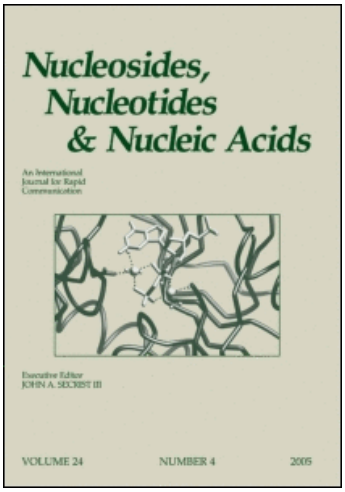


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

KINETICALLY SELECTIVE BINDING OF SINGLE STRANDED RNA OVER DNA BY A PYRROLIDINE-AMIDE OLIGONUCLEOTIDE MIMIC (POM)

David T. Hickman; Paul M. King[a]; Jonathan M. Slater[a]; Matthew A. Cooper[b]; Jason Micklefield
[a] Department of Chemistry, Birkbeck College, University of London, London, United Kingdom [b] University Chemical Laboratory, Cambridge, United Kingdom

Online publication date: 31 March 2001

To cite this Article Hickman, David T. , King, Paul M. , Slater, Jonathan M. , Cooper, Matthew A. and Micklefield, Jason(2001) 'KINETICALLY SELECTIVE BINDING OF SINGLE STRANDED RNA OVER DNA BY A PYRROLIDINE-AMIDE OLIGONUCLEOTIDE MIMIC (POM)', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 1169 — 1172
To link to this Article: DOI: 10.1081/NCN-100002512
URL: http://dx.doi.org/10.1081/NCN-100002512

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 1169–1172 (2001)

KINETICALLY SELECTIVE BINDING OF SINGLE STRANDED RNA OVER DNA BY A PYRROLIDINE-AMIDE OLIGONUCLEOTIDE MIMIC (POM)

David T. Hickman,[1] Paul M. King,[2] Jonathan M. Slater,[2] Matthew A. Cooper,[3] and Jason Micklefield[1,*]

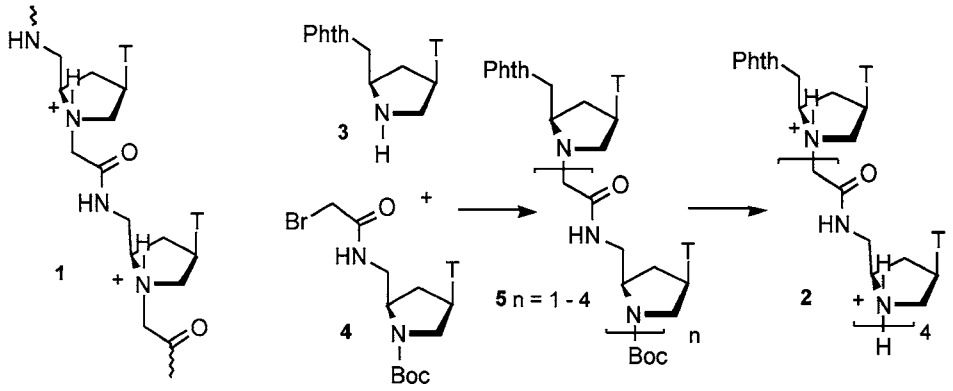
[1]Department of Chemistry, UMIST, PO Box 88, Manchester M60 1QD, United Kingdom
[2]Department of Chemistry, Birkbeck College, University of London, Gordon House, 29 Gordon Square, London WC1H 0PP, United Kingdom
[3]University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, United Kingdom

ABSTRACT

Replacing the sugar-phosphodiester backbone of nucleic acids with a pyrrolidine-amide backbone results in an oligonucleotide mimic POM **1** which binds with high affinity and specificity to complementary DNA and RNA. Unlike other modified oligonucleotides, POM binds much more rapidly to single stranded RNA than DNA.

Backbone modified oligonucleotides are of considerable interest as potential therapeutic agents, probes in molecular biology as well as models to study nucleic acid structure, recognition, function and evolution. To date only a few *de novo* modified oligonucleotides have been reported with positively charged backbones (1). In this paper we introduce a novel pyrrolidine-amide oligonucleotide mimic (POM) **1** (scheme 1) which is cationic at physiological pH due to protonation of the backbone pyrrolidine ring. X-ray crystal structures and molecular modelling

*Corresponding author.



Scheme 1.

studies reveal that the protonated pyrrolidine ring in POM adopts a preferred conformation which closely resembles a typical C3′-endo ribose ring in native RNA (2). In addition, the rigid amide linkage has been shown to be a good replacement for the phosphodiester linkage in DNA (3).

Initial investigations were carried out with a pentamer, T_5–POM **2**, which was synthesised, in solution, by alkylation of the phthalimide (Phth) protected pyrrolidine **3** with bromoacetamide **4**, both of which were derived from *trans*–4–hydroxy– L–proline (4) (scheme 1). Boc deprotection of the resulting dimer **5** (n = 1) and a second coupling with **4** gave the trimer **5** (n = 2). Repeating these steps to the pentamer **5** (n = 4), followed by treatment with dilute HCl resulted in T_5–POM **2**. UV thermal denaturation experiments were then carried out with an equimolar mixture of T_5–POM **2**, and poly(rA) (42 μM each in bases) with initial cooling from 93°C to 15°C followed by heating to 93°C at 0.2°C/min. At pH 7, 0.12 M K^+ a melting temperature (T_m) of 49°C was determined from the first derivative of the slow heating curve. In comparison, native $d(T)_5$ showed no hyperchromic shift with poly(rA), above 8°C under identical conditions, whilst $d(T)_{20}$ formed a duplex with poly(rA) with a T_m of 42°C. Peptide nucleic acid (PNA) lys–T_5–lysNH_2 exhibited only slightly higher affinity for poly(rA) (T_m 56°C). In addition, no melting was observed between T_5–POM **2** and non–complementary poly(rC), (rG) and (rU), whilst Job plots of **2** with poly(rA) revealed a 1:1 binding stoichiometry consistent with the formation of a Watson–Crick base paired duplex.

Upon increasing the ionic strength, whilst maintaining the pH at 7, slightly higher T_ms for **2** with poly(rA) of 52, 54 and 55°C were observed at 0.22, 0.62 and 1.20 M K^+ respectively. In contrast, other cationic modified oligonucleotides have been shown (1) to form less stable duplexes and triplexes with RNA and DNA at higher salt concentration, which is attributed to a reduction in the electrostatic attraction between the oppositely charged backbones (1). The T_ms of **2** with poly(rA) were also highly dependent on pH (T_ms = 45, 46, 54 and 57°C at pH 8.0, 7.5, 6.5 and 6.0 respectively with constant ionic strength 0.12 M K^+). This indicates that more stable duplexes are formed at lower pH, suggesting that the extent of protonation of

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

the nitrogen atom of the pyrrolidine ring, is important for binding to RNA. However, factors other than electrostatic attraction, perhaps conformational changes brought about by protonation at lower pH, are more likely to be the cause of increased duplex stability.

Notably no hyperchromic shift was observed between T_5–POM **2** and equimolar poly(dA) under identical conditions. Only after increasing the concentration of both **2** and poly(dA) five fold (210 μM each in bases) followed by an extended period of incubation at room temperature (48–96h) was it possible to observe melting. This suggests that T_5–POM binds much more slowly to poly(dA) than poly(rA). Conversely the affinity of **2** for poly(dA) (T_m 57°C, pH 7, 0.12 M K^+) was considerably higher than for poly(rA) whilst lys–T_5–lysNH_2 PNA exhibited a lower affinity for poly(dA) (T_m 48°C, pH 7, 0.12 M K^+). Noticeably with **2** and poly(dA) increasing the ionic strength (pH 7, 0.62 M K^+) resulted in two transition melting temperatures 42 and 66°C consistent with the melting of a triple helix to a duplex to single strands. Similarly at lower pH (pH 6, 0.12 M K^+) two transition T_ms, 35 and 64°C, were observed. Job plots of **2** with poly(dA) indicated a 2:1 (T:A) binding stoichiometry consistent of triplex formation.

The difference in the association kinetics of T_5–POM **2** with poly(dA) and poly(rA) was investigated by monitoring the change in A_{260} with time immediately following mixing of equimolar amounts of the polyadenylates with **2** (Fig. 1a). At pH 7, 0.12 M K^+ and a base concentration of 42 μM for both poly(rA) and **2** (●) a 29% hypochromic shift was observed with a $t_{1/2}$ for association of *ca*. 7 min. In contrast no hypochromic shift was observed with poly(dA), under identical conditions (○), even after 15 h. Upon increasing the concentration of both T_5–POM **2** and poly(dA) fivefold (□) only a moderate 6% hypochromic shift was observed with a $t_{1/2}$ of at least 30 min. Clearly this shows that T_5–POM **2** binds

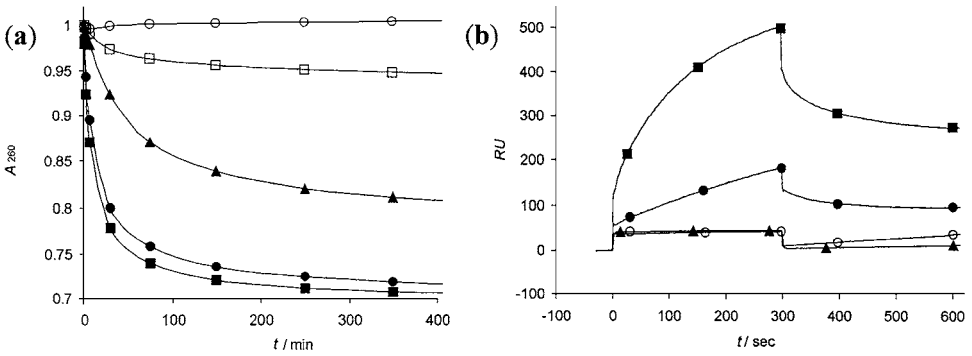


Figure 1. **a**) Normalised UV absorbance (A_{260}) of T_5–POM **2** with poly(rA) and (dA) *vs*. time at 25°C. **2** and Poly(dA) (42 μM each in bases), 0.12 M K^+, pH 7 (○); **2** and Poly(dA) (210 μM), 0.12 M K^+, pH 7 (□); **2** and Poly(rA) (42 μM), 0.22 M K^+, pH 7 (▲); **2** and Poly(rA) (42 μM), 0.12 M K^+, pH 7 (●); **2** and Poly(rA) (42 μM), 0.12 M K^+, pH 6 (■). b) SPR response (*RU*) *vs*. time for T_5–POM **2** injected across $r(A)_{20}$ (■), $d(A)_{20}$ (●), d(AGC TTC AGA GAT CGA TCG GAG AGA GTA GTG-3′) (▲) derivatised surfaces and an underivatised control surface (○).

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

much more slowly to poly(dA) than poly(rA). From these experiments it was also apparent that T_5–POM binds faster to poly(rA) at lower pH and salt concentration, indicating that electrostatic attraction increases the rate of association.

Surface plasmon resonance (BIAcore 2000 instrument) was used to confirm the observed high affinity, sequence specific binding and relative rates of association of T_5–POM **2** with DNA and RNA. In these experiments $d(A)_{20}$, $r(A)_{20}$ and a mixed sequence DNA 30–mer, biotinylated at the 5′–end, were immobilised via streptavidin into a dextran matrix upon a gold sensor chip. The SPR response was then measured against time following injection of T_5–POM **2** (40 μM strand concentration, pH 7, 0.12 M K^+) across each surface (Fig. 1b). This revealed that **2** does bind strongly to both $r(A)_{20}$ and $d(A)_{20}$ but associates faster with $r(A)_{20}$ than $d(A)_{20}$. Significantly, the response sensogram of the mixed sequence DNA was identical to the control non-derivatised surface, confirming that no non-specific interactions occur between POM and non-complementary DNA.

In conclusion we have introduced a novel class of modified nucleic acids with a pyrrolidine-amide backbone and shown that the pentamer T_5–POM **3** binds sequence specifically to both ssDNA and ssRNA with an affinity that is much higher than native nucleic acids and is similar to PNA. However unlike PNA, T_5–POM binds much faster to ssRNA than ssDNA. Other oligonucleotides such as 2′,5′–linked RNA and DNA exhibit a thermodynamic binding selectivity for native ssRNA over ssDNA (5), but as far as we are aware T_5–POM is the first modified oligonucleotide that can kinetically discriminate between the two. As a consequence of this POM may have significant advantages as a therapeutic agent, as an hybridisation probe in gel shift assays, in arrays for measuring levels of cellular mRNAs (trancriptomics), or in the affinity purification of RNA.

REFERENCES

1. R. O. Dempcy, K. A. Browne and T. C. Bruice, *J. Am. Chem. Soc*., **1995**, *117*, 6140; B. A. Linkletter, I. E. Szabo and T. C. Bruice, *J. Am. Chem. Soc*., **1999**, *121*, 3888; D. P. Arya and T. C. Bruice, *J. Am. Chem. Soc*., **1998**, *120*, 12419; M. D'Costa, V. A. Kumar and K. N. Ganesh *Org. Lett*., **1999**, *1*, 1513.
2. C. Altona and M. Sundaralingam, *J. Am. Chem. Soc*., **1972**, *94*, 8205; W. Saenger, *Principles of Nucleic Acid Structure* (Ed. C. R. Cantor), Springer–Verlag, New York, **1984**.
3. A. De Mesmaeker, A. Waldner, J. Leberton, P. Hoffmann, V. Fritsch, R. M. Wolf and S. M. Freier, *Angew. Chem. Int. Ed. Engl*., **1994**, *33*, 226.
4. G. L. Baker, S. J. Fritschel, J. R. Stille and J. K. Stille, *J. Org. Chem*., **1981**, *46*, 2954; G. Lowe, T. Vilaivan, *J. Chem. Soc. Perkin Trans. 1*, **1997**, 539.
5. P. A. Giannaris and M. J. Damha, *Nucleic Acids Res*., **1993**, *21*, 4742; T. L. Sheppard and R. C. Breslow, *J. Am. Chem. Soc*., **1996**, *118*, 9810.

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

Request Permission or Order Reprints Instantly!