

Retractable bivalent polypeptide inhibitors

SUMMARY

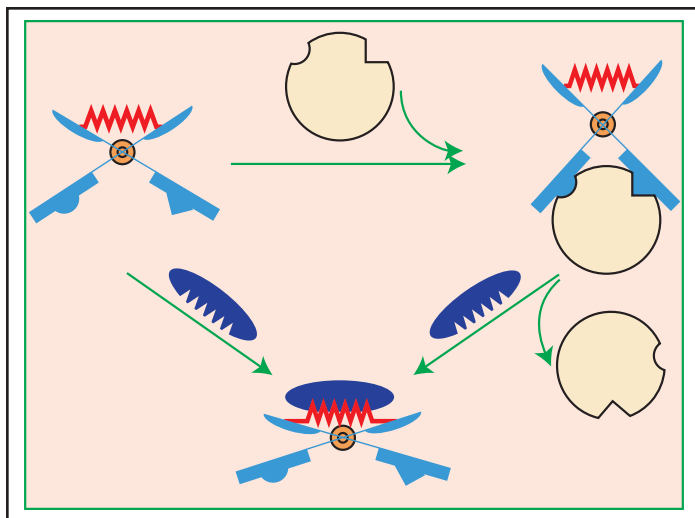
Bivalent polypeptides are particularly suited for the inhibition of difficult pharmaceutical targets such as protein-protein interactions. When constructed from weaker binding moieties, the high-affinity binding of bivalent molecules is intrinsically inducible or retractable. This approach was successfully demonstrated by developing a new generation of thrombin inhibitors. These retractable inhibitors controlled through polypeptide-protein interactions with the flexible peptide bridge that link the two binding fragments may represent an alternative approach to antidote-reversible anticoagulant therapy.

APPLICATIONS

- Controlling biological pathways such as the blood clotting cascades or cell signaling networks.
- Localized release of drug action of the bivalent molecules.
- Stabilizing enzymes such as proteases.
- Novel screening assays for drug discovery targeting protein-protein interactions.
- Development of affinity purification systems complete with agents to release the targeted proteins or enzymes.

CONCEPT

Multivalent molecular designs have emerged as an important tool for protein and polypeptide engineering complementary to the traditional methods of medicinal chemistry and recombinant antibody technology. Taking it one step further, multivalent molecules can be conferred with the ability to bind a target under certain conditions and releasing it under others. These molecules consist of two binding moieties (BM) having an affinity for different sites on the target. The BMs are joined by an



oligomeric or polymeric linker, creating a bivalent ligand having higher binding affinity than each BM taken separately. The flexible polypeptide linker is adapted to respond to a change in its environment. As such, the flexible linker adopts a well-defined conformation, upon binding of a linker-specific protein (e.g. an antibody), which prevents the ligand from acting in a bivalent fashion. This general approach was illustrated by the design of a new generation of bivalent thrombin inhibitors, using moieties binding to the fibrinogen-binding exosite I and the active site of thrombin. The linkers were either one of two bioactive peptides, one of which binds to an SH₂ protein and the second recognizes an antibody. Activation of inhibited thrombin was successfully realized through SH₂-peptide and antibody-peptide interactions.

FEATURES AND BENEFITS

High-affinity binding

Multivalent molecular constructs achieve high-affinity binding by linking together monovalent constituents that have only weak affinities when acting alone.

Versatility in design

The binding “heads” and the connecting linker are in principle interchangeable independently of one another. As such, changing the linker potentially provides numerous ways to control the inhibitory activities. Furthermore, multivalent binding allows the affinity and binding kinetics to be manipulated at will by the presence of constituent monovalent molecules.

Controllable binding

A change in flexibility and/or conformation of the linker that connect binding fragments in bivalent ligands allows their transformation into retractable inhibitors. These inhibitors are responsive to and can be neutralized by polypeptide-protein interactions with the respective protein “antidotes” or by an external trigger such as temperature and radiofrequency waves. Such control of binding could provide, for example, the opportunity to selectively cause drug activation in a localized area of the body.

Validated concept

The effectiveness of the retractable bivalent inhibitor approach was demonstrated using human thrombin, an important target for pharmaceutical design as well as Cdc42, a ubiquitous GTPase controlling cell-signaling networks. Activities of these potent bivalent inhibitors were reversed by the specific, but much weaker, binding of the linker moiety to protein “antidotes”.

PROTECTION STATUS

Polypeptide ligands containing controllable flexible linkers (NRC no. 11615).

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