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# In vitro selection of DNA aptamers binding ethanolamine

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**Abstract**

We have identified aptamers (synthetic oligonucleotides) binding to the very small molecule ethanolamine with high affinity down to the low nanomolar range. These aptamers were selected for their ability to bind to ethanolamine immobilised on magnetic beads, from an 96mer library of initially about $1 \times 10^{16}$ randomised ssDNA molecules. The dissociation constants of these aptamers range between $K_D = 6$ and $K_D = 19$ nmol $L^{-1}$. The aim of the development of ethanolamine aptamers is their use for the detection of this substance in clinical and environmental analysis. Ethanolamine is associated with several diseases. Moreover, ethanolamine and its derivatives di- and tri-ethanolamine are used in chemical and cosmetic industries. The use of biosensors with ethanolamine aptamer as new molecular recognition element could be an innovative method for an easy and fast detection of ethanolamine.


*Keywords:* Aptamer; Ethanolamine; FluMag-SELEX; Ethanolaminosis; Small target; Magnetic beads; Biosensor

Aptamers are now available for more than a decade. They have been using successfully in very different in vitro and in vivo applications like protein quantitation, protein purification, and as inhibitors of receptors or enzyme activities. Their advantages are the reproducible manufacture by chemical synthesis giving good availability, the easy insertion of modifications, e.g., functional groups for immobilisation or stabilisation as well as reporter molecules for detection. Moreover, the SELEX conditions may be adjusted to the application conditions ensuring very little matrix effects to selectivity, affinity, sensitivity, and stability. In contrast to antibody development, aptamer development for toxic targets or targets with low or no immunogenicity is possible at all.

The development of the SELEX process (systematic evolution of ligands by exponential enrichment) has provided a new alternative to generate ssDNA or RNA oligonucleotides in order to bind tightly and specifically to a given ligand [1,2]. The diversity of structures exhibited by a random DNA oligonucleotide library allows selection of tight binding aptamers for simple targets, such as a single amino acid [3], or for complex targets such as a virus [4]. Aptamers with high affinity and specificity have been selected for many different ligands and are emerging as a new class of molecules that contest antibodies in therapeutics, imaging, diagnosis, analysis, and affinity enrichment (Fig. 1).

Here, we describe first results of the in vitro selection of novel ssDNA aptamers that bind tightly to the very small molecule ethanolamine ($M_r = 61.08$) (Fig. 2). This molecule seems to be the smallest aptamer target so far. The in vitro selection of those aptamers was processed in a so-called FluMag-SELEX, which uses fluorescent labels for DNA quantification and magnetic beads for target immobilisation [5]. One round of this SELEX process contained the following steps: 1. Binding of ssDNA to the target, immobilised on magnetic beads; 2. Washing of the DNA–target complex; 3. Heat elution of bound ssDNA from the target; 4. Amplification of eluted ssDNA by PCR; 5. Preparation/purification of the relevant ssDNA out of the dsDNA PCR products (Fig. 3).

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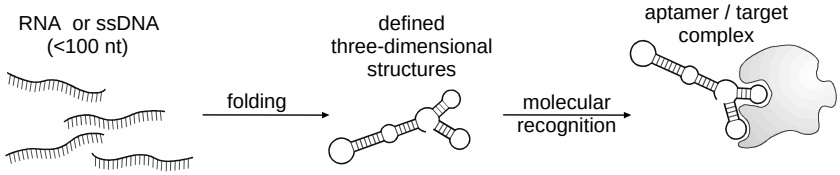


Fig. 1. Schematic explanation of functionality of aptamer-target binding.

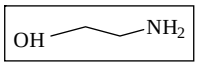


Fig. 2. Chemical structure of ethanolamine ($M_w = 61.08$).

## Materials and methods

Materials, instruments, random oligonucleotide library, and primers were used as described in detail in Stoltenburg et al. [5].

*Ethanolamine magnetic bead preparation*. Aliquots of Dynabeads M-270 Epoxy or M-280 Tosylactivated (Dynal Biotech ASA, Norway) were handled according to manufacturer's instructions. One millilitre of 1 M ethanolamine was incubated with approximately $1 \times 10^9$ magnetic beads overnight at 37 °C by mild shaking, followed by washing steps ($4 \times 1$ mL PBS, pH 7.3; $1 \times 1$ mL PBS, pH 7.3 + 1% Tween 20; $1 \times 1$ mL PBS, pH 7.3. PBS: 1.47 mM $KH_2PO_4$, 8.1 mM $Na_2HPO_4$, 137 mM NaCl, and 2.7 mM KCl). Afterwards the ethanolamine-modified beads were resuspended and stored in 500 μL PBS, pH 7.3, at 4 °C. The separation of the magnetic beads from the buffer solution after washing steps was performed by using a magnetic separation stand (Promega, Germany).

*In vitro selection (FluMag-SELEX)*. The selection of ethanolamine binding aptamers occurred within a FluMag-SELEX process with laminarin as main target, immobilised on magnetic beads (Dynabeads M-270 Epoxy), and ethanolamine was used to block free binding sites on these beads during the immobilisation procedure. Thus, ethanolamine and laminarin were present on the surface of the magnetic beads as potential targets for the aptamer selection.

Starting point of the FluMag-SELEX process was a random ssDNA oligonucleotide library designated as BANK and prepared by Invitrogen, UK (chemical synthesis, purified by PAGE): 5′-ATACCAGCTTAT TCAATT—N60—AGATAGTAAGTGCAATCT-3′. The following modified primers that anneal to the 5′-end and 3′-end of the library were used for amplification of the selected oligonucleotides during the aptamer selection process: primer AP60 5′ fluorescein-ATACCAGCTTATTCAAT T-3′ and primer TER-AP20 5′ $dA_{20}$HEGL-AGATTGCACTTACTATC T-3′ (Invitrogen, UK and IBA GmbH, Germany). Iterative rounds of aptamer selection and amplification during this FluMag-SELEX process were performed as described previously [5]. In each round, $10^8$ target-modified magnetic beads were used. After the ninth round, the selected oligonucleotides were tested for their binding specificity to laminarin and ethanolamine. We found an explicit enrichment of ssDNA that binds to ethanolamine immobilised on magnetic beads.

*Cloning and sequencing*. The aptamer pool from the ninth SELEX round was given to a final 10th SELEX round using ethanolamine-modified magnetic beads as target. In this last round, ssDNA was amplified subsequently with unmodified primers and cloned into the vector pCR2.1-TOPO. *Escherichia coli* TOP10 cells (TOPO TA Cloning Kit; Invitrogen, UK) were transformed by taking up this vector constructs. After preparing plasmid DNA using the 'Plasmid DNA Purification Kit' (Macherey-Nagel, Germany) and the 'QIAprep SPIN Miniprep Kit' (Qiagen, Germany), the inserted aptamer DNA of several clones was sequenced (AGOWA Sequencing Service GmbH, Germany).

Sequence analysis and alignments were performed using the Vector NTI Suite 8 (Invitrogen, UK). Analysis of secondary structure of several

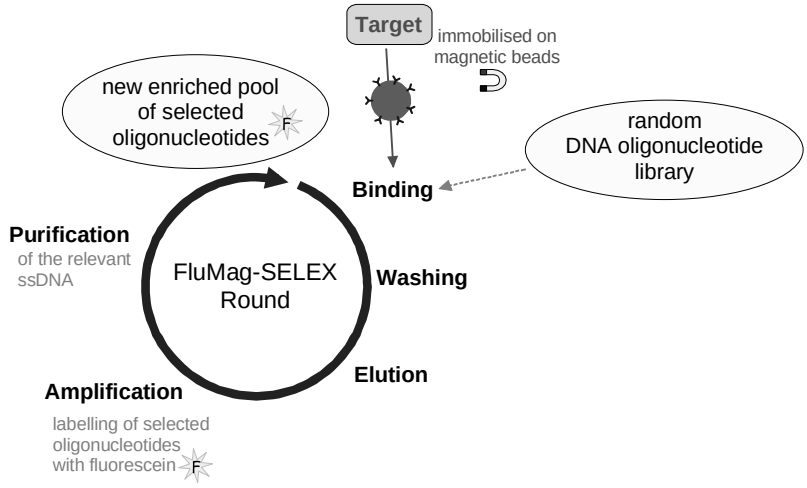


Fig. 3. Procedure of the FluMag-SELEX process. Schematic representation of the fluorescence controlled in vitro selection protocol, the so-called FluMag-SELEX, for generating DNA aptamers for specific target molecules immobilised on magnetic beads. The starting random ssDNA oligonucleotide library was incubated with the target beads (ethanolamine-coated magnetic beads) for *binding*. Unbound oligonucleotides were *washed* away and the bound oligonucleotides were *eluted* from the target beads by heat treatment. The selected oligonucleotides were *amplified* by PCR using fluorescein-modified primers. The relevant ssDNA was subsequently *purified* from the PCR products and the resulting enriched DNA pool was used in the next SELEX round for binding with target beads. The fluorescein labelling (F, fluorescein) of the selected oligonucleotides after the first SELEX round permitted their quantification in further rounds and thus the control of the enrichment of target specific aptamers. After the last round the selected aptamers were cloned and several 'monoclonal' aptamers were characterised.

aptamers was performed by free energy minimisation algorithm according to Zuker [6] using the internet-tool *mfold* [7].

*Binding assays*. All sequenced aptamer clones were tested for their individual binding characteristic to ethanolamine immobilised on tosyl-activated magnetic beads.

For each binding assay, a fresh aliquot of the prepared ethanolamine-modified magnetic beads ($1 \times 10^7$ beads for each binding reaction) was washed five times in binding buffer (BB) (100 mmol $L^{-1}$ NaCl/20 mmol $L^{-1}$ Tris/HCl, pH 7.6/2 mmol $L^{-1}$ $MgCl_2$/5 mmol $L^{-1}$ KCl/1 mmol $L^{-1}$ $CaCl_2$/0.02% Tween 20).

Approximately 15 pmol aptamer ssDNA was incubated in 500 μL BB at 90 °C for 5 min, afterwards 15 min on ice and 5 min at RT for thermal equilibration. This prepared aptamer was incubated with the washed aliquot ethanolamine-modified magnetic beads for 30 min at 21 °C while shaking. After the binding reaction, the unbound aptamers were removed and the binding complex was washed five times in 500 μL BB each.

The bound aptamers were eluted twice in 100 μL BB by 7 min incubation at 80 °C while shaking and got pooled.

The amounts of the three fractions of applied, non-bound, and eluted ssDNA were determined by fluorometry (Wallac Victor$^2$V Multilabel Counter; Perkin-Elmer Life Sciences, Germany) under the following measuring conditions: excitation 485 nm/emission 535 nm; time 1 s; CW-lamp energy 15,000. The sample volume was 100 μL/well in 96-well black microtitre plates (NUNC, Germany). The calculation of the ssDNA concentration in each fraction was done by using a calibration plot.

*Determination of dissociation constants ($K_D$) of individual aptamers*. To determine the binding affinity of selected aptamers (best binding aptamers of groups I and II) to their target ethanolamine, we accomplished binding assays as described above but with increasing amounts of a single aptamer clone (between 2 and 70 nmol $L^{-1}$ fluorescein-labelled ssDNA) and a constant amount of ethanolamine-coated magnetic beads ($1 \times 10^7$ beads) for each assay. The amount of bead-bound aptamers was calculated after elution from the beads, followed by fluorescence measurement. Saturation curves were calculated by use of these data and the dissociation constant $K_D$ was calculated by non-linear regression analysis.

*Determination of the specificity of individual aptamers by affinity elution assays*. Selected aptamers were tested for their ability to bind to the free (non-immobilised) target ethanolamine as well as to the ethanolamine derivatives di- and tri-ethanolamine. Moreover, the physiologically relevant targets serine and choline, which—like ethanolamine—occur as head groups in the chemical structure of phosphoglycerides, were involved. Glycine was also tested as the basic amino acid and as a molecule with chemical structure similar to that of ethanolamine. These tests were arranged as affinity elution assays. The aptamers were bound to the ethanolamine-modified magnetic beads ($1 \times 10^7$ beads) as described above, followed by washing steps. The aptamer–bead complex was incubated with 500 μL BB containing 5 μmol of free target (10 mmol $L^{-1}$) for 15 min by shaking at 21 °C. The supernatant containing the eluted aptamer (after treatment of the beads by the similar target) was removed by using the magnetic separation stand. The beads were washed three times with 200 μL BB, all washing solutions were pooled. After that, all of the remaining aptamers were eluted from the beads by heat treatment as described above. The amounts of aptamers in each elution fraction (affinity elution, washing pool, and heat elution) were determined by fluorescence measurement and calculation using a calibration plot as described above.

## Results

### *Selection of DNA aptamers binding ethanolamine*

DNA aptamers binding ethanolamine were selected by use of the previously described FluMag-SELEX process. The target ethanolamine was immobilised on epoxy functionalised magnetic beads together with a second target laminarin. The target immobilisation on the surface of magnetic beads allows easy and efficient handling during the SELEX process.

After selection round nine, an enrichment of ssDNA binding explicitly to ethanolamine was detected. Subsequently, an additional round with ethanolamine as the only target, immobilised on magnetic beads (without laminarin), was performed. The selected oligonucleotides were amplified by PCR using unmodified primers for following cloning of the aptamer pool. As a result, 37 individual aptamer clones were sequenced (Table 1). Three groups of sequences were identified. Within group I and II the aptamer sequences were mostly identical with only single base variations. Within group I, we identified 21 sequences and in group II 11 sequences. Between the groups I and II the sequences were different to a wide extent, but they showed a common conserved guanosine-rich section of 16 nucleotides at the 5′-end of the core region of the aptamers. This characteristic region (consensus sequence: GAGGT/$CG_3TG_3TG_3$) was built of four G-triplets which were separated from each other by additionally one thymidine or cytidine, respectively. The first triplet was interrupted by an adenosine after the first guanosine. The only aptamer representing the group III was completely different to the aptamers of group I and II, but we could also identify a G-rich region in this aptamer, which is similar to the consensus sequence (Table 1).

Four other clones neither showed homologies to the sequences of group I, II or III, nor contained the consensus sequence (data not shown).

Analyses of the secondary structure of one representative aptamer of each group I and II showed that the primer regions were always involved in a stem–loop structure (Fig. 4A). The consensus sequence was always found in a single-stranded loop. Therefore, it seems possible that three-dimensional folding of the consensus sequence leads to a three-layer G-quadruplex structure (Fig. 4B).

### *Binding studies*

Each of the 37 sequenced aptamer clones was tested for its ability to bind to ethanolamine immobilised on tosylactivated magnetic beads, according to SELEX conditions. All aptamers of group I, II, and III are able to bind to immobilised ethanolamine. $1 \times 10^7$ ethanolamine-modified beads were used for each assay. The average amount of bound aptamers ranged in group II (~2 pmol) about 50% higher than in group I and III (~1 pmol) (Fig. 5). The random oligonucleotide library (BANK) and the four clones without any sequence homologies to the aptamers of group I, II, and III (mentioned above) were not able to bind to the target.

### *Determination of dissociation constants ($K_D$) of individual aptamers*

The dissociation constants $K_D$ of six selected aptamers with good binding abilities (#14.3 and #11.4 of group I,

Table 1
Core regions of the aptamer sequences (5′–3′ direction, without primers)

| Core regions of the aptamer sequences | Aptamer clone | Subgroup | Identical sequences | $K_D^*$ (nmol $L^{-1}$) |
|---|---|---|---|---|
| *Group I* | | | | |
| TGAGGCGGGTGGGTGGGTTGAATACGCTGATTACCCCATCGTAGAACGTTAAGGCGCTTC | #5.3 | I.1 | 5 | n.d. |
| TGAGGCGGGTGGGTGGGTTGAATATGCTGATTACCCCATCGCAGAACGTTAAGGCGCTTC | #14.3 | I.2 | 6 | 6 ± 3 |
| TGAGGCGGGTGGGTGGGTTGAATACGCTGATTACCCCACCGGAGAACGTTAAGGCGCTTC | #11.2 | I.3 | 2 | n.d. |
| TGAGGCGGGTGGGTGGGTTGAATACGCTGATCACCCCATCGGAGAACGTTAAGGCGCTTT | #11.4 | | 1 | 9 ± 5 |
| TGAGGCGGGTGGGTGGGTTGAATACGCTGATTACCCCATCGTATAACGTTAAGGCGCTTC | #5.7 | | 1 | n.d. |
| GGAGGCGGGTGGGTGGGTTGAATACGCTGATTACCCCAACGGAGAACGTTAAGGCGCTTC | #2.7 | | 1 | n.d. |
| TGAGGCGGGTGGGTGGGTTGAATACGCTGATCACCCCATCGGAGAACGTTAAGGCGCTTC | #9.7 | | 1 | n.d. |
| TGAGGCGGGTGGGTGGGTTGAATACGCTCATTACCCCATCGGAGAACGTTAAGGCGCTTC | #2.2 | | 1 | n.d. |
| AGTGGCGGGTGGGTGGGTTGAATACGCTGATTACCCCATCGTAGAACGTTAAGGCGCTTC | #9.9 | | 1 | n.d. |
| CGAGGCGGGTGGGTGGGTTGAATACGCTGATTACCCCATCGGAGAACGCTAAGGCGCTTC | #11.8 | | 1 | n.d. |
| GGAGGCGGGTGGGTGGGTTGAATACGCTGATTACCCCACCGGAGAACGTTAAGGCGCTAC | #2.4 | | 1 | n.d. |
| *Group II* | | | | |
| GCTGCGAGGTGGGTGGGTGGGAGCAATTGGTCCTCGCTTAGCTTCTACGGTGGGCTATCT | #2.3 | II.1 | 2 | 17 ± 4 |
| GCTGTGAGGTGGGTGGGTGGGAGCAATTGGTCCTCGCTTAGCTTCTACGGTGGGCTATCT | #9.6 | II.2 | 2 | 15 ± 4 |
| GCTGCGAGGTGGGTGGGTGGGAGCAATTGATCCTCGCTTAGCTTCTACGGTGGGCTATCT | #9.4 | II.3 | 2 | 10 ± 2 |
| GCTGCGAGGTGGGTGGGTGGGAGCAATTGGTCCTTGCTTAGCTTCTACGGTGGGCTATCT | #9.10 | | 1 | 19 ± 7 |
| GCTGTGAGGTGGGTGGGTGGGAGCAATT-GTCCTCGCTTAGCTTCTACGGGGGGCTATCT | #2.9 | | 1 | n.d. |
| GCTGCGAGGTGGGTGGGTGGGAGCAATTGGTCTTCGCTTAGCTTCTACGGTGGGCTATCT | #14.1 | | 1 | n.d. |
| GCTGCGAGGTGGGTGGGTGGGAGCAATTGGTTCTCGCTTAGCTTCTACGGTGGGCTATCT | #14.6 | | 1 | n.d. |
| GCTGCGAGGTGGGTGGGTGGGAGCAATTGATCTTCGCTTAGCTTCTACGGTGGGCTATCT | #9.1 | | 1 | n.d. |
| *Group III* | | | | |
| CCGAGTTTGGGTGGGAGTGGTGGGTTCGGAATTGTTAGTTATTTGGGTTTATGCGAGGTG | #14.5 | III | 1 | n.d. |

Three main groups of aptamer sequences were identified. Group I and II were divided into subgroups, each subgroup contains identical sequences. Variable base positions within one group are marked by black background. One strongly conserved region (consensus sequence), containing several G-triplets, was identified for all aptamers of group I and II (underlined). The framed region in the sequence of the group III aptamer was similar to the underlined consensus sequence. The calculated dissociation constants ($K_D$) of some aptamer clones are listed on the right (n.d., not determined) and also the number of identical sequences in each subgroup, as well as the clone and subgroup name.

#2.3, #9.6, #9.4, and #9.10 of group II) were determined. Binding reactions with a constant number of ethanolamine-coated magnetic beads ($1 \times 10^7$ beads) as target beads and a concentration series of each fluorescein-labelled aptamer (1–60 pmol) were conducted. After several washing steps, the bead-bound aptamers were eluted and the amount was calculated by fluorescence measurement and plotted against the concentration of the aptamers used in the binding reaction. Fig. 6 shows the non-linear regression analysis of the binding data of the selected aptamers. The dissociation constants $K_D$ of the six selected aptamers were in the low nanomolar range of 6–19 nmol $L^{-1}$ (Table 1).

*Binding of the aptamers to free ethanolamine in solution and determination of the specificity of the aptamers by affinity elution assays*

The aptamers were selected for binding to immobilised ethanolamine. They were also able to bind to ethanolamine in solution as free, non-immobilised target. Moreover, it was checked if the aptamers also bind to other substances with similar chemical structure like mono-, di-, and tri-ethanolamine, D-/L-serine, choline, and glycine. Therefore, we accomplished affinity elution assays by use of one aptamer of group I (#14.3) and II (#9.4), respectively. We chose the aptamers with the best dissociation constants from each group I and II. We found that both tested aptamers were able to bind to free ethanolamine in solution and also to the ethanolamine derivatives di- and tri-ethanolamine (Fig. 7). Moreover, the results revealed significant differences between the binding ability to the ethanolamine derivatives. During the affinity elution steps in these experiments, di-ethanolamine was able to elute the highest amount of aptamers bound to ethanolamine-modified magnetic beads (99%), whereas a descending amount was eluted by mono-ethanolamine (90%) and tri-ethanolamine (70%), respectively, for aptamer #14.3. A similar affinity elution profile was found for aptamer #9.4, but the amounts of aptamers eluted by mono-, di-, and tri-ethanolamine were generally smaller. In contrast, binding of the aptamers #14.3 and #9.4 to choline, serine, and glycine could not be observed. The buffer solution (BB) without any target (as a reference) led to an aptamer elution of $\leqslant$20%.

## Discussion

We have shown first results of aptamer development for ethanolamine. Ethanolamine is a very small molecule ($M_r = 61.08$) and as far as we know it is the smallest aptamer target described. Therefore, our results may be inter-

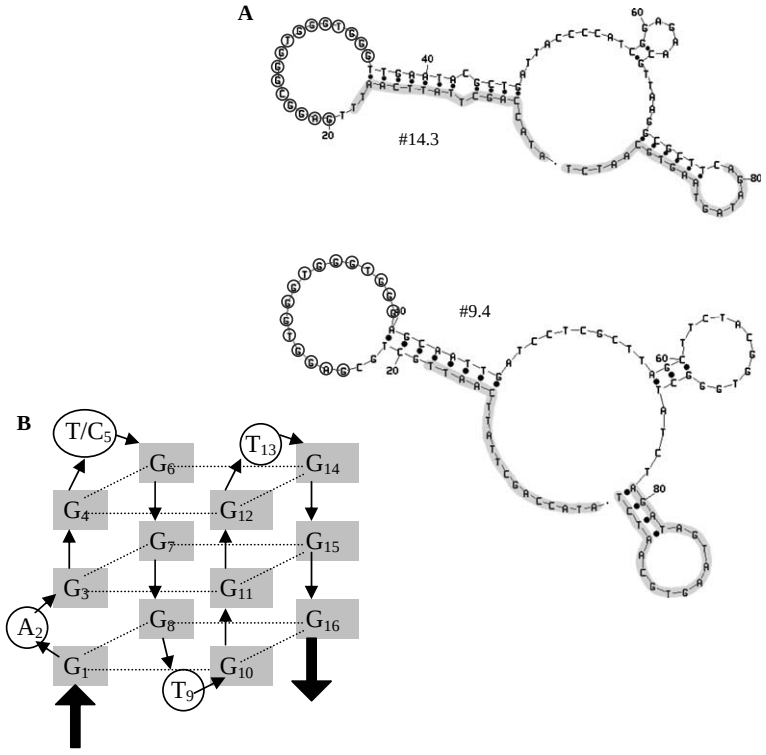


Fig. 4. Structure analysis. (A) Secondary structure. The energetic favourable secondary structures of aptamer #14.3 (group I) and #9.4 (group II), determined by the internet-tool *mfold*, are shown. The primer regions (nucleotides 1–18 and 79–96) are highlighted in grey and the consensus sequence (nucleotides 20–35 for #14.3 and 24–39 for #9.4) is marked by circles. The primer regions were always arranged in stem–loop structures, whereas the consensus sequences were found in a single-stranded loop. (B) G-quadruplex structure. The consensus sequence of 16 nucleotides was characterised by several G-triplets. Thus, the three-dimensional shape of this consensus sequence could be a threefold stacked G-quadruplex structure. The picture shows one possible conformation of the nucleotides.

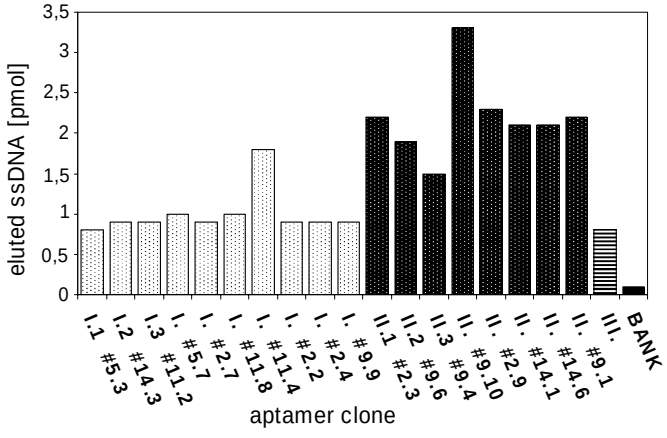


Fig. 5. Comparison of the binding abilities of all aptamer clones of group I, II, and III. The binding ability of all individual aptamers was tested in single binding assays with ethanolamine-coated magnetic beads as target. After binding of the aptamers to the target beads, followed by several washing steps, the bound aptamers were eluted by heat treatment. The diagram shows the amounts of eluted ssDNA of each binding assay with individual aptamer clones in comparison. The unselected random oligonucleotide library (BANK) served as reference. It was shown that all aptamers of group I–III bind to the ethanolamine-coated magnetic beads. In group II, the amounts of target-bound aptamers were about twice as high as in group I and III.

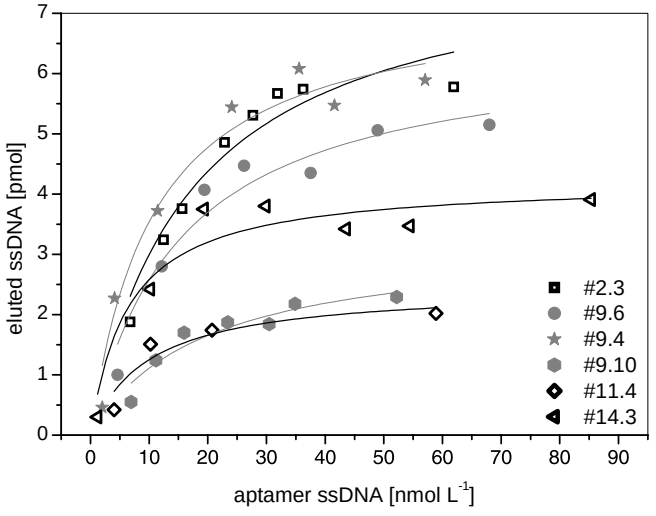


Fig. 6. Saturation curves and determination of dissociation constants ($K_D$). Binding studies were performed with a defined amount of ethanolamine-coated tosylactivated magnetic beads ($1 \times 10^7$) and a concentration series (1–60 pmol) of the fluorescein-labelled aptamers. Based on the amounts of aptamers bound to the target, saturation curves were obtained and the dissociation constants ($K_D$) were calculated by non-linear regression analysis.

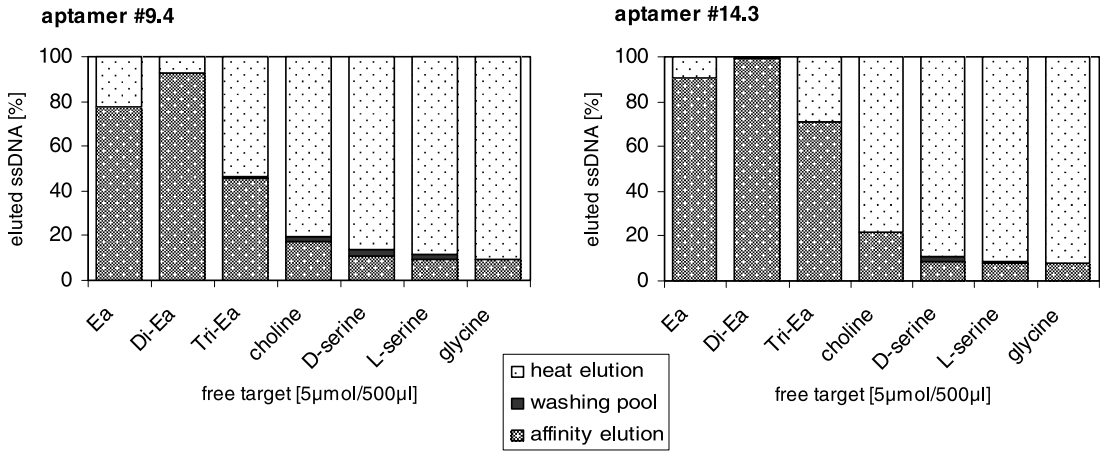


Fig. 7. Affinity elution assays with aptamers #9.4 and #14.3 The ability of aptamers #9.4 and #14.3 to bind to free ethanolamine and other structurally related targets was tested in several affinity elution assays. For these assays the aptamers were first bound to ethanolamine-coated magnetic beads, washed, and aliquoted to $1 \times 10^7$ beads. Each aliquot was then incubated with one of the targets in solution (5 µmol target/500 µL BB). Afterwards the beads were washed and the remaining aptamers were completely removed from the beads by heat elution. The amounts of aptamers measured in the affinity elution fraction, washing fractions, and heat elution fraction are shown as percentages of the starting amount of bound aptamers to the target beads in a stacked bar graph. (Ea, ethanolamine; Di-Ea, diethanolamine; Tri-Ea, triethanolamine.)

esting for other SELEX experiments with small targets. The detection of ethanolamine is relevant both for clinical and environmental analyses. Ethanolamine seems to be connected with diseases like Schizophrenia [8], Alzheimer's disease [9] as well as the storage disease ethanolaminosis [10]. Ethanolamines are also environmentally relevant targets [11], because they are used as fatty acid derivatives in detergents and cosmetics and are starting substances for organic synthesis. Di-ethanolamine is classified as a carcinogenic substance.

We observed a highly conserved G-rich region of 16 nucleotides in the sequences of 32 out of 37 aptamer clones. Moreover, the secondary structure prediction showed a wide structural similarity for these 32 aptamers belonging to group I and II. The conserved region, also referred to as consensus sequence, was always located in a single-stranded loop. Therefore, it seems possible that it folds into a G-quadruplex structure, which is described in literature for numerous examples of G-rich sections in aptamers [12–14]. G-quadruplexes are thought to be biologically relevant and are often found in eukaryotic genomes. G-quadruplexes can be stabilised by cations like $K^+$, which fit well into the built quadruplex cave [15]; this increases the thermodynamic and kinetic stability of the structure. The binding buffer (BB) used for the aptamer selection in this work did in fact contain potassium cations. All selected aptamers which show the G-rich section and therefore might be able to form G-quadruplexes (group I, II, and III) bound to the target ethanolamine. The other four aptamer sequences were not able to bind to the target. This is another point that convinces us that the consensus sequence found is a major structural element for target recognition and stabilisation of the aptamer–target complex. For detailed information about that, we will have to perform structure analysis by X-ray or NMR.

The aptamers described in this work bind to ethanolamine immobilised on magnetic beads with very high affinity. The dissociation constants were in a low nanomolar range ($K_D = 6$–$19$ nmol $L^{-1}$). Other aptamers, which were also selected for small molecules, are characterised by much higher $K_D$ values, e.g., arginine: 0.3 µmol $L^{-1}$ [16] or citrulline: 62 µmol $L^{-1}$ [17]. This illustrates a good affinity of these aptamers to their very small target ethanolamine. We could show that the aptamers also bind to the free target ethanolamine in solution. Furthermore, the selected aptamers were able to bind to the ethanolamine derivatives di- and tri-ethanolamine, but not to structurally and functionally similar molecules like serine, choline or glycine. For detailed information of the specificity, further experiments will have to be performed testing targets containing different structural features (amino-, ethyl-, and hydroxyl-groups, elongation of C-chain, phosphorylated ethanolamines, etc.). A sequence optimisation by truncation is another further aim. The development of an aptamer biosensor for the detection of ethanolamine is the ultimate objective of these investigations.

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