Interaction of nucleic acid polymers with the large and small forms of the hepatitis delta antigen protein

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INTRODUCTION AND AIMS

- Nucleic acid polymers (NAPs) are phosphorothioated oligonucleotides (PS-ONs) that inhibit HBV[1,2] and HDV[3] infection.
- REP 2139 is only weakly active in blocking the entry of HBV[4] and cannot block entry of HDV[5] yet has potent antiviral activity against both infections.
- In HBV, NAPs act via a post-entry mechanism to block the assembly and or release of HBV subviral particles[6,7], effectively clearing HBsAg from the circulation[1-3].
- Distinct antiviral mechanisms for NAPs in HDV are present but uncharacterized[3].
- Antiviral investigations of NAP activity in tissue culture models are complicated by altered intracellular trafficking of PS-ONs *in vitro* compared to that occurring *in vivo*[7-9].
- A fluorescence polarization (FP)-based, cell-free interaction assay was validated for NAPs based on known PS-ON protein interactors[10, 11] and was used to examine interactions of NAPs with the hepatitis B surface antigen (HBsAg) and the small (S-HDAg) and large (L-HDAg) isoforms of the hepatitis delta antigen protein.

MATERIAL & METHODS

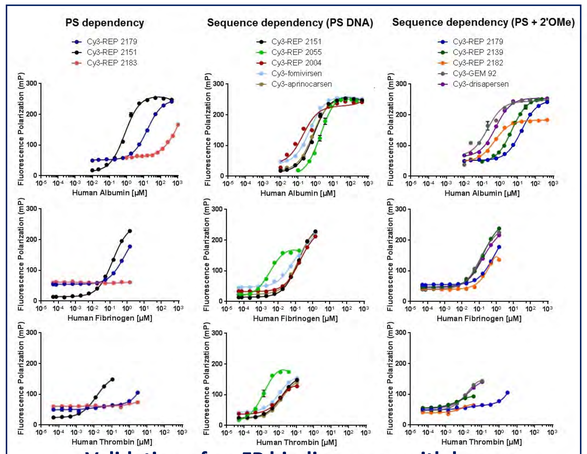
- Fluorescent labelling of various NAP species was done by 5' conjugation of Cy3.
- FP binding assays were realized by adding increasing concentrations of the tested proteins and 3nM Cy3-labelled PS-ONs in a total volume of 50µL assay buffer (80mM NaCl, 1mM EDTA, 10mM ß-mercaptoethanol (ß-MCE), 0.1% Tween-20 in PBS). The samples were mixed and incubated for 50 min at room temperature. For each plate, (Model 3686; Corning, Acton, MA), FP measurements were performed using a ClarioStar apparatus (BMG, Germany) with wells containing buffer only and PS-ONs only as controls for background fluorescence and basal FP level determination, respectively. The polarization degrees (FP) were measured with an excitation filter of at 540nm (bandwidth 20 nm), LP566 dichroic mirror and an emission filter of 590 nm (bandwidth 20 nm)
- Proteins used in this assay;
  - Purified human albumin, thrombin and fibrinogen (Sigma Aldrich).
  - Full length HBAg (strain awy, immuoreactrive with serum from HBV-infected subjects), recombinant preS1 and preS2 (Prospec-Tany Technogene Ltd., Israel).
  - Full length, recombinant S-HDAg and L-HDAg (produced in mammalian cells , MyBioSource, USA).

NAPs used in this study

Oligonucleotide	Sequence (5'-3')	Length	PS	2'OMe	5-MeC
fomiversen[a]	GCGTTTGCTCTTCTTCTTGCG	21	+	-	-
aprinocarsen[a]	GTTCTCGCTGGTGAGTTTCA	20	+	-	-
GEM 92[a]	UCGCACCCATCTCTCTCCUUC	21	+	+	-
drisapersen[a]	UCAAGGAAGAUGGCAUUUCU	20	+	+	-
REP 2004	NNNNNNNNNNNNNNNNNNNN	20	+	-	-
REP 2182	NNNNNNNNNNNNNNNNNNNN	20	+	+	-
REP 2183	ACACACACACACACACAC	20	-	+	+
REP 2151	ACACACACACACACACAC	20	+	-	-
REP 2055	**ACACACACACACACACACACACACACACACACACAC**	**40**	+	-	-
REP 2184	ACACACACAC	10	+	+	+
REP 2179	ACACACACACACACACAC	20	+	+	+
REP 2169	ACACACACACACACACACACACACAC	30	+	+	+
REP 2139	**ACACACACACACACACACACACACACACACACACAC**	**40**	+	+	+
REP 2147	ACACACACACACACACACACACACACACACACACAC	40	-	+	+

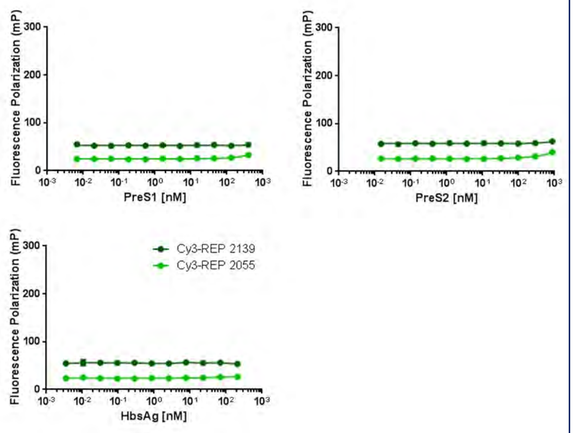
[a] Clinically evaluated PS-ONs for other indications.
N = random incorporation of A, G, T (or 2'OMeT) and C.
Nucleotide positions with 2'O-methyl modified ribose are underlined.
NAPs outlined in green have clinically validated antiviral activity against HBV infection and or HBV / HDV co-infection.

RESULTS



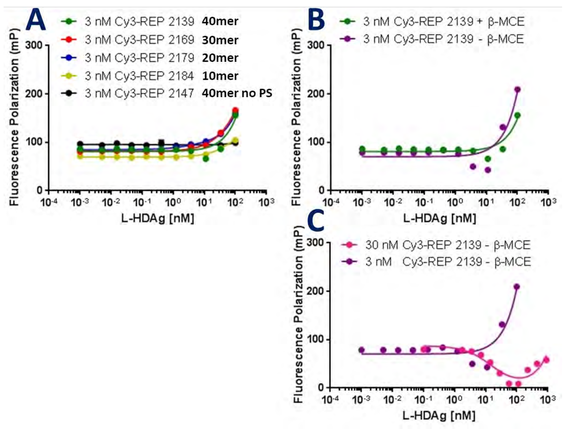
Validation of an FP binding assay with known PS-ON interactors

FP-generated PS-ON protein binding isotherms replicate previously described, class conserved phosphorothioate-dependent, sequence-independent interactions for a variety of clinically evaluated PS-ONs and NAPs with the known PS-ON interactors albumin, thrombin and fibrinogen. The clinical NAPs REP 2055 and REP 2139 are provided for reference in green.



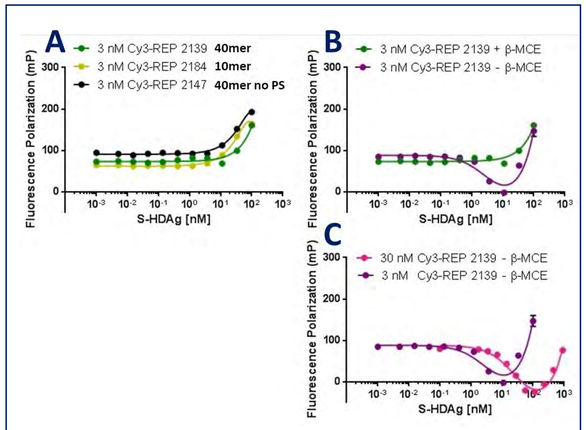
Interaction of REP 2055 and REP 2139 with HBsAg

FP-generated PS-ON protein binding isotherms show no interaction between REP 2055 or REP 2139 and preS1, preS2 or full length strain ayw HBsAg.



REP 2139 interaction with L-HDAg

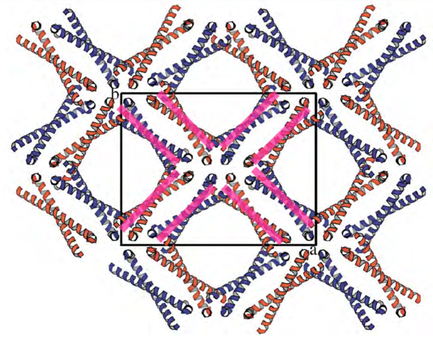
FP-generated NAP - L-HDAg binding isotherms demonstrate a size dependent, phosphorothioation dependent interaction for REP 2139 (A) in which a highly unique FP quenching is observed and is more pronounced in the absence of β-MCE (B). Increased REP 2139 concentration leads to complete FP quenching and blocks the FP supershift (C).



REP 2139 interaction with S-HDAg

FP-generated NAP - S-HDAg binding isotherms demonstrate a size independent, phosphorothioation independent interaction for REP 2139 (A) in which a highly unique FP quenching is observed and is more pronounced in the absence of β-MCE (B). Increased REP 2139 concentration leads to complete FP quenching and blocks the FP supershift (C).

Model for REP 2139 - HDAg interaction



NAPs (in pink) anneal to perpendicularly oriented amphipathic alpha helices in oligomerizing HDAg, resulting in FP quenching. NAP annealing destroys biochemical functionality of these amphipathic alpha helices similar to that observed for NAPs with other viruses[12,13] and may inhibit further assembly of HDAg. (Crystal structure of HDAg From 14).

CONCLUSIONS

- **A FP assay has been developed and validated for the cell-free examination of protein interactions with NAPs.**
- **No interaction with HBsAg, preS1 or preS2 was observed for REP 2055 or REP 2139, suggesting that inhibition of SVP assembly and or release by NAPs is mediated by host interactions.**
- **REP 2139 interacts in a size and phosphorothioation dependent manner with L-HDAg.**
- **REP 2139 interacts in a size and phosphorothioation independent manner with S-HDAg.**
- **The unique signature of FP quenching is consistent with the annealing of NAPs with the perpendicular orientation of amphipathic alpha helices found in L-HDAg and S-HDAg[14,15] and may act to block HDAg oligomerization.**
- **REP 2139 interactions with S-HDAg are consistent with previously reported non-specific interactions of nucleic acids with the chaperone domain of HDAg[16] and may act to block HDAg ribozyme activity.**

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DISCLOSURES

AV is an employee and shareholder in Replicor Inc. All other authors have no conflicts of interest to disclose.

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