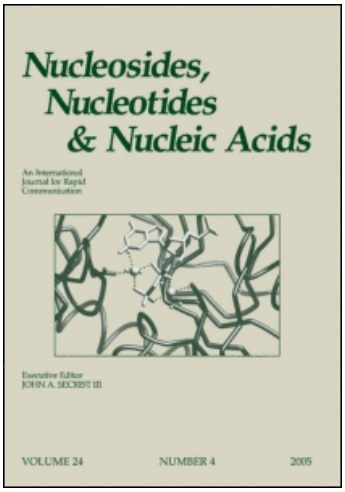

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

In Vitro Selection, Structural Characteristics, and Photocleavage of an Isoalloxazine Binding Rna Aptamer

C. Huber[a]; P. Burgstaller[a]; M. Famulok[a]
[a] Institut für Biochemie der LMU Munchen, München, Germany

To cite this Article Huber, C. , Burgstaller, P. and Famulok, M.(1997) 'In Vitro Selection, Structural Characteristics, and Photocleavage of an Isoalloxazine Binding Rna Aptamer', Nucleosides, Nucleotides and Nucleic Acids, 16: 5, 717 — 720
To link to this Article: DOI: 10.1080/07328319708002939
URL: http://dx.doi.org/10.1080/07328319708002939

NUCLEOSIDES & NUCLEOTIDES, 16(5&6), 717-720 (1997)

IN VITRO SELECTION, STRUCTURAL CHARACTERISTICS, AND PHOTOCLEAVAGE OF AN ISOALLOXAZINE BINDING RNA APTAMER

C. Huber, P. Burgstaller and M. Famulok*
Institut für Biochemie der LMU München, Feodor-Lynen-Strasse 25, 81377 München, Germany

ABSTRACT: An RNA motif which binds FMN was isolated by *in vitro* selection of an RNA library. The secondary structure of this RNA contains an internal loop forming an isoalloxazine binding site through non-canonical base pairing. Irradiation with light in presence of FMN induces photocleavage at G·U base pairs outside the aptamer binding site.

RNA molecules are capable of forming binding pockets and clefts for the specific molecular recognition of substrates, similar to those found in proteins or antibodies. Notable examples include the self-splicing group I intron from *Tetrahymena*, which has a defined binding pocket for the guanosine cofactor[1]. RNA aptamers for the specific recognition of numerous low molecular weight guest molecules have been isolated from large combinatorial RNA libraries[2]. In many cases, RNAs exhibited extraordinary selectivity and affinity for their respective cognate substrates, such as adenosine triphosphate (ATP)[3], FMN[4], theophylline[5], amino acids[6], vitamins[7], and antibiotics[8]. For some of those aptamers, detailled structural studies exist, which confirmed that binding occurs in well defined complementary binding pockets[9].

We have previously applied the in vitro selection ("SELEX") technology to screen a combinatorial RNA library consisting of 10^{15} different sequences, for individual RNA molecules which are able to specifically recognize various flavine derivatives in solution[4a]. This screen identified two different RNA motifs, designated as "aptamers" which bind with different affinities to the isoalloxazine moiety of flavin-adenine dinucleotide (FAD) **1**, flavine mononucleotide (FMN) **2**, riboflavin **3**, and isoalloxazine **4** (FIG. 1).

An RNA-pool that had a core of 74 random-sequence positions, flanked by defined primer binding sites was chosen as a starting point for the selections (FIG. 2).

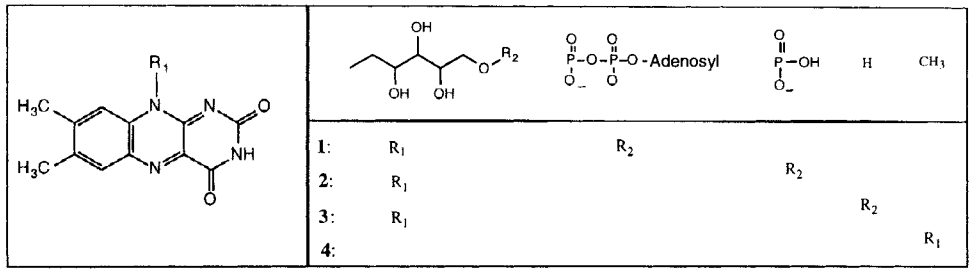

FIGURE 1: Chemical structure of the isoalloxazine derivatives.

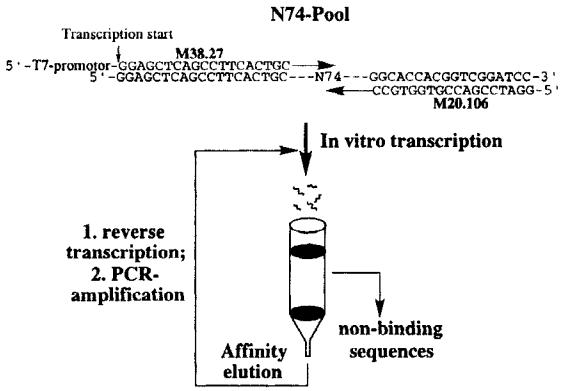

FIGURE 2 Pool and selection scheme. Sequences of the pool and primers are shown at the top. The RNA pool was loaded onto the column. Non-binding RNAs were removed by washing with binding buffer, bound RNAs were affinity eluted with a solution of 3.0 mM FMN in binding buffer. Eluted RNAs were reverse transcribed, the cDNA subjected to PCR amplification, the PCR-DNA was transcribed yielding an enriched RNA pool as the input for the next cycle[4a].

After six cycles of iterative selection and amplification, two different sets of sequences were obtained from which two different minimal RNA motifs could be defined. One of the selected RNA motifs contained an internal loop which consisted of 6 conserved bases in the upper and 5 in the lower strand, flanked by Watson-Crick helices. This RNA recognizes exclusively the isoalloxazine moiety of the cofactor with values of K_d between 300 and 500 nM (FIG. 3). To refine the secondary structure model obtained by sequence comparisons, we used chemical probing to examine the secondary structure of the FMN binding site of this RNA aptamer with the specific structure probes dimethylsulfate (DMS), kethoxal (KE), and 1-cyclohexyl-3-[2-(morpholino ethyl)-carbodiimide]-*p*-toluene sulfonate (CMCT)[10]. We found that in the free RNA every base within the two conserved consensus sequences A12-G13-G14-A15-U16-A17-U18 and A25-G26A27-A28-G29-G30 is accessible for chemical modification.

In accordance with the secondary structure model shown in FIG. 3, the bases upstream of the binding site are not modified, due to Watson-Crick pairing and helix formation. A15 (A11 in FIG. 4) is the only position within the invariant consensus sequence which is not conserved among different isolates and can be substituted by the three other bases without altering the strength of binding[4a].

The 3D-structure of the aptamer-FMN complex was resolved by NMR spectroscopy of ^{15}N- and ^{13}C-labeled RNA[11]. This structural analysis revealed that extensive non-canonical base pairing occurs in the internal bulge region. In addition, a base triple formed by residues G10, U12, and A25 is formed on which the isoalloxazine rings are stacked. The N3-H and O4 of the isoalloxazine moiety form hydrogen bonds with A26 of the aptamer. The binding mode of the complex is shown in FIG. 4.

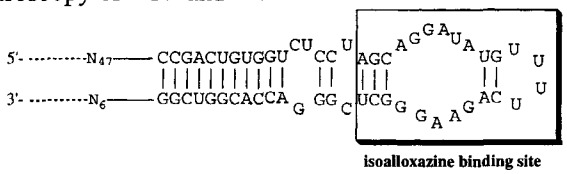

FIGURE 3. Secondary structure of the RNA aptamer FMN-2. The minimal cofactor binding site of this RNA is shown in the boxed region. The total length of the RNA aptamer was 109 nucleotides[4a, 11].

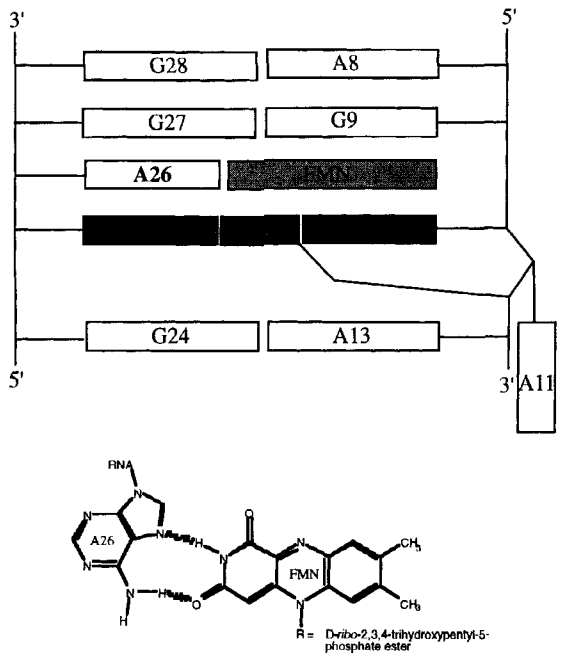

FIGURE 4. Tertiary interactions and hydrogen bonding scheme in the FMN/RNA-aptamer complex based on Fan *et al.*[11].

During the structural characterization of the isoalloxazine binding aptamer FMN-2, we observed that the aptamer is cleaved by FMN and riboflavine at a defined cleavage site, a G·U wobble base pair located within a 11-bp stem region in the aptamer[12]. This cleavage reaction requires the photosensitizers FMN, or riboflavine at concentrations between 100 μM and 500 μM, and does not occur with the non-photosensitizing molecules FAD or lumichrome. In addition, the cleavage reaction requires divalent cations such as Mg^{2+}, Ca^{2+}, Ba^{2+} or Zn^{2+}, but does not occur with Mn^{2+}, or Cu^{2+}. We also showed that the photooxidative cleavage reaction of RNA at G·U base pairs is a general mechanism and does not require the isoalloxazine aptamer binding site. The G·U-specific photocleavage reaction thus represents the first example in which a structure motif made of an unusual base pair in an RNA is specifically recognized and affected by a low-molecular-weight molecule. This observation might open up the possibility of probing in folded RNAs the accessibility of G·U base pairs, which are endowed with specific structural and functional roles in numerous structured and catalytic RNAs.

ACKNOWLEDGEMENT: This work was supported by grants from the Deutsche Forschungsgemeinschaft. We thank T. Tiedchen for helpful discussions.

REFERENCES

1. Michel, F., Hanna, M., Green, R., Bartel, D.P., Szostak, J.W. *Nature* **1989**, *342*, 391.

2. Gold, L.; Polisky, B.; Uhlenbeck, O.; Yarus, M. *Annu. Rev. Biochem.* **1995**, *64*, 763; Hirao, I.; Ellington, A.D. *Curr. Biol.* **1995**, *5*, 1017; Gold, L. *J. Biol. Chem.* **1995**, *270*, 13581; Klug, S. J.; Famulok, M. *Mol. Biol. Rep.* **1994**, *20*, 97; Joyce, G. F. *Curr. Opin. Struct. Biol.* **1994**, *4*, 331; Ellington, A. D. *Curr. Biol.* **1994**, *4*, 427; Famulok, M.; Szostak, J. W. In *Nucleic Acids and Molecular Biology*; Eckstein, F.; Lilley, D.M.J., Eds. Springer Verlag: New York, **1993**; Vol. 7, pp. 271; Famulok, M.; Szostak, J. W. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 979.

3. Sassanfar, M.; Szostak, J. W. *Nature* **1993**, *364*, 550; Huizenga, D. E.; Szostak, J. W. *Biochemistry* **1995**, *34*, 656.

4. Burgstaller, P.; Famulok, M. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1084; Lauhon, C. T.; Szostak, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 1246.

5. Jenison, R. D.; Gill, S. C.; Pardi, A.; Polisky, B. *Science* **1994**, *263*, 1425.

6. Famulok, M. *J. Am. Chem. Soc.* **1994**, *116*, 1698; Connell, G.J., Illangesekare, M., Yarus, M. *Biochemistry* **1993**, *32*, 5497; Tao, J., Frankel, A.D. *Biochemistry* **1996**, *35*, 2229; Majerfeld, I., Yarus, M. *Nature Struct. Biol.* **1994**, *1*, 287; Geiger, A.; Burgstaller, P.; Von der Eltz, H.; Roeder, A.; Famulok, M. *Nucleic Acids Res.* **1996**, *24*, 1029.

7. Lorsch, J. R.; Szostak, J. W. *Biochemistry* **1994**, *33*, 973.

8. Lato, S. M.; Boles, A. M.; Ellington, A. D. *Chem. Biol.* **1995**, *2*, 291; Wang, Y.; Rando, R. R. *Chem. Biol.* **1995**, *2*, 281; Wallis, M. G.; von Ahsen, U.; Schroeder, R.; Famulok, M.*Chem. Biol.* **1995**, *2*, 543.

9. Burgstaller, P.; Kochoyan, M.; Famulok, M. *Nucleic Acids Res.* **1995**, *23*, 4769; Yang, Y., Kochoyan, M., Burgstaller, P., Westhof, E., Famulok, M. *Science* **1996**, *727*, 1343.

10. Burgstaller, P.; Famulok, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1157-1162.

11. Fan, P.; Suri, A. K.; Fiala, R.; Live, D.; Patel, D. *J. Mol. Biol.* **1996**, *258*, 480-500.

12. Burgstaller, P.; Famulok, M. *J. Am. Chem. Soc.* **1997**, *119*, in press.