Five noncovalent peptidic ligands show different affinity rankings in solution and gas phase

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Introduction

Study of noncovalent protein-ligand complexes by Electrospray Ionization (ESI) Mass Spectrometry (MS) is a relatively new and fast developing field. Mass spectrometry stands out against conventional techniques because the system under study (namely, protein-ligand complex) is handled and analyzed in vacuum. Thus, it is possible to study properties of the system, eliminating the effect of the solvent. It can provide valuable thermodynamical and structural information about the system. However, it is difficult to correlate the protein-ligand binding affinity data, acquired in the gas phase, with the "real" values, obtained from the study of the complex in its natural environment. The present work is a comparison of binding affinities of 5 noncovalent peptidic ligands, differing from each other in chirality of one certain amino acid, to the V E G F protein in solution and gas phase.

Protein

VEGF is a member of the cysteine-knot growth factors family. It is a homodimer, stabilized with 2 intermolecular disulfide bridges. Its function as a key mediator of angiogenesis made it a highly validated drug target for anti-angiogenic research.

For our study we used a 11-109 construct of $VEGF_{121}$ [1], the smallest biologicaly active form of VEGF (Fig.1). In our experience it is a very stable protein, which makes it suitable as a model system.

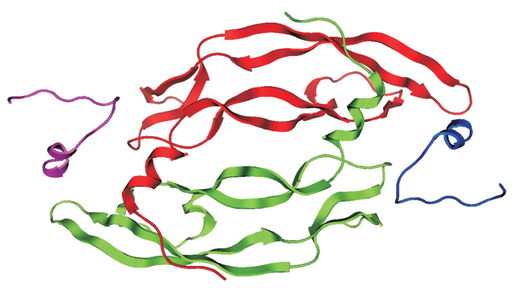

Figure 1. The NMR structure of 11-109 construct fo $VEGF_{121}$ in complex with two v107 ligands.

Ligands

The 19 amino acids long peptide v107, previously reported to be a strong ligand to VEGF [2], was taken as a starting point and synthesized together with 4 mutants (Fig.3). Each one differs from the "wild-type" peptide in chirality of one amino acid. Residues with different solvent accessible surface buried in the binding site, were picked for mutations (Fig.2).

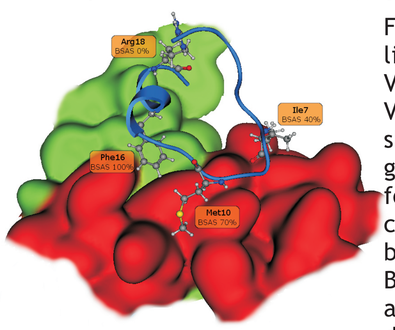

Figure 2. The wild-type ligand, attached to VEGF binding pocket. VEGF monomers are shown in red and green. The residues, for which chirality was changed, are shown as ball-and-stick models. Burried surface accessible area is denoted for them.

v107 (wild type): GGNECDIARMWEWECFERL
v107DPhe16: GGNECDIARMWEWECD-PheERL
v107DMet10: GGNECDIARD-MetWEWECFERL
v107DIle7: GGNECDD-IleARMWEWECFERL
v107DArd18: GGNECDIARMWEWECFED-ArgL

Fig 3. List of peptides, used as ligands to VEGF

MS experiments

The Collision Induced Dissociation (CID) experiments were carried out on Waters SYNAPT HDMS TOF mass spectrometer with Advion nanoMate nanoESI ion source. VEGF-ligand complex was dissolved in a 200 mM Ammonium Acetate volatile buffer (pH 6.9) and introduced in the mass-spectrometer. Concentrations of 10 uM (VEGF) and 30 to 100uM (ligands) were used.

For all the ligands, $[VEGF + 2L]^{+10}$ ions were isolated in the quadrupole region of the instrument and activated by collisions with neutral buffer gas (Argon). The voltage bias between the trap and the quadrupole was varied, changing the energy of the ion beam, entering the trap. A dissociation curve for each complex was obtained (Fig.4).

The two-step Impulsive Collision Transfer (ICT) model [2] was adapted and used to calculate the energy, transferred to the complex on its way through the collision cell. The results of the calculations are presented in Table 2.

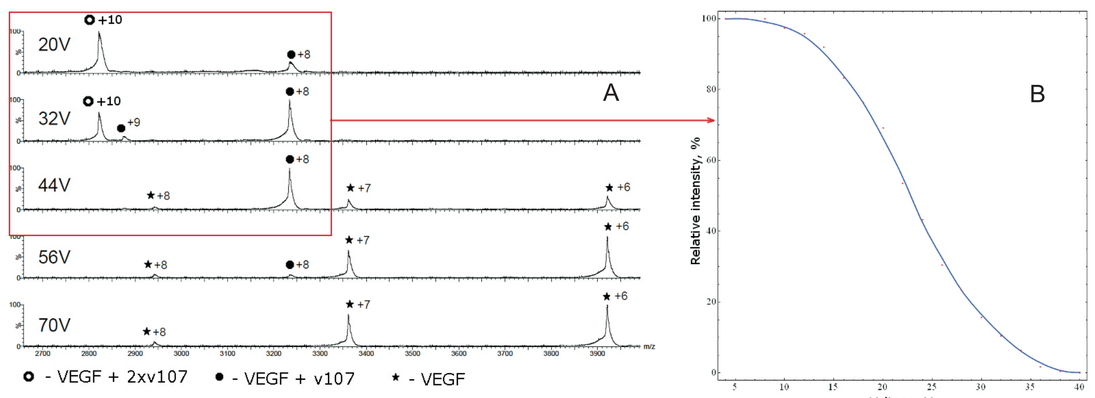

Figure 4. A. The set of mass-spectra, showing the evolution of ion distribution with increase of energy of collisions between the complex and the buffer gas. B. The smoothed dissociation curve for the dissociation reaction

Results

NMR			MS		
-ΔG, kJ/mol	K_D, uM	Ligand	Ligand	ΔU, *V*	ΔE_{int}, e*V*
36.4 ± 13.0	1.02 ± 0.18	v107	v107	22	36.29
31.0 ± 2.5	3.50 ± 0.60	v107DArg18	v107DIle8	24	39.58
21.3 ± 6.2	252.05 ± 76.96	v107DIle8	v107DArg18	25.5	42.06
19.9 ± 3.4	312.60 ± 53.23	v107DPhe16	v107DPhe16	29	47.83
16.7 ± 5.1	1811.62 ± 147.17	v107DMet10	v107DMet10	34	56.08

Increase of stability (↓ in solution) / Increase of stability (↑ in gas phase)

Table 2. Values of K_D, free Gibbs energy, voltage and internal energy increase, obtained for complexes of VEGF with 5 peptidic ligands. The left part corresponds to the "solution behavior", right part - for the "gas phase behavior". Rankings in terms of stability are practically opposite.

NMR

Solution state affinity for the ligands and VEGF was determined by observing the chemicals shifts of the backbone signals induced upon titration with the ligands by NMR CSP (Fig.5). Titration points were selected to cover the optimal range of the curve. For every peptide four signals with the strongest shift were evaluated. The data was fitted to the Two Equal Binding Sites model and an average K_D calculated (Table 2).

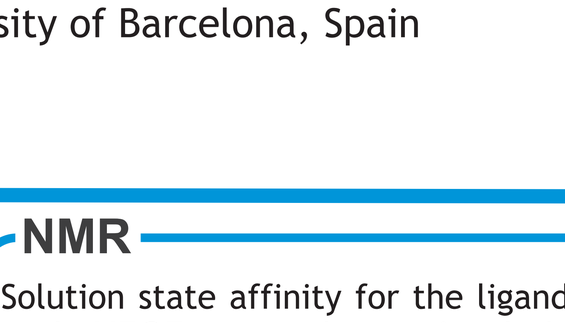

Figure 5. A. ^{15}N-^{1}H HSQC spectra of a 100 uM sample of (methyl^{13}C)-Met- all-^{15}N $VEGF_{11-109}$ at 45 °C titrated with v107DIle7 (600 MHz with cryoprobe). B. Zoom of Lys48 shifts. C. Fititing of 2D shifts to the model function in order to calculate the K_D

ITC

Isothermal Titration Calorymetry (ITC) experiments were carried out for two of five ligands, v107 (Fig.6) and v107D-Arg18, in order to provide a control for the NMR CSP experiments. Being able to measure the heat of the reaction directly, ITC provides the most accurate dissociation constant values. The experiments showed good agreement between K_D values obtained from NMR CSP and ITC (Table 1).

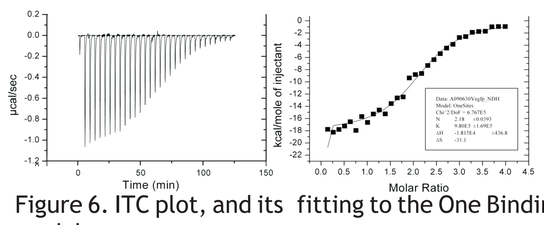

Figure 6. ITC plot, and its fitting to the One Binding Site model.

Ligand	K_D by NMR	K_D by ITC
v107	1.02 ± 0.18	1.04 ± 0.37
v107DArg18	3.50 ± 0.60	7.91 ± 2.31

Table 1. Dissociation constant values for v107 and v107DArg18, obtained from NMR CSP and ITC experiments

Discussion

It appears that trend of stability of the studied noncovalent complexes is reversed in the gas phase relatively to the solution. One possible explanation of this behavior is schematically shown on Fig.7 and discussed below.

Binding of wild type peptide (v107) to VEGF is maintained almost exclusively by hydrophobic interactions. Thus, introducing point mutations to residues with different value of BSAS, we disturb the hydrophobic surface of the peptide, making the favorable entropic effect of hydrophobic interactions less prominent for the in-solution binding.

On the other hand, in the gas phase hydrophobic interactions play a very poor role. Thus, well organized hydrophobic surface will be unfavorable for the complex stability in the gas phase, making in more difficult to form highly-energetic electrostatic interactions. Disturbing this by point mutations lets electrostatic interactions to be formed easier, thus increasing stability of the complex in the gas phase.

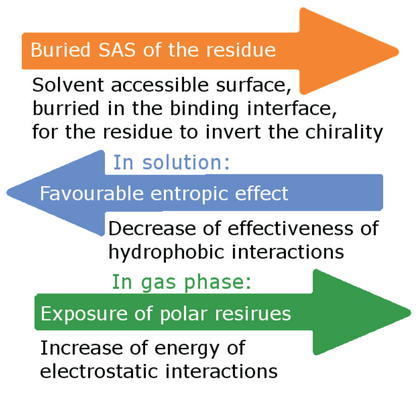

Figure 7. Scheme, illustrating the discussion section.

Conclusion

In this work stability of VEGF in complex with 5 noncovalent peptidic ligands was studied, both in liqiid phase by NMR and ITC methods and in gas phase by MS. It was shown, that in this particular case the ligands show reversed stability rankings for liquid and gas phases. An explanation of this behavior is presented.

Acknowledgement

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