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A study of the non-covalent interaction between flavonoids and DNA triplexes by electrospray ionization mass spectrometry

Cuihong Wan[a,b], Meng Cui[a], Fengrui Song[a], Zhiqiang Liu[a], Shuying Liu[a,*]

[a] Changchun Center of Mass Spectrometry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 5625 Renmin Street, Changchun 130022, PR China
[b] Graduate School of the Chinese Academy of Sciences, Beijing 100039, PR China

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ABSTRACT

The binding interactions of 22 flavonoids (9 aglycones and 13 glycosides) with DNA triplexes were investigated using electrospray ionization mass spectrometry (ESI-MS). The results revealed that the hydroxyl positions of aglycones, the locations and numbers of saccharide, as well as the aglycone skeletons play roles in the triplex-binding properties of flavonoids. The presence of 3-OH, or 3′-OH, or replacement of 4′-OH with methoxy group in aglycones decreased the fraction of bound DNA sharply. Flavonoid glycosides exhibit higher binding affinities towards the DNA triplexes than their aglycone counterparts. Glycosylations of flavones at the 8-C position and isoflavones at the 7-O position show higher binding affinities than those on the other positions of ring A of aglycones. Glycosylation with a disaccharide on C3 position of flavonol results in higher binding affinity than that with monosaccharide. Flexibility of the ring B is favorable for its interaction with DNA triplex. According to sustained off-resonance irradiation collision-induced dissociation (SORI-CID) experiments, glycosylation and non-planarity of flavonoid aglycones lead to different dissociation pathways of the flavonoid/triplex complexes. The differences between dissociation patterns suggest different DNA-binding modes or DNA-binding affinities. Although the exact binding geometry of the flavonoid–triplex complexes cannot be specified, the results may be helpful for understanding the triplex-binding properties of flavonoids and give a clue to design of triplex-binding ligands.

1. Introduction

Binding of small molecules to DNA has been studied intensively, since these small molecules are effective pharmaceutical agents, and exhibit a high potential as chemotherapeutic drugs, especially in anticancer chemotherapy. Besides the DNA duplexes, a number of alternative DNA structures have been described to date [1]. The association of more than two strands of DNA may lead to the formation of triplexes and quadruplexes [2,3]. Alternative DNA structures play important roles in transcriptional regulation and probably have profound biological implications. Therefore, unusual DNA structures, such as triplexes and quadruplexes, may serve as valid targets for therapeutic agents.

A single-strand oligonucleotide binds to the major groove of a double helix, forming a triple helix through Hoogsteen or reverse Hoogsteen hydrogen bonds. (C, T)-containing oligonucleotide binds in a parallel orientation to the purine strand of target duplex DNA [3,4]. The binding is pH-dependent because cytosine must be protonated in acidic condition to form two hydrogen bonds with guanine to stabilize the triple helix [4,5]. Triplex DNA is unstable under physiological conditions and this limitation has led to the design of small molecules that interact with the triplex and stabilize it or of chemical modifications that increase the affinity of the third strand for its target. So far, many ligands have exhibited considerable potential as triplex-specific agents. Intercalating reagents and their derivatives, such as BePI (**3-methoxy-7H-8-methyl-11[(3′-amino)propylamino]-benzo[e]pyrido[4,3-b]indole**), BQQ (**benzoquinoquinoxaline**), naphthylquinoline, coralyne, and disubstituted amidoanthraquinones, have been proven to stabilize triplex [6–9].

Among various DNA-binding organic compounds, natural products persist to be valuable and attract considerable interest since most clinical anticancer drugs are natural products or their derivatives, and most exert their effects by acting on DNA [10,11]. Flavonoids, an important class of natural products, are abundant in fruits and vegetables, and known to have many beneficial health effects and wide-ranging biological properties, such as antiviral, anti-inflammatory, antitumor, scavenging free radical, and mutagenic [12,13]. These biological activities are mostly attributed to their notable antioxidant properties as well as their inhibition of several enzymes in the human body [12,13]. Meanwhile, the interaction of flavonoids with nucleic acid structures plays an

* Corresponding author. Tel.: +86 431 85262613; fax: +86 431 85262886.
E-mail addresses: syliu@ciac.jl.cn, Syliu19@yahoo.com.cn (S. Liu).

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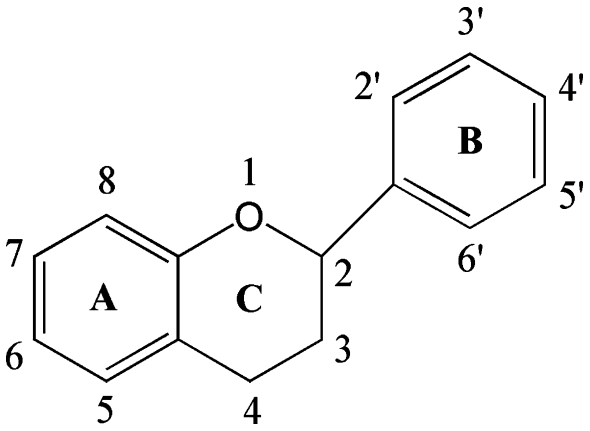

Fig. 1. General structure of flavonoid.

important role in mechanism of their actions [14–18]. For these reasons, the noncovalent interactions between flavonoids and DNA duplexes have attracted considerable interest and have been investigated using several solution-phase techniques including absorption, fluorescence, NMR spectroscopies, electrochemical and linear dichroism [19–23]. Electrospray ionization mass spectrometry (ESI-MS) has proved to be a powerful tool for drug discovery and examining drug/DNA complexes with advantages such as determination of precise stoichiometry, low sample consumption and short analysis time [24,25]. Many drug/DNA complexes have been extensively studied by ESI-MS, and the results have agreed well with those obtained using solution phase techniques [26–37]. The flavonoid/duplex DNA complexes were first investigated using ESI-MS in our laboratory [26].

Recently, the flavonoids daidzin, quercetin and rutin have been found to interact with G-quadruplexes [31,38,39]. The natural alkaloids berberine, sanguinarine, cryptolepine and aristololactam-β-D-glucoside, as well as plant pigment anthocyanins have been shown to form complexes with triplex DNA [34,40–42]. These discoveries motivated us to test the potential interaction of flavonoid with triplex DNA. As far as we know, the study of flavonoid/triplex complex has not been reported yet, neither in solution nor in gas phase. Thousands of different flavonoids have been found in plants with a common phenyl-benzopyrone skeleton (Fig. 1), their differences arise from the positions of hydroxyl and methoxy groups, and the number and location of saccharides involved in glycosylation. Studies have demonstrated that subtle changes in structures have significant effects on the biological activity [14,16,18]. Five sets of triplexes with different base contents and sequences (Table 1), and 22 flavonoids (Fig. 2, classified as flavonol, flavone, isoflavone and flavanone) were selected in this work. The purpose of the present work is to characterize the binding of flavonoids to DNA triplexes for the first time using ESI-MS, and to evaluate the influence of structural features on their DNA-binding properties.

2. Materials and methods

2.1. Chemical reagents

Quercetin, quercetrin, hyperin, rutin, luteolin, apigenin, vitexin, daidzein, daidzin, puerarin, genistein and genistin were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Kaempferol, orientin, luteolin-7-O-glucoside and isovitexin were purchased from Shanghai Winherb Medical Science Co. Ltd. (Shanghai, China). Isoorientin was purchased from Shanghai Tauto Biotech Co. Ltd. (Shanghai, China). All the above compounds were used without further purification. All the stock solutions (1 mM) of the above flavonoids were prepared in methanol. Milli-Q™ water (Millipore, Bedford, MA, USA) and HPLC grade methanol from Fisher Chemicals (Fair Lawn, NJ, USA) were used throughout the experiments.

Single strand oligodeoxynucleotides (Table 1) were purchased from Takara Biotechnology Co. Ltd. (Dalian, China) and used without further purification. Stock solutions of each single strand were prepared at 1 mM concentration in Milli-Q water. Concentrations of the single strands were estimated by their UV absorbance at 260 nm wavelength and the extinction coefficients provided by website (http://scitools.idtdna.com/scitools/Application/OligoAnalyzer). The annealing solutions containing three single strands in 150 mM ammonium acetate (pH 5.5) were heated to 90 °C for 15 min and slowly cooled at 4 °C overnight to form the DNA triplexes.

2.2. Mass spectrometry

For MS analysis, a mixture containing 1:2 ratio of each triplex DNA and flavonoid (10 and 20 μM, respectively) was prepared in 20 mM ammonium acetate solution. The solutions were allowed to equilibrate at room temperature, and 20% MeOH was added just before the sample injection.

All negative-ion ESI-MS experiments were performed on an IonSpec HiRes Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS) (Lake Forest, CA), equipped with a Micromass Z-spray electrospray source and a 7-T shielded superconducting magnet. Samples were directly infused into the source region at a rate of 4 μL/min. The spray voltage, source and probe temperature were set at 2400 V, 80 and 100 °C, respectively. All spectra were single acquisition scanned from *m/z* 600 to 2500 at data points of 1024 K and an ADC rate of 1 MHz. The sustained off-resonance irradiation collision induced dissociation (SORI-CID) was performed at +2000 Hz offset frequency with SORI voltages varied from 3 to 15 V.

3. Results and discussion

Though the interactions between flavonoids and DNA duplexes have been widely investigated by solution-phase methods and mass spectrometry [19–23,26], the interactions between flavonoids and DNA triplexes have not been reported until now. Our work focuses on the noncovalent interactions between flavonoids and DNA triplexes and evaluation of their binding abilities by using negative ion mode ESI-MS

Table 1
Oligodeoxynucleotides used in this study. TD: triplex DNA for short, and the third strand was in bold.

Name	Sequence	Mass	GC%
TD 1	5′-TCTTCTTCTTCTTC-3′ 5′-GAAGAAGAAGAAGA-3′ **5′-CTTCTTCTTCTTCT-3′**	4119.690 4400.825	35.7
TD 2	5′-CTCTCTCTTTCTCT-3′ 5′-AGAGAAAGAGAGAG-3′ **5′-TCTCTTTCTCTCTC-3′**	4104.690 4416.820	42.8
TD 3	5′-TCTCTCTCTCTCTC-3′ 5′-GAGAGAGAGAGAGA-3′ **5′-CTCTCTCTCTCTCT-3′**	4089.691 4432.815	50.0
TD 4	5′-TCTCTCTCCCTCTC-3′ 5′-GAGAGGGAGAGAGA-3′ **5′-CTCTCCCTCTCTCT-3′**	4074.691 4448.810	57.1
TD 5	5′-CTCCTCCTCCTCCT-3′ 5′-GGAGGAGGAGGAG-3′ **5′-TCCTCCTCCTCCTC-3′**	4059.691 4151.747	64.3

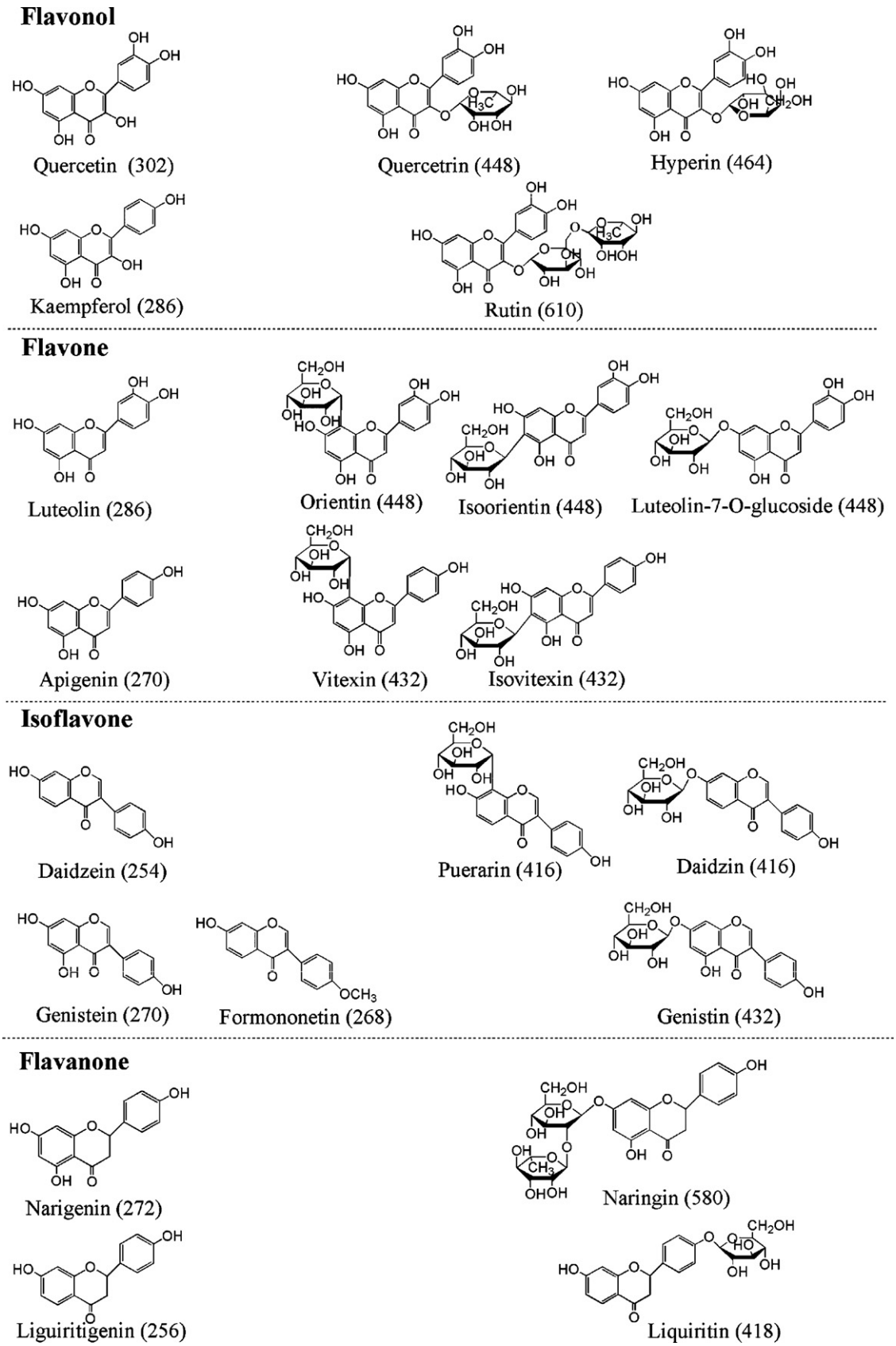

Fig. 2. Flavonoid structures and relevant molecular weights.

3.1. Binding of flavonoid aglycones to DNA triplexes

Mixtures of one flavonoid aglycone and one triplex at a 2:1 molar ratio (20 and 10 μM, respectively) were analyzed using ESI-MS and selected mass spectra are shown in Fig. 3. Quercetin and luteolin have been confirmed as efficient topoisomerase I poisons and bind to duplexes by intercalation and external binding [16,43]. Similar results were obtained for these two compounds in the negative ion mass spectra. Take quercetin (Fig. 3(a)) as an example, intact DNA in the form of triplex, duplex and single strand were observed. Peaks corresponding to the flavonoid/triplex complexes were not detected, indicating no or quite weak interactions between quercetin or luteolin and the triplexes. Similar results were obtained for kaempferol, which differs from quercetin by the absence of 3-OH group and weak duplex interaction [19]. Genistein is topoisomerase II poison [14], and ions corresponding to the 1:1 flavonoid/triplex complex were clearly observed in the mass spectrum (Fig. 3(b)). The same binding stoichiometries with varying relative intensities were obtained for other flavonoid aglycones (Fig. 3(c)) except for formononetin, whose complex with duplex were not detected in gas phase as well according to our previous results [26].

Those aglycones, such as quercetin and luteolin, show complexation with DNA duplexes in both solution and gas phase [20,26], but no flavonoid/triplex complexes were detected for those aglycones, indicating that they have structure selectivities with a preference for duplex DNA (see discussion in following section). The binding results of these flavonoid aglycones with the other four triplexes were similar to those obtained for TD 2.

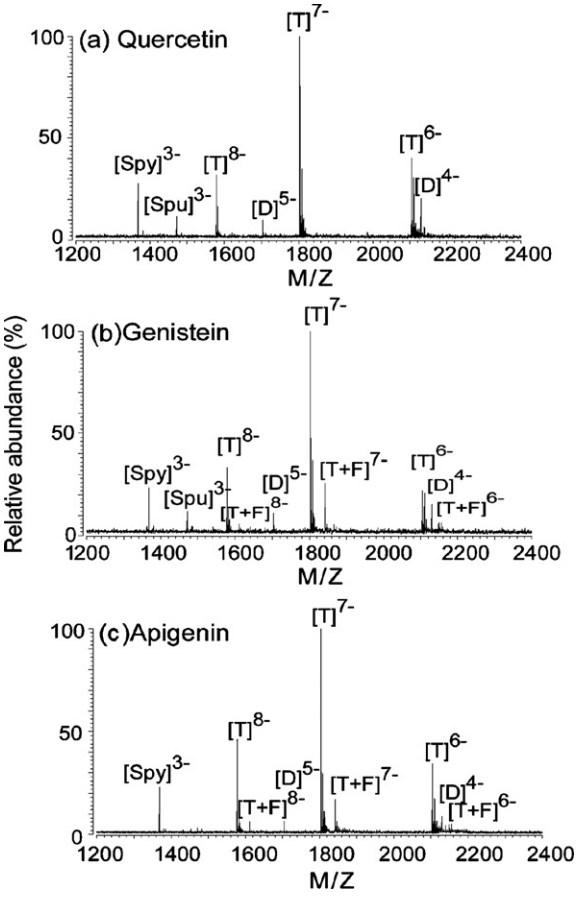

Fig. 3. Negative-ion ESI-MS spectra of complexes of flavonoid aglycones with TD 2 (a) quercetin, (b) genistein and (c) apigenin. (T: triplex, D: duplex, F: flavonoid, Spy: single pyrimidine strand, Spu: single purine strand).

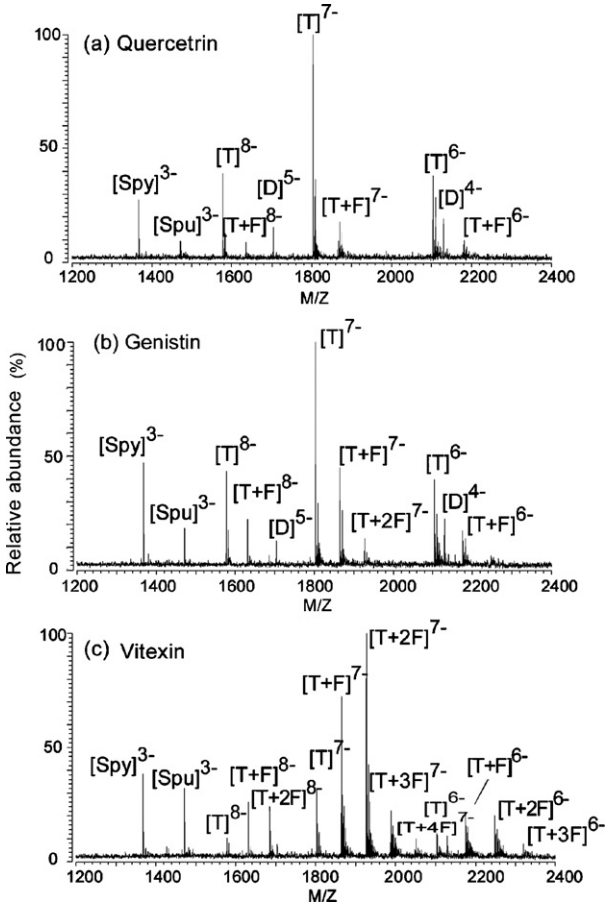

Fig. 4. Negative-ion ESI-MS spectra of complexes of flavonoid glycosides with TD 2, (a) quercetrin, (b) genistin and (c) vitexin.

3.2. Binding of flavonoid glycosides to DNA triplexes

Besides flavonoid aglycones, the flavonoid glycosides also abound in nature and have extensive biological activities including anticancer, anti-HIV, and antiviral properties [12,44]. There are two main types of flavonoid glycosides: flavonoid O-glycosides with glycosidic O–C bond formed by one or more hydroxyl group of the aglycone linked with a sugar chain and flavonoid C-glycosides with glycosylation by direct linkage of the sugar chain to the flavonoid's basic nucleus via a C–C bond.

Mixtures of one flavonoid glycoside and one triplex at a 2:1 molar ratio (20 and 10 μM, respectively) were analyzed using negative-ion ESI-MS. All the flavonoid glycosides studied were found to form complexes with DNA triplexes. The representative mass spectra were shown in Fig. 4. For quercetrin (quercetin-3-O-rhamnoside), only 1:1 binding stoichiometry was observed (Fig. 4(a)). The same binding stoichiometries were detected for hyperin, isoorientin, luteolin-7-O-glucoside and puerarin (the spectra were not shown). The 1:1 and 2:1 flavonoid/triplex complexes were observed in the mass spectrum for genistin (genistin-7-O-glucoside, Fig. 4(b)). The monoglycosides orientin, isovitexin, daidzin, rutin, liquirtin and the diglycoside naringin have similar binding stoichiometries to genistin, differing from each other in the relative peak intensities in spectra (the spectra were not shown). For all the above flavonoid glycosides, abundances of the complexes were lower than those of the free intact triplexes. How two flavonoid molecules bind to DNA is currently unclear, however, it might be attributed to the self-association of flavoniods as well, because dimers of flavonoids are detected in acidic solutions [45]. Only vitexin (apigenin-8-C-glucoside) shows 3:1 and

small amounts of 4:1 flavonoid/triplex complexes in the spectrum (Fig. 4(c)). The 2:1 vitexin/triplex complex was the most abundant in the spectrum while the 1:1 complex was of higher abundance than that of free triplex. The abundance of 3:1 complex was slightly lower than that of free triplex. Similar results were obtained for the flavonoid glycosides binding to the other four DNA triplexes.

3.3. Concentration effects

When the concentration of analyte is increased, nonspecific complexes may be detected in ESI mass spectra [29,37,46]. The concentration effects were investigated with mixtures where the DNA concentration was fixed at 10 μM and the flavonoid concentrations were varied (5, 10, 20, 40 or 80 μM). As the flavonoid/DNA molar ratios were increased from 0.5:1 to 8:1, higher binding stoichiometries were observed and the relative abundances of flavonoid/DNA complexes increased. For example, when the genistin/DNA ratio was 0.5:1, only a 1:1 complex was observed (Fig. 5). As the genistin/DNA ratio was increased, the abundance of 1:1 complex increased, and a 2:1 genistin/DNA complex emerged when the genistin/DNA ratio was increased to 2:1. The free intact triplex ion was the most abundant when the genistin/DNA ratio was below 4:1. When the genistin/DNA increased to 4:1, the 3:1 genistin/DNA complex was observed, and most abundant peak in the spectrum corresponds to the 1:1 genistin/DNA complex. Finally, as the molar ratio reached 8:1, the distribution of peaks was unaltered with the emergence of 4:1 genistin/DNA complex in low abundance. Similar results were obtained for the other flavonoids. The concentration-dependent binding of ligands were obtained for some intercalators and flavonoids to duplexes [26,29,36], which suggested some non-specific binding of ligands to DNA or self-aggregation of the ligands under high concentration. It should be noted that even at quercetin/DNA ratio of 8:1, no quercetin/DNA complex was observed. This suggests that the non-specific binding of flavonoids to the triplexes is not substantial under the experimental condition. To maximize interaction of the flavonoids with the triplexes and to minimize non-specific binding, a 2:1 molar ratio of each flavonoid and DNA was applied in this work.

3.4. Relative binding affinities of the flavonoids to DNA triplexes

Values of the fraction of bound DNA, calculated using the following equation, were used to evaluate the relative binding affinity of the flavonoids to the DNA triplexes.

$$\text{Fraction of bound DNA} = \frac{I_{(1:1)} + I_{(2:1)} + I_{(3:1)} + \cdots}{I_{(\text{DNA})} + I_{(1:1)} + I_{(2:1)} + I_{(3:1)} + \cdots} \quad (1)$$

where I represents the relative abundance of free DNAs or drug–DNA complexes. Assuming that the addition of drug dose not changes the electrospray response factor of the DNA triplexes, the relative intensities of intact or bound DNA in the mass spectra are proportional to their relative abundance in the solution [35]. The value of fraction can be used to estimate the binding affinities and selectivities of drugs to DNA, and in many studies, results obtained using ESI-MS agreed well with those obtained in solution phase experiments [27,29]. The higher values of the fraction of bound DNA indicate greater DNA/drug binding affinities. Relative binding affinities of the flavonoids to TD2 are shown in Fig. 6(a) as an example. The results of the other four triplexes followed the same trend (data not shown).

The hydroxyl groups of flavonoid aglycones have crucial influence on their DNA-binding properties, especially the OH group on 3, 3′ and 4′ positions. The replacement of an OH group by an OCH_3 group at the 4′-position of daidzein, giving the compound formononetin led to a notable decrease in the fraction of bound DNA. Previous studies have suggested that the 4′-OH group was of primary importance for duplex and quadruplex binding of flavonoid aglycones, as it may form a hydrogen bond with the DNA structures and stabilize the complexes [16,21,26,31]. It may play the same role when binding to the triplex DNA. The difference between kaempferol and apigenin is the presence of OH group on C3 position for kaempferol whereas this group is absent for apigenin. However, this subtle difference in structure resulted in measurable increase in fraction of DNA bound to apigenin. In the case of luteolin, which also has a similar structure to apigenin but with an additional 3′-OH group, no drug/DNA complex was observed. These results indicated that both 3- and 3′-OH groups destabilized triplex-binding, although they were found to increase the binding of flavonoid aglycones to duplex DNA [16,18,26]. The 3′-OH group on B ring can form intramolecular hydrogen bond with 4′-OH group [47] to enhance the duplex-binding and results in the duplex preferences of quercetin and luteolin. The duplex-versus-triplex selectivity and different effects of substituents might be attributed to the presence of the third strand, which may change the helical parameter and width of the grooves, thus altering the energetics of the drug–DNA

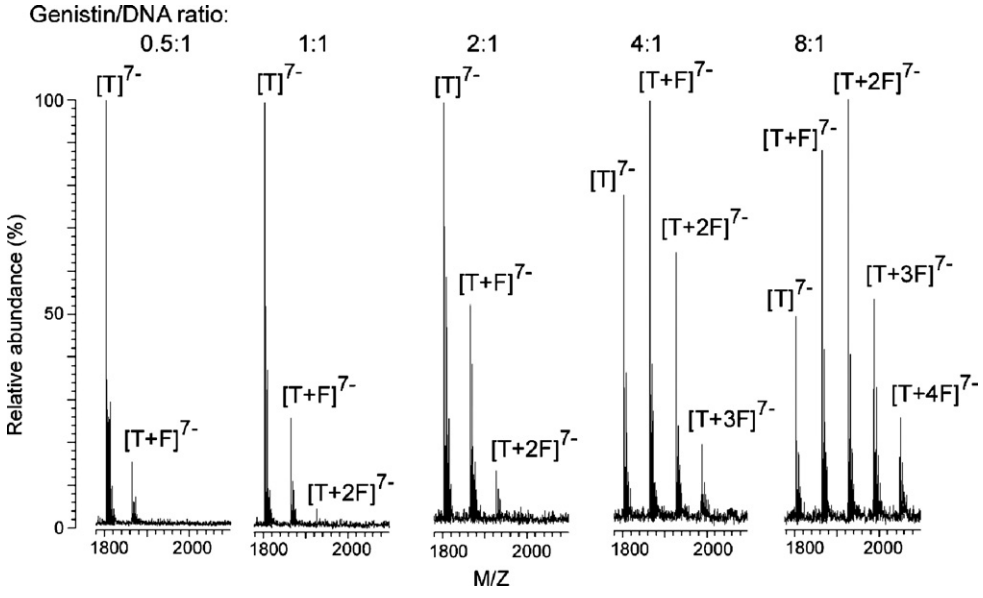

Fig. 5. ESI-MS spectra of genistin/DNA complexes at different molar ratios in mass range m/z 1800–2100.

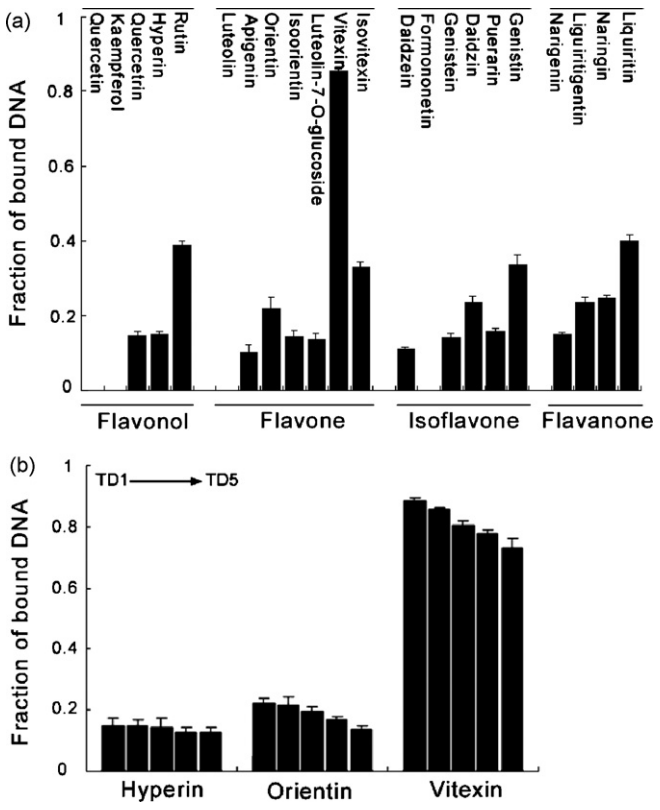

Fig. 6. Relative binding affinities (average of five measurements) based on ESI-MS measurements of (a) the flavonoids to TD 2 and (b) hyperin, orientin and vitexin to the five triplexes.

interaction by changing the alignment of the interacting functional groups [2,48]. Based on the above results, the greatest effects of substituents occur when they are on the B ring and the C2–C3 part of C ring, suggesting that these positions of flavonoid aglycone are essential for triplex binding of flavonoids.

Besides the 3, 3′ and 4′-OH groups, hydroxyl substitutions at the C5 position of the flavonoid aglycones can also affect their DNA-binding properties. The 5-OH group exerts distinct effects on triplex binding of different flavonoid aglycones. In the case of isoflavone, the presence of the 5-OH group correlated with increased DNA-binding affinity, as genistein bound a higher fraction of DNA than daidzein. It has previously been suggested that the presence of 5-OH in genistein facilitates the formation of a strong intramolecular hydrogen bond with the 4-keto group on the C ring and adds a six-membered ring moiety to the structure, which may further promote the interactions between the isoflavones and duplex [21], and might enhance the triplex-binding in the same way. The 5-OH group of flavanone showed the reverse effect: liquiritigenin without a 5-OH group had a higher fraction of bound DNA than naringenin with 5-OH group. However, the effect of 5-OH group is weaker than those of the hydroxyls on the 3, 3′ or 4′ positions.

Each flavonoid glycoside bound a higher fraction of DNA than its corresponding aglycone. The influence of glycosylation varies with the location and number of saccharide moieties. The presence of a disaccharide at the C3 position of flavonol shows more enhancement than a monosaccharide at the same position, referring to rutin compared with quercetrin and hyperin. The 3-rutinoside of rutin, which proved to cause steric hindrance to interaction with duplex DNA [16,23], enhanced the interaction with triplex, indicating potential structure selectivity and triplex preference. For flavone glycosides, glycosylation at the C8 position significantly increased the binding affinity: vitexin (apigenin-8-C-glucoside) has a higher fraction of bound DNA than isovitexin (apigenin-6-C-glucoside) and the fraction of bound DNA for orientin is higher than for isoorientin and luteolin-7-O-glucoside. For isoflavones, glycosylation on the 7-O position is important since daidzin shows a higher fraction of bound DNA than puerarin. The C-7–OH of genistein may engage in a rather strong hydrogen bond when it interacts with biomolecules [49]. The sugar ring at the 7-O position may act as a strong hydrogen bond former, as the saccharide can lie in the DNA minor groove resulting in sugar–phosphate and sugar-base binding [50–52]. Interactions of glycosylated compounds and DNA triplexes have been studied, but the function of the glucoside ring was not clear yet [40,43]. Groove binding of side chains of intercalators can enhance the triplex-binding [7,9]. It is also possible that the sugar chain of flavonoids in the DNA groove may enhance interactions with DNA triplex.

The effects of location of the saccharides and 5-OH group of the aglycone on triplex DNA binding with flavonoids vary with the position of ring B. In addition, the flavanone narigenin bound a higher fraction of DNA than the isoflavone genistein, and the latter bound a higher fraction of DNA than the flavone apigenin. Another example is the liquirigenin/daidzein pair: the flavanone liquirigenin present quite higher fraction of bound DNA than the isoflavone daidzein. Taken together, these observations suggest that the binding of flavonoid aglycones to DNA triplex follows the order flavanone > isoflavone > flavone. The difference between narigenin and apigenin is a single versus double bond between C2 and C3, while the difference between genistein and apigenin is the position of the B ring. The single bond between C2 and C3 of the skeleton allows free rotation of ring B relative to the benzoyl plane of rings A and C, while the conjugation of ring B on the C3 position introduces non-planarity to the isoflavone skeleton [21,47]. The results indicated that flexibility of the ring B, which is unfavorable for its duplex interaction [16,21,22,26], is favorable for its interaction with DNA triplex. The presence of a third strand in the major groove of the duplex has induced modifications that certainly change the binding of ligands to the triplex structure.

The relative binding affinities of each flavonoid towards the five triplexes with different GC contents and base sequences (Table 1) were used to probe sequence selectivities. In Fig. 6(b), the fractions of bound DNA for AT rich triplexes were slightly higher than that for the GC-rich triplexes, but overall flavonoids bound to the triplexes without any prominent preference, suggesting these flavonoids do not have distinct sequence selectivities.

3.5. SORI-CID of the flavonoid/DNA complexes

The relative binding affinities, binding stoichiometries and sequence selectivities of drug interaction with DNA can be deduced from ESI-MS, however, additional information can be obtained by analysis of CID spectra of the drug/DNA complexes (ESI-MS/MS). Sustained off-resonance irradiation collision-induced dissociation (SORI-CID) experiments of 7-charged flavonoid/DNA complexes were performed to examine their dissociation patterns. Fig. 7 shows the ESI-MS/MS spectra for flavonoid aglycones and glycosides with triplex TD2. Three characteristic dissociation patterns were summarized as following equations:

$$[Triplex + F]^{7-} \rightarrow [Triplex]^{7-} + F$$
$$\hookrightarrow [Duplex]^{4-} + [Single\ pyrimidine\ strand]^{3-} \quad (2)$$

$$[Triplex + F]^{7-} \rightarrow [Triplex]^{6-} + F^{-} \quad (3)$$

$$[Triplex + F]^{7-} \rightarrow [Duplex]^{4-} + [Single\ pyrimidine\ strand]^{3-} + F \quad (4)$$

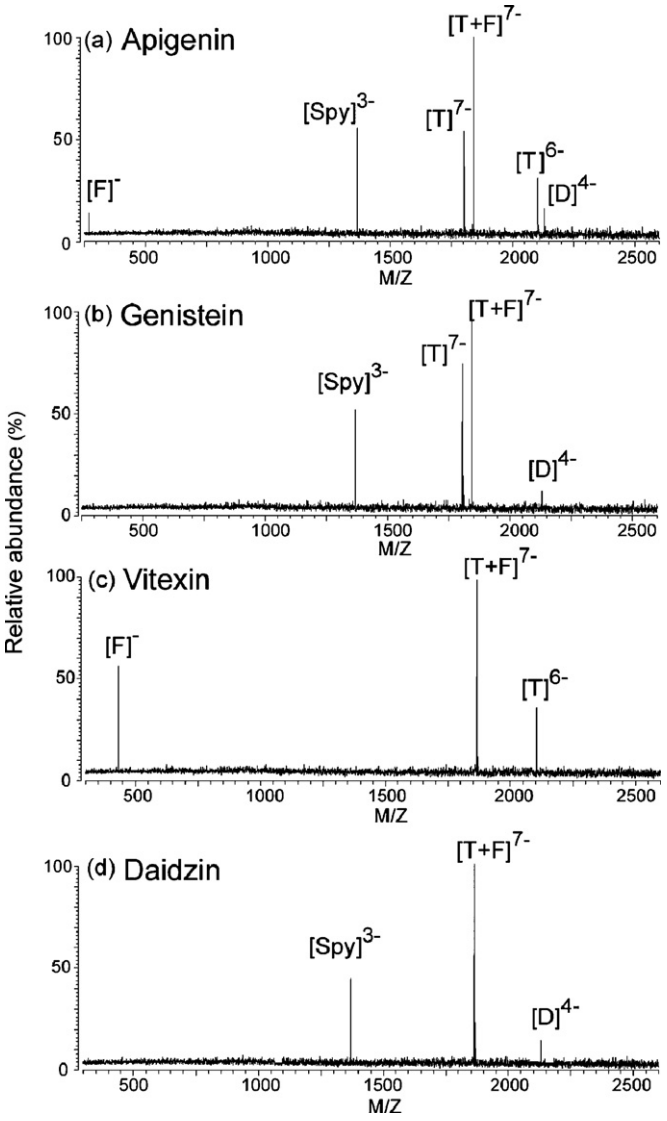

Fig. 7. SORI-CID mass spectra of complexes of triplex TD2 and flavonoids (a)apigenin, (b)genistein, (c)Vitexin or (d) Daidzin at 7-charge state (5 V for apigenin, 10 V for others).

Taking apigenin/DNA and genistein/DNA (shown in Fig. 7(a and b)) as examples, two dissociation patterns were detected for the 7-charged flavonoid aglycones and DNA complexes. DNA complexes with isoflavone and flavanone aglycones dissociate via the loss of neutral drug, and companion with the loss of the antigene strand (Fig. 7(b) and Eq. (2)). The predominant dissociation pathway of the flavone aglycone apigenin was the same as for isoflavone and flavanone aglycones (loss of neutral drug and $[Spy]^{3-}$), however, the deprotonated flavone and 6-charged triplex were observed as well (Fig. 7(a) and Eq. (3)). It should be noted that, the dissociation of the apigenin/DNA complex occurs at lower energy than DNA complexes with isoflavone and flavanone aglycones. At the SORI voltage of 5 V, the relative abundance of daughter ions $[Spy]^{3-}$ and $[F]^{7-}$ of apigenin/DNA were nearly equal to that of genistein/DNA at the SORI voltage of 10 V (Fig. 7(a and b)).

The dissociation of 7-charged DNA complexes with flavonoid glycosides also occurred via two pathways. No matter the position of glycosylation of the flavonoids, the dissociation pathways for those flavonoid glycosides are different from their corresponding aglycones, which varied with aglycones. For flavone and flavonol glycosides, flavonoid anions and 6-charged triplexes were observed in the spectra (Fig. 7(c) and Eq. (3)). Fig. 7(d) shows the SORI-CID of 7-charged daidzin/triplex complex as an example of the characteristic dissociation pathway of isoflavone and flavanone. The third strand and the neutral drug dissociated from the triplex DNA, giving a 3-charged single strand and the 4-charged duplex (see Eq. (4)).

In general, duplex DNA binding ligands with same binding mode exhibited the same dissociation patten [25,30]. For unknown duplex DNA binding-mode, it can be deduced by comparing drug–DNA complex dissociation pattern with those of known binding-modes of traditional major-groove binders or intercalators or drugs using ESI-MS [30,35–37]. However, because few MS/MS studies of drug/triplex complexes have been performed [28], no dissociation pattern of drug/triplex complex is available as comparison. Furthermore, the dissociation pathways for drug–DNA complexes can vary with charge states and instruments [29,32], making justification of the binding mode based on the dissociation pattern only problematic. Some solution phase tools, such as UV and NMR, are needed to help unravel the binding modes of these ligands.

Though the particular DNA-binding mode and geometry of flavonoid/DNA complex cannot be deduced from the tandem mass spectra of flavonoid/DNA complexes, some valuable results were acquired. The dissociation of the flavone aglycone from triplex DNA occurs at lower collision voltage than isoflavone and flavanone aglycones, which suggests a different DNA-binding mode or weaker DNA-binding interaction of the flavone compared to the isoflavone and flavanone. Flavone and flavonol give the same dissociation pattern, which is different from that of isoflavone and flavanone, indicating that the aglycone skeletons play an important role on the binding mode of flavonoid to triplex DNA. The single bond between C2 and C3 of the flavanone or the ring B position of isoflavone makes the B ring depart from the plane of rings A and C [21,47], which may change the binding mode or affinity of flavonoid to DNA triplex. As triplex DNA has base triplets not base pairs, the curved or unfused aromatic ring seems to stack better with the bases of triplex than the duplex [7,8]. The departure of ring B might change the binding of the saccharide as well and consequently lead to a different dissociation pathway, as the binding of the side chain influences the dissociation pattern [28].

The dissociation patterns of flavonoid glycosides differ from those for their corresponding aglycones, suggesting different DNA-binding modes or DNA-binding interactions evoked by glycosylation. Different dissociation patterns for flavonoid glycosides and aglycones bound to DNA duplexes have also been observed [26]. Both the fraction of bound DNA and SORI-CID results suggest the glycosylation and non-planarity of aglycones introduce change to the triplex interaction with the flavonoids.

4. Conclusion

The nonconvalent complexes of 22 flavonoid compounds with triplexes were studied using negative-ion ESI-MS. Although solution phase methods are still necessary for a better understanding of complete binding modes, ESI-MS has its unique advantage in speed, stoichiometries and sensitivity in analysis of noncovalent nucleic acid complexes with a view to primary drug screening. From the negative-ion ESI-MS and tandem mass spectrometry some important information can be obtained, such as binding stoichiometries, relative binding affinities and sequence selectivities of the flavonoids towards the DNA triplexes. The hydroxyls on 3, 3′ and 4′ positions of aglycone, the location and numbers of saccharide, as well as the aglycone skeletons affect the triplex-binding properties of flavonoids, which play different roles in triplex-binding and duplex-binding. The presence of a third strand in the major groove of the duplex has resulted in different binding interactions of

flavonoids with triplexes and duplexes. These results are expected to be helpful for the understanding of triplex-binding properties of flavonoids and give a clue to design of triplex-binding ligands. However, further investigation of flavonoids and DNA triplexes is needed to be done in the future.

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