DOI: 10.1002/chem.200801282

Multifunctional G-Quadruplex Aptamers and Their Application to Protein Detection

Tao Li,[a] Lili Shi,[b] Erkang Wang,[a] and Shaojun Dong*[a]

Abstract: Two significant G-quadruplex aptamers named AGRO100 and T30695 are identified as multifunctional aptamers that can bind the protein ligands nucleolin or HIV-1 integrase and hemin. Besides their strong binding to target proteins, both AGRO100 and T30695 exhibit high hemin-binding affinities comparable to that of the known aptamer (termed PS2M) selected by the in vitro evolution process. Most importantly, their corresponding hemin–DNA complexes reveal excellent peroxidase-like activities, higher than that of the reported hemin–PS2M DNAzyme. This enables these multifunctional aptamers to be applied to the sensitive detection of proteins, which is demonstrated by applying AGRO100 to the chemiluminescence detection of nucleolin expressed at the surface of HeLa cells. Based on the specific AGRO100–nucleolin interaction, the surface-expressed nucleolin of HeLa cells is labeled in situ with the hemin–AGRO100 DNAzyme, and then determined in the luminol–H_2O_2 system. Through this approach, the sensitive detection of total nucleolin expressed at the surface of about 6000 HeLa cells is accomplished. Our results suggest that exploiting new functions of existing aptamers will help to extend their potential applications in the biochemical field.

Keywords: DNA · DNAzyme · G-quadruplex · hemin · multifunctional aptamers · proteins

Introduction

Nucleic acid aptamers are generally obtained through an in vitro evolution process termed SELEX (systematic evolution of ligands by exponential enrichment).[1,2] Some of them have G-rich consensus sequences and can fold into stable four-stranded structures known as G-quadruplexes.[3–9] Such specific secondary structures play an important role in the binding of aptamers to ligands.[5,10–13] A G-quadruplex formed by a G-rich DNA aptamer named PS2M[10] has been found to bind hemin (an anion porphyrin) to form a complex with high catalytic activity towards the H_2O_2-mediated oxidation of 2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS).[14,15] This hemin–aptamer complex with the peroxidase-like activity, in fact, is a new kind of deoxyribozyme (i.e. DNAzyme).[16] Several related aptamers of PS2M are all found to form high-activity DNAzymes with hemin.[14,15,17] Another family of such aptamers with relatively low DNAzyme function has also been reported.[18,19] As a general DNA ligand with the porphyrin ring, hemin should bind to more G-quadruplexes, not just the above two families. So, it is interesting and significant to explore the interactions between hemin and other G-quadruplex aptamers, which will help us to discover new functions of those aptamers selected by the SELEX process.

Some G-quadruplex aptamers have shown great biomedical significance due to their potential as potent therapeutic agents.[20,21] A G-rich DNA aptamer named AGRO100[22] can fold into an intermolecular G-quadruplex, which specifically bind nucleolin (a protein marker expressed at high levels in human cancer cells) and strongly inhibit DNA replication and cell proliferation.[22] Thus it is considered as an advanced anticancer aptamer.[21] Two G-quadruplex aptamers termed T30695[23] and 93del[24] have been proved effective for inhibiting HIV-1 integrase in vitro, and so they are regarded as potential anti-HIV therapeutic agents.[20] The re-

[a] T. Li, Prof. Dr. E. Wang, Prof. S. Dong
State Key Laboratory of Electroanalytical Chemistry
Changchun Institute of Applied Chemistry
Chinese Academy of Sciences, Changchun
Jilin, 130022, China
and
Graduate School of the Chinese Academy of Sciences
Beijing, 100039 (China)
Fax: (+86) 431-8568-9711
E-mail: dongsj@ciac.jl.cn

[b] L. Shi
Institute of Functional Material Chemistry
Department of Chemistry, Northeast Normal University
Changchun, Jilin, 130024 (China)

WILEY InterScience DISCOVER SOMETHING GREAT
© 2009 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

lated aptamers of AGRO100 and T30695 are all found to have similar secondary structures and exhibit strong anticancer or anti-HIV activity as well.[6,25–28] Exploiting the undiscovered functions of these families of aptamers will extend their biomedical applications.

In view of the G-quadruplex structures of AGRO100, T30695, and 93del, here we explore their potential as hemin binders, aiming to exploit new families of DNAzymes and their bioanalytical application. With the hemin-binding aptamer PS2M as the control, these G-quadruplex aptamers were incubated with hemin and then characterized by using UV/Vis absorption spectroscopy to evaluate their binding affinities and DNAzyme functions. According to the comparison results, AGRO100 and T30695 are identified as new hemin-binding aptamers. They can bind both hemin and the protein ligand (nucleolin or HIV-1 integrase). Such multiple functions give these G-quadruplex aptamers great potential in bioanalysis, which is demonstrated by applying AGRO100 to the chemiluminescence (CL) detection of nucleolin expressed at the surface of HeLa cells.

Results and Discussion

To demonstrate the binding of AGRO100, T30695, and 93del to hemin, these aptamers are under investigation together with the control PS2M. Figure 1 depicts the colorimetric analyses of hemin-aptamer interactions by using UV/Vis absorption spectroscopy. The uncomplexed hemin has the Soret absorption band centered at 397 nm (curve a). After incubation with PS2M, a sharp hyperchromicity is observed in the Soret band of hemin (curve b). The center of Soret band shifts to 404 nm, while the maximal absorption intensity increases dramatically. This indicates the strong interactions between PS2M and hemin, as reported.[15] The same phenomenon is also observed after incubation of hemin with AGRO100, T30695, or 93del. Under same conditions, both AGRO100 and T30695 give rise to a sharp hyperchromicity of the hemin Soret band (curves c and d), whereas 93del causes a much smaller one (curve e). These data suggest that there is a strong interaction between hemin and AGRO100 (or T30695). Although 93del is able to bind hemin as well, from the hyperchromicity it is estimated that the affinity of 93del is much lower than that of AGRO100, T30695, or PS2M, which is confirmed by the determination of dissociation constants (K_d) of different hemin–aptamer complexes (see the Experimental Section).

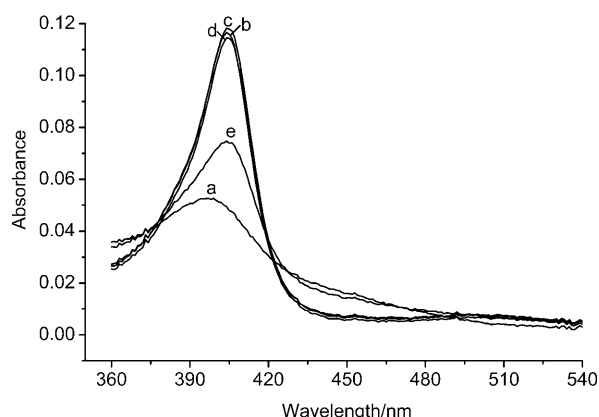


Figure 1. Colorimetric analyses of hemin–aptamer interactions by using UV/Vis absorption spectroscopy. a) uncomplexed hemin (1 μM); b) incubation of a) with PS2M (1 μM); c) incubation of a) with AGRO100 (1 μM); d) incubation of a) with T30695 (1 μM); e) incubation of a) with 93del (1 μM). Experimental conditions: HEPES (25 mM), pH 7.4, KCl (20 mM), NaCl (200 mM), Triton X-100 (0.05% w/v), and DMSO (1% v/v).

However, for hemin–DNA complexes, the binding action is not always consistent with the catalytic behavior.[15] Travascio et al. have confirmed that several hemin-binding aptamers including PS2M are capable of forming DNAzymes with hemin, whereas a folded *Oxytricha* telomeric DNA does not, although this G-quadruplex binds hemin as well.[15] To demonstrate the DNAzyme function of AGRO100, T30695, and 93del, their catalytic behaviors were investigated in the ABTS–H_2O_2 system, with PS2M as the control. The H_2O_2-mediated oxidation of ABTS can produce the free-radical cation $ABTS^{\bullet+}$, which has a maximal absorption at 421 nm. Thus, the absorbance at this wavelength can be employed to quantitatively analyzed the product of peroxidation, and then assess the peroxidase-like activities of different hemin–aptamer complexes. Figure 2 shows AGRO100, T30695, and 93del are all capable of forming catalytic complexes with hemin, like PS2M. Surprisingly, the hemin–AGRO100 complex exhibits an ultrahigh catalytic activity towards the oxidation of ABTS by H_2O_2 (curve c), which is much higher than that of the reported DNAzyme

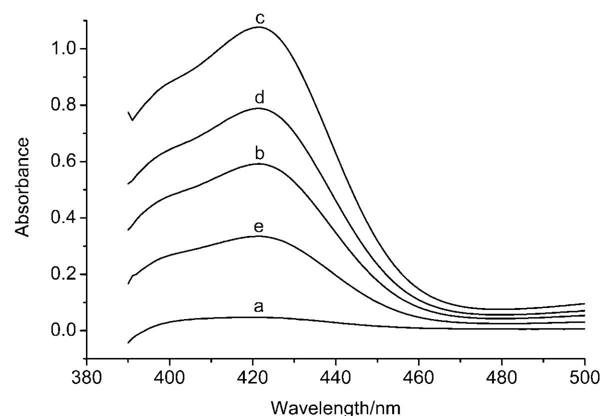


Figure 2. UV/Vis absorption spectra for analyzing the peroxidation product ($ABTS^{\bullet+}$) of the ABTS–H_2O_2 system catalyzed by several catalysts for 4 min: a) uncomplexed hemin (0.25 μM), b) sample a) plus PS2M (0.25 μM), c) sample a) plus AGRO100 (0.25 μM), d) sample a) plus T30695 (0.25 μM), e) sample a) plus 93del (0.25 μM). Experimental conditions: [ABTS] = 5.9 mM, [H_2O_2] = 0.6 mM, in HEPES buffer (25 mM, pH 8.0) containing KCl (20 mM), NaCl (200 mM), Triton X-100 (0.05%), and DMSO (1%).

 © 2009 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

hemin–PS2M (curve b). The hemin–T30695 complex also exhibits an excellent catalytic behavior (curve d), while the enzyme activity of hemin–93del is relatively low (curve e). According to the above comparisons of binding action and DNAzyme function between the three aptamers and PS2M, it can be concluded that AGRO100 and T30695 are two new hemin-binding aptamers comparable to (or better than) PS2M, whereas 93del does not. Therefore, 93del is no longer discussed below.

To evaluate the binding parameters of T30695 and AGRO100, hemin is titrated with two aptamers in the HEPES buffer, as shown in Figure 3. The absorbance at 404 nm increases with the concentrations of aptamers, and ultimately changes no longer, indicating the saturated binding of aptamers to hemin. It is found that the binding isotherms of T30695 and AGRO100 to hemin are largely similar, suggesting the binding stoichiometry (n) and affinity (K_d) of these two aptamers are very close. Scatchard analysis shows both T30695 and AGRO100 bind hemin with $n \approx 1$. That is, T30695 can form a 1:1 complex with hemin, whereas AGRO100 binds hemin to form a 2:2 complex owing to its bimolecular G-quadruplex structure. According to the binding assays, two new hemin-binding aptamers T30695 and AGRO100 all possess high affinities ($K_d \approx 241$ and 129 nM, respectively), which are higher than that of PS2M ($K_d \approx 439$ nM) selected by the SELEX process.

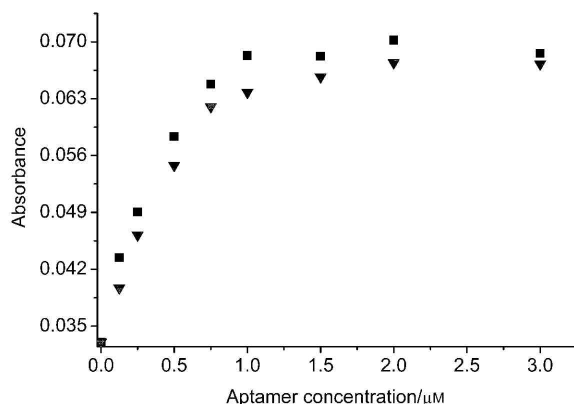


Figure 3. Absorbance (at 404 nm) changes in response to the titration of hemin (0.5 μM) with different concentrations of a) T30695 and b) AGRO100. Experimental conditions: HEPES (25 mM), pH 7.4, KCl (20 mM), NaCl (200 mM), Triton X-100 (0.05%), and DMSO (1%). The Scatchard analysis was then performed according to the saturated binding data.

Further experiments reveal the ionic conditions significantly influence the binding of T30695 and AGRO100 to hemin (Figure 4). In general, the G-quadruplex structures can be stabilized by alkali cations such as K^+ and Na^+.[29] It has been proved that K^+ is absolutely required for the catalytic behavior of the hemin–PS2M complex.[14] Unexpectedly, we find T30695 can form a catalytic complex with hemin without any coordination cation, whereas the addition of alkali metal ions especially K^+ gives rise to a small decrease in the enzyme activity (Figure 4 A). Previous studies[23,28] have indicated that T30695 can fold into the G-quadruplex structure without K^+ or Na^+, which well explains why T30695 can bind hemin in the absence of K^+ or Na^+, as observed from Figure 4 A. In contrast, AGRO100 does not form a catalytic complex with hemin in the absence of coordination cations, whereas the presence of K^+ even at 10 μM significantly promotes the enzyme activity (Figure 4B). The addition of Na^+ reveals a much smaller promotion on the catalytic activity. As the concentration of Na^+ increases, the effect of this alkali cation on hemin–AGRO100 DNAzyme becomes more and more evident (Figure 4B, curves d–f). These observations indicate the coordination ion (e.g., K^+ or Na^+) is required for AGRO100 folding and hemin binding. Among alkali cations, K^+ has the highest efficiency at coordinating to G-quadruplex,[29] consistent with its greatest promotion on the formation of hemin–AGRO100 DNAzyme (Figure 4B, curve c).

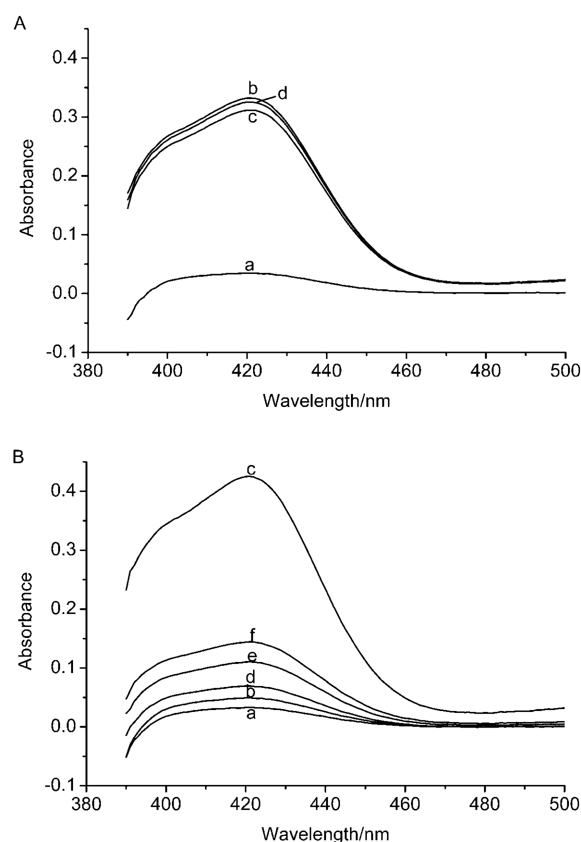


Figure 4. Effect of coordination ions on the hemin–aptamer complexes. A) UV/Vis absorption spectra (after 4 min) for analyzing: a) uncomplexed hemin (0.125 μM), b) hemin–T30695 complex (0.125 μM), c) sample b) plus K^+ (10 μM), d) sample b) plus Na^+ (10 μM). B) UV/Vis absorption spectra (after 4 min) for analyzing: a) uncomplexed hemin (0.125 μM), b) mixture of hemin and AGRO100 (0.125 μM), c) sample b) plus K^+ (10 μM), d) sample b) plus Na^+ (10 μM), e) sample b) plus Na^+ (500 μM), f) sample b) plus Na^+ (1 μM). Experimental conditions: [ABTS] = 5.9 mM, $[H_2O_2]$ = 0.6 mM, in Tris-HCl (25 mM, pH 8.0) containing Triton X-100 (0.05%), and DMSO (1%).

It has been reported that some planar aromatic ligands (e.g., cation porphyrins) strongly bind to G-quadruplexes through stacking on the terminal G-tetrads.[30–32] As an anion

© 2009 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

metalloporphyrin, hemin has been also proposed to bind to G-quadruplex aptamers through end stacking,[33] rather than intercalation between two adjacent G-tetrads.[10] In fact, although several DNA ligands are proposed to bind G-quadruplexes through the intercalative mode,[34–36] the true intercalation is generally considered unlikely in terms of energy.[37] So, hemin is reasonably proposed to bind to T30695 or AGRO100 by end stacking, as reported.[33] In the early reports,[23,28] T30695 is thought to adopt a chair-like antiparallel G-quadruplex structure consisting of two G-tetrads, just like a 15-mer thrombin-binding aptamer.[38] However, more recent research has indicated that this aptamer and other closely related sequences only folds into the propeller-type parallel structures with three consecutive G-tetrads linked by three single residue loops.[39–42] The T loop residue points toward the G-tetrads and forms hydrogen bonds with guanines,[39] making this structure extremely stable. In terms of stability, this parallel-stranded structure should be the most favorable conformation in the hemin–T30695 complex, in which hemin stacks on one terminal G-tetrad of folded T30695 (Figure 5 A). As reported,[33] hemin

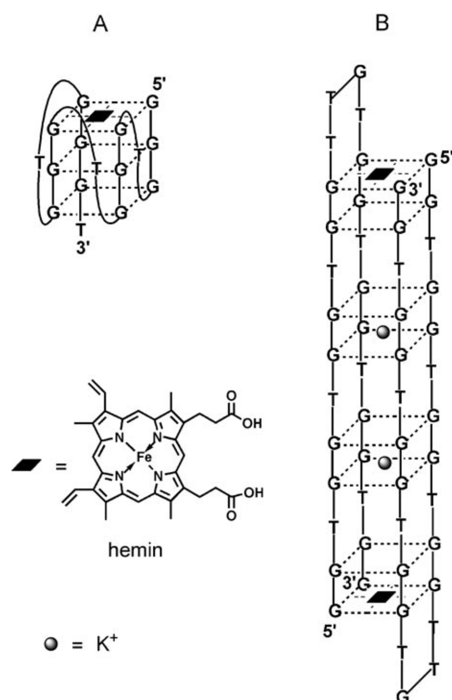


Figure 5. Proposed structural models for A) hemin–T30695 and B) hemin–AGRO100 complexes. Hemin is thought to bind to the folded T30695 and AGRO100 through end stacking to form 1:1 and 2:2 complexes, respectively.

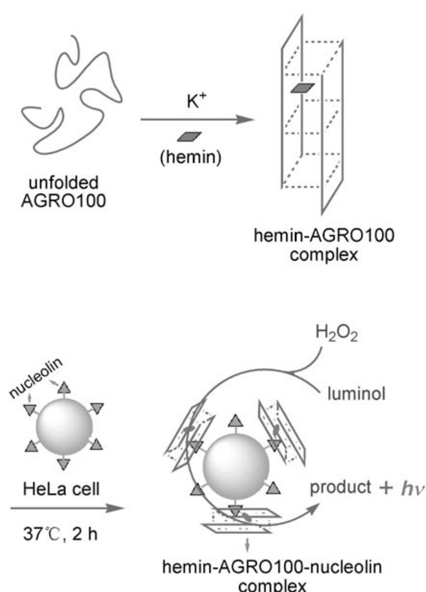
binding to T30695 may operate through an induced fit mechanism. That is, the hemin binding itself contributes to the formation of favorable binding sites. The ligand-induced formation of G-quadruplex is also observed in other ligand–DNA interactions.[43] In the presence of K^+, AGRO100 can dimerize to form a highly symmetric bimolecular G-quadruplex consisting of four pairs of stacked G-quartets.[22] Two hemin molecules can stack two terminal G-tetrads of folded AGRO100, respectively, to form a 2:2 complex (Figure 5B). This intermolecular structure requires the coordination cations, especially K^+, to keep it stable, as observed from Figure 4B. Such a structural model suggests that the hemin–AGRO100 complex possesses two active sites for catalysis, consistent with its excellent enzyme activity (Figure 2).

Another significant function of T30695 and AGRO100 is their binding to the corresponding protein ligand (HIV-1 integrase or nucleolin), which gives them potential as anti-HIV or anticancer therapeutic agents.[20,21] A mechanistic study of inhibiting HIV-1 integrase by T30695 has indicated a "face-to-face" interaction between these two molecules.[13] Similarly, a molecular model of a related aptamer of AGRO100 (named GRO29A)[27] has indicated that nucleolin binds to the G-quadruplex groove of GRO29A. Because AGRO100 has a G-quadruplex core the same as that of GRO29A, the groove binding mode is also applicable to the AGRO100–nucleolin interaction. That is, both T30695 and AGRO100 can bind hemin as well as target protein. Hence, they can be defined as multifunctional aptamers capable of binding two or more ligands. The related aptamers of T30695 and AGRO100, including T30177[23] and GRO29A,[27] are all found to possess such multiple functions (data not shown). Most importantly, the hemin–T30695 and hemin–AGRO100 complexes reveal excellent peroxidase-like activities even higher than that of the known DNAzyme hemin–PS2M. This gives T30695 and AGRO100 great potential as a platform for the sensitive detection of protein ligands (nucleolin and HIV-1 integrase).

To demonstrate the potential application of multifunctional aptamers in bioanalysis, we chose AGRO100 as an example and applied it in the chemiluminescence (CL) detection of the protein marker nucleolin expressed at the surface of HeLa cells (Scheme 1). As a nucleolar protein, nucleolin is primarily expressed in the nucleus, but it is found to shuttle between the surfaces and nuclei of human cancer cells cultured in serum-containing media.[37,38] Hence, the cell-surface-expressed nucleolin can be captured in situ by AGRO100 complexed with hemin, as described in a previous report.[22] After being labeled with hemin–AGRO100, the formed hemin–AGRO100-nucleolin catalytic complex at the cell surface was analyzed in the luminol–H_2O_2 system, which promotes the generation of CL emission.

The integral curves of CL signal for analyzing different number of HeLa cells are shown in Figure 6 A. It is found that the addition of 2×10^3 cells labeled with DNAzyme does not elevate the signal above background noise (curve b), whereas a noticeable increase in integrated CL is observed after addition of 6×10^3 cells (curve c). As the number of cells increases, the intensity of generated CL emission is gradually increased, suggesting the formation of more hemin–AGRO100–nucleolin at the cell surface. Figure 6B indicates the dependence of integrated CL intensity (after 150 s) on the number of HeLa cells. From this figure it is concluded that the total surface-expressed nucleolin of

 © 2009 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Scheme 1. Multifunctional aptamer AGRO100 as a DNAzyme-based platform for the CL detection of nucleolin expressed at the surface of HeLa cells. With the multifunction of simultaneously binding hemin and nucleolin, the folded AGRO100 can label the cell-surface-expressed nucleolin in situ after being complexed with hemin, providing an approach to sensing this protein marker in the luminol–H_2O_2 system.

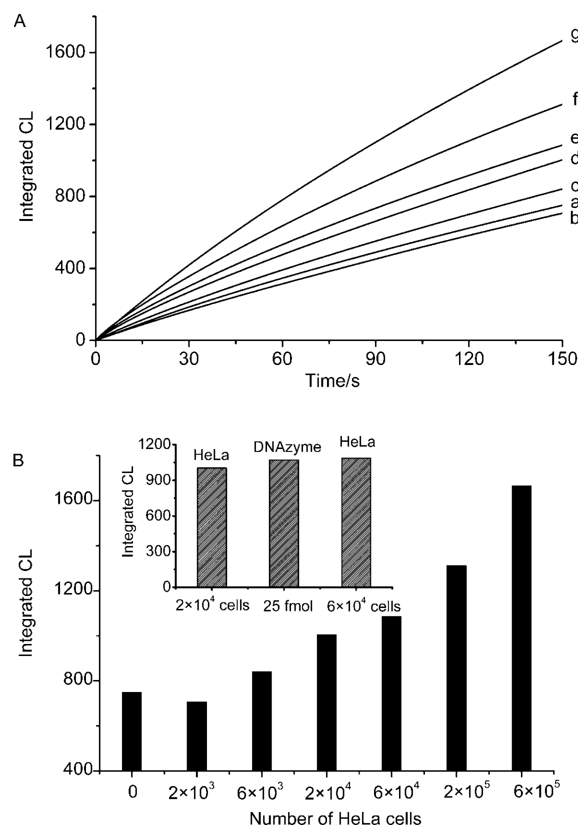


Figure 6. Chemiluminescence detection of surface-expressed nucleolin of HeLa cells with hemin–AGRO100 as the *in vitro* catalytic label. A) Integral curves of CL signal for analyzing different number of HeLa cells in the luminol-H_2O_2 system: a) background, b) 2×10^3 cells, c) 6×10^3 cells, d) 2×10^4 cells, e) 6×10^4 cells, f) 2×10^5 cells, g) 6×10^5 cells. B) Dependence of integrated CL (after 150 s) on the number of HeLa cells. Experimental conditions: [luminol] = 0.5 mM, [H_2O_2] = 2 mM, in HEPES buffer (25 mM, pH 8.0) containing KCl (20 mM) and NaCl (200 mM). The voltage of photomultiplier tube was set at 700 V. The inset shows a comparison between the CL signals originated from HeLa cells and from hemin–AGRO100 DNAzyme.

about 6000 HeLa cells can be detected by this sensing platform. The inset in Figure 6B shows a comparison between the intensities of CL signals originated from HeLa cells and from hemin–AGRO100 DNAzyme. It is found that the CL signal from 60000 HeLa cells is largely similar to that from 25 fmol DNAzyme. This means the cell-surface-expressed nucleolin can be detected at the fmol level, indicating a high sensitivity of our developed DNAzyme-based method for protein detection. However, it should be noted that nucleolin is a shuttle protein and not expressed at the surface of serum-starved cells;[37] that is, the amount of nucleolin expressed at the cell surface changes as the culture conditions change. So, keeping cells well-nourished is always required for sensing the surface-expressed nucleolin of human cancer cells.

Conclusion

Two families of G-quadruplex aptamers, AGRO100, T30695, and various related aptamers, have shown great biomedical significance due to their potential as anticancer or anti-HIV therapeutic agents. Exploring some new functions of these significant aptamers will help to extend their biomedical applications. In view of the secondary structures of AGRO100 and T30695, their potential as hemin binders are explored here, to discover their potential DNAzyme function. We find that both AGRO100 and T30695 bind hemin with high affinities comparable to that of PS2M. Most importantly, their corresponding hemin–DNA complexes exhibit higher peroxidase-like activities than the known DNAzyme hemin–PS2M. Accordingly, the anticancer aptamer AGRO100 and the anti-HIV aptamer T30695 are also considered as two new hemin-binding aptamers. They are capable of simultaneously binding a protein ligand (nucleolin or HIV-1 integrase) and hemim through two different modes, and related aptamers also do so. Such multiple functions enable these aptamers to serve as a novel platform for sensing protein markers in a facile way. As an example, AGRO100 has been applied to the CL detection of surface-expressed nucleolin of HeLa cells. Based on the specific AGRO100–nucleolin interaction, the cell-surface-expressed nucleolin is labeled in situ with hemin–AGRO100 complex and then determined in the luminol–H_2O_2 CL system. This sensing platform can be used to detect the total surface-expressed nucleolin of about 6000 HeLa cells. Our findings show that exploiting the undiscovered functions of existing aptamers will extend their potential applications in bio analysis.

© 2009 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Experimental Section

Sequences and pretreatment of DNA aptamers: Six G-quadruplex aptamers (PS2M: 5′-GT GGG TA GGG C GGG TT GG-3′, AGRO100: 5′-GGT GGT GGT GGT TGT GGT GGT GGT GG-3′, T30695: 5′-GGGT GGGT GGGT GGGT-3′, 93del: 5′-GGG GT GGG A GGA GGGT-3′, T30177: 5′-GT GGT GGGT GGGT GGGT-3′, GRO29A: 5′-TTT GGT GGT GGT GGT TGT GGT GGT GGT GG-3′) were synthesized by Sangon Biotechnology (Shanghai, China). Before use, these oligonucleotides were dissolved in the TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4), and quantified by using UV/Vis absorption spectroscopy with the following extinction coefficients (ε_{260nm}, $M^{-1}cm^{-1}$): A = 15400, G = 11500, C = 7400, T = 8700. The DNA solutions were then heated at 88 °C for 10 min, and gradually cooled to room temperature.

Investigation of hemin–aptamer interactions and DNAzyme functions: An equal volume of the hybridization buffer (50 mM HEPES, pH 7.4, 40 mM KCl, 400 mM NaCl, 0.1% Triton X-100, 2% DMSO) was added to the above DNA solutions, allowing aptamers to fold for 40 min to form the G-quadruplex structures. Then an equal volume of same concentration of hemin dissolved in the binding buffer (25 mM HEPES, pH 7.4, 20 mM KCl, 200 mM NaCl, 0.05% Triton X-100, 1% DMSO) added to the DNA solutions. The mixtures were kept at room temperature for 1 h, allowing the aptamers to bind hemin properly. Finally, the formed hemin–aptamer complexes were characterized by using UV/Vis absorption spectroscopy, to evaluate the binding actions and catalytic behaviors of several investigated G-quadruplex aptamers. The absorption spectra were recorded with a Cary 500 Scan UV/Vis/NIR Spectrophotometer (Varian, USA).

Binding assays: Based on the peroxidase-like activities of hemin and hemin–aptamer complexes, a new method for determination of the affinity (K_d) of hemin-binding aptamers was developed by using UV/Vis absorption spectroscopy. Briefly, hemin (0.1 μM) was incubated with different concentrations of aptamers for 1 h in the aqueous buffer consisting of ABTS (5.45 mM), HEPES (25 mM, pH 8.0), KCl (20 mM), NaCl (200 mM), Triton X-100 (0.05%), and DMSO (1%). Then, H_2O_2 (10 μL, 60 mM) was added to the hemin and aptamer mixture (990 μL) to initiate the reaction. The absorbance at 421 nm within 4 min was recorded in different cases. According to the literature,[14,15,44] we deduced a formula for K_d calculation [Eq. (1)] in which $[\text{aptamer}]_0$ and $[\text{hemin}]_0$ are the initial concentrations of aptamer and hemin; A_0, A_8, and A_x are the absorbance (at 421 nm) for analyzing uncomplexed hemin (in the absence of aptamers), fully bound hemin (in the presence of extremely excess aptamers), and hemin bound partially by aptamers (in the presence of appropriate concentrations of aptamers), respectively.

$$[\text{aptamer}]_0 = K_d(A_x - A_0)/(A_8 - A_x) + [\text{hemin}]_0(A_x - A_0)/(A_8 - A_0) \qquad (1)$$

Cell treatment and CL detection of surface-expressed nucleolin: After passage, HeLa cells in logarithmic growth phase were incubated with hemin–AGRO100 complex (10 μM) in serum-containing culture media at 37 °C for 2 h, allowing the complex to properly label nucleolin expressed at the cell surface. The intact cells were separated from excess DNAzyme by centrifugation, and thoroughly washed with the detection buffer (25 mM HEPES, pH 8.0, 20 mM KCl, 200 mM NaCl), then dispersed in buffer to prepare different concentrations of cell suspensions (the cell concentrations were counted by using a hemocytometer). Finally, H_2O_2 (10 μL, 60 mM) followed by different concentrations of cell suspensions (10 μL) was added to luminol (280 μL, 0.5 mM). The hemin–AGRO100–nucleolin complex at the cell surface catalyzed the oxidation of luminol by H_2O_2 to generate CL emission. The CL signal was recorded with a Model MCDR-A Luminescence System (Remex, Xi'An, China). The voltage of photomultiplier tube was set at 700 V.

Acknowledgements

We are grateful to Ms. Jin'e Wang for providing the HeLa cells. This work is supported by the National Natural Science Foundation of China with the Grants 20675078 and 20735003, 973 project 2007CB714500, and the Chinese Academy of Sciences KJCX2.YW.H11.

[1] A. D. Ellington, J. W. Szostak, *Nature* **1990**, *346*, 818–822.
[2] C. Tuerk, L. Gold, *Science* **1990**, *249*, 505–510.
[3] L. C. Bock, L. C. Griffin, J. A. Latham, E. H. Vermaas, J. J. Toole, *Nature* **1992**, *355*, 564–566.
[4] Y. Li, C. R. Geyer, D. Sen, *Biochemistry* **1996**, *35*, 6911–6922.
[5] D. E. Huizenga, J. W. Szostak, *Biochemistry* **1995**, *34*, 656–665.
[6] A. Mazumder, N. Neamati, J. O. Ojwang, S. Sunder, R. F. Rando, Y. Pommier, *Biochemistry* **1996**, *35*, 13762–13771.
[7] F. Pileur, M. L. Andreola, E. Dausse, J. Michel, S. Moreau, H. Yamada, S. A. Gaidamakov, R. J. Crouch, J. J. Toulme, C. Cazenave, *Nucleic Acids Res.* **2003**, *31*, 5776–5788.
[8] M. L. Andreola, F. Pileur, C. Calmels, M. Ventura, L. Tarrago-Litvak, J. J. Toulme, S. Litvak, *Biochemistry* **2001**, *40*, 10087–10094.
[9] D. M. Tasset, M. F. Kubik, W. Steiner, *J. Mol. Biol.* **1997**, *272*, 688–698.
[10] P. Travascio, P. K. Witting, G. Mauk, D. Sen, *J. Am. Chem. Soc.* **2001**, *123*, 1337–1348.
[11] K. Y. Wang, S. H. Krawczyk, N. Bischofberger, S. Swaminathan, P. H. Bolton, *Biochemistry* **1993**, *32*, 11285–11292.
[12] A. T. Phan, V. Kuryavyi, J. B. Ma, A. Faure, M. L. Andreola, D. J. Patel, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 634–639.
[13] N. Jing, C. Marchand, J. Liu, R. Mitra, M. E. Hogan, Y. Pommier, *J. Biol. Chem.* **2000**, *275*, 21460–21467.
[14] P. Travascio, Y. Li, D. Sen, *Chem. Biol.* **1998**, *5*, 505–517.
[15] P. Travascio, A. J. Bennet, D. Y. Wang, D. Sen, *Chem. Biol.* **1999**, *6*, 779–787.
[16] D. Sen, C. R. Geyer, *Curr. Opin. Chem. Biol.* **1998**, *2*, 680–687.
[17] V. Pavlov, Y. Xiao, R. Gill, A. Dishon, M. Kotler, I. Willner, *Anal. Chem.* **2004**, *76*, 2152–2156.
[18] Y. Xiao, V. Pavlov, R. Gill, T. Bourenko, I. Willner, *ChemBioChem* **2004**, *5*, 374–379.
[19] T. Li, S. Dong, E. Wang, *Chem. Commun.* **2007**, 4209–4211.
[20] S. H. Chou, K. H. Chin, A. H. Wang, *Trends Biochem. Sci.* **2005**, *30*, 231–234.
[21] C. R. Ireson, L. R. Kelland, *Mol. Cancer Ther.* **2006**, *5*, 2957–2962.
[22] A. C. Girvan, Y. Teng, L. K. Casson, S. D. Thomas, S. Juliger, M. W. Ball, J. B. Klein, W. M. Pierce, Jr., S. S. Barve, P. J. Bates, *Mol. Cancer Ther.* **2006**, *5*, 1790–1799.
[23] N. Jing, R. F. Rando, Y. Pommier, M. E. Hogan, *Biochemistry* **1997**, *36*, 12498–12505.
[24] V. R. de Soultrait, P. Y. Lozach, R. Altmeyer, L. Tarrago-Litvak, S. Litvak, M. L. Andreola, *J. Mol. Biol.* **2002**, *324*, 195–203.
[25] P. J. Bates, J. B. Kahlon, S. D. Thomas, J. O. Trent, D. M. Miller, *J. Biol. Chem.* **1999**, *274*, 26369–26377.
[26] X. Xu, F. Hamhouyia, S. D. Thomas, T. J. Burke, A. C. Girvan, W. G. McGregor, J. O. Trent, D. M. Miller, P. J. Bates, *J. Biol. Chem.* **2001**, *276*, 43221–43230.
[27] V. Dapic, P. J. Bates, J. O. Trent, A. Rodger, S. D. Thomas, D. M. Miller, *Biochemistry* **2002**, *41*, 3676–3685.
[28] N. Jing, M. E. Hogan, *J. Biol. Chem.* **1998**, *273*, 34992–34999.
[29] D. Sen, W. Gilbert, *Nature* **1990**, *344*, 410–414.
[30] R. T. Wheelhouse, D. K. Sun, H. Y. Han, F. X. G. Han, L. H. Hurley, *J. Am. Chem. Soc.* **1998**, *120*, 3261–3262.
[31] E. Izbicka, R. T. Wheelhouse, E. Raymond, K. K. Davidson, R. A. Lawrence, D. Sun, B. E. Windle, L. H. Hurley, D. D. Von Hoff, *Cancer Res.* **1999**, *59*, 639–644.
[32] H. Han, D. R. Langley, A. Rangan, L. H. Hurley, *J. Am. Chem. Soc.* **2001**, *123*, 8902–8913.
[33] D. J. Chinnapen, D. Sen, *Biochemistry* **2002**, *41*, 5202–5212.
[34] I. Haq, J. O. Trent, B. Z. Chowdhry, T. C. Jenkins, *J. Am. Chem. Soc.* **1999**, *121*, 1768–1779.

 © 2009 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

[35] N. V. Anantha, M. Azam, R. D. Sheardy, *Biochemistry* **1998**, *37*, 2709–2714.
[36] L. R. Keating, V. A. Szalai, *Biochemistry* **2004**, *43*, 15891–15900.
[37] J. L. Mergny, C. Helene, *Nat. Med.* **1998**, *4*, 1366–1367.
[38] R. F. Macaya, P. Schultze, F. W. Smith, J. A. Roe, J. Feigon, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3745–3749.
[39] P. Hazel, J. Huppert, S. Balasubramanian, S. Neidle, *J. Am. Chem. Soc.* **2004**, *126*, 16405–16415.
[40] P. A. Rachwal, I. S. Findlow, J. M. Werner, T. Brown, K. R. Fox, *Nucleic Acids Res.* **2007**, *35*, 4214–4222.
[41] A. Risitano, K. R. Fox, *Biochemistry* **2003**, *42*, 6507–6513.
[42] A. Bugaut, S. Balasubramanian, *Biochemistry* **2008**, *47*, 689–697.
[43] T. Murashima, D. Sakiyama, D. Miyoshi, M. Kuriyama, T. Yamada, T. Miyazawa, N. Sugimoto, *Bioinorg. Chem. Appl.* **2008**, 294756.
[44] Y. Wang, K. Hamasaki, R. R. Rando, *Biochemistry* **1997**, *36*, 768–779.

Received: June 27, 2008
Published online: December 3, 2008

© 2009 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim