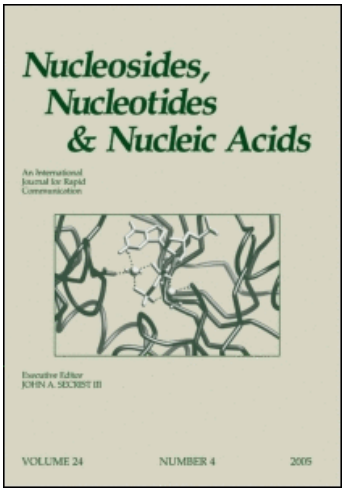

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Assay of Random RNA Oligomerisation in Buffers with High Concentrations of Divalent Metal Ions

Alexander Vlassov[a]
[a] Somagenics, Inc., Santa Cruz, California, USA

Online publication date: 10 July 2004

To cite this Article Vlassov, Alexander(2004) 'Assay of Random RNA Oligomerisation in Buffers with High Concentrations of Divalent Metal Ions', Nucleosides, Nucleotides and Nucleic Acids, 23: 6, 999 — 1001
To link to this Article: DOI: 10.1081/NCN-200026054
URL: http://dx.doi.org/10.1081/NCN-200026054

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 6 & 7, pp. 999–1001, 2004

Assay of Random RNA Oligomerisation in Buffers with High Concentrations of Divalent Metal Ions

Alexander Vlassov*

Somagenics, Inc., Santa Cruz, California, USA

ABSTRACT

Certain conditions, such as high concentrations of divalent metal ions in the reaction buffer or low pH, can cause aggregation and precipitation of RNA species with complementary sequences. If oligomerisation has gone unnoticed, some sequences from the pool of random RNA may be underrepresented or even lost at the very beginning of the selection experiment. Two simple assays for RNA oligomerisation are suggested. One is based on electrophoresis in non-denaturing gels, and the other uses gel-filtration.

Key Words: Random RNA; Oligomerisation; Aggregation; SELEX.

Currently combinatorial library selections through the systematic evolution of ligands by exponential enrichment (SELEX) technique is widely used to identify nucleic acid molecules with desired properties.[1,2] This powerful technique have numerous applications ranging from basic research reagents to the identification of novel diagnostic and therapeutic reagents based on aptamers and ribozymes.[3,4] Since the systems used are very complex and sensitive, even seemingly slight changes can severely affect the outcome of selection, and thus reaction conditions should be chosen wisely. To select for some specific activities, one sometimes has to use buffers with

*Correspondence: Alexander Vlassov, Somagenics, Inc., 2161 Delaware Ave., Santa Cruz, CA 95060, USA; E-mail: avlassov@somagenics.com.

DOI: 10.1081/NCN-200026054 1525-7770 (Print); 1532-2335 (Online)

moderate and high concentrations of divalent metal ions. It is widely known that certain multivalent metals, such as Zn^{2+}, Pb^{2+}, Mn^{2+}, Mg^{2+}, can cause nucleic acid degradation. However fewer people are aware of the fact that RNA species with complementary sequences can also aggregate and precipitate in these conditions. In experiments with RNAs containing random sequences, more than half of the pool RNA taken for selection was found to aggregate when incubated in buffer containing 50 mM $MgCl_2$, 500 mM KCl and 0.5 μM RNA.[5] If oligomerisation has gone unnoticed, some sequences may be underrepresented or even lost at the very beginning of the selection experiment. Here two simple assays for RNA oligomerisation are described.

The first method used for analysis of complex formation was electrophoresis in non-denaturing gels. A 102-nt internally ^{32}P-labeled RNA pool with 60-nt random region was generated from T7 promoter sequence by in vitro transcription. 100 pmol (30 μl) of RNA was heated in water at 65°C for 3 min, then 10 × selection buffer was added (1 × : 50 mM HEPES pH 7.0, 100 mM NaCl, 40 mM KCl, 0–20 mM $MgCl_2$, 0–10 mM $CaCl_2$), solution was cooled to room temperature over 15 min, then mixed with 6 × loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol in water) and loaded on a 8% non-denaturing polyacrylamide gel. The gel was run in selection buffer at room temperature using a 20 V/cm field for 1–2 h and analysed by phosphorimager (Molecular Imager FX, Bio-Rad).

It was found that random RNA is monomeric in buffer containing up to 5 mM Ca^{2+} and 5 mM Mg^{2+} (Fig. 1A, left). However increase of Mg^{2+} and Ca^{2+} concentration up to 20 mM and 10 mM, respectively, results in considerable oligomerisation of RNA molecules. Under these salt conditions, about 90% of RNA aggregate in complexes large enough to completely prevent migration in 8% polyacrylamide gel (Fig. 1A, right). Similar results were obtained with 2% agarose gels and using RNA libraries (10–1000 pmol) containing different length of the random region (40–80 nt). Aggregation is even more pronounced at low pH (< 5.0) since nucleic acids are known to precipitate under acidic conditions.

The second method used for analysis of the complex formation was gel-filtration. 100 pmol (30 μl) of the folded internally ^{32}P-labeled RNA were loaded on a Sephacryl

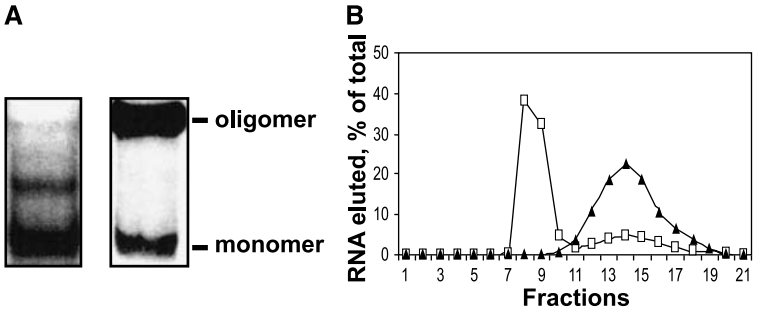

Figure 1. Analysis of random RNA pool for oligomerisation. (A) Random RNA analysis in 8% polyacrylamide gel. Left, buffer contains 5 mM $MgCl_2$, 5 mM $CaCl_2$; right, buffer contains 20 mM $MgCl_2$, 10 mM $CaCl_2$. (B) Random RNA analysis on a Sephacryl S-1000 gel-filtration column. (▲), buffer contains 5 mM $MgCl_2$, 5 mM $CaCl_2$; (□), buffer contains 20 mM $MgCl_2$, 10 mM $CaCl_2$.

S-1000 gel-filtration column (65 × 2.2 mm, Amersham Pharmacia), the column was run in selection buffer, fifty-microliter fractions were collected and radioactivity measured. The obtained results were identical to above discussed results from non-denaturing gel electrophoresis. At high concentration of Mg^{2+} and Ca^{2+} RNA aggregate and complexes appear in the column void volume while monomeric RNA is retarded (Fig. 1B). The gel-filtration method has the following advantages: 1) high salt concentration does not interfere with the analysis as in case of native gel electrophoresis; 2) fractionation is much faster—it takes 20–30 min as compared to 1–2 h in case of electrophoresis assay.

In summary, if selection is to be performed at high concentrations of divalent metal ions, analysis of RNA oligomerisation in the experimental conditions should be done. Of the two methods tested gel-filtration on Sephacryl S-1000 column is preferable. In case of intensive complex formation RNA can be immobilized on agarose beads before the addition of divalent ions to minimize problem of aggregation.[5]

ACKNOWLEDGMENT

The author would like to thank Dr. Michael Yarus for helpful discussions.

REFERENCES

1. Ellington, A.D.; Szostak, J.W. In vitro selection of RNA molecules that bind specific ligands. Nature **1990**, *346*, 818–822.
2. Tuerk, C.; Gold, L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science **1990**, *249*, 505–510.
3. Gold, L.; Polisky, B.; Uhlenbeck, O.; Yarus, M. Diversity of oligonucleotide functions. Annu. Rev. Biochem. **1995**, *64*, 763–797.
4. Jayasena, S.D. Aptamers: an emerging class of molecules that rival antibodies in diagnostics. Clin. Chem. **1999**, *45*, 1628–1650.
5. Bartel, D.P.; Szostak, J.W. Isolation of new ribozymes from a large pool of random sequences. Science **1993**, *261*, 1411–1418.

Received December 2, 2003
Accepted April 23, 2004