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Molecular interaction of gp120 and B40 aptamer: A potential new HIV-1 entry inhibitor drug

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INTRODUCTION

HIV-1 infection and its concomitant disease – Aids, remain major public health problems in southern Africa. While current antiretroviral drugs have prolonged the quality of life for many HIV-positive individuals, they do not eliminate the virus (2, 5). This is compounded by the rapid emergence of drug resistant HIV-1 strains. For these reasons, a search for novel antiretroviral agents with modalities different from those currently in use remains a high priority. As a novel strategy to combat the HIV/Aids epidemic, we have recently discovered and described small nucleic acid ligands called aptamers with antiviral activity (1, 4). These aptamers prevent HIV-1 infection by binding to gp120, which is the viral surface envelope glycoprotein necessary for the earliest stage of infection called entry. In this study we used *in silico* molecular modelling coupled with biochemical experiments to delineate the interaction of one of the aptamers called B40 and gp120; with a view of using the information to develop the aptamer as a novel HIV-1 entry inhibitor drug.

RESULTS AND DISCUSSION

B40 aptamer interferes with gp120 binding to host cell natural receptors.
Using real time surface plasmon resonance technology (BIAcore®), we showed that the B40 aptamer interfered with interaction of gp120 with CCR5 and soluble human CD4 receptors (Figure 1). CD4 and CCR5 are physiological receptors expressed on target host cells that are exploited by the virus for entry and infection.

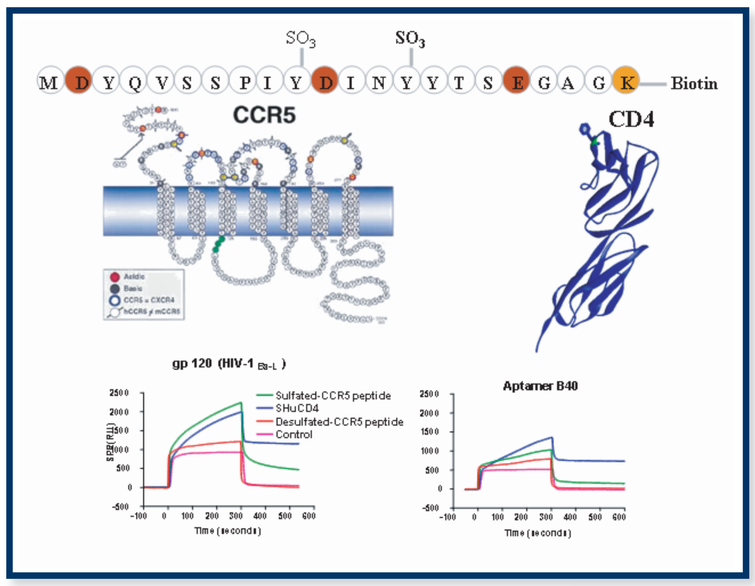


Figure 1. Aptamer B40 significantly reduce binding of gp120 to soluble human CD4 receptor and sulphated CCR5 peptide

Point mutations of core residues on gp120 disrupt binding of B40 aptamer

Mutating a series of amino acid residues, one at a time, on the predicted gp120 interface, disrupt binding of the B40 aptamer as measured by the BIAcore® (Figure 2). The B40 aptamer binds to core conserved residues on gp120 (Table 1). This data suggests that the aptamer binds to functional core conserved residues on gp120 that the virus cannot afford to mutate without loosing fitness and selective advantage.

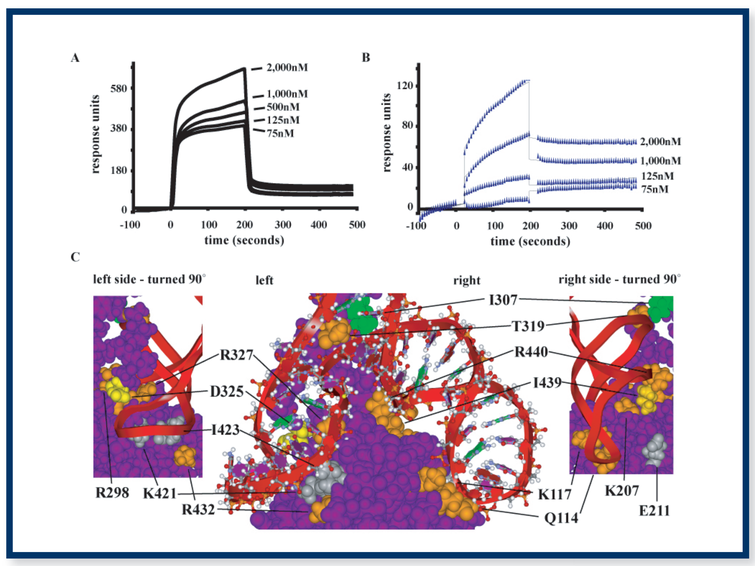


Figure 2. Point mutations in the B40-gp120 interface disrupt binding

B40 aptamer induces distant conformational changes on gp120

B40 aptamer reduced binding of B6, B12 and 2G12 monoclonal antibodies (Figure 3). Conversely, the B40 aptamer enhanced binding of the 19b antibody to the V3 loop and reciprocally, gp120 I307A (a mutation in the V3 loop) increased binding of B40 aptamer. B40 aptamer may therefore prevent HIV-1 infection of host cells by also causing distant non-productive conformational changes in gp120 that affect virus-receptor interactions.

TABLE 1: Gp120 alanine mutations and their effect on B40t77 binding.

Mutation[a]	k_a (1/Ms)[b]	k_d (1/s)[c]	K_d (M)[d]	Chi^2[e]	Effect[f]
Wild-type	1.52E+4	5.82E-4	6.66E-8	1.01	/
Q114A	1.48E+4	4.85E-3	3.28E-7	3.46	*
K117A	4.97E+3	3.32E-3	6.69E-7	0.644	*
K207A	9.81E+3	2.71E-3	2.76E-7	2.57	*
E211A	4.72E+4	2.71E-3	4.58E-8	0.412	/
R298A	6.80E+3	3.62E-3	5.32E-7	0.620	*
I307A	1.22E+6	7.76E-3	6.99E-9	1.39	+
T319A	1.41E+4	6.03E-3	4.27E-7	2.65	*
D325A	6.61E+1	2.40E-2	3.63E-4	3.76	***
R327A	7.33E+3	2.46E-3	3.36E-7	5.27	*
K421A	3.33E+5	4.68E-3	1.41E-8	1.43	/
I423A	4.20E+5	5.85E-3	1.39E-8	1.70	/
R432A	4.66E+3	3.89E-3	8.34E-7	1.63	*
I439A	1.06E+4	3.22E-3	3.03E-7	1.63	*
R440A	8.03E+3	3.49E-3	4.35E-7	0.645	*

[a] Amino acid numbering is based on the HIV-1 $_{Ba-L}$ gp120 sequence.
[b] k_a represents the association rate constant.
[c] k_d represents the dissociation rate constant.
[d] $K_d = k_d / k_a$, and represents the equilibrium dissociation constant.
[e] Chi^2 = residual square sum.
[f] The effect of B40t77 binding to mutant gp120 as compared to wild-type. / = no change, * ≤ 10^1 decrease in binding, *** ≥ 10^3 decrease in binding, + = increase in binding

The B40-gp120 interface is stabilised by a network of hydrogen bonds

The 10 gp120 amino acids that reduce B40 aptamer binding upon mutation (Table I), 7 (Q114, K117, T319, D325, R327, K432, and R440) are within 2.5Å of a B40 hydrogen bonding partner (Figure 4A-C), 2 (K207 and R298) are approximately 6Å from a hydrogen-bonding (h-bonding) partner, and the remaining 1 (I439) is next to a h-bond donor (R440) and thus may cause a conformational change that disrupts h-bonding (Figure 4C). Arginine may be the most common amino acid to participate in h-bonding with B40 due to its potential to form 3 h-bonds (Figure 4C), as seen with other aptamer-protein complexes (3). HIV-1$_{Ba-L}$ gp120 amino acids that h-bond appear to donate a hydrogen to B40 in all but 2 cases, E322 and D325, where gp120 then becomes the h-bond acceptor. D325A also had the largest reduction in binding, which may be explained in part by the different nature of this hydrogen bond (Figure 4B). These results suggest that the B40-gp120 interface is primarily stabilised by a hydrogen bonding network between charged amino acids of gp120 and the oxygen atoms in the backbone of B40 aptamer.

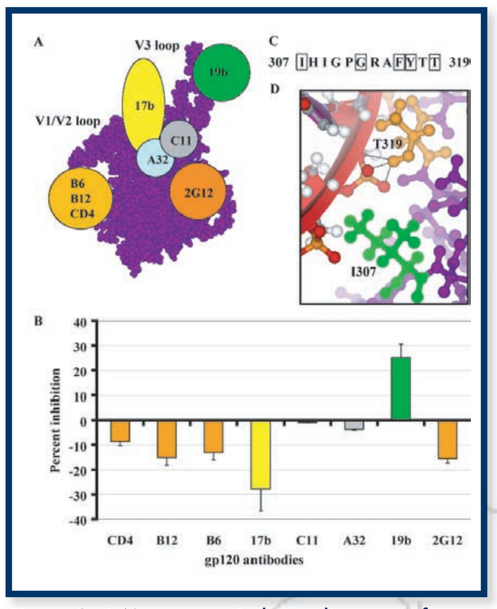


Figure 3. B40 aptamer induces distant conformational changes in gp120

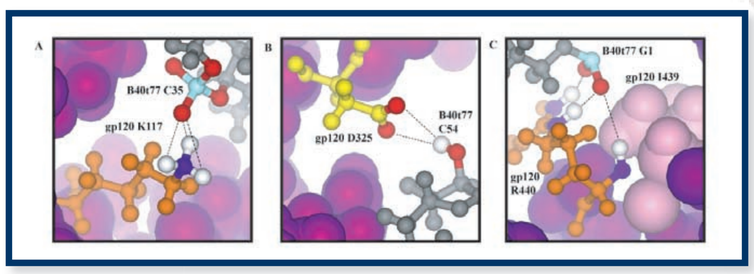
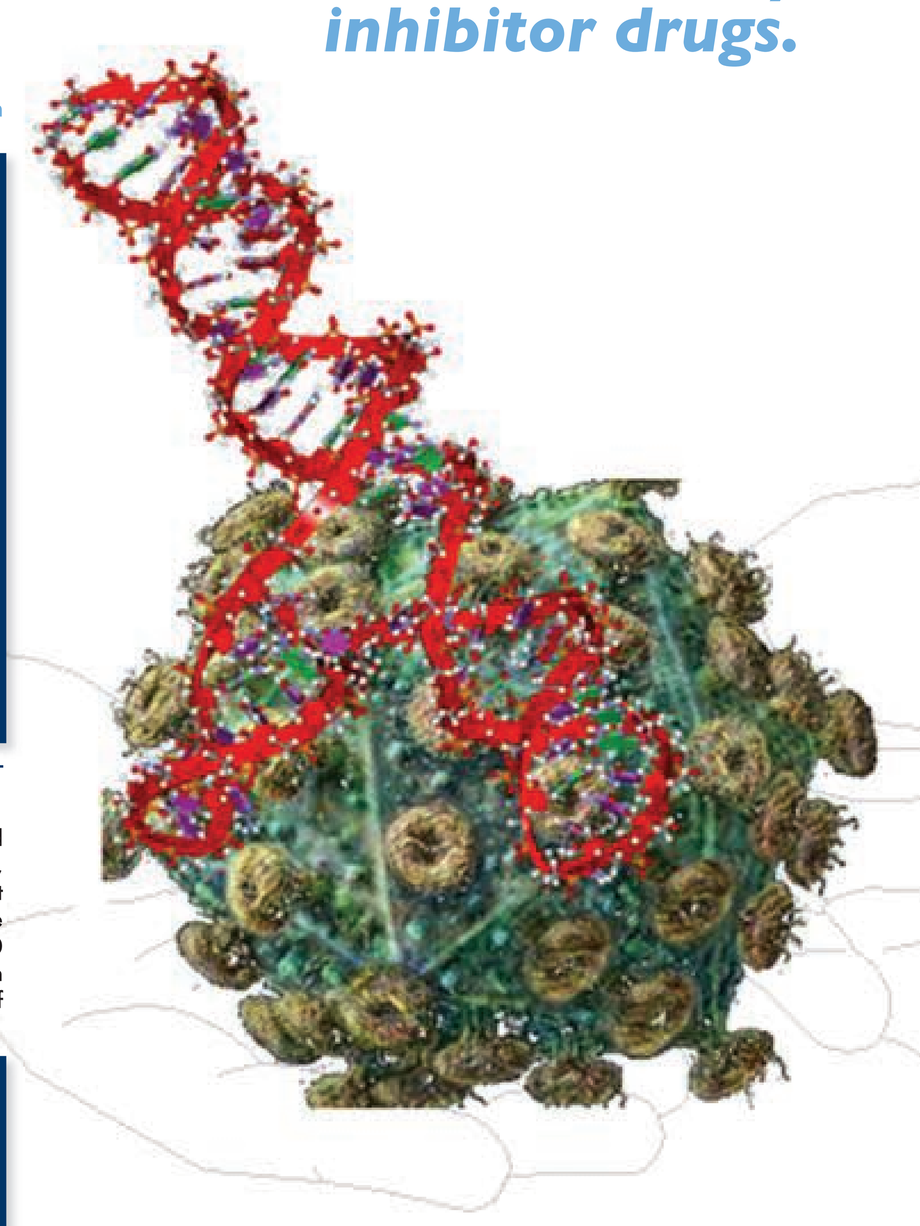


Figure 4. The B40t77-gp120 interface is stabilised by a network of hydrogen bonds

SUMMARY AND CONCLUSION

- The B40 aptamer that potently and broadly prevents HIV-1 infection binds to core conserved residues on gp120
- The aptamer binds to functional core conserved residues on gp120 that the virus cannot afford to mutate without loosing fitness and selective advantage
- The aptamer prevents HIV-1 infection by direct steric hindrance and also by causing distant non-productive conformational changes in gp120 that affect virus-receptor interactions
- The next step is to assay the utility of the aptamer in more dynamic animal models in preclinical studies and eventually in clinical trials with a view of using the information gained to develop the aptamer as a novel HIV-1 entry inhibitor drug.

CSIR scientists are developing small, artificially-engineered molecules called aptamers as potential new HIV-1 entry inhibitor drugs.



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