Binding Kinetics of Aptamers to gp120 derived from HIV-1 Subtype C

LAURA MILLROY[1,2], GRACE LONDON[1,3], ROBIN VEALE[2], MARCO WEINBERG[4], MAKOBETSA KHATI[1,3]
[1]CSIR, Aptamer Technology, Drug Discovery and Development, Biosciences, Pretoria, South Africa [2]School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa.
[3]Department of Medicine, Groote Schuur Hospital and University of Cape Town, Cape Town, South Africa. [4]School of Pathology, University of the Witwatersrand, Johannesburg, South Africa.
Email: lmillroy@csir.co.za

INTRODUCTION

Aptamers are artificial nucleic acid ligands that can be engineered to bind with high specificity to a macromolecule. Their binding specificity and small size allow for a range of therapeutic applications. One avenue of research is to develop aptamers with specific and strong affinity to the HIV-1 envelope glycoprotein gp120 and act as novel HIV-1 entry inhibitor drugs or as targeted drug delivery systems to HIV-1 infected cells. Prior to any downstream applications, novel gp120 aptamers need to be biophysically characterised with regards to their target binding characteristics.

METHODS

Eight aptamers previously isolated against gp120 that effectively block HIV-1 entry into target cells were analysed in this study. Secondary structures and the relative stability of the aptamers were computationally determined and analysed using MFOLD (**mfold**.rna.albany.edu). Using surface plasmon resonance technology (with a BIAcore) aptamer binding was tested and binding kinetics was determined **(Figure 1-3)**.

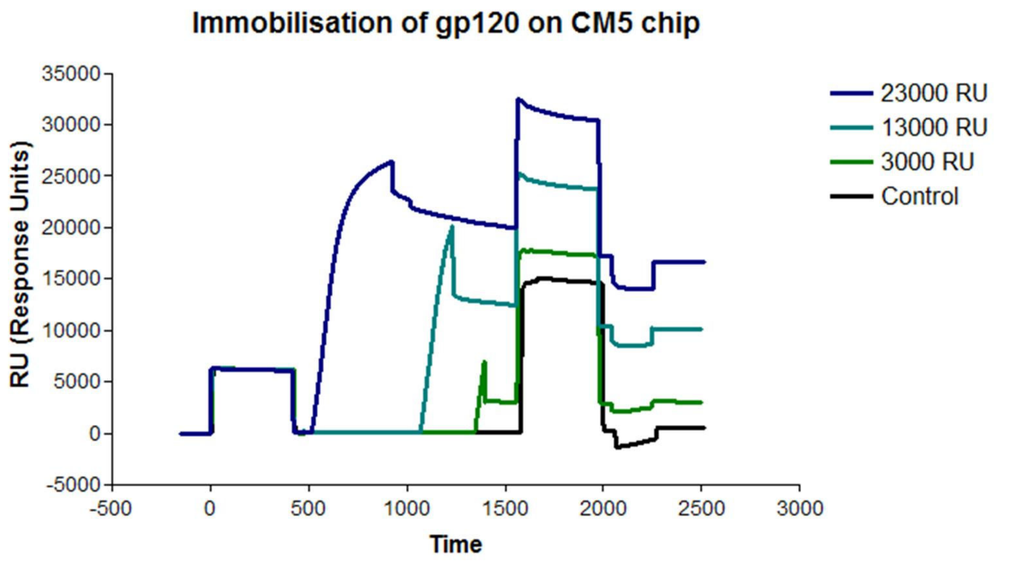

Figure 1 : Preparation of CM5 chip for binding and kinetics

To prepare the BIAcore chip for binding and kinetic analysis, it was activated with EDC:NHS before protein was injected into three flow cells. Protein binds to the activated chip by amine coupling. Ethanolamine was then used to block remaining active sites and glycine-HCl to removed weakly bound protein. Chips were prepared using this method for kinetic and binding analysis.

Five aptamers (a1-5) raised against gp120 derived from a subtype B isolate were tested for binding to monomeric HIV-1_{DU151} gp120 followed by kinetic analysis. The remaining three aptamers (p1-3) were raised against a subtype C HIV-1 isolate and were tested for binding on monomeric HIV-1_{CAP45} gp120. All three were used for binding kinetic assessment on monomeric HIV-1_{CAP45} gp120 .

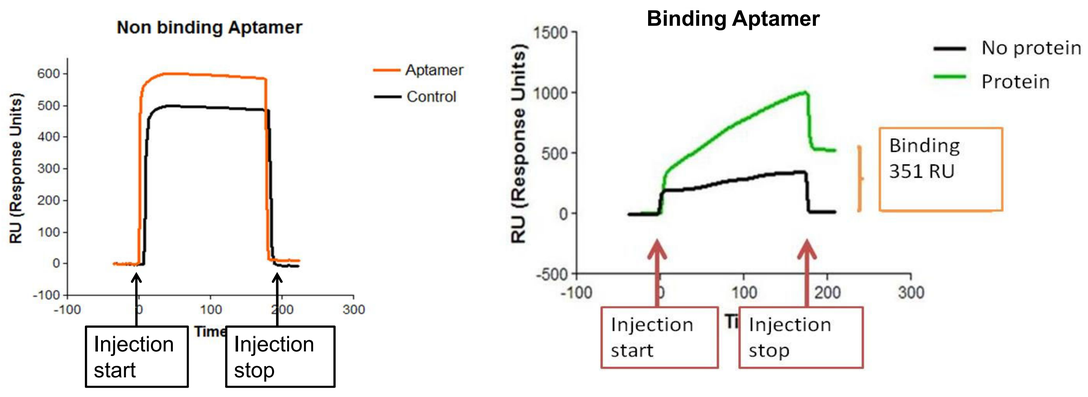

Figure 2 : Aptamer binding to gp120 determined on the BIAcore 3000

Aptamer injected at 200nM concentration over the prepared BIAcore chip. The aptamer injection start and stop points are indicated. After injection the relative binding of the aptamers was determined in response units. Aptamers found to bind with significant response units were used for determining the kinetics of the binding interaction while those that did not bind were excluded.

RESULTS & DISCUSSION

The dissociation constants were found to be similar to that of previously characterised anti-gp120 aptamers within the nanomolar range of 16.9 nM to 221 nM **(Table 1).** Using the dissociation constant and predicted structure, two aptamers were identified as having the greatest potential for further characterisation and utilization.

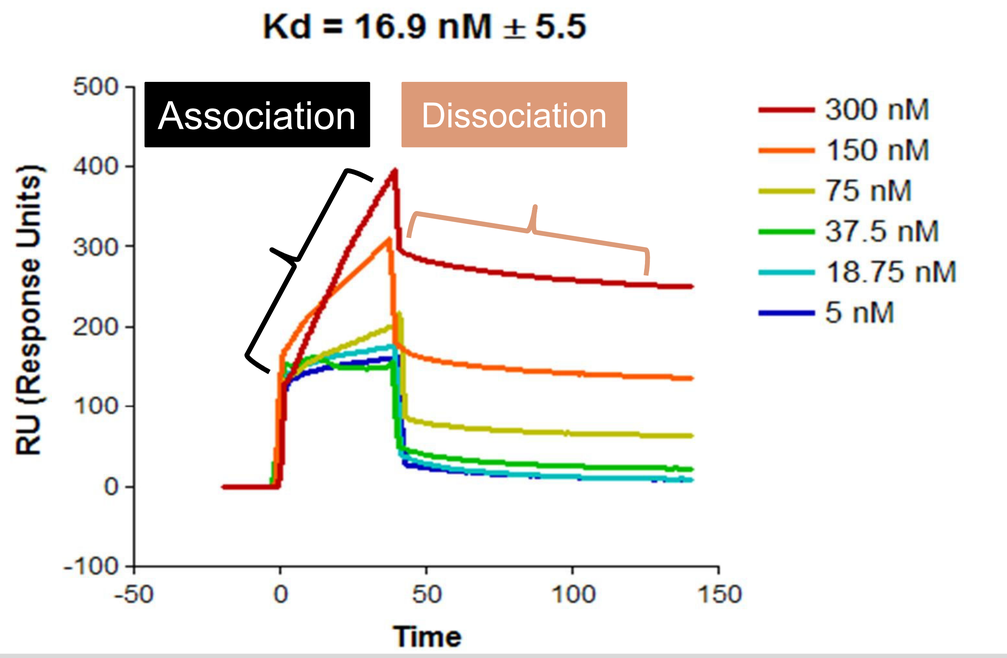

Figure 3 : Aptamer binding to gp120 determined on the BIAcore 3000

Aptamer injected at different concentrations over the prepared CM5 chip. The association and dissociation phases of the aptamer are indicated. The upper concentration aptamers remain bound after the dissociation phase. The lower concentration aptamers dropped close to the base line. The resultant K_d was 16.9 nM ± 5.5.

Table 1: Summary table of aptamer binding response (RU) units, K_d for gp120 binding and ΔG for structural stability

Aptamer	K_d	Binding response units	ΔG
p3	16.9 nM ± 5.5	351	-19.72
a2	23 nM ± 8	85	-19.05
B40t77*	**31.2 nM ± 2**		**-23.79**
p2	33 nM ± 14	739	-18.4
UCLA	34.5 nM ± 0.7	106 / 597	
a3	51 nM ± 8.5	147	-11.3
a4	179 nM ± 81	147	-26.8
p1	221 nM ± 81	90	-23.13

*Determined by Dey (2005)

Aptamers listed in green were raised against a subtype C virus while those in orange were raised against a subtype B virus. Aptamers shown to bind gp120 were used for kinetic analysis. UCLA was used as a control for subtype B and subtype C raised aptamers and so both RU of binding are shown in the table. Two aptamers showed a stronger kinetic than B40t77, an anit-gp120 aptamer identified as a strong binder. The ΔG value indicates the relative stability of suboptimal predicted structures using MFOLD.

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