Allosteric inhibition of VIMs metallo-β-lactamases by a camelid single-domain antibody fragment.

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Metallo-β-lactamases (MβLs) are zinc dependent enzymes able to hydrolyze a broad spectrum of clinically useful β-lactam antibiotics, including the most powerful carbapenems. These enzymes are not susceptible to classical β-lactamase inhibitor so that their widespread use, especially amongst multiresistant Gram-negative strains, may urgent a better understanding of these enzymes in order to discover new drugs. The selection of broad spectrum inhibitors against metallo-β-lactamases is made difficult first, by the diversity among MβLs which is classified in 3 subclasses, secondly by their mechanism which does not include any highly populated metastable reaction intermediates and finally by the fact that the compound must remain attractive similar human proteins.

In this context, we have selected from two immune libraries of phages, 55 heavy chain antibody fragments (VH) able to bind the clinically relevant MβL VIM-4. A phage display experiment that was performed in solution by using biotinylated VIM-4 allowed us to select one inhibiting dromedary VH termed CA1838. This inhibiting VH was fused to the ‘cherry’ protein in order to overproduce in E.coli. The inhibition is in the μM range for all the β-lactamases assayed and, with cephalothin, has been found to be mixed hyperbolic with a predominant uncompetitive component. Moreover, a substrate inhibition occurred only when the VH is bound.

With the aim of identifying binding hot-spots of CA1838, VIM-4 residual activities were measured in presence of 17 alanine mutants of the VH. The main binding determinant of the paratope is a stretch of 6 hydrophobic amino acids in the CDR3 (T107, Y108, V109, F110, T112.2 and L114) Peptide-arrays allowed us to identify a conformational epitope on VIM-4 which corresponds mainly to the hydrophobic loop L6 and the C-terminal end of the helix α2. As this binding site is distant from the active-site and alters both substrate binding and catalytic properties of VIM-4, this VH quality to the definition of an allosteric effector. Therefore, the binding of cherry-CA1836 could inhibit the enzyme by interfering with molecular motion required for efficient catalysis. We hypothesized that the amino-acids stretch whose dynamic would be affected could correspond to the N-terminal part of helix α2 which is in direct connection with the active site loop L7. Thus, this work is another indication of the dynamic nature of metallo-β-lactamases, and the different VHs that have been selected could be useful for the resistance typing of pathogenic strains.

I. Phage Display

A selection of VHs that was performed in solution allows the identification of CA1838 as an inhibitor of VIM-4.

II. Paratope analysis

The main part of the interaction is driven by the hydrophobic stretch T107VFGVD, which is in the N-terminal part of the CDR3 loop. These residues are probably present at the interface between the two proteins.

III. Steady state kinetics

The inhibition is in the μM range for all the β-lactamases assayed. By using cephalothin, Michaelis-Menten curves fit to a mixed hyperbolic inhibition model with a predominant uncompetitive component. A substrate inhibition occurred only when the VH is bound.

IV. Epitope mapping

Epitope mapping: scanning and truncation peptide arrays allowed us to define the conformational epitope of CA1838 as being composed of two amino-acids stretches that are in close proximity, solvent exposed and distant from the active site. (i.e. LPVTRA and VLRAAG)

V. Epitope

Substituting the epitope by the corresponding sequence of BlaB results in a chimeric functional MβL that is not recognized by the VH.

Because the VH CA1838 binds to a site distant from the active-site and alters both substrate binding and catalytic properties of VIM-4, this VH quality to the definition of an allosteric effector with kinetic parameters α and β (table 1) quantifying the allosteric coupling.

MD studies have identified a local flip of the active site loop L7 that would be a consequential response to the increase in Zn-Zn distance upon binding of a ligand in the active-site (Salsbury et al. 2009). Binding of CA1838 could prevent such a dynamic flip, thereby acting as an allosteric inhibitor of VIMs MβLs.

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