Engineering the NK1 fragment of HGF/SF as a MET receptor antagonist

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Introduction

The growth and motility factor hepatocyte growth factor/scatter factor (HGF/SF) and its receptor, the tyrosine kinase encoded by the c-MET proto-oncogene, exert major roles in cancer invasion and metastasis and are key targets for therapy. Specifically, HGF/SF and MET cause tumour cells to loose contacts with neighbours and become highly motile initiating tumour invasion of adjacent tissue and, ultimately, the growth of distant tumours. As a result, a major effort is underway in order to develop inhibitors of HGF/SF-MET signalling for cancer therapy.

MET antagonists may constitute effective inhibitors of HGF/SF-MET signalling and both the single chain form of HGF/SF and the chain of the mature form, a fragment also known as NK4, inhibit the assembly of the active signalling complex and have antagonistic activity in vitro and in vivo. Here we report that dimerisation deficient mutants of a small fragment of HGF/SF containing the N-terminal and the first kringle domain (NK1) represent novel and effective MET antagonists.

Results

NK1 consistently crystallises as a dimer in a head to tail orientation [1]. The dimer interface buries in excess of 2,000 Å² and consists of a central area in which the inter-domain linkers (K122, D123, Y124, I125, R126 and N127) make extensive contacts. We have introduced an extensive number of individual or cluster alanine mutations in the inter-domain linker of NK1. These included single mutations (LA: K122A, LB: D123A, LC: Y124A, LD: R126A and LE: N127A) double mutations: (LF: D123A:Y124A and LG: D123A:N127A) and a triple mutation: (LH: D123A:Y124A:N127A). The single linker mutants LC (Y124A) and LE (N127A) bound MET with affinities indistinguishable from wild type NK1 in surface plasmon resonance experiments. In marked contrast, the double mutants LF (D123A:Y124A) and LG (D123A:N127A) displayed >100 fold reduced MET binding affinity (data not shown).

The stoichiometry of complexes formed by the NK1 mutants with MET567, a fragment of MET ectodomain carrying the ligand-binding -propeller domain, was studied next by small angle X-ray scattering (SAXS). Fig 1a shows experimental SAXS scattering profiles of wild type NK1 and the LC mutant in complex with MET567. The overall structural parameters computed from the SAXS experiments indicate that, in the presence of 12mer heparin, the complex formed by NK1 and MET567 is dimeric and has a 2:2 stoichiometry in solution whereas that formed by the linker mutant LC and MET567 has 1:1 stoichiometry. This conclusion is further supported by ab initio shape determination.
Low resolution bead models of the complexes formed by wild type NK1 or the LC mutant, reconstructed with MONSA [2], neatly fit the experimental data and are shown in Fig 1b (LC-MET567) and 1c (NK1-MET567). The latter model accommodates two copies of a 1:1 NK1-MET567 complex with the dimer built around the NK1 dimerisation interface and the two MET567 located at the periphery.

Figure 1: The complexes formed in solution by wild type NK1 or the linker mutant LC with MET567 by small angle X-ray scattering. (a) scattering intensities as functions of momentum transfer ($s=4\pi \sin(\theta)/\lambda$, where $2\theta$ is the scattering angle and $\lambda=0.15$ nm is the X-ray wavelength). Experimental data are shown as black dots, fits from ab initio models are presented by red solid lines. (b and c) ab initio models of the complexes formed by the LC (Y124A) mutant and wild-type NK1 (yellow beads) and MET567 (grey beads) in the presence of 12mer heparin. The complex formed by the LC mutant has 1:1 stoichiometry, the one formed by wild-type NK1 which has 2:2 stoichiometry. The models were generated with MONSA [2].

Conclusions

We have succeeded in converting the NK1 receptor binding fragment of HGF/SF, a partial agonist, into a protein that retains receptor binding but fails to assemble a dimeric complex with the receptor by introducing mutation in the inter-domain linker (Fig 1a and 1b). Because dimerisation is a prerequisite for receptor activation we envisage that these new NK1 mutants may act as receptor antagonists and may find useful applications in cancer therapy.

References