DNA-Aptamers

DOI: 10.1002/ange.200502979

# Abasic-Site-Containing Oligodeoxynucleotides as Aptamers for Riboflavin**

*N. B. Sankaran, Seiichi Nishizawa, Takehiro Seino, Keitaro Yoshimoto, and Norio Teramae**

Aptamers, short nucleic acid binding species, have emerged as promising candidates for molecular-recognition events on account of their significant ability to bind large numbers of ligands,[1–10] including amino acids such as arginine[3] and citrulline;[4] ribose-containing cofactors, such as adenosine triphosphate[5] and flavins;[6–8] and drugs, such as theophylline[9] and cocaine.[10] The high affinity and specificity of these aptamers toward target ligands are generally achieved by a combination of complementary molecular shapes, hydrogen-bonding, and stacking interactions.[1] Such binding events typically involve ligand-induced structural changes in the aptamers, which result in the formation of unique secondary structures that are responsible for ligand binding; the internal- or stem-loop structures are representative of the active sites for binding events. However, in the further design of both RNA and DNA aptamers for small ligands, little attention has been paid to other types of non-base-pairing moieties, despite their potential as binding pockets.

Herein, we report a new class of DNA-duplex aptamers for selective and strong binding to a biologically important ligand, where an abasic (AP) site[11–15] in the duplex is utilized as an active cavity for binding events. While it is well known that AP sites are formed by spontaneous hydrolysis of an N-

[*] Dr. N. B. Sankaran, Dr. S. Nishizawa, T. Seino, Prof. Dr. N. Teramae
Department of Chemistry
Graduate School of Science
Tohoku University
and
CREST, Japan Science and Technology Agency (JST)
Aoba-ku, Sendai 980-8578 (Japan)
Fax: (+81) 22-795-6552
E-mail: tera@anal.chem.tohoku.ac.jp

Dr. K. Yoshimoto
Bioengineering Laboratory
Discovery Research Institute
The Institute of Physical and Chemical Research
Wako, Saitama 351-0198 (Japan)

[**] This work was partially supported by Grants-in-Aid for Scientific Research (A) (no. 17205009), for Encouragement of Young Scientist (B) (no. 16750058), and for the COE Project "Giant Molecules and Complex Systems" from the Ministry of Education, Culture, Sports, Science and Technology, Japan. Partial support by RFTF from the Japan Society for the Promotion of Science (JSPS) is also acknowledged. N.B.S. would like to thank the JSPS for a fellowship. We thank Dr. Takeyoshi Kondo (Instrumental Analysis Center for Chemistry, Graduate School of Science, Tohoku University) for performing the $^1$H NMR spectroscopic measurements.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

glycosidic bond[11] or by the removal of damaged or inappropriate bases by N-glycosylases,[12] we intentionally incorporate such lesion sites into the duplex, for which a normal DNA is complementarily hybridized with an AP-site-containing DNA. For our DNA aptamers, we strongly expect that, by stacking with two nucleotides flanking the AP site, a target ligand will be intercalated into the AP site and this will be accompanied by the formation of hydrogen bonds with a receptor nucleotide opposite the AP site (Figure 1). In

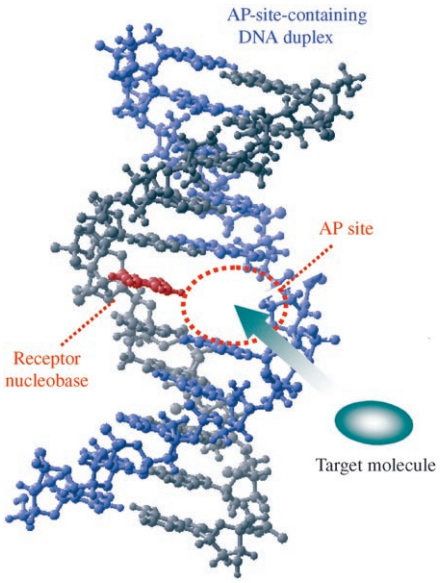
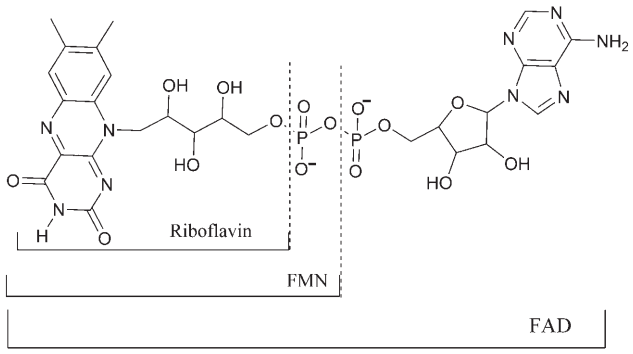



***Figure 1.*** Illustration of the binding motif of an AP-site-containing DNA duplex as an aptamer for small ligands. The pale-blue strand represents the AP-site-containing strand and the gray strand represents the complementary strand with the receptor nucleobase (red).

contrast to the relatively unspecific intercalation of aromatic ligands into double-stranded nucleic acids, this kind of binding motif would allow strong and selective binding to target ligands. Indeed, we have recently demonstrated that small aromatic ligands, such as naphthyridines, are able to bind with high affinity and selectivity to nucleotides opposite the AP site, and we have proposed a ligand-based fluorescence assay for single-nucleotide-polymorphism (SNP) typing.[16–18] Herein, we focus on the development of AP-site-containing DNA aptamers for riboflavin (vitamin $B_2$; Scheme 1), which is a biological redox cofactor that is essential for human and animal health because of its crucial role in metabolism.[19] A 23-mer duplex containing thymidine as a receptor nucleotide (**23T**) is found to strongly recognize riboflavin with a 1:1 binding stoichiometry based on examinations of the receptor nucleotides, the length of the duplexes, and the nucleotides flanking the AP site. Despite the simplicity of the binding motif, the 1:1 binding affinity of **23T** for riboflavin reaches $5.3\times10^5\,\text{M}^{-1}$ (at 20 °C, pH 7.0, $I=0.11$ M), which is comparable to the binding affinities of RNA aptamers developed previously for flavin derivatives, in which the formation of the loop structures[6,8] or intramolecular G quartets[7] was responsible for the flavin binding. Moreover, the aptamer **23T** is found to show selectivity for riboflavin




***Scheme 1.*** Chemical structures of riboflavin, flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD).

over flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These features of the riboflavin–duplex interactions are discussed as a potential basis for the further development of AP-site-containing DNA aptamers.

Firstly, the effect of nucleobases opposite the AP site on the riboflavin–duplex interactions was examined by means of melting temperature ($T_m$) measurements. For this, we prepared four kinds of 11-mer oligodeoxynucleotide (ODN) duplexes that contain guanine (**11G**), adenine (**11A**), cytosine (**11C**), or thymine (**11T**) as the respective receptor nucleobase for binding with riboflavin. The sequences of these duplexes are given in Figure 2, together with those of other duplexes (**23C**, **23T**, **23BT**, **41T**, and **51T**) that are discussed later.

**11G** 5′-TCC AG**X** GCA AC-3′ / 3′-AGG TCG CGT TG-5′

**11A** 5′-TCC AG**X** GCA AC-3′ / 3′-AGG TCA CGT TG-5′

**11C** 5′-TCC AG**X** GCA AC-3′ / 3′-AGG TCC CGT TG-5′

**11T** 5′-TCC AG**X** GCA AC-3′ / 3′-AGG TCT CGT TG-5′

**23C** 5′-TCT GCG TCC AG**X** GCA ACG CAC AC-3′ /3 ′-AGA CGC AGG TCC CGT TGC GTG TG-5′

**23T** 5′-TCT GCG TCC AG**X** GCA ACG CAC AC-3′ / 3′-AGA CGC AGG TCT CGT TGC GTG TG-5′

**23BT** 5′-TCT GCG TCC AGG CAA CGC ACA C-3′ / 3′-AGA CGC AGG TCT CGT TGC GTG TG-5′

**41T** 5′-CAG CCG ATA ACG AAG TCC AG**X** GCA ACG ACA CGC ATA TGT GC-3′ / 3′-GTC GGC TAT TGC TTC AGG TCT CGT TGC TGT GCG TAT ACA CG-5′

**51T** 5′-GTG ACA CGC GAT TAA GAC AGT CCA G**X**G CAA CGC ACA CCA ATG CAA GGT CCA-3′ / 3′-CAC TGT GCG CTA ATT CTG TCA GGT CTC GTT GCG TGT GGT TAC GTT CCA GGT-5′

***Figure 2.*** The names and sequences of the ODN duplex aptamers. **X** = the AP site (spacer $C_3$, a propylene residue).

As shown in Table 1, the changes in $T_m$ value strongly depend on the nucleobase opposite the AP site. In solutions buffered to pH 7.0 ($I=0.11$ M), the largest change in $T_m$ value is observed for the 11-mer duplex containing the thymine base as the receptor (**11T**), for which the $T_m$ value of the duplex (30 μM) increases by 4.0 °C in the presence of riboflavin (46 μM). An increase in $T_m$ value is also observed for the

***Table 1:*** Melting temperatures $T_m$ [°C] for the aptamers in the absence and presence of riboflavin.[a]

| Aptamer | $T_{m(-)}$ | $T_{m(+)}$ | $\Delta T_m$ |
|---|---|---|---|
| **11G** | 34.6 | 33.9(±0.1) | −0.7 |
| **11A** | 33.9 | 33.4(±0.1) | −0.5 |
| **11C** | 30.9 | 32.9(±1.0) | +2.0 |
| **11T** | 29.1 | 33.1(±0.8) | +4.0 |
| **23C** | 68.3 | 68.7(±0.8) | +0.4 |
| **23T** | 66.5 | 67.3(±1.0) | +0.8 |

[a] The DNA samples were annealed before the $T_m$ measurements. $T_m$ measurements were done on a Shimadzu UV-2450 UV/Vis spectrophotometer equipped with a thermoelectrically temperature-controlled micromulticell holder (8 cells; optical pathlength = 1 mm). [DNA] = 30 μM, [riboflavin] = 46 μM, [NaCl] = 100 mM, [sodium cacodylate buffer] = 10 mM, [ethylenediamine tetraacetate] = 1 mM; pH 7.0; heating rate = 1.5 °C min$^{-1}$. $T_{m(-)}$ = Melting temperature of the riboflavin-free DNA, $T_{m(+)}$ = melting temperature of the DNA with riboflavin added, $\Delta T_m = T_{m(+)} - T_{m(-)}$.

***Table 2:*** Binding constants $K_{1:1}$ [M$^{-1}$][a] at 20 °C for the binding of riboflavin with various aptamers and the corresponding thermodynamic parameters calculated by using a van't Hoff plot.[b]

| Aptamer | $K_{1:1}$ | $-\Delta H$ [kcal mol$^{-1}$] | $-\Delta S$ [e.u.][c] | $-\Delta G_{25\,°C}$ [kcal mol$^{-1}$] |
|---|---|---|---|---|
| **11G** | $3.3\times10^3$ | 18.1 | 45.3 | 4.6 |
| **11A** | $1.3\times10^4$ | 10.0 | 15.3 | 5.5 |
| **11C** | $8.1\times10^4$ | 17.6 | 37.6 | 6.4 |
| **11T** | $2.0\times10^5$ | 23.6 | 56.2 | 6.9 |
| **23C** | $2.0\times10^5$ | 12.4 | 18.1 | 7.0 |
| **23T** | $5.3\times10^5$ | 16.2 | 29.0 | 7.5 |
| **23BT** | $1.3\times10^4$ | 5.1 | −1.6 | 5.5 |
| **41T** | $6.7\times10^5$ | 13.2 | 18.5 | 7.7 |
| **51T** | $5.3\times10^5$ | 12 | 14.8 | 7.6 |

[a] Value ±10%. [b] The DNA samples were annealed before the fluorescence experiments. Fluorescence spectra were measured with a Jasco FP-6500 spectrofluorophotometer. [riboflavin] = 23 μM, [sodium cacodylate buffer] = 10 mM, [NaCl] = 100 mM, [ethylenediamine tetraacetate] = 1 mM; pH 7.0. The concentration of ODN duplex was varied from 5–80 μM. The measurement temperatures were 293, 288, 283, and 278 K. [c] e.u. = entropy unit = 4.184 J K$^{-1}$ mol$^{-1}$.

cytosine-containing duplex **11C** (+2.0 °C), but it is not as significant as that for **11T**. By contrast, the $\Delta T_m$ values are almost negligible when the receptor nucleobases are adenine (**11A**: −0.5 °C) or guanine (**11G**: −0.7 °C). Apparently, among these four kinds of 11-mer duplex, the thermal stability of **11T** is effectively increased in the presence of riboflavin, a result that indicates the incorporation of riboflavin into the AP site. The strongest binding with riboflavin is therefore expected when the receptor nucleobase opposite the AP site is thymine.

The binding affinity of the 11-mer duplexes with riboflavin was further examined quantitatively by fluorescence titration experiments, for which complexation-induced changes in the fluorescence of riboflavin were utilized (see the Supporting Information). While riboflavin (1.0 μM) shows no obvious changes for the normal 11-mer duplexes containing no AP sites (2.0 μM), its fluorescence is significantly quenched in the presence of AP-site-containing duplexes (2.0 μM). In accordance with the $T_m$ measurements, the largest quenching is observed when the receptor nucleobase at the AP site is thymine (**11T**), followed by cytosine (**11C**), adenine (**11A**), and guanine (**11G**). For all cases, the resulting changes in the fluorescence spectra can be explained by the formation of a 1:1 complex, a result that gives a 1:1 binding constant ($K_{1:1}$; at 20 °C, pH 7.0, $I = 0.11$ M) of $2.0\times10^5$ for **11T**, $0.81\times10^5$ for **11C**, $0.13\times10^5$ for **11A**, and $0.033\times10^5$ M$^{-1}$ for **11G** (Table 2). For effective binding with riboflavin, thymine is therefore the most suitable nucleobase as the receptor in the AP-site-containing duplex.

Interestingly, the binding affinity for riboflavin is further strengthened by the use of longer duplexes. As given in Table 2, the 1:1 binding constant with the 23-mer duplex containing thymine opposite the AP site, **23T** (see Figure 2), reaches $5.3\times10^5$ M$^{-1}$, which is at least two times larger than that with the corresponding 11-mer duplex, **11T** ($2.0\times10^5$ M$^{-1}$). Similarly, the 1:1 binding affinity with the 23-mer duplex **23C** ($2.0\times10^5$ M$^{-1}$) is stronger than its shorter counterpart, **11C** ($0.81\times10^5$ M$^{-1}$). On a further increase in the duplex length, from 23- to 51-mer, however, the increasing trend in $K_{1:1}$ values is rather insignificant (see Table 2), so the use of longer duplexes is not necessary. We therefore conclude that the 23-mer ODN duplex **23T** qualifies as an ideal aptamer in our investigations.

To understand such an effect of the ODN length on the riboflavin–duplex interactions, the nature of the thermodynamic profile was examined by construction of a van't Hoff plot from temperature-dependant fluorescence titration experiments (see Table 2 and the Supporting Information). As can be seen in Table 2, the riboflavin–duplex interaction is enthalpy driven for all the duplexes containing AP sites. However, the enthalpy term becomes destabilizing as the duplex length is increased from an 11-mer to a 23-mer. This is highly compensated for by less negative values of entropy that result in a more favorable free energy change ($\Delta G_{25\,°C}$). In the case of ODN duplexes containing thymine as the receptor base (**11T** and **23T**), the loss of enthalpy from the 11-mer to the 23-mer is approximately 7 kcal mol$^{-1}$, whereas the gain in entropy is approximately 27 e.u. Similarly, the increase in the binding affinity from **11C** to **23C** is due to the favorable gain in entropy ($\Delta\Delta H = 5.2$ kcal mol$^{-1}$; $\Delta\Delta S = -19.5$ e.u.). For the higher homologues, such as **41T** and **51T**, the overall loss of entropy reaches the minimum values among those of the series of thymine-containing duplexes (**11T**–**51T**); the reduced gain in enthalpy is compensated for by this. These favorable gains in entropy between complexes of different length may be a result of minimal conformational changes associated with the ODN duplexes on binding with riboflavin. Indeed, as revealed by melting temperature measurements (Table 1), the complexation-induced change in the $T_m$ value is almost negligible for 23-mer ODN duplexes ($\Delta T_m$: **23C**: +0.4 °C; **23T**: +0.8 °C), a fact that indicates that the thermal stabilities of these longer duplexes hardly change upon binding with riboflavin. It is therefore likely that, as compared to the binding affinities of 11-mer duplexes, the enhanced binding ability of the longer duplexes towards riboflavin is accompanied by rather less conformational change of the aptamers. This kind of binding event contrasts with those of typical RNA/DNA aptamers containing 30–40 nucleotides,

wherein effective binding is generally achieved by folding and conformational reorientation of the aptamers.[20]

Another interesting feature of the binding events compared to those with the previous flavin-binding aptamers[6,20] is the difference in the receptor nucleotides responsible for effective binding with riboflavin. In the RNA aptamers developed for flavins earlier,[6,20] an adenine that is coplanar with the flavin recognizes the polar groups along the edge of the isoalloxazine ring through hydrogen bonding. The uracil-like edge of isoalloxazine hydrogen bonds to the Hoogsteen edge of an adenine residue in such aptamers. However, in the present case, as is evident from the melting temperature and fluorescence studies, the binding of riboflavin with **11T**, in which a thymidine moiety locates opposite the AP site, is stronger than with **11A**, where the receptor nucleobase is adenine. The formation of hydrogen bonding between the receptor thymine base and riboflavin is confirmed by $^1$H NMR spectroscopic measurements by using the Watergate technique.[21,22] As shown in Figure 3, the signals for the imino

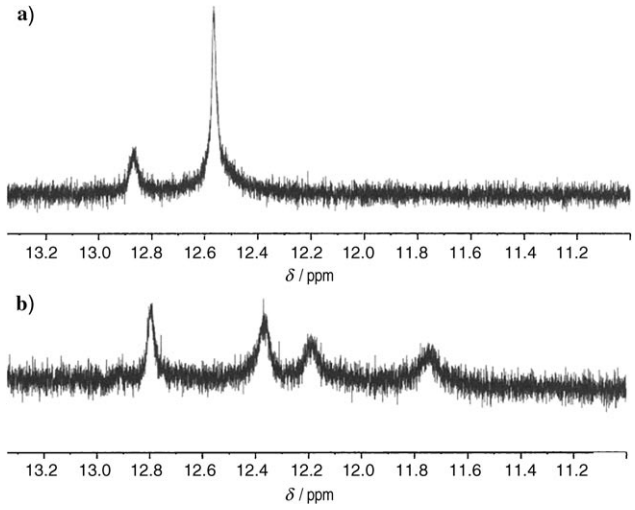



***Figure 3.*** $^1$H NMR spectra showing the imino proton region of the spectrum for the AP-site-containing DNA duplex **11T** in the absence (a) and in the presence (b) of riboflavin. The results support the formation of hydrogen bonds between the thymine receptor nucleobase and riboflavin. NMR spectra were measured with an Avance 600 spectrometer (600 MHz, Bruker) by employing the Watergate method. [DNA duplex] = 0.80 mM, [riboflavin] = 0.82 mM, [tris(hydroxymethyl)aminomethane buffer] = 10 mM, [NaCl] = 100 mM, [ethylenediamine tetraacetate] = 1 mM; pH 8.3, $T$ = 288 K. 5% $D_2O$ was added to lock the signals.

protons due to G–C base pairing are observed at $\delta = 12.86$ and 12.57 ppm for the 11-mer ODN duplex (spectrum a), whereas riboflavin alone does not generate any signals (spectrum not shown). Upon complexation, new signals appear at $\delta = 12.18$ and 11.75 ppm accompanied by upfield shifts of the signals for the imino protons due to G–C base pairing (spectrum b). The new signals can be assigned to imino protons due to binding of a thymine base with riboflavin, as has been observed for mismatched base pairings, such as T–T, that give signals in a more upfield region than those from fully matched base pairs.[22] It is therefore likely that at the AP site a pseudo base pair is formed with a thymine base along the uracil-like edge of the isoalloxazine ring of riboflavin, as shown in the energy-minimized structure of the complex obtained by molecular-modeling studies (see the Supporting Information). From the calculated structure (see the Supporting Information), we also see that the isoalloxazine ring is located at the AP site, in which the ring moiety is stacked with two guanines flanking the AP site. This indicates that, as has previously been revealed for the binding events of some RNA aptamers,[1] the high stability of the AP-site-based aptamer is achieved by a cooperative binding event, that is, not only by hydrogen bonding with target molecules but also by stacking with nucleobases flanking the AP site. Indeed, examination of the influence of the nucleobases flanking the AP site on fluorescence quenching of riboflavin with **23T** reveals that the fluorescence of riboflavin is quenched strongly when the nucleobases flanking the AP site were guanines, whereas other nucleobases flanking the AP site have considerably less influence on the fluorescence of riboflavin (see the Supporting Information). Apparently, the binding events at the AP site depend on the flanking nucleobases and an appropriate environment can be provided for riboflavin to effectively bind with the receptor thymine base when the flanking nucleobases are both guanines (G**X**G, **X** = AP site).

In connection with these effects of flanking nucleobases on the binding events, it is also of interest to note that the binding ability of the thymine base toward riboflavin depends on the size of the binding pockets in ODN duplexes. The 1:1 binding constant of riboflavin with **23BT**, a 23-mer ODN duplex where a single bulge structure is produced with thymine as the receptor nucleobase but with no AP sites (see Figure 2), is only $0.13 \times 10^5\,\text{M}^{-1}$, which is considerably less than that of the corresponding AP-site-containing duplex **23T** ($5.3 \times 10^5\,\text{M}^{-1}$). The examination of the thermodynamic profile of **23BT** shows that the interaction of the bulged thymine with riboflavin is both enthalpy and entropy driven, with a $\Delta G$ value of $-5.5$ kcal mol$^{-1}$, a $\Delta H$ value of $-5.1$ kcal mol$^{-1}$, and a $T\Delta S$ value of 0.4 kcal mol$^{-1}$ at 25 °C (Table 2). The observed nature of the binding is clearly different from the enthalpy-driven binding at the AP site and is rather similar to that for outside-edge- and partial-insertion-binding drugs.[23] It is therefore likely, probably for steric reasons, that the isoalloxazine ring of riboflavin is not able to intercalate deeply between the nucleotides opposite the bulge structure and the effective formation of key molecular interactions, including stacking and hydrogen bonding, is not allowed with binding to the bulged thymine in **23BT**.

Finally, we examined the binding ability of **23T** with other flavin cofactors, such as FMN and FAD. As shown in Figure 4, the aptamer **23T** is found to quench fluorescence of these ligands, and the resulting changes in fluorescence can be analyzed based on the formation of a 1:1 complex. These results indicate that, similarly to the binding mode for riboflavin, **23T** also recognizes the isoalloxazine ring of FMN and FAD and the tricyclic ring intercalates into the AP site in the duplex. However, the binding affinity for FMN or FAD is significantly lower than the affinity for riboflavin. The 1:1 binding constants for FMN and FAD are $0.21 \times 10^5$

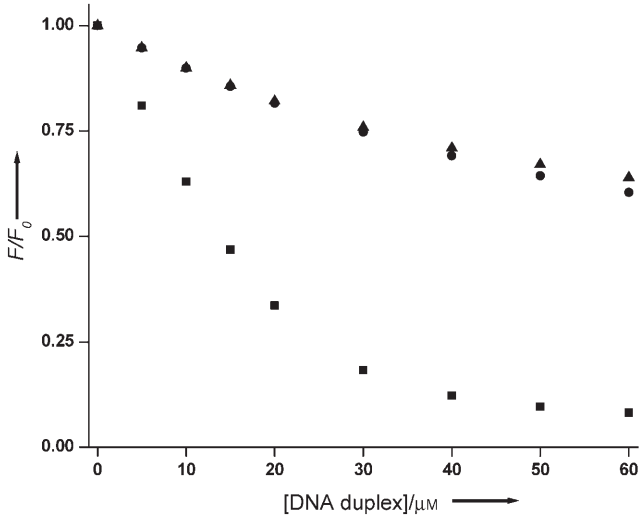



***Figure 4.*** Fluorescence titration curves of riboflavin (■), FMN (●), and FAD (▲) with **23T**. [flavins] = 23 μM, [**23T**] = 0, 5, 10, 15, 20, 30, 40, 50, or 60 μM, [sodium cacodylate buffer] = 10 mM, [NaCl] = 100 mM, [ethylenediamine tetraacetate] = 1 mM; pH 7.0. Fluorescence spectra were measured with a Jasco FP-6500 spectrofluorophotometer; $\lambda_{ex} = 448$ nm. $F_0$ is the fluorescence intensity (at 530 nm) of flavins before adding the ODN duplex and $F$ is the fluorescence intensity after addition of the ODN duplex.

and $0.25 \times 10^5\,\text{M}^{-1}$, respectively, under identical conditions to those in which riboflavin binding was examined. Such high selectivity for riboflavin over FMN and FAD was not obtained by the RNA aptamers developed previously, where the formation of intramolecular G quartets[7] or the internal-loop structure[8] was utilized as the binding motif for the flavin portion of these ligands.[24]

A simple explanation for the lower affinity of FMN and FAD is that of charge repulsion of the negatively charged phosphate groups of both ligands with the ODN phosphate backbone. In the case of riboflavin, this repulsive interaction is apparently absent and, even in the uncharged form, a favorable gain from the electrostatic free-energy contribution $\Delta G_{pe}$ can also be expected, as has been observed for the interaction of electrically neutral echinomycin with DNA duplexes.[25] This is generally attributed to lengthening and unwinding of the DNA, both of which increase the phosphate spacing along the helix axis. This results in a decrease in the charge density of the duplex, thereby releasing condensed counterions and providing an entropically favorable contribution to the binding free energy.[25] Indeed, in the present case, from the examination of the salt dependence of the binding constants, the effective number of charges on riboflavin is found to be $+0.52$ when binding to **11T**. At 110 mM $Na^+$ concentration (pH 7.0, at 5 °C), the polyelectrolyte contribution $\Delta G_{pe}$ is calculated to be $-0.56\,\text{kcal mol}^{-1}$, a result that reveals an effective contribution of $\Delta G_{pe}$ to the overall free energy of the riboflavin–ODN binding ($-7.97\,\text{kcal mol}^{-1}$). Under the same conditions, the apparent charge of riboflavin on binding with **23T** is $+0.60$ and $\Delta G_{pe}$ is $-0.65\,\text{kcal mol}^{-1}$ leads to a favorable entropy contribution to the overall binding free-energy ($-8.14\,\text{kcal mol}^{-1}$) of the longer duplex.

However, other possibilities should also be taken into account when it is considered that **23T** binds to FMN and FAD with equal affinity, despite the difference in the number of negative charges. In the case of FAD, for example, the difference in the hydrate shell from the other two ligands is evident since FAD is known to exist predominantly in a stacked conformation. The thermodynamic profile of the FAD–**23T** interaction shows that $\Delta G = -5.7\,\text{kcal mol}^{-1}$, $\Delta H = -16.0\,\text{kcal mol}^{-1}$, and $T\Delta S = -10.3\,\text{kcal mol}^{-1}$ at 25 °C, thereby indicating that the significant loss of binding entropy is responsible for the lower affinity as compared to the riboflavin–**23T** interaction. By contrast, loss of binding enthalpy is responsible for the lower affinity to FMN ($\Delta G = -5.7\,\text{kcal mol}^{-1}$, $\Delta H = -10.8\,\text{kcal mol}^{-1}$, and $T\Delta S = -5.1\,\text{kcal mol}^{-1}$ at 25 °C). We are currently investigating the flavin–ODN interactions in more detail to obtain a deeper insight into the origin of the binding affinity and selectivity.

In summary, we have demonstrated a new class of DNA aptamer in which AP sites in the duplex were utilized as the binding pockets for riboflavin recognition. By optimizing structural parameters, such as the receptor nucleotide, the length of the duplexes, and the flanking nucleotides to the AP site, the aptamer **23T** selectively bound to riboflavin over FMN and FAD, with a 1:1 binding constant of $5.3 \times 10^5\,\text{M}^{-1}$ (at 20 °C, pH 7.0, $I = 0.11$ M). The binding affinity of **23T** up to the micromolar range was indeed comparable with binding affinities of RNA aptamers developed earlier for flavin derivatives[6–8] and was significantly better than the millimolar affinity of polymer-bound zinc(II) azamacrocycles for riboflavin.[26] Although the fact that only four nucleobases are available for our design scheme may necessitate some synthetic modification of the nucleobases in order to make it suitable for binding with a large number of ligands, our DNA aptamer may have some advantages, such as its easy and low-cost synthesis and its higher chemical stability relative to typical RNA aptamers. These features of the AP-site-based DNA aptamer would allow various kinds of analytical applications, including the isolation of riboflavin from natural sources[26] and the determination of riboflavin in aqueous samples.[27–29] Moreover, our system can also be applied to the analysis of thymidine-related SNPs, for which riboflavin functions as a thymidine-selective fluorescence ligand and the complexation-induced quenching of its fluorescence is utilized to detect the single-base mutation (see the Supporting Information). We are now undertaking further studies in these directions.

Received: August 22, 2005
Revised: October 27, 2005
Published online: January 30, 2006

**Keywords:** abasic sites · aptamers · flavins · fluorescence probes · nucleic acids

[1] T. Hermann, D. J. Patel, *Science* **2000**, *287*, 820.
[2] A. D. Ellington, J. W. Szostak, *Nature* **1990**, *346*, 818.
[3] K. Harada, A. D. Frankel, *EMBO J.* **1995**, *14*, 5798.
[4] M. Famulok, *J. Am. Chem. Soc.* **1994**, *116*, 1698.

[5] a) S. D. Jhaveri, R. Kirby, R. Conrad, E. J. Maglott, M. Bowser, R. T. Kennedy, G. Glick, A. D. Ellington, *J. Am. Chem. Soc.* **2000**, *122*, 2469; b) S. Jhaveri, M. Rajendran, A. D. Ellington, *Nat. Biotechnol.* **2000**, *18*, 1293; c) E. J. Merino, K. M. Weeks, *J. Am. Chem. Soc.* **2003**, *125*, 12370.
[6] P. Burgstaller, M. Famulok, *Angew. Chem.* **1994**, *106*, 1163; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1084.
[7] C. T. Lauhon, J. W. Szostak, *J. Am. Chem. Soc.* **1995**, *117*, 1246.
[8] M. Roychowdhury-Saha, S. M. Lato, E. D. Shank, D. H. Burke, *Biochemistry* **2002**, *41*, 2492.
[9] R. D. Jenison, S. C. Gill, A. Pardi, B. Polisky, *Science* **1994**, *263*, 1425.
[10] M. N. Stojanovic, P. de Prada, D. W. Landry, *J. Am. Chem. Soc.* **2001**, *123*, 4928.
[11] T. Lindahl, B. Nyberg, *Biochemistry* **1972**, *11*, 3610.
[12] H. E. Krokan, R. Standal, G. Slupphaug, *Biochem. J.* **1997**, *325*, 1.
[13] T. Lindahl, *Nature* **1993**, *362*, 709.
[14] J. H. J. Hoeijmakers, *Nature* **2001**, *411*, 366.
[15] E. C. Friedberg, *Nature* **2003**, *421*, 436.
[16] K. Yoshimoto, S. Nishizawa, M. Minagawa, N. Teramae, *J. Am. Chem. Soc.* **2003**, *125*, 8982.
[17] K. Yoshimoto, C.-Y. Xu, S. Nishizawa, T. Haga, H. Satake, N. Teramae, *Chem. Commun.* **2003**, 2960.
[18] S. Nishizawa, K. Yoshimoto, T. Seino, C.-Y. Xu, M. Minagawa, H. Satake, A. Tong, N. Teramae, *Talanta* **2004**, *63*, 175.
[19] The importance of flavins in various biological processes has been highlighted; see, for example: a) *Flavins and Flavoproteins* (Eds.: S. K. Chapman, R. N. Perham, N. S. Scrutton), Rudolf Weber Agency for Scientific Publications, Berlin, **2002**; b) *Chemistry and Biochemistry of Flavoenzymes, Vol. 1* (Ed.: F. Müller), CRC, Boca Raton, **1991**.
[20] P. Fan, A. K. Suri, R. Fiala, D. Live, D. J. Patel, *J. Mol. Biol.* **1996**, *258*, 480.
[21] M. Piotto, V. Saudek, V. Sklenar, *J. Biomol. NMR* **1992**, *2*, 661.
[22] P. K. Bhattacharya, J. Cha, J. K. Barton, *Nucleic Acids Res.* **2002**, *30*, 4740.
[23] I. Haq, J. Ladbury, *J. Mol. Recognit.* **2000**, *13*, 188.
[24] The binding of RNA aptamers with 7,8-dimethylalloxazine, FMN, and FAD has been examined,[6] whereby aptamers for FMN or FAD were isolated by in vitro selection. While the RNA aptamers (FMN-2 and 35FMN-2) based on the internal-loop structure were not able to distinguish between these flavin derivatives, FAD-1 or 27FAD-1 containing the stem-loop structure as the binding motif showed moderate selectivity for 7,8-dimethylalloxazine over FMN and FAD: the dissociation constants of 27FAD-1 with 7,8-dimethylalloxazine, FMN, and FAD were reported to be 35, 280, and 273 μM, respectively.
[25] F. Leng, J. B. Chaires, M. J. Waring, *Nucleic Acids Res.* **2003**, *31*, 6191.
[26] B. König, H.-T. Gallmeier, R. Reichenbach-Klinke, *Chem. Commun.* **2001**, 2390.
[27] J. A. Tillotson, M. M. Bashor, *Anal. Biochem.* **1980**, *107*, 214.
[28] J. Becvar, G. Palmer, *J. Biol. Chem.* **1982**, *257*, 5607.
[29] I. Caelen, A. Kalman, L. Wahlström, *Anal. Chem.* **2004**, *76*, 137.