Biol. Chem., Vol. 384, pp. 1497 – 1500, October/November 2003

Short Communication

Aptamers That Recognize the Lipid Moiety of the Antibiotic Moenomycin A

Heike Betat[1,a], Stefan Vogel[2], Marc Struhalla[3], Hans-Heinrich Förster[3], Michael Famulok[4,*], Peter Welzel[2,*] and Ulrich Hahn[1,*]

[1] Universität Hamburg, Abteilung Biochemie und Molekularbiologie, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany
[2] Universität Leipzig, Institut für Organische Chemie, Johannisallee 29, D-04103 Leipzig, Germany
[3] Universität Leipzig, Institut für Biochemie, Brüderstr. 34, D-04103 Leipzig, Germany
[4] Universität Bonn, Kekulé-Institut für Organische Chemie und Biochemie, Gerhard-Domagk-Str. 1, D-53121 Bonn, Germany

*Corresponding authors

Moenomycin A is an amphiphilic phosphoglycolipid antibiotic that interferes with the transglycosylation step in peptidoglycan biosynthesis. The antibiotic consists of a branched pentasaccharide moiety, connected to the moenocinol lipid *via* a glycerophosphate linker. We have previously described the selection of aptamers that require the lipid group and the disaccharide epitopes of the oligosaccharide moiety for moenomycin binding. Here we report that the enriched moenomycin-binding library contains sequences that evolved for specific recognition of the unpolar lipid group of the antibiotic. These results suggest that the evolution of hydrophobic binding pockets in RNA molecules may be much more common than previously assumed.

Key words: Antibiotics / Aptamers / Moenomycin A / RNA / SELEX.

One particular method of directed molecular evolution enables the selection of nucleic acids (called aptamers) that bind specifically to target compounds with high affinity. For RNA aptamers the selection process starts with a mixture of about 10^{14} different RNA oligonucleotides and consists of (i) affinity-selection and (ii) amplification of the selected members of the library by cDNA synthesis, polymerase chain reaction, and *in vitro* transcription leading to an enriched RNA pool. The process is iteratively repeated until aptamers of high affinity are obtained. Aptamers have a large potential for analytical purposes, as diagnostics and as drugs due to their capability of interfering with potential targets, such as nucleic acids or proteins, even within the cell (Osborne *et al.*, 1997; Famulok, 1999).

Intermolecular interactions such as electrostatic forces and hydrogen bonds and, in addition, π-stacking interactions, have been shown to contribute to the stability of the complexes between aptamers and their targets (Hermann and Patel, 2000).

Here we describe RNA aptamers that recognize the unpolar region of a compound that, in addition, offers the opportunity of a multitude of polar interactions. The work started with the intention to select aptamers specific for epitopes of the antibiotic moenomycin A (Figure 1, compound **1**; El-Abadla *et al.*, 1999; Goldman and Gange, 2000). Moenomycin A is one of the rare compounds known to interfere with the transglycosylation step in peptidoglycan biosynthesis (Kosmol *et al.*, 1997; Ritzeler *et al.*, 1997; van Heijenoort, 2001) and, thus, is a lead compound for the development of novel antiinfectives with a new mode of action (Ritter and Wong, 2001). We had in mind to employ the aptamers for the identification of biosynthetic moenomycin intermediates (Schürer *et al.*, 2001b) and to use these as scaffolds for libraries of semisynthetic analogs.

2'-Aminopyrimidine RNA aptamers, which are known to be less prone to enzymatic degradation than naturally occurring RNAs, were selected by affinity chromatography making use of a Sepharose matrix to which the moenomycin-derived thiol **2** was coupled *via* a disulfide bridge (Schürer *et al.*, 2001b). All isolated aptamers were shown to be G-rich and to contain either of the two consensus motifs, a [GGAGG(N)nGGAGG] and b (GGUGUGGUG). For two of the moenomycin aptamers (C2 with motif a and A6 with motif b) K_d values of about 400 nM could be determined (Schürer *et al.*, 2001a). By loading moenomycin-carrying Sepharose with the aptamer and performing a competitive elution of the aptamers with moenomycin analogs it was demonstrated that the disaccharide analog **3a** (Figure 2; El-Abadla *et al.*, 1999; Goldman and Gange, 2000) is recognized almost as efficiently as moenomycin A itself. In continuing the epitope mapping we studied the binding of compounds **4**, **5**, and **7** to aptamer C2. Neither **5** nor **7** showed significant binding whereas compound **4**, containing the lipid and the

[a] Present address: Max-Planck-Institut für evolutionäre Anthropologie, Deutscher Platz 6, D-04103 Leipzig, Germany

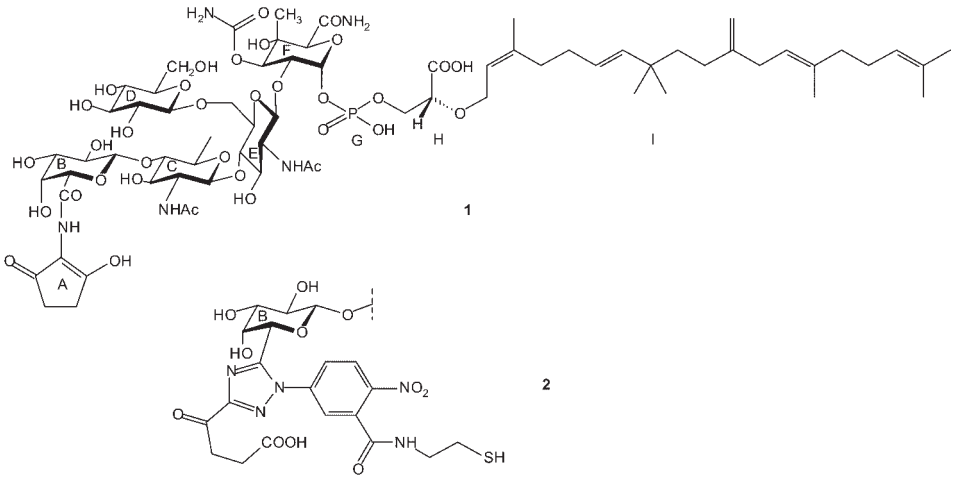

Fig. 1 Structures of Moenomycin A (**1**) and the Thiol Derivative **2**.

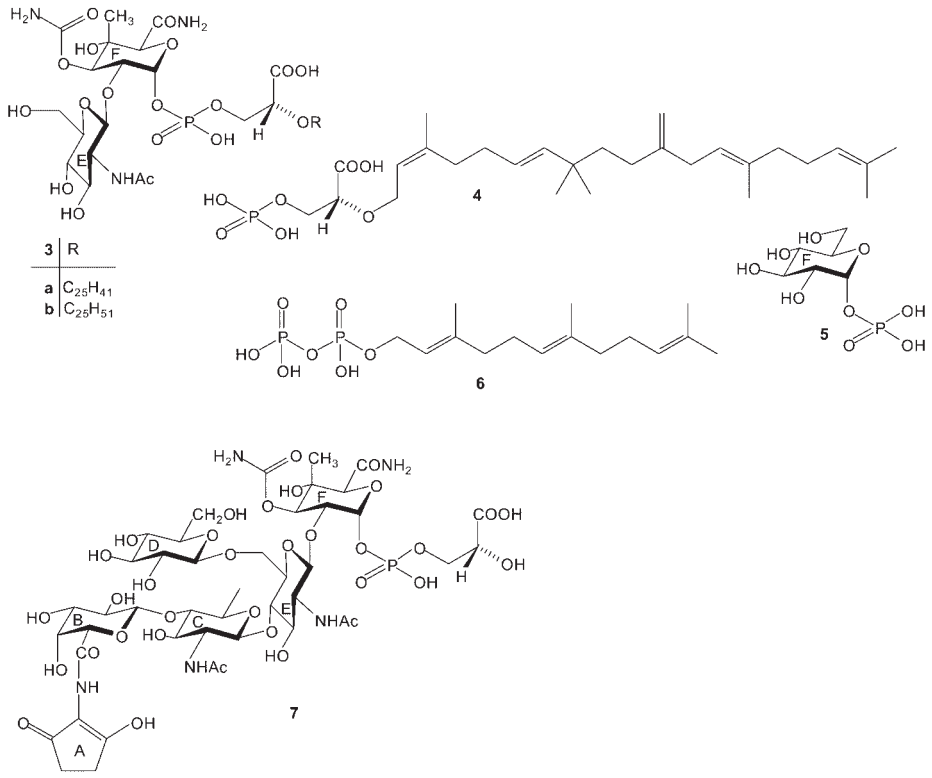

Fig. 2 Structures of Compounds Tested in Competition Assays with Moenomycin Aptamers.

phosphoglycerate part of moenomycin, showed a remarkable binding to the aptamer (Figure 3A). A further important observation was that compound **3b**, which differs from the strong binder **3a** only by the lack of the five double bonds in the lipid part, is recognized to a much lower degree by the aptamer (Figure 3B). Irrespective of the role of the polar units in **3a** and **4** it is quite obvious that the unsaturated lipid part plays a crucial role in the binding of moenomycin A and analogs such as **3a** and **4**. Similar results were found for aptamer A6 (data not shown).

The fact that the aptamers preferentially bind to an unsaturated lipid chain is remarkable as one intuitively would assume that binding of aptamers, a class of intrinsically polar molecules, would occur on highly polar parts of the bound ligand. Comparing the aptamers described here with those that have been shown to bind to the farnesyl moiety of the farnesylated Ras peptide (Gilbert *et al.*, 1997) reveals the remarkable fact that in both cases very similar consensus sequences were selected (Table 1). The farnesyl-Ras aptamers contain a part of

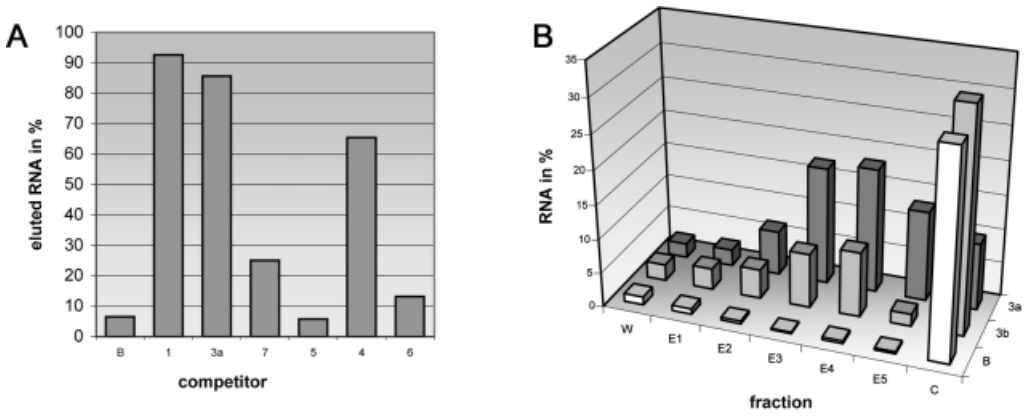

Fig. 3 Results of *in vitro* Competition Experiments.
The assay was performed by loading moenomycin-Sepharose with [^{32}P]-labeled aptamers as previously described (Schürer *et al.*, 2001b). After washing with buffer to remove unbound RNA, aptamers were affinity eluted by different moenomycin analogs. The competitor-mediated elution of aptamers was detected by Čerenkov-counting. (A) The diagram shows the amount of RNA which was recognized by moenomycin or its analogs (**1**, **3a**, **7**, **5**, **4**, **6**) and could be eluted from the moenomycin-matrix. The amount of RNA to obtain 100% corresponds to that amount of RNA which could not be displaced from the matrix and remained bound to the moenomycin-column after competitive elution. (B) Elution profiles for compounds **3a** (unsaturated lipid chain) and **3b** (saturated lipid chain). 'W' corresponds to the radioactivity in the last washing fraction to remove unbound RNA. Fractions E1 to E5 each represent the amount of RNA that could be eluted from the affinity column, whereas the last fraction C corresponds to that part of RNA that remained bound to the column; 'B' denotes the buffer control in both panels.

motif a: GGAGG, and a GU-rich motif which differs only slightly from the GU-rich motif b of the moenomycin aptamers.

The structural similarity of these aptamers is remarkable in view of the differences between the two classes of lipid-binding aptamers: the farnesylated Ras aptamers consist of unmodified RNA whereas the moenomycin aptamers contain 2'-amino modified pyrimidines. Furthermore, the two classes of aptamers were selected from completely different pools. The fact that yet similar sequences were selected may imply that there exist sequence-based structure motifs enabling the recognition of such hydrophobic epitopes. To investigate whether there are cross-reactions of the moenomycin aptamers with the farnesyl moiety of the farnesylated Ras peptide, farnesyl diphosphate (compound **6**; Zahn *et al.*, 2000) was used as binding competitor as described above. In this experiment farnesyl diphosphate (**6**) turned out to be a much weaker binding competitor than compound **4** (Figure 3A). These results were corroborated by another experiment, in which the binding affinity of a farnesyl-Ras

Table 1 Comparison of Lipid-Binding Aptamers.

Moenomycin aptamers[a]		Farnesyl-Ras aptamers[b]	
Consensus motif	K_d [nM]	Consensus motif	K_d [nM]
C2: GGAGG	350	GGGAGG	139
A6: GUGGUGUGGUG	320	GGGUGG	930

[a] Arnold and Blanch (1986).
[b] Gilbert *et al.* (1997).

Table 2 Sequences of Tested Aptamers.

Moenomycin-binding aptamers
Primer binding sites
5'-CUAUAGGGAGAGACAAGCUUGGGUC-**40N**-AGAAGAGAAAGAGAAGUUAAUUAAGGAUCCUCAC-3'

Aptamer C2 (motif a)
5'-UCGACCUCGCGCGA**GGAGG**GU**GGAGG**GUCGUAGAGCGCGUA-3'

Aptamer A6 (motif b)
5'-UCAUC**GGAGG**AGC**GGUGUGGUG**AGGUGACGCGGAAAAGGU-3'

Farnesylated Ras peptide-binding aptamer
Primer binding sites
5'-GGGAGAAUUCCGACCAGAAGCCU-**N**-CAUAUGUGCGUCUACAUGGAUCCUCA -3'

Aptamer G4
5'GCUCA**GGGGUGGG**UCGUAUAAUUAGCGUA**GGGAGG**UAGUGGAUGAGUAAGCCUGGGAUGU-3'

aptamer (Table 2) to immobilized moenomycin was studied. In this case, too, no significant binding was observed. These findings lead to the conclusion that despite the similar sequence motifs of both aptamer classes the selected aptamers are highly specific for their respective cognate ligands.

How do ribonucleic acids interact with such hydrophobic targets? It has previously been predicted (based on interactions between aptamers and the aliphatic amino acids valine and isoleucine) that conservation of G/U motifs in RNA may indicate a hydrophobic binding site (Yarus, 1998). Another example of an interplay of G-rich motifs and hydrophobic targets was shown by the *in vitro* selection of RNA sequences that bind and disrupt phospholipid bilayers (Khvorova *et al.*, 1999; Vlassov *et al.*, 2001). Since the moenomycin aptamers preferentially recognize compounds with the unsaturated side chain of the moenomycin analogs (cf **3a** *versus* **3b**) it is conceivable that π-stacking interactions with the nucleobases play an important role in stabilizing these complexes (see Puglisi and Williamson, 1998). GGAGG sequences may also adopt a unique structure involving A(GGGG)A hexad formation and the lipid moiety could be sandwiched between two hexad platforms (Kettani *et al.*, 2000). The surprising selectivity of the moenomycin and the farnesyl-Ras aptamers for their respective ligands may be the result of the different polar groups, but could also originate from the different configuration around the double bond between C-2 and C-3 of the isoprenoid moieties.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (Innovationskolleg 'Chemisches Signal und biologische Antwort' and priority program 'RNA Biochemistry'), the Fonds der Chemischen Industrie, and the Karl-Ziegler foundation. We thank K. Richter and U. Schuricht, J. Zotzmann for a sample of farnesyl diphosphate, and M. Blind for helpful discussions.

References

Arnold, F. H., and Blanch, H. W. (1986). Analytical affinity chromatography. II. Rate theory and the measurement of biological binding kinetics. J. Chromatogr. *355*, 13–27.

El-Abadla, N., Lampilas, M., Hennig, L., Findeisen, M., Welzel, P., Müller, D., Markus, A., and van Heijenoort, J. (1999). Tetrahedron *55*, 699–722.

Famulok, M. (1999). Oligonucleotide aptamers that recognize small molecules. Curr. Opin. Struct. Biol. *9*, 324–329.

Gilbert, B. A., Sha, M., Wathen, S. T., and Rando, R. R. (1997). RNA aptamers that specifically bind to a K Ras-derived farnesylated peptide. Bioorg. Med. Chem. *5*, 1115–1122.

Goldman, R. C., and Gange, D. (2000). Inhibition of transglycosylation involved in bacterial peptidoglycan synthesis. Curr. Med. Chem. *7*, 801–820.

Hermann, T., and Patel, D. J. (2000). Adaptive recognition by nucleic acid aptamers. Science *287*, 820–825.

Kettani, A., Gorin, A., Majumdar, A., Hermann, T., Skripkin, E., Zhao, H., Jones, R., and Patel, D. J. (2000). A dimeric DNA interface stabilized by stacked A.(G.G.G.G).A hexads and coordinated monovalent cations. J. Mol. Biol. *297*, 627–644.

Khvorova, A., Kwak, Y. G., Tamkun, M., Majerfeld, I., and Yarus, M. (1999). RNAs that bind and change the permeability of phospholipid membranes. Proc. Natl. Acad. Sci. USA *96*, 10649–10654.

Kosmol, R., Hennig, L., Findeisen, M., Müller, D., Markus, A., and van Heijenoort, J. (1997). J. Prakt. Chem. *339*, 340–358.

Osborne, S. E., Matsumura, I., and Ellington, A. D. (1997). Aptamers as therapeutic and diagnostic reagents: problems and prospects. Curr. Opin. Chem. Biol. *1*, 5–9.

Ritter, T. K., and Wong, C.-H. (2001). Angew. Chem. Int. Ed. *40*, 3508–3533.

Ritzeler, O., Hennig, L., Findeisen, M., Welzel, P., Müller, D., Markus, A., Lemoine, G., Lampilas, M., and van Heijenoort, J. (1997). Tetrahedron *53*, 1675–1694.

Schürer, H., Buchynskyy, A., Korn, K., Famulok, M., Welzel, P., and Hahn, U. (2001a). Fluorescence correlation spectroscopy as a new method for the investigation of aptamer/target interactions. Biol. Chem. *382*, 479–481.

Schürer, H., Stembera, K., Knoll, D., Mayer, G., Blind, M., Förster, H.-H., Famulok, M., Welzel, P., and Hahn, U. (2001b). Aptamers that bind to the antibiotic Moenomycin A. Bioorg. Med. Chem. *9*, 2557–2563.

van Heijenoort, J. (2001). Formation of the glycan chains in the synthesis of bacterial peptidoglycan. J. Glycobiol. *11*, 25R–36R.

Vlassov, A., Khvorova, A., and Yarus, M. (2001). Binding and disruption of phospholipid bilayers by supramolecular RNA complexes. Proc. Natl. Acad. Sci. USA *98*, 7706–7711.

Yarus, M. (1998). Amino acids as RNA ligands: a direct-RNA-template theory for the code's origin. J. Mol. Evol. *47*, 109–117.

Zahn, T. J., Eilers, M., Guo, Z., Ksebati, M. B., Simon, M., Scholten, J. D., Smith, S. O., and Gibbs, R. A. (2000). Evaluation of isoprenoid conformation in solution and in the active site of protein-farnesyl transferase using carbon-13 labeling in conjunction with solution- and solid-state NMR. J. Am. Chem. Soc. *122*, 7153–7164.

Received January 10, 2003; accepted August 12, 2003