SUPPORTING INFORMATION

Light-regulation of aptamer activity: An anti-thrombin aptamer with caged thymidine nucleobases

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DNA synthesis

The synthesis of the DMTr-protected phosphoramidite of T^{NPP} has already been published.[7] Thymidine phosphoramidites were purchased from Applied Biosystems, "Ultramild©"-protected guanosine phosphoramidites from Glen. Oligonucleotides were synthesized on an ABI 394 DNA/RNA-synthesizer using standard protocols (DMTr on) with columns containing "Ultramild©"-protected guanosine on CPG as first residue (200 µmol-scale). Cleavage was performed using 200 µl of a mixture of MeOH and 33% NH_3 (1:1) for 4 h at r.t. Then the suspension was filtered with a spin filter column and the remaining CPG was washed with additional 50µl of the cleavage mixture. After evaporation of the solvents under reduced pressure a precipitate formed which was redissolved with 50µl MeCN and 50 µl of a 1M aqueous solution of triethylammonium acetate (pH 7.0). The product mixture was then purified by RP-HPLC (0.1 M aqueous triethylammonium acetate (pH 7.0)/acetonitrile, 0'→4' 5% MeCN, 4'→35' 5%-50% MeCN, 1 ml min^{-1}, Nucleosil C18 5 µm 250mm*4mm). After lyophilization and coevaporation with 50 µl water, deprotection was performed by addition of 75 µl 80% HOAc and keeping the solution at r.t. for 20 min. Then 25 µl of a 1M aqueous solution of triethylammonium acetate (pH 7.0) were added and the crude product again purified by HPLC (same conditions as before). The pure fractions were lyophilized and the product coevaporated with 100 µl water. Thus for example 44 nmol of $\mathbf{A_2}$ have been isolated. The identity of the products was confirmed by ESI-MS:

oligonucleotide	determined mass	expected mass
$\mathbf{A_2}$	4886.83	4886.86
$\mathbf{A_3}$	4886.85	4886.86
$\mathbf{A_4}$	5049.95	5049.92
$\mathbf{A_5}$	4886.87	4886.86

upon irradiation of the caged aptamers (for example in phosphate buffer pH 11.0) the uncaged aptamer $\mathbf{A_1}$ was formed: determined mass 4723.77, expected mass 4723.79.

The aptamers $\mathbf{A_1}$ and $\mathbf{A_6}$ were purchased from Metabion (www.metabion.com) and purified under the same conditions as described above.

Filterbinding analysis

ssDNA aptamers were 5'-end labeled with γ^{32}P-ATP using T4 PNK (Stratagene) for 45 minutes at 37 °C in ligase buffer (Stratagene). Subsequently, the reaction mixture was applied to G25 microspin columns (Amersham Biosciences) to remove unreacted γ^{32}P-ATP and the resulting ssDNA was analyzed on 12% polyacrylamide gels to check the integrity. Radioactively-labeled ssDNA was incubated at a final concentration of 0.5 nM with increasing amounts of bovine α-thrombin (Sigma) as indicated in PBS pH 7.4, supplemented with 1 µg/µl BSA (Calbiochem), 10 µM tRNA from E.Coli (Sigma) and 3 mM $MgCl_2$. After incubation at 37 °C for 30 minutes the reaction mixture was filtered through 0.45 µm nitrocellulose (Schleicher & Schuell) membranes (pretreated with 0.4 M KOH and rinsed with water) and washed with 1 ml PBS/3 mM $MgCl_2$. The membrane was exposed on a storage phosphor screen and analyzed on a FUJIFILM FLA-3000 with the AIDA Imagequant software. The dissociation constants were calculated using the program Origin and the logistic fit function assuming a 1:1 binding stoichiometry of ssDNA-thrombin complexes. Light-activated ssDNA was generated by irradiation with UV light (Desaga MinUvis Hg low-pressure, 366 nm, 8W, 285 mm*15 mm, 1.2 mW cm^{-2}) for 25 minutes in water or 10mM phosphate buffer prior to incubation with bovine α-thrombin. All experiments were performed at least in duplicates.

Blood clotting assays

Thrombin-dependent blood clotting times were measured with an AMAX CS 190 pro coagulometer (Amelung, Lemgo, Germany). 50µl of frozen-thawed plasma were pre-incubated at 37 °C for 2 minutes. Then 50µl of PBS (pH 7.4, 3 mM $MgCl_2$), containing 0.25 NIH units thrombin and the indicated concentration of aptamers, were added. Light-activated ssDNA was generated by irradiation with UV light (Desaga MinUvis Hg low-pressure, 366 nm, 8W, 285 mm*15 mm, 1.2 mW cm^{-2}) for 25 minutes in 10mM phosphate buffer pH 7.4 or pH 11.0 (as indicated) prior to the incubation with bovine α-thrombin. All experiments were performed at least in triplicate.