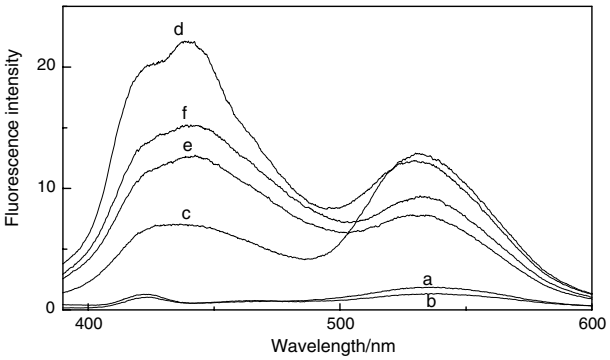

Figure 3. Fluorescence spectra of 24 μM quercetins (a) without and (c) with 0.2 mM monomeric G-quadruplexes (5′-TTAGGG-3′, in strand), and (e) with 0.12 mM G-quadruplexes (5′-TTAGGGT-3′, in strand) in PB (pH 7.04) containing 3.3% (v/v) ethanol; (b) without and (d) with 0.2 mM dimeric G-quadruplexes (5′-TTAGGG-3′, in strand) and (f) with 0.12 mM G-quadruplexes (5′-TTAGGGT-3′, in strand) in PB (pH 7.04) containing 100 mM KCl and 3.3% (v/v) ethanol.

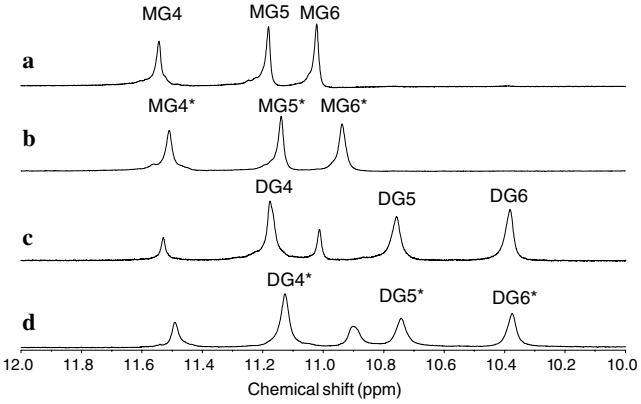

Figure 4. ^{1}H NMR spectra of 1.43 mM G-quadruplexes (in strand) (a) without and (b) with 0.4 mM quercetins in 80% H_2O/20% DMSO, PB (10 mM K_2HPO_4/KH_2PO_4, 1 mM EDTA, pH 7.04), and without (c) and with (d) 0.4 mM quercetins in PB with 100 mM KCl. Guanine imino proton signal assignments are indicated as MG_n and DG_n, where n represents residue number and superscript M and D are monomer and dimer, respectively.

with maxima at about 422 and 533 nm. It is reported that the emission band at 422 nm is assigned to normal tautomer fluorescence ($S_1 \rightarrow S_0$), while other band at 533 nm to the fluorescence emission of the proton transfer (PT) tautomer ($S'_1 \rightarrow S'_0$).[12,20,21] Proton transfer occurs between C(4)═O···HO–C(3) of quercetins, and it could occur either intermolecularly or intramolecularly. In general, the proton transfer process occurs in the excited state of quercetins[22,23] and is easily affected by the environments, such as viscosity, solvent, and temperature.[22]

After quercetin formed complex with G-quadruplex, the fluorescence intensity was increased greatly and the relative fluorescence intensity ratio between two emission bands was also changed. The fluorescence intensity of band at 533 nm was higher than that at 422 nm when quercetins were bound with monomeric G-quadruplexes, which was just opposite when quercetins were bound

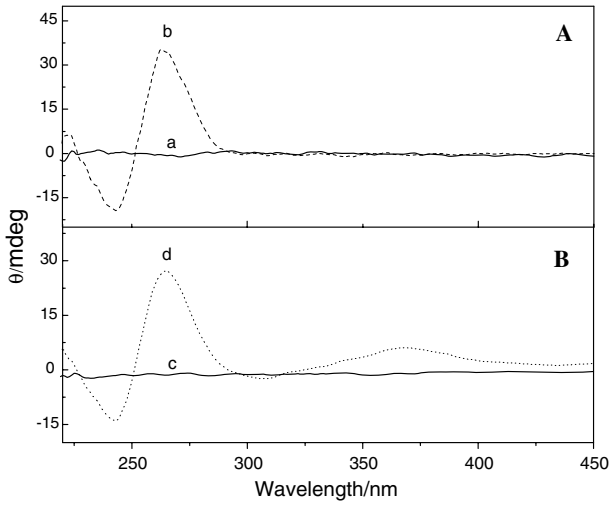

Figure 5. CD spectra of 25 μM quercetins (A) (a) without and (b) with 25 μM monomeric G-quadruplexes (in strand) in PB (pH 7.04), and (B) (c) without and (d) with 25 μM mainly dimeric G-quadruplexes (dashed line) in PB containing 100 mM KCl and 3.3% (v/v) ethanol.

with dimeric G-quadruplexes. These results suggest that when quercetins interacted with monomeric G-quadruplexes, proton transfer more easily occurred, and once quercetins were bound to dimeric G-quadruplexes, proton transfer was limited. The different fluorescence behaviors of quercetins in two solutions indicate that quercetins interacted with monomeric and dimeric G-quadruplexes differently. When small organic molecules interact with DNA, there are two major binding modes: intercalation and groove binding. In intercalation mode (Fig. 6A), quercetin is thought to be stacked between two G-tetrads or onto the end of the G-tetrad. This mode could not destroy proton transfer easily. If quercetins interacted with dimeric G-quadruplexes adopting groove binding mode (Fig. 6B), protonated carbonyl groups of quercetins would form hydrogenous bonds with phosphate anions of DNA,[14] which would inhibit proton transfer. The larger fluorescence intensity

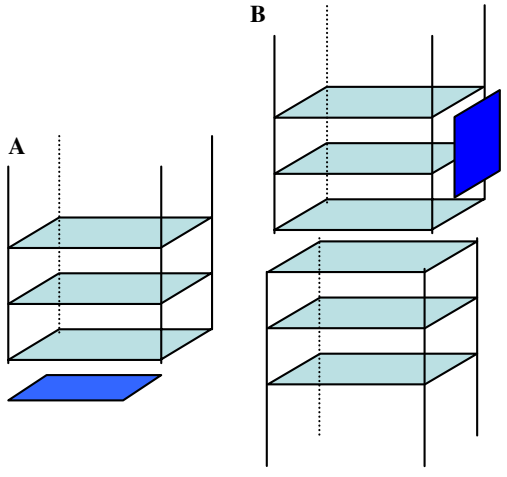

Figure 6. Schematic drawings of parallel quadruplex/porphyrin complex with (A) intercalating mode and (B) groove binding mode. Quercetins are illustrated as blue squareness.

ratio of normal tautomer when it was bound to dimeric G-quadruplex should be due to the limitation of proton transfer, implying quercetin interaction with dimeric G-quadruplexes by groove binding and with monomeric G-quadruplexes by intercalation.

Moreover, the fluorescence spectra of quercetin interacting with DNA sequences (5′-TTAGGGT-3′) (TDTs) which could not form dimeric G-quadruplex[2] with 100 mM KCl were also studied (Fig. 3). Whether KCl was present or not, after DNA was titrated in, the relative fluorescence intensity ratio between two emission bands of quercetins was almost the same, indicating that the influence of KCl on the spectra of quercetins could be neglected. The relative fluorescence intensity ratio between two emission bands of quercetins bound to TDTs G-quadruplexes was mostly the same as that of quercetin bound to dimeric G-quadruplex. G-Quadruplex formed by TDTs just like dimeric G-quadruplex had no tail G-tetrad, which was opposite to monomeric G-quadruplex. Quercetins could not easily stack to the G-tetrads of TDTs like quercetins stacking to the tail G-tetrads of monomeric quadruplexes, and probably were bound to the grooves of G-quadruplexes, which caused the fluorescence spectral features of quercetins bound to TDTs G-quadruplexes the same to that of quercetins grooving bound to dimeric G-quadruplex. The results further support our conclusion.

Above conclusion that quercetins have different binding modes when bound with monomeric and dimeric G-quadruplexes could be further proved by ^{1}H NMR spectra (Fig. 4). From upfield to downfield, three peaks belonged to exchangeable imino protons of monomeric G6, G5, and G4 tetrads in PB, and three bigger peaks attributed to those of dimeric G6, G5, and G4 tetrads, respectively. When quercetins were present, ^{1}H NMR spectra of G-quadruplexes were changed greatly: all the peaks shifted toward upfield. The peaks of the monomeric G-quadruplexes shifted more than those of dimeric G-quadruplexes. The chemical shift of G6 imino protons was changed the most among these monomeric G-tetrad imino protons, while that of the dimeric G5 was changed the most indicated monomeric G6 and dimeric G5 were the major binding sites.

Based on the above results, a conclusion could be drawn that interaction sites between quercetin and monomeric G-quadruplex should be G6 tetrads. At this interaction site, G6 tetrad was the nearest group away from quercetins. Therefore, the π electronic cloud in the quercetin would affect the chemical shift of the imino protons the most in G6 tetrad. In monomeric G-quadruplex and quercetin solution, quercetins would stack onto the terminal G6 tetrads of monomeric G-quadruplexes with their molecular planes parallel with G-tetrads. Between quercetins and G6 tetrads, there would be π–π interaction. The strong π–π stacking was likely to be the major energetic factor in stabilizing the ligand–G-quadruplex complex.[1] Whereas the chemical shift of dimeric G-quadruplexes did not change as the rule of monomeric G-quadruplexes changing, implying the

different interaction modes between monomeric and dimeric G-quadruplexes with quercetins.

The binding mode of quercetin with duplex DNA has been studied by linear dichroism (LD), and a negatively induced LD band in the Soret region indicated intercalation.[13,14,24] Here, CD spectra were used to study the binding mode of quercetin with G-quadruplex. Figure 5 exhibits the CD spectra of free and bound quercetins. Quercetins are achiral, thus do not exhibit any CD signal in solution.[13,24] When bound to monomeric G-quadruplexes, there was still no signal from quercetin. However, when quercetins were bound to dimeric G-quadruplexes, an induced positive anisotropic signal, which retraces the corresponding isotropic absorption band at 376 nm, was observed. The results suggest the different interaction modes between monomers and dimers with quercetins, consistent with other spectroscopic results. Intercalators will often exhibit lower intensity CD spectra compared with groove binders due to the fact that a groove binder contacts a larger part of the DNA and twists to follow the groove.[25] According to above conclusion and CD results, quercetin-dimers binding mode should be groove binding, and that of quercetin-monomers should be intercalation, which is in agreement with the results of fluorescence and NMR spectra.

In conclusion, by using absorption, fluorescence, ^{1}H NMR, and CD spectroscopies, we have identified that quercetins interact with monomeric and dimeric G-quadruplexes differently exhibiting two different kinds of modes: stacking mode for monomeric G-quadruplexes and quercetins with the molecular plane being parallel with G-tetrads and groove binding mode for dimeric G-quadruplexes and quercetins with the molecular plane of quercetins being vertical with the planes of G-tetrads. It is reported that telomerase inhibition activity of drugs is strongly related to the stabilization of quadruplex structure. Therefore, quercetin might be a promising candidate for potent anti-cancer drugs. Further biochemical studies using telomerase and cancer cells will reveal the effectiveness of quercetins for cancer chemotherapy.

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