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Dependence of aptamer activity on opposed terminal extensions: improvement of light-regulation efficiency

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ABSTRACT

Aptamers that can be regulated with light allow precise control of protein activity in space and time and hence of biological function in general. In a previous study, we showed that the activity of the thrombin-binding aptamer HD1 can be turned off by irradiation using a light activatable 'caged' intramolecular antisense-domain. However, the activity of the presented aptamer in its ON state was only mediocre. Here we studied the nature of this loss in activity in detail and found that switching from 5′- to 3′-extensions affords aptamers that are even more potent than the unmodified HD1. In particular we arrived at derivatives that are now more active than the aptamer NU172 that is currently in phase 2 clinical trials as an anticoagulant. As a result, we present light-regulatable aptamers with a superior activity in their ON state and an almost digital ON/OFF behavior upon irradiation.

INTRODUCTION

Aptamers are short single-chain nucleic acids that fold into well-defined three-dimensional structures. They can be identified by an *in vitro* selection approach, also termed SELEX (systematic evolution of ligands by exponential enrichment) (1,2). To a large variety of target molecules this approach has been applied, yielding selective aptamers. One of the most prevalent classes of target molecules is proteins. Due to their sophisticated inhibitory capabilities, aptamers promise to be an excellent novel class of therapeutic molecules and one aptamer, namely Macugen, which binds to vascular endothelial growth factor (VEGF) thereby inhibiting its interaction with the VEGF-receptor is approved by the FDA for the treatment of the wet form of age-related macular degeneration (3).

Since the first description of aptamers, many efforts have been undertaken to develop thrombin-targeting aptamers for the use as anticoagulants (4–6). In this regard, the 15 nucleotides minimal motif of an anti-thrombin aptamer (**HD1**, Figure 1a), initially described by Bock *et al*. in 1992, raised huge attention and represents today one of the most intensively studied aptamers (7). Albeit clinical trials to verify the aptamer's effectiveness as anticoagulant have been halted after phase I, the aptamer has been used by many researchers for structural and diagnostic investigations what is mainly due to its simple composition and G-quadruplex structure. The latter has led to many studies analyzing the structure, and NMR, circular dichroism (CD) spectroscopy, native polyacrylamide gel electrophoresis, molecular dynamic simulation and crystallography have been used to address the conformation of this G-quadruplex and the basis for its interaction with thrombin. These show that **HD1** folds into an intramolecular quadruplex with an antiparallel orientation of the strands in a chair-like conformation (Figure 1a).

We have recently started to develop generic approaches aiming at the control of nucleic acid function by light in space and time. This generic approach allows interfering with gene expression and protein function in arbitrary regions and at well-defined points in time and profits from the fact that light is an 'orthogonal' and harmless trigger signal—if applied correctly—and that many relevant living samples are light-accessible (8–12). One way to introduce light-control is by attaching photolabile

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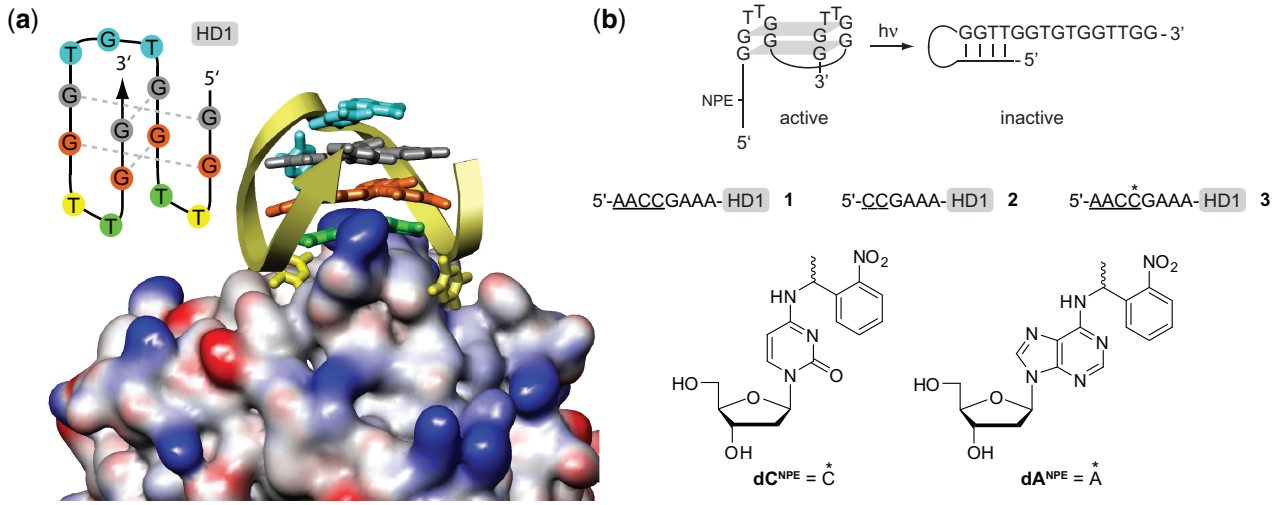

Figure 1. (**a**) Cartoon representation of the antiparallel quadruplex structure of the thrombin-binding aptamer **HD1** and of **HD1** bound to thrombin (PDB code: 1HAO). Thrombin is shown as surface with negatively charged areas colored in red and positively in blue. (**b**) Top: Cartoon representation of the light-induced inactivation of **HD1**. Middle: The aptamers **1–3** with designed intramolecular antisense residues (underlined) have been part of a previous study in which we already showed that it is possible to turn the activity of **HD1** OFF by irradiation with light (18). Shown are also the residues $\mathbf{dC^{NPE}}$ and $\mathbf{dA^{NPE}}$ (bottom). The former has been introduced in our previous study (18) while the latter is introduced here. Both residues have photolabile NPE groups (o-nitrophenylethyl) that keep them from forming normal nucleobase interactions until irradiation and can thus mask nucleotide activity that can be restored with control of space and time.

groups and thus obtain 'caged' compounds. Applying this concept to nucleic acids we have for example already developed light activatable siRNAs (13) and showed that it is possible to induce transcription with light (14). To demonstrate the concept of controlling protein function in time and space using the aptamer approach, we defined and synthesized variants of **HD1** whose thrombin interacting activity can be controlled by light. For the reasons given above, **HD1** is an ideal candidate. To make **HD1** light-inducible, based on the co-crystal structure of the **HD1**–thrombin complex (PDB code: 1HAO, Figure 1a), we introduced a photolabile group at a strategic position of the aptamer to temporarily inhibit the interaction with thrombin. The modification of T4 in **HD1** with such a photolabile caging group that prevents this residue from forming the natural nucleobase interaction led to a derivative that could be effectively turned ON upon irradiation with UV-A light (15). Later, we showed that this is also possible by light-induced formation of the G-quadruplex conformation (16,17). In an ongoing endeavor, we also started designing variants of the aptamer that could be turned OFF by UV-A light exposure. Therefore, we extended the 5′-terminal region of **HD1** with sequences supposed to form a hairpin-like structure stabilized by a GNRA-tetraloop (aptamers **1** and **2**, Figure 1b, residues that are intended antisense residues are underlined in the sequences) (18). It could be shown that four antisense residues (such as in aptamer **1**) were sufficient to make **HD1** completely inactive. To make this conformational transition light-inducible, we subsequently placed a caged residue at C4 in the aptamer sequence (aptamer **3**). Figure 1b shows the caged residue $\mathbf{dC^{NPE}}$ that has been used in that study and that is abbreviated as C* in a one-letter code representation here. The nucleobase carries a photolabile o-nitrophenylethyl (NPE) group that makes C* a mismatch until irradiation upon which an unmodified cytidine is formed. Thus we constructed a novel anticoagulant with a built-in antidote activity that can be released upon light irradiation. However, although we demonstrated for the first time that an aptamer can be inactivated with light the inhibitory potential of aptamer **3** in coagulation assays did not reach the one of the unmodified **HD1**. Here we systematically address this observation by the comparison of **HD1** variants that bear various extensions either at their 5′- or 3′-terminal. Unexpectedly, we found that 5′-extensions lead to a dramatic loss in activity whereas the same extensions at the 3′-end resulted in even more potent aptamers compared with the parent aptamer **HD1**. Ultimately, these findings led to the design and synthesis of improved second generation caged aptamers that were found to be more active than **HD1** and could be efficiently turned OFF upon light irradiation.

MATERIALS AND METHODS

Oligonucleotide synthesis

Unmodified oligonucleotides were purchased from Microsynth, Balgach (Switzerland) (www.microsynth.ch), and had been HPLC purified. The amidites $\mathbf{dC^{NPE}}$ and $\mathbf{dA^{NPE}}$ were used with regular solid phase synthesis protocols on an ABI-392 synthesizer. For the cleavage either aqueous ammonia (65°C, 4 h) or a 1:1 mixture of aqueous ammonia and methyl amine (1:1, room temperature, overnight) was used. The resulting crude product was purified by RP-HPLC (Nucleosil 100-5, C18, 0.1 M triethylammonium acetate pH 7, acetonitrile), detritylated and again purified by RP-HPLC (same protocol). The identity of the oligonucleotides has been established by ESI: aptamer **3**: 7350.0 Da (expected

average: 7348.8 Da), aptamer **26**: 7767.2 Da (expected exact: 7767.4 Da), aptamer **27**: 8056.6 Da (expected exact: 8056.4 Da), aptamer **28**: 8588.3 Da (expected exact: 8588.5 Da), aptamer **29**: 8886.1 Da (expected exact: 8886.6 Da), aptamer **30**: 8742.3 Da (expected average: 8741.8 Da).

Irradiation

For the uncaging the respective aptamers were irradiated for 3 min with three UV-LEDs (Nichia NCCU033, 360 nm, 100 mW each) operated at 0.5 A.

Plasma coagulation assay

The anticoagulant activity of the aptamers was measured in a one-stage plasma-based coagulation assay [thrombin-time (TT)] using an ACL Top coagulation analyser (IL, Kirchheim, Germany). In brief, human α-thrombin (CellSystems, St. Katharinen, Germany) was diluted in the assay buffer (1 ×PBS, pH 7.4, 3 mmol/l $MgCl_2$, 1 μg/μl BSA) to reach a final concentration of 10 NIH U/ml (75 nmol/l). This thrombin solution was spiked with aptamers at a final concentration of 1 μmol/l and 75 μl of the reaction mixtures were added to prewarmed cuvettes of the ACL-analyzer. After incubation for 1 min at 37°C, 75 μl of pooled normal human plasma was added and clotting times measured. For comparative determination of the thrombin-inhibiting capacity of the tested aptamers, clotting times were normalized to standard curves as determined by serial dilutions of α-thrombin in a concentration range from 10 NIH U/ml (100%) down to 0.625 NIH U/ml (6.25%). To assess the anticoagulant activity of the aptamers relative to that of **HD1**, clotting times were normalized to that of **HD1**-standard curves [0 (0%) to 2 μmol/l (200%)] processed in parallel.

CD spectroscopy

To obtain CD spectra 1 nmol of the respective aptamers was dissolved in 110 μl of PBS buffer (final concentrations 137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4). The spectra were measured on a JASCO J-715 and are background-subtracted and smoothed.

Filter retention assay

For filter retention analysis, aptamers were 5′-end labeled with γ^{32}P-ATP using T4 polynucleotide kinase (Stratagene, Amsterdam, Netherlands) for 45 min at 37°C. Subsequently, the reaction mixture was applied to G25 microspin columns (GE Healthcare, Munich, Germany) to remove unreacted γ^{32}P-ATP and the resulting ssDNA was analyzed on 12% polyacrylamide gels to check the integrity. For determination of dissociation constants, radioactively labeled aptamers at a final concentration of 0.5 nM were incubated with increasing amounts of bovine thrombin (0–1 μM). Generally, experiments were performed in PBS, pH 7.3, containing 2 μM tRNA. After incubation at 37°C for 30 min, the reaction mixtures were filtered through a wet 0.45 μm nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany) and washed with 0.8 ml washing buffer (PBS, pH 7.3). After drying, the membrane was exposed on a storage phosphor screen and analyzed on a FUJIFILM FLA-3000 with the AIDA Imagequant software (Fujifilm, Düsseldorf, Germany). The dissociation constants were calculated using a logistic fit function assuming a 1:1 binding stoichiometry of aptamer–protein complexes.

RESULTS AND DISCUSSION

Figure 2 shows the performance of the various derivatives of **HD1** in the coagulation assays. The results are given in two scales. For one the activity of the aptamer variants is displayed as percentage of the activity of the parent aptamer **HD1** (Figure 2, black bars). The other scale represents the percentage of residual thrombin activity in the presence of the indicated aptamers (Figure 2, grey bars). Both scales are reciprocal to each other. Normalization of raw data (clotting-times) to corresponding thrombin- and **HD1**-activites was a prerequisite for accurate evaluation of the tested aptamer variants. This is due to the typical non-linear correlation between both thrombin- or inhibitor-activity and resulting clotting times (Supplementary Data). Under the conditions chosen, the application of unmodified **HD1** yielded a residual thrombin activity of 20%. Aptamer **1** in turn, with a four-nucleotide 5′-antisense region and the GAAA-tetraloop sequence had no anti-thrombin activity—as intended and as previously reported (18). A shorter antisense sequence was not sufficient to block aptamer activity, as aptamer **2** with only two complementary residues retained 12% of the parental **HD1** activity. In the previously mentioned caged aptamer **3**, the antisense region should be blocked and hence the aptamer should reach 100% **HD1** activity before irradiation. However, it does not, as it only shows 14% activity compared to **HD1**. Irradiation of **3** on the other hand results in almost the same values as observed with aptamer **1** indicating that the uncaging process is complete. This is also what can be seen in HPLC traces (Supplementary Data).

On the way to an explanation why aptamer **3** is less efficient before irradiation as **HD1** we tested aptamer **4** with only the GAAA loop region and found that already by attaching these four nucleotides to the 5′-end the aptamer suffers by a reduction of its activity down to 34% on the **HD1** scale. Assuming that the adenosine residues might interfere with the thymidine residues of **HD1**, we chose different loop sequences (aptamers **5** and **6**) and found that the **HD1** activity could be somewhat improved. An aptamer with an AAAA loop (aptamer **7**) performed again similarly as aptamer **4**, supporting that desoxyadenosines are particularly activity impairing at the 5′-end but their possible interaction with the thymidine residues of **HD1** cannot be the only explanation. Assuming that the nucleotide 5′-extension might be detrimental due to their pi stacking interactions we tried non-nucleosidic linkers such as abasic sites (aptamer **8**), aliphatic spacers (aptamer **9**) and ethylene glycol-derived spacers (aptamer **10**) but 100% **HD1** activity could again not be reached by far.

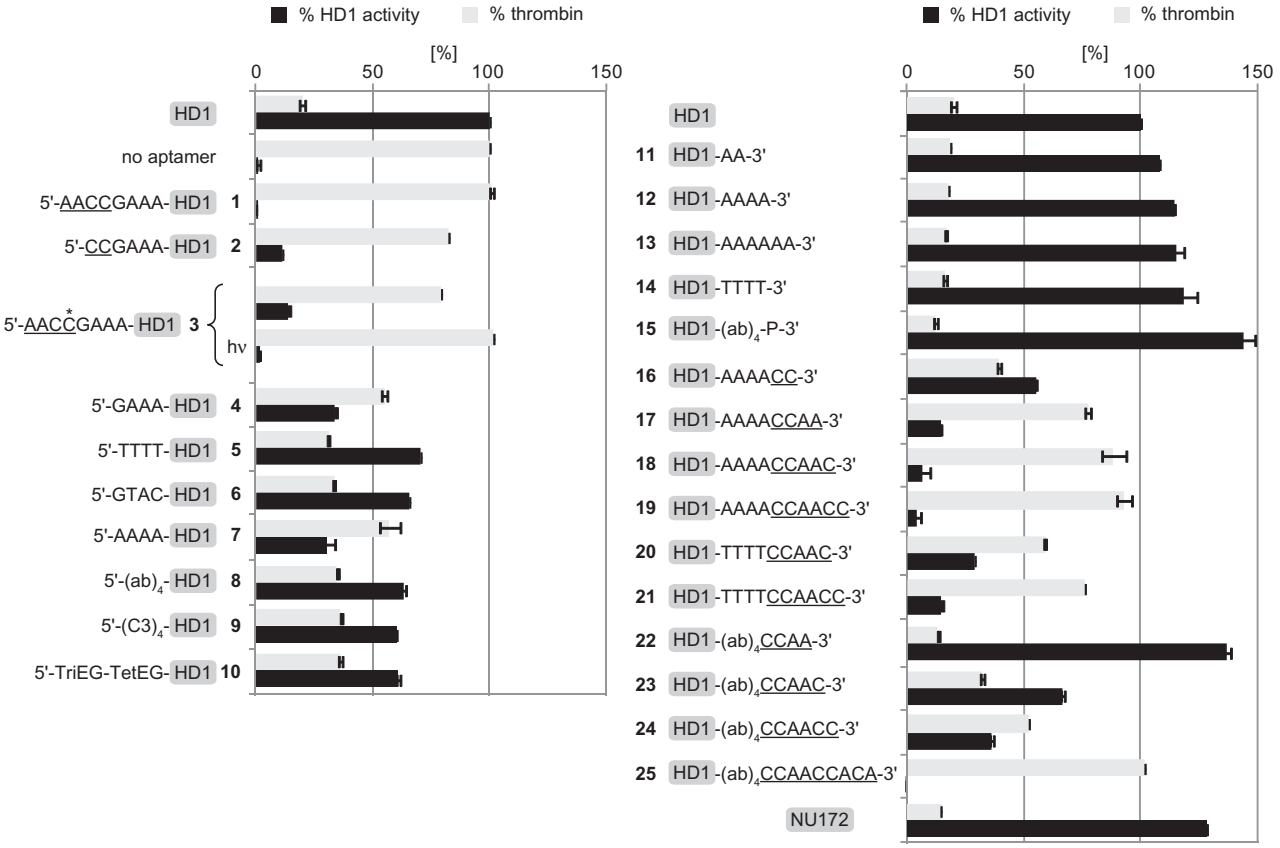

Figure 2. Results of coagulation assays with the listed aptamers without or with (hv) irradiation. Clotting times have been normalized toward the one of **HD1**. A second—reciprocal—scale is given that shows how much thrombin remained active. The sequence of the aptamer **NU172** (used in this study for comparison reasons) is CGCCTAGGTTGGGTAGGGTGGTGGCG (20). (ab = abasic site, C3 = n-propyl spacer nucleotide, TriEG = triethylene glycol nucleotide, TetEG = tetraethylene glycol nucleotide).

Initially purely for reference, we also attached linkers to the 3′-end of **HD1** (aptamer **11**). To our surprise, however, an aptamer with two more adenosines on the 3′-end even showed an activity that was slightly better than the one of **HD1**—significant within error limits. The anticoagulant performance could even be increased by adding more adenosines and reached a plateau at around six (aptamers **12** and **13**). For reference it shall be noted that in a previous study, an **HD1**-derivative with a 3′-dA_{15}-extension showed similar activities compared to **HD1** in our assays (19). With four thymidines at the 3′-end (aptamer **14**), a similar picture was found. Exchanging the nucleoside linkers on the 3′-end for abasic sites (aptamer **15** with a phosphate on the 3′-end due to synthesis reasons) resulted in the most potent aptamer in the entire study and a 1.5-fold increase of activity compared to **HD1** was observed. Therefore, aptamer **15** is superior to a different anti-thrombin aptamer that also targets exosite I and that is currently in phase II clinical trials, namely **NU172** (Figure 2) (20). This different behavior of opposed terminal extensions of **HD1** was especially surprising to us since in the co-crystal structure of the **HD1**–thrombin complex the 5′- and the 3′-ends reside right next to each other (Figure 1a). Encouraged by these findings, we started to address the question whether the activity could again be turned off by adding antisense residues—this time to the 3′-end. In the 3′-adenosine-linker series (aptamers **16–19**), it turned out that adding five to six antisense residues reduced the activity of **HD1** almost to background levels. With thymidines as 3′-linkers (aptamers **20** and **21**), a significant activity remained even with six complementary residues. With abasic sites as 3′-linkers (and thus starting from a higher **HD1** activity) nine antisense residues afforded an aptamer that was cleanly OFF (aptamers **22–25**).

To now obtain caged aptamers with significantly improved difference in activity between the ON and OFF state and improved performance in the ON state, caged residues were introduced in the antisense region. The caged $\mathbf{dC}^{\mathbf{NPE}}$ had already been introduced previously; however, a similarly caged $\mathbf{dA}^{\mathbf{NPE}}$ had never been used before. Figure 3 shows the synthesis route that we chose in analogy to the synthesis of the caged ribonucleotide analogue that had been used in a study by Silverman *et al.* (21). In brief, the hydroxyl groups of deoxyinosine were TBDMS-protected and then O^6 was transferred into a leaving group by reaction with triisopropylbenzenesulfonyl chloride (TPSCl). After nucleophilic attack with the amine of the caging group (NPE-NH_2) and

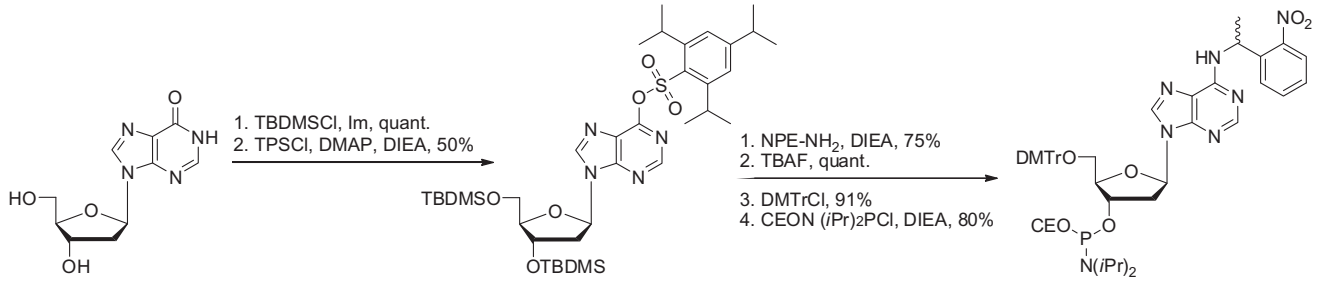

Figure 3. Synthesis of the caged phosphoramidite for the introduction of a $\mathbf{dA}^{\mathbf{NPE}}$ residue into an oligonucleotide with standard solid phase synthesis technology (TBDMS = tert. butyldimethylsilyl, TPS = triisopropylbenzenesulphonyl, DMAP = *N*,*N*-dimethylaminopyridine, DIEA = Hünig's Base, TBAF = tetrabutylammonium fluoride).

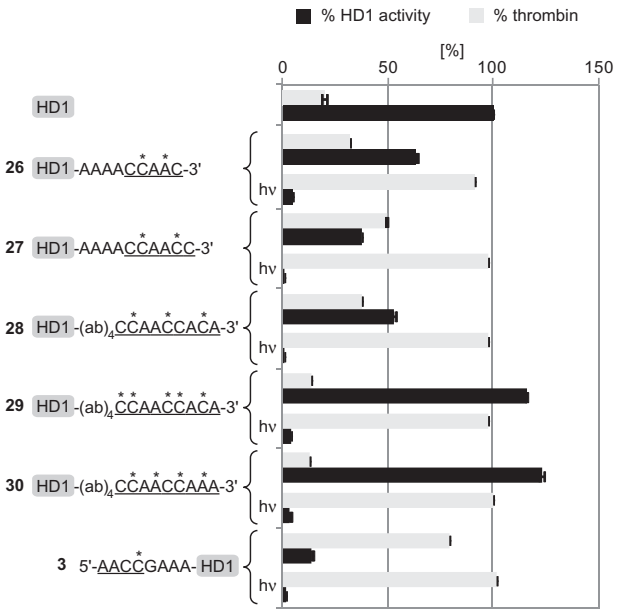

Figure 4. Results of coagulation assays with the listed aptamers without or with (hν) irradiation. Clotting times have been normalized toward the one of **HD1**. A second—reciprocal—scale is given that shows how much thrombin remained active.

deprotection with tetrabutylammonium fluoride (TBAF) selective protection of the primary 5′-OH group with dimethoxytrityl chloride (DMTrCl) and introduction of the phosphoramidite at the 3′-OH afforded the protected building block that could be used in a regular solid phase synthesis (for more details of the synthesis, see the Supplementary Data).

The performance of the aptamers with caged 3′-extensions are shown in Figure 4. Aptamer **26** with an adenosine linker, five antisense residues and two cages only showed an **HD1** activity of 64%. This clearly demonstrates that the effect of the antisense strand is insufficiently masked by the two cages in this case. This is even more pronounced in aptamer **27** with six antisense nucleotides and still two cages. Due to the better performance of the aptamers with 3′-abasic site linkers we then switched to this series and found that five or four cages in the nine-nucleotide antisense region (aptamers **28–30**) performed best. Notably, compared to the aptamer **3** in our original study, with aptamers **29** and **30**, we now present caged aptamers that even outperform **HD1** before irradiation and only show background activity after irradiation. With four or five caged residues HPLC analysis did not provide evidence for incomplete uncaging (Supplementary Data).

The question remains about the reason for the significantly improved performance of the 3′-extended aptamers. In order to address this question, we performed CD measurements with the aptamers presented here. Figure 5a shows the CD spectra of the 5′-extended aptamers. It can clearly be seen that these fall into three clusters. On the one side there is the spectrum of only **HD1** that shows the strong expected signal of the antiparallel G-quadruplex for example with its maximum around 290 nm and its minimum around 270 nm (22). The second cluster consists of the spectra of aptamers **5**, **8**, **9** and **10**, while the third cluster comprises the spectra of aptamers **4** and **7**. These clusters correlate nicely with the performance of the respective aptamers in the coagulation assays with **HD1** being the best, aptamers **5**, **8**, **9** and **10** being mediocre and aptamers **4** and **7** being worst. It appears that especially the latter two aptamers have a structure in solution that is significantly different from the structure of **HD1**. On the contrary almost all spectra of the aptamers in the 3′-extended series (without antisense sequence) are very close to the one of **HD1** and aptamer **15**—the best in the coagulation series—has a CD spectrum that comes closest (Figure 5b). The 3′-extended aptamers with antisense residues **18**, **19** and **25** do show completely different CD spectra—supporting the expected conformational transition and explaining the intended loss in activity.

The CD spectra of the caged aptamers **26–28** look surprisingly different from the one of **HD1** and almost no change upon irradiation is observed (Figure 6a). The same was true for our original caged aptamer **3**. From these findings it is surprising that these aptamers still showed a significant anti-thrombin activity before irradiation. However, it might be speculated that upon adaptive binding to thrombin the conformational equilibrium between active and inactive structure is still shifted toward the active one (23). This would not be unexpected as **HD1** has even been used as an aptamer beacon that folded from a stem–loop structure into a G-quadruplex structure upon addition of thrombin and was propagated for the detection of the presence of thrombin (24).

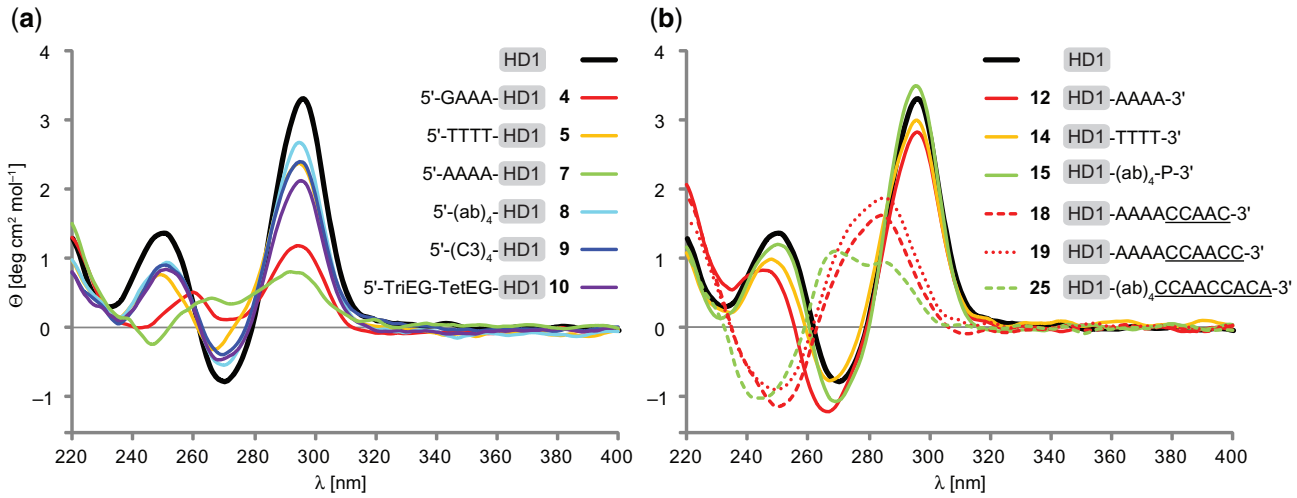

Figure 5. CD spectra of the listed aptamers (ab = abasic site, C3 = n-propyl spacer nucleotide, TriEG = triethylene glycol nucleotide, TetEG = tetraethylene glycol nucleotide).

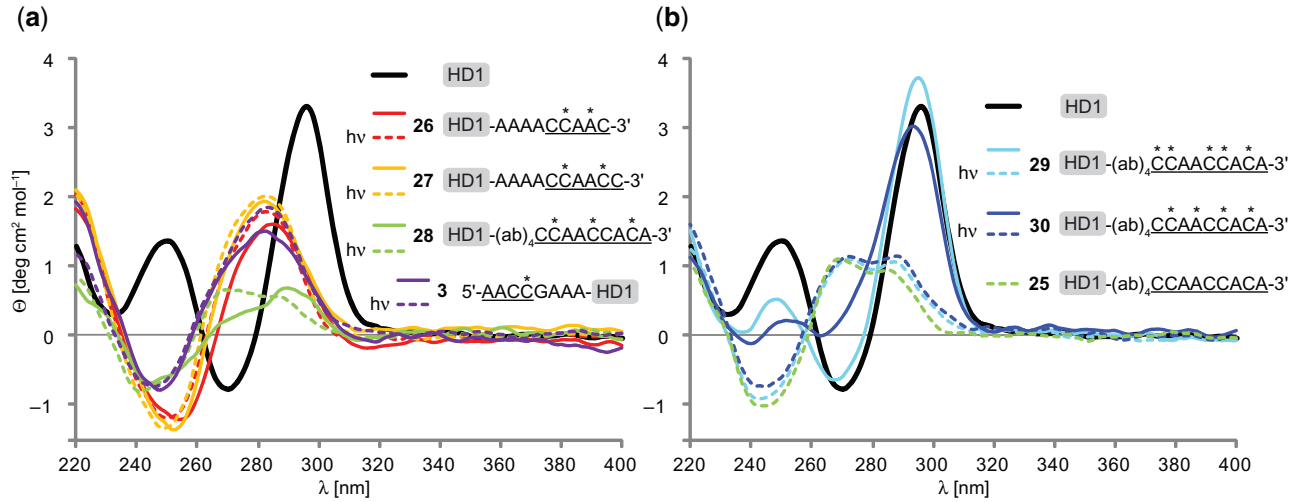

Figure 6. CD spectra of the listed aptamers without or with (hν) irradiation (ab = abasic site, C3 = n-propyl spacer nucleotide, TriEG = triethylene glycol nucleotide, TetEG = tetraethylene glycol nucleotide).

The caged aptamers **29** and **30**, finally, show a strong G-quadruplex CD signal before irradiation that correlates nicely with the findings in the performed clotting assays. After irradiation, the CD spectrum changes completely and results in the same as for aptamer **25** that is consistent with the observation of the clean uncaging process by HPLC.

Thus, CD spectroscopy shows that 5′- and 3′-extended aptamers (without antisense region) differ significantly in their solution structure already in the absence of thrombin. As noted previously, this was unexpected since in the antiparallel structure of the G-quadruplex both ends are relatively close. To further assess the source of the different behavior of 5′- and 3′-extensions, we determined the dissociation constants for some of the aptamers toward thrombin using filter retention analyses. The results are summarized in Table 1. **HD1** had a dissociation constant of 88 nM toward thrombin while for aptamer **1** with the 5′-antisense region and -linker no interaction could be detected, as expected. It is apparent that the 5′-extended aptamers **5**, **7** and **8** all afforded dissociation constants similar to the one of **HD1**. Notably, aptamer **7** with four adenosines at the 5′-end has literally the same affinity toward thrombin as **HD1** even though in the functional coagulation assays it performed worst in the 5′-extended series. On the other hand with filter retention assays in the case of a mixture of conformations the dissociation constants of the individual conformers can be measured in principle even if they are only present in small quantities. Given the results of the CD measurements it is likely that in these cases another binding-incompetent conformation is present in the equilibrium. The 3′-extended aptamers **12**, **14** and **15** again showed a very similar dissociation constant to the one of **HD1**. One possible reason for the increased efficiency of the 3′-extensions could have been the addition of negative

Table 1. Dissociation constants determined from filter binding assays with the listed aptamers without or with (hν) irradiation

	Aptamer	K_D [nM]
	HD1	88 ± 5
1	5'-AACCGAAA-HD1	none determinable
5	5'-TTTT-HD1	44 ± 4
8	5'-$(ab)_4$-HD1	84 ± 7
7	5'-AAAA-HD1	81 ± 5
12	HD1-AAAA-3'	55 ± 3
14	HD1-TTTT-3'	53 ± 3
15	HD1-$(ab)_4$-P-3'	41 ± 4
18	HD1-AAAACCAAC-3'	none determinable
19	HD1-AAAACCAACC-3'	none determinable
23	HD1-$(ab)_4$CCAAC-3'	38 ± 2
24	HD1-$(ab)_4$CCAACC-3'	56 ± 2
25	HD1-$(ab)_4$CCAACCACA-3'	none determinable
26	HD1-AAAA$C^{*}C^{*}$AAC-3'	41 ± 1 hν none determinable
27	HD1-AAAA$C^{*}CAAC^{*}$C-3'	80 ± 2 hν none determinable
28	HD1-$(ab)_4C^{*}CAAC^{*}CAC^{*}$A-3'	26 ± 1 hν none determinable
29	HD1-$(ab)_4C^{*}C^{*}CAAC^{*}CAC^{*}$A-3'	33 ± 2 hν none determinable
30	HD1-$(ab)_4C^{*}CAAC^{*}CAC^{*}$A-3'	26 ± 4 hν none determinable

charges to the aptamer that enhances the interaction with thrombin electrostatically. Looking at Figure 1a it is apparent that in the vicinity of the 3′-end of the aptamer, thrombin displays quite a number of positively charged residues. However, they should also be reachable by negatively charged 5′-extensions and indeed the findings of the K_D-measurements suggest that electrostatic interaction is not the source for the enhanced affinity. For the 3′-extended aptamers with antisense regions **18**, **19** and **25**, again no interaction with thrombin was observed, as expected. The results with aptamers **23** and **24** demonstrate once more what has just been mentioned. A K_D value in the same region as the one of **HD1** was determined even though both aptamers are already significantly under the influence of the antisense region in the coagulation assay. However, here the transition from the binding-competent conformation to an incompetent one is intended and only the K_D value of the binding-competent conformation in the equilibrium is determined. In the set of caged aptamers (**26–30**), the K_D values have a tendency to be somewhat lower in most of the cases in comparison with **HD1**—again despite the fact that aptamers **28** and **29** are in fact the best caged aptamers of the entire study. This demonstrates that the reason for the improved performance is not the affinity.

In summary we have demonstrated that there is a substantial difference in anti-thrombin aptamer performance between 5′- and 3′-extended derivatives—even if the same sequences are used. 3′-extended aptamers even improve the activity of **HD1** while 5′-extensions are detrimental. According to our data, the reason is not a significant change in affinity toward thrombin but rather a structural transition that happens in the aptamer itself even in the absence of thrombin that cannot be rescued by adaptive binding. The data presented here provide strong evidence that modifications of aptamers or any other functional nucleic acid can have a very strong impact on their performance. Previous studies already pointed toward this direction for aptamer **HD1** but did not investigate this in greater detail (25,26). Thus, the data presented here provide a comprehensive view on aptamer dependence on opposed terminal extensions and give rise to variants that were 1.5-fold more active as the parental aptamer (**HD1**) and even more active as a second generation exosite I targeting aptamer (**NU172**) currently under investigation in clinical trials (20). As a further result, we present now caged **HD1** derivatives that are light-responsive and that before irradiation are more active than **HD1** and after irradiation are cleanly turned OFF and do not show any interaction with thrombin anymore. Thereby we are able to efficiently design aptamers with an inherent antidote function without loss of aptamer activity and a clean OFF behavior. The design of antidote molecules is a major task in molecular medicine and within this field potent strategies to control aptamer activity have been developed (4,27). Our findings have a strong impact on the design of hemostyptic and tumor occluding agents, which seek to employ aptamers to temporarily control thrombin activity but upon light irradiation releasing native thrombin quantitatively. In contrast to recently reported reversible light-dependent approaches that result only in marginal regulation efficiency (28), the findings here can be considered almost digital (as 1 and 0). The dichotomy as to why 5′-extensions lead to different conformations as opposed to 3′-extensions cannot be fully explained in the current study and suggests thorough investigation for example by NMR in solution. Our study therefore suggests further investigation by dynamic NMR to investigate the relationship between extensions, aptamer conformation and adaptive target binding in greater detail.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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