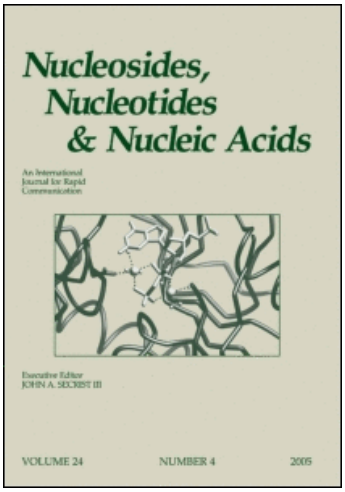
This article was downloaded by:
On: *26 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



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

PNA-RELATED OLIGONUCLEOTIDE MIMICS AND THEIR EVALUATION FOR NUCLEIC ACID HYBRIDIZATION STUDIES AND ANALYSIS

V. Efimov[a]; M. Choob[a]; A. Buryakova[a]; D. Phelan; O. Chakhmakhcheva[a]
[a] Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia

Online publication date: 31 March 2001

To cite this Article Efimov, V. , Choob, M. , Buryakova, A. , Phelan, D. and Chakhmakhcheva, O.(2001) 'PNA-RELATED OLIGONUCLEOTIDE MIMICS AND THEIR EVALUATION FOR NUCLEIC ACID HYBRIDIZATION STUDIES AND ANALYSIS', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 419 — 428
To link to this Article: DOI: 10.1081/NCN-100002316
URL: http://dx.doi.org/10.1081/NCN-100002316

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 419–428 (2001)

PNA-RELATED OLIGONUCLEOTIDE MIMICS AND THEIR EVALUATION FOR NUCLEIC ACID HYBRIDIZATION STUDIES AND ANALYSIS

V. Efimov,[1,*] M. Choob,[1,2] A. Buryakova,[1] D. Phelan,[2] and O. Chakhmakhcheva[1]

[1]Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, 117871 Moscow, Russia
[2]Active Motif, Carlsbad, California 92008

Downloaded At: 11:58 26 January 2011

ABSTRACT

DNA mimics containing phosphonate analogues of PNAs (pPNAs), particularly PNA-pPNA hybrids as well as hetero-oligomers consisted of pPNA units and PNA-like molecules on the base of *trans*-4-hydroxy-*L*-proline (HypNA) have been synthesized. The evaluation of their effectiveness in assays based on the hybridization technique in the comparison with natural oligonucleotides and classical PNAs has shown a high potential of these mimics as sensor molecules for nucleic acid based diagnostics and as molecular probes for mRNA isolation.

INTRODUCTION

Last years, synthetic oligonucleotides (ODNs) attracted increasing interest as effective tools for many molecular biology applications based on the hybridization technique. However, some problems still remain, which are connected with their biological instability and a relatively low thermal stability of their duplexes. Among DNA analogues developed to improve the affinity and specificity of the target-probe interaction, peptide nucleic acids (PNAs) and their derivatives are currently of much interest due to their excellent binding properties and enzymatic stability (1,2). PNAs consist of achiral monomer units derived from N-(2-aminoethyl)glycine bearing

*Corresponding author.

Copyright © 2001 by Marcel Dekker, Inc. www.dekker.com

ORDER REPRINTS

nucleoside heterocyclic bases and connected by amide bonds. PNAs can hybridize to complementary DNA or RNA chains forming very stable duplexes and triple helixes, and their application as sensor molecules and molecular probes leads to a simultaneous increase in both specificity and sensitivity of the analysis, because of their higher affinity and ability to discriminate single base pair difference better than DNA probes (3). Moreover, PNAs can hybridize to complementary regions of nucleic acids in low salt concentrations that prevents a target nucleic acid from intra-strand folding and increases the accessibility of target sequences. Nevertheless, the biological applications of PNAs are limited by their poor water solubility, tendency to self-aggregation and sedimentation in low salt concentrations, as well as inability to activate RNAase H (4,5). To improve the potency of PNAs, we have developed novel oligonucleotide mimics representing phosphonate analogues of peptide nucleic acids (pPNAs), a set of chimeric oligomers containing pPNA and PNA residues (6,7) as well as hetero-oligomers constructed of pPNA units and PNA-like monomers on the base of *trans*-4-hydroxy-*L*-proline (HypNA) (8) (Fig. 1). The investigation of their properties revealed that these PNA-related mimics are fully stable to the action of nucleases, and the introduction of negative charges into the PNA backbone led to the excellent solubility characteristics. The combination these properties with high hybridization characteristics makes PNA-pPNA

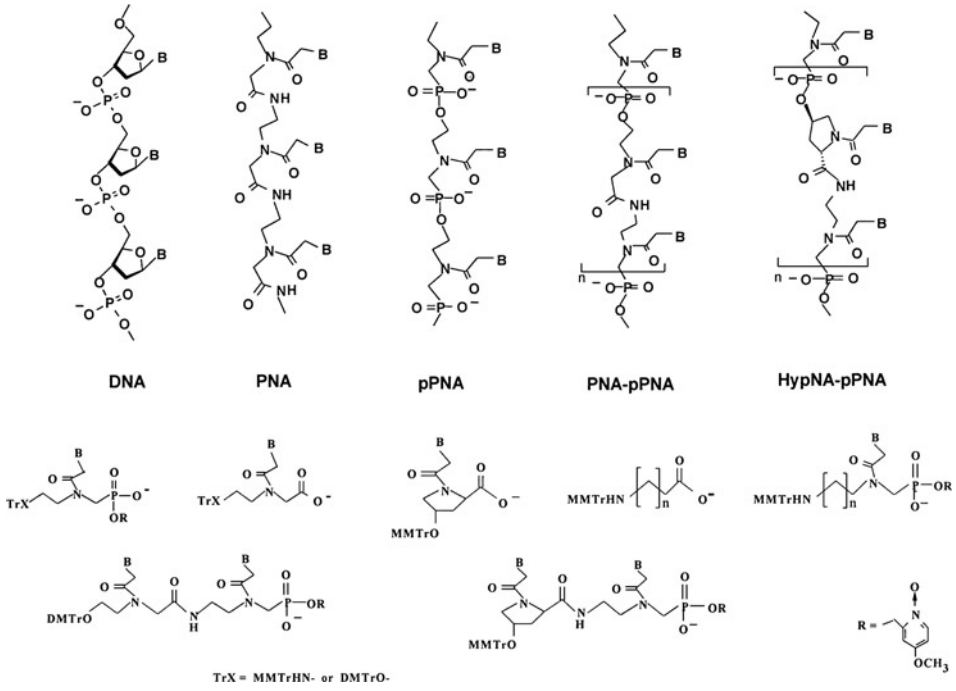


Figure 1. General chemical structures of PNA-related oligonucleotide mimics and the building blocks for their solid phase synthesis.

Downloaded At: 11:58 26 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

ORDER REPRINTS

and HypNA-pPNA chimeras very promising for evaluation as potential anti-sense and anti-gene therapeutic agents as well as specific molecular probes and sensor molecules.

RESULTS AND DISCUSSION

To construct PNA-related oligomers, the procedures to obtain corresponding pPNA, HypNA, PNA monomers as well as PNA-pPNA and HypNA-pPNA dimer building blocks has been developed (7–9). The building units depicted in Figure 1 have a combination of blocking groups compatible with the solid phase DNA phosphotriester synthesis using the intra-molecular O-nucleophylic catalysis (7), and the dimers with mixed PNA-pPNA or HypNA-pPNA backbone contain the internal amide bond between the corresponding monomer residues. A synthetic route, which was used to assemble PNA-pPNA or HypNA-pPNA chimeric oligomers on a CPG support included stepwise coupling these units with the necessary changing a unit type during the chain elongation (8,9).

In the hybridization experiments, PNA-pPNA and HypNA-pPNA chimeras demonstrated strong binding to complementary nucleic acid chains (7,8). Thus, HypNA-pPNA chimeras with 1:1 ratio of alternating monomer units exhibit a high thermal stability of their complexes with DNA (RNA) targets, which was very close to that of complexes formed by pure PNA oligomers (8). The especially interesting aspect of the complex formation between PNA-related mimics and nucleic acids is the influence of a salt concentration on melting temperatures (T_m) of their complexes. It is known that, by virtue of a neutral backbone of classic PNAs, the stability of their complexes with nucleic acids is fairly independent on ionic strength, in contrast to DNA/DNA or RNA/DNA duplexes (3). Moreover, the T_m of PNA/DNA (RNA) duplexes has a tendency to decrease with increasing salt concentration ($0.01 \rightarrow 0.5$ M NaCl), whereas the T_m of natural DNA and RNA duplexes increases (10). Similar to classic PNAs, T_m values of complexes between DNA(RNA) and hetero-oligomers composed of alternating PNA (HypNA) and pPNA residues in 1:1 or 1:2 ratio are not dependent on ionic strength. The decrease of a salt concentration has only a weak destabilizing effect on duplexes and triplexes formed by these mimics that, probably, is a consequence of the partial intra-molecular neutralization of negative pPNA charges by the interaction with amide groups of a backbone. Nevertheless, the hybridization properties of pure pPNA oligomers and their chimeras containing less than 30% PNA (or HypNA) residues were similar to those of natural oligonucleotides, and at low salt concentrations we observed the decreasing T_m values of their complexes (Fig. 2) (11).

Recently, we have tested the PNA-related mimics as sensor molecules in the construction of arrays for nucleic acid hybridization analysis using assay formats utilizing polyacrylamide-based technique as a test system (12,13). For the attachment of bio-molecules to polyacrylamide (PAA) supports, we used an approach

Downloaded At: 11:58 26 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

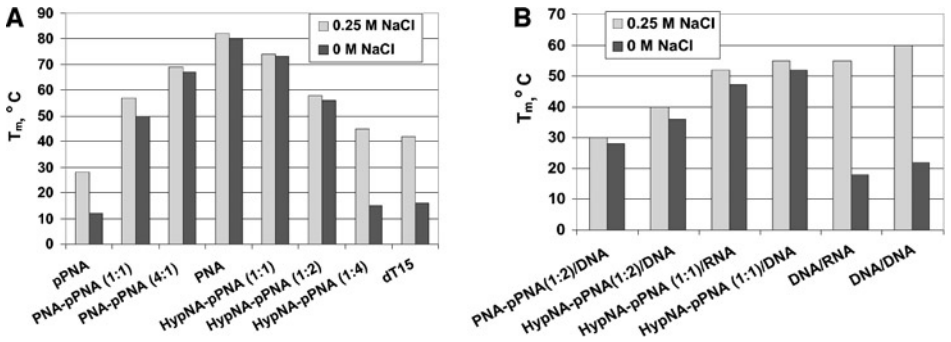


Figure 2. Influence of a salt concentration on the stability of complexes formed by mimic oligomers with their complementary targets. A - Thy_{15}/rA_{15} complexes; B -.complexes of oligomers with mixed purine-pyrimidine sequences.

including the polymerization of acrylamide-modified oligomer probes into acrylamide-based copolymers. A convenient scheme for the solid phase synthesis of 5′- and 3′-acrylamide conjugates of oligonucleotides and mimic oligomers via an universal intermediate have been developed (12). According to this scheme, mimic oligomers were synthesized from the appropriate monomer and dimer units using a CPG support derivatized with the properly protected 1-deoxy-D-ribofuranose as schematically shown in Figure 3.

The acrylamide-containing mimics were used for the construction of soluble PAA-oligomer conjugates as well as polyacrylamide gels (PAAG) with tethered oligomer probes. To obtain soluble polymers, an acrylamide-mimic conjugate and acrylamide were co-polymerized in 1:100 molar ratio. The addition into the reaction mixture of bis-acrylamide allowed to obtain PAAGs containing mimic probes. To obtain polymers modified with specific functional groups, the apropriate activated

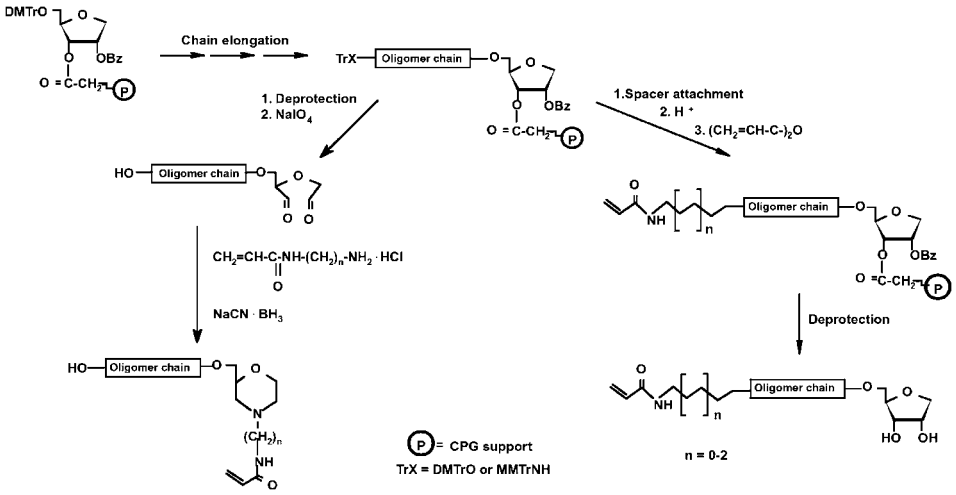


Figure 3. Scheme for the synthesis of acrylamide conjugates with ODNs and mimic oligomers.

Downloaded At: 11:58 26 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

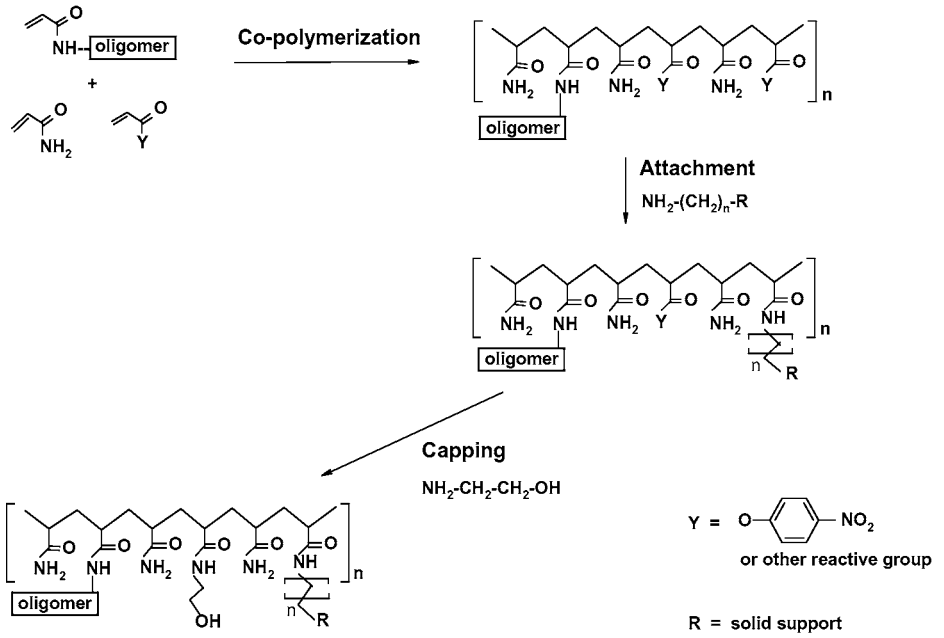


Figure 4. Approaches to the construction of PAA-oligomer conjugates and their attachment to a solid surface with the use of activated acrylic acid derivatives.

Downloaded At: 11:58 26 January 2011

acrylic acid derivatives, particularly p-nitrophenyl ester, or N-substituted acrylamides, N-aminoalkylacrylamide or its N-bromoacetamide derivative, were added into the polymerisation reaction along with other components (Fig. 4) (12,13). These functional groups can be used for the introduction of fluorescent labels or side chains of other types to obtain multiple functionalized polymer, and for a modulation of the polymer properties with respect to hydrophilicity and charge. The reactive groups connected to PAA chains can be also used for the attachment of polymers to solid surfaces. Thus, we used an approach to the derivatization of solid supports with oligomer probes, which involves the attachment to the activated surface of a preliminary prepared comb-type PAA precursor containing the tethered oligomer in multiple copies and the corresponding reactive groups in side chains. This technique provides connection of a polymer with a surface through the stable chemical bonds (C–N and C–S) and results in high loading of an oligomer probe (for example, 350–500 fmol/mm^2 in the case of glass supports) (12).

The examination of the binding selectivity of PNA-related mimics to nucleic acids using melting experiments on solid phase has shown that the hybridization of PAA-mimic conjugates occurred in a sequence specific manner, and we did not detect the formation of complexes between mimic probes and non-complementary targets. The introduction of one mismatch in the center of a mimic sequence gave a significant drop in the melting temperature (17–23°C). Oligomers with two separately situated mismatches were not able to form stable complexes with the targets. The stability of duplexes formed by DNA target and pPNA-containing mimics can

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

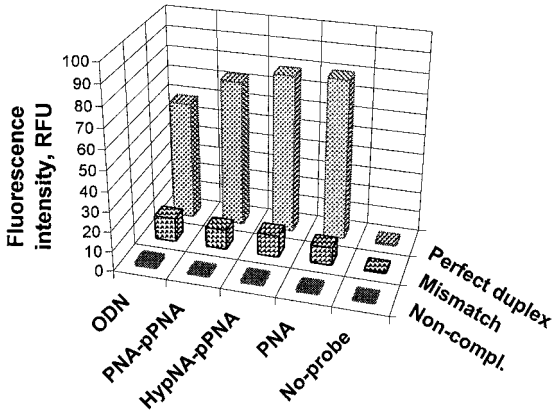


Figure 5. Analysis of a mismatch effect in solid phase hybridization of the fluorescently labeled oligonucleotide d(TCATGGTGTCCTTTGCAG) to the surface-immobilized PAA-oligomer conjugates with the complementary, mismatched in the 9th position and non-complementary sequences.

be described as being in general 0.72–0.89°C higher per base pair than that of the corresponding DNA/DNA duplex, whereas for PNAs this value was 1.05°C at the same conditions (Fig. 5). In general, PNA-pPNA and HypNA-pPNA chimeric probes showed in ~1.5 times better mismatch discrimination factors than ODNs, and their characteristics were similar to those for PNAs (12).

For the examination of hybridization properties of mimics, we used affinity-electrophoresis also. Oligomer capture probes were immobilized within fixed regions of non-denaturing PAAG by co-polymerization similar to the procedure described earlier (14). Electrophoresis of ^{32}P-labelled complementary DNA samples through the gel resulted in hybridization-mediated capture of dilute complementary targets by the immobilized mimic oligomer probes, whereas ODNs with non-complementary sequences had a migration behavior as in a gel lacking the probe. The mismatched ODNs did not bind tightly to the probe and moved on a gel on the bases of their decreasing affinity for recognition sequence. Following electrophoresis, hybridized samples were detected by autoradiography or imaging (15).

We have found that some intercalating fluorescent dyes, particularly ethidium bromide and ethidium homodimer, are not able to bind single-stranded PNA-related mimics in contrast to natural DNA(RNA) fragments. At the same time, in contrast to PNAs (16), the duplexes and triplexes of the pPNA-containing mimics with DNA(RNA) targets exhibit fluorescence under UV-light after the staining with these dyes (15). Thus, in the case of gel-immobilized mimic probes the visualization of a duplex formation between a probe and its migrating target can be readily achieved by staining the gel with some dyes. Another dye tested by us for visualization and recognition of mimic-target complexes was a symmetrical cyanine dye 1-ethyl-2-[3-(1-ethylnaphtho[1,2-*d*]thiazolin-2-ylidene)-2-methylpropenyl]naphtho[1,2-d]thiazolium bromide ("Stains all"). Practically, it does not stain single-stranded PNAs and PNA-related mimics, whereas the successful binding of the mimics to the target DNA sequence is reported by the dark blue

Downloaded At: 11:58 26 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

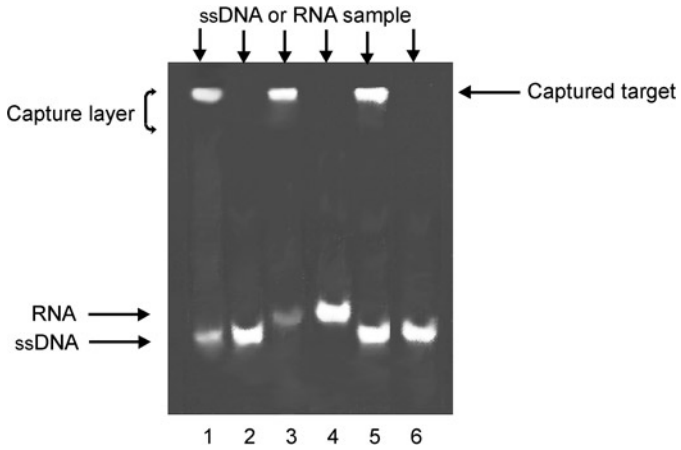


Figure 6. Analysis of target DNA or RNA samples using the affinity PAGE. The PAAG contains a layer with immobilized HypNA-pPNA mimic oligomer as a capture probe. Detection by staining with ethidium bromide. The probes contain: a complementary target-1,3; a mismatched target-2,4,6; a mixed comp./mismatched target-5.

(or violet) color of a band. The application of a such visualization method in conjunction with PNA-related mimics can be especially useful for the analysis of long nucleic acid targets by affinity electrophoresis as it is shown in Figure 6, because it does not require the preliminary radioactive or fluorescent target labeling.

PNA-related mimics were also tested for the construction of soluble branched detection probes for signal amplification in sandwich hybridization assays (12). The assay formats for sandwich hybridization consisted of a comb-type PAA-oligomer capture probe immobilized on a glass surface and a comb-type PAA-based detection-amplification probe. In general, this assay system with PNA-pPNA and HypNA-pPNA probes capable of detecting 1–5 amol of a target with the use of radioactively labeled amplifier probes (12) that is suitable for a variety of biology testing applications. As a variant of the detection probe for sandwich hybridization, we tested a mimic conjugate with the double-stranded DNA fragment of ~1000 bp-length obtained by PCR. This detection format is based on the unique property of single-stranded PNAs and the above-described PNA-relative mimics, which were used as capture probes, do not interact with some intercalating dyes, particularly ethidium homodimer, homodimeric oxazole yellow (YOYO) and thiazole orange (TOTO) dyes. In contrast, these dyes selectively stain captured target DNA or RNA fragments giving stable fluorescent complexes (17). Utilizing this nucleic acid assay format and ethidium homodimer, the detection limit was estimated in the range of 0.01–0.1 fmol of a target, whereas with YOYO and TOTO the detection sensitivity was ~10-fold higher (15).

Further extending the range of PNA-relative mimics applications is connected with the optimization of mRNA isolation from cells or tissues for cDNA arrays, libraries and RT-PCR (11). Current techniques for the isolation of mRNA include the hybridization of homo-T ODNs to the 3′ poly-A tailed mRNA. High

Downloaded At: 11:58 26 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

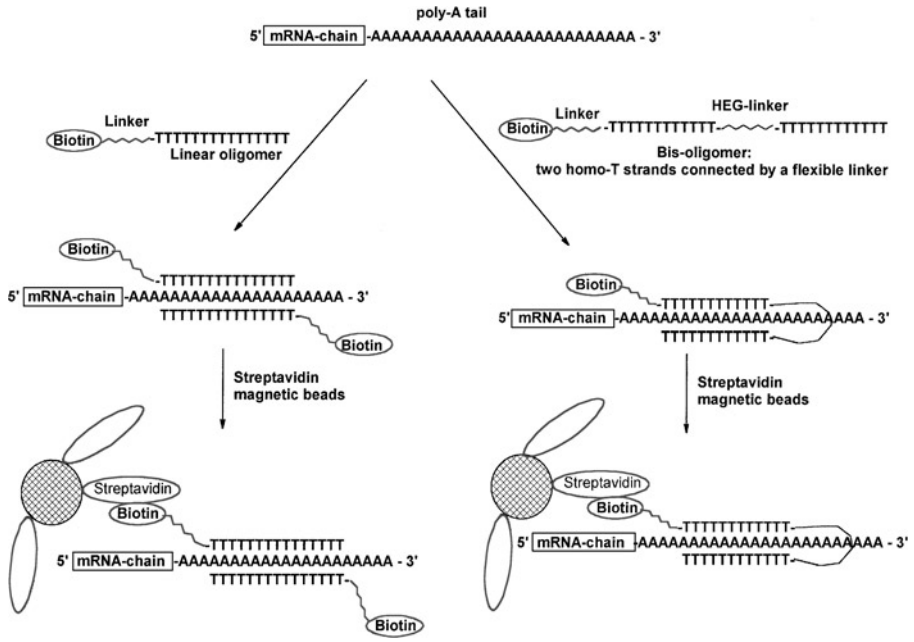


Figure 7. Principal scheme for mRNA isolation with the use of "linear" and "clamping" homo-Thy mimic oligomers bearing terminal biotin residue.

affinity to nucleic acid targets, good water solubility and nuclease resistance of PNA-pPNA and HypNA-pPNA chimeras allow to improve the isolation procedure. For this purpose, two types of triplex forming chimeric homo-Thy oligomers representing "linear" and "clamping" molecules have been designed and synthesized (Active Motif, patent pending). The latter molecule represented two linear homo-Thy mimic chains connected with a neutral flexible linker (Fig. 7). Also, these oligomers contained the N-terminal biotin residue, which allows the conjugation to a streptavidin-coated surface, particularly magnetic beads, to isolate mimic/RNA

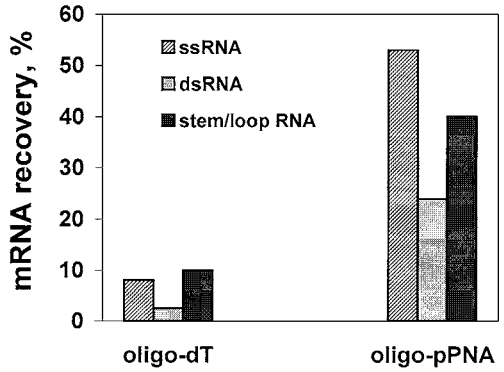


Figure 8. Comparison of the purification efficiency of mRNA templates with short poly-A tails using biotinylated oligo-dT and HypNA-pPNA homo-Thy oligomer using streptavidin magnetic beads.

Downloaded At: 11:58 26 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

complexes. It was demonstrated that both the "linear" and "clamping" chimeras hybridize to poly-A sequence with a high degree of specificity and give very low background binding of the probe to unwanted rRNA (18). The application of pPNA-containing mimics in mRNA isolation has shown that these oligomers allow for the isolation of high quality mRNA ensuring a representative mRNA population including mRNA with short poly-A tracks (Fig. 8). Additionally, their advantages over natural ODNs include reduced DNA contamination that is a consequence of possibility of DNase treatment during mRNA preparation.

CONCLUSION

The investigation on the properties of PNA-relative mimics bearing negative charges, particularly PNA-pPNA and HypNA-pPNA chimeras, demonstrated their high potential as probes for nucleic acid isolation and analysis. In view of high hybridization properties, biological stability and good water solubility, their use gives rise to the sensitivity and effectiveness of the processes as compared to that of natural ODNs without the increasing a background.

REFERENCES

1. Nielsen, P.E.; Egholm, M.; Berg, R.H.; Buchardt, O. *Science*, **1991**, *254*, 1497–1500.
2. Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S.M.; Driver, D.A.; Berg, R.H.; Kim, S.K.; Norden, B.; Nielsen, P.E. *Nature*, **1993**, *365*, 566–568.
3. Geiger, A.; Lester, A.; Kleiber, J.; Orum, H. *Nucleosides & Nucleotides*, **1998**, *17*, 1717–1724.
4. Weiler, J.; Gausephohl, H.; Hauser, N.; Jensen, O.N.; Hoheisel, J.D. *Nucleic Acids Res*., **1997**, *25*, 2792–2799.
5. Bergman, F.; Bannwarth, W.; Tam, S. *Tetrahedron Lett*., **1995**, *36*, 6823–6826.
6. Van der Laan, A.; Stromberg, R.; van Boom, J.; Kuyl-Yeheskiely, E.; Efimov, V.; Chakhmakhcheva, O. *Tetrahedron Lett*., **1996**, *37*, 7857–7860.
7. Efimov, V.; Choob, M.; Buryakova, A.; Kalinkina, A.; Chakhmakhcheva, O. *Nucleic Acids Res*., **1998**, *26*, 566–575.
8. Efimov, V.; Buryakova, A.; Choob, M.; Chakhmakhcheva, O. *Bioorgan. Khim*., **1999**, *25*, 613–624.
9. Efimov, V.; Buryakova, A.; Choob, M.; Chakhmakhcheva, O. *Bioorgan. Khim*., **1998**, *24*, 696–709.
10. Tomas, S.; Sarkar, M.; Ratilainen, T.; Wittung, P.; Nielsen, P.; Norden, B.; Graslund, A. J. Am. Chem. Soc., **1996**, *118*, 5544–5552.
11. Efimov, V.; Chakhmakhcheva, O. *Bioorgan. Khim*, **2000**, *26*, in press.
12. Efimov, V; Buryakova, A.; Chakhmakhcheva, O. *Nucleic Acids Res*., **1999**, *27*, 4416–4426.
13. Efimov, V.; Chakhmakhcheva, O. *Bioorgan. Khim*., **1999**, 25, 848–854.
14. Rehman, F.; Audeh, M.; Abrams, E.; Hammond, P.; Kenney, M.; Boles, T. *Nucleic Acids Res*., **1999**, *27*, 649–655.

Downloaded At: 11:58 26 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

15. Efimov, V.; Chakhmakhcheva, O. *In: Solid phase synthesis and combinatorial libraries*, Ed. Empton, R., **2000**, in press.
16. Witting, P.; Kim, S.; Buchardt, O.; Nielsen, P.; Norden, B. *Nucleic Acids Res*., **1994**, 22 5371–5377.
17. Rye, H.; Yue, S.; Wemmer, D.; Quesada, M.; Haugland, R.; Mathies, R.; Glazer, A. *Nucleic Acids Res*., **1992**, *20*, 2803–2812.
18. Phelan, D.; Hondorp, K.; Choob, M.; Efimov, V.; Fernandez, J. *Nucleosides, Nucleotides and Nucleic Acids*, **2001**, *20*, this issue.

Downloaded At: 11:58 26 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Downloaded At: 11:58 26 January 2011

Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Reprints Here" link below and follow the instructions. Visit the U.S. Copyright Office for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on Fair Use in the Classroom.

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our Website User Agreement for more details.

Order now!

Reprints of this article can also be ordered at

http://www.dekker.com/servlet/product/DOI/101081NCN100002316