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Potent Enzyme Inhibitors

Tokuna Josti*

Editorial office, Biochemistry: An Indian Journal, India

***Corresponding author:** Tokuna Josti, Editorial office, Biochemistry: An Indian Journal, India, E-Mail: tokunajosti@gmail.com

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Abstract

There is evidence that dromedary heavy-chain antibodies, which have been in vivo-matured without light chains, constitute a unique source of inhibitory antibodies. After immunizing a dromedary with bovine erythrocyte carbonic anhydrase and swine pancreatic amylase, it was discovered that a significant amount of heavy-chain antibodies circulate in the bloodstream, acting as real competitive inhibitors. Conventional antibodies, on the other hand, do not appear to interact with the enzyme's active region. We next showed that lymphocytes from the peripheral blood may be used to clone variable domain segments in a phage-display vector in a single step. Several antigen-specific single-domain fragments for both enzymes may be easily obtained using bio-panning. We further show that active site binders are significantly represented among the isolated fragments. When examined in chromogenic experiments, these active site binders appear to be strong enzyme inhibitors when generated as a recombinant protein in Escherichia coli. The antigen-binding site of these single-domain antibodies, which consists of only three loops, may be useful in the development of smaller synthetic inhibitors.

Keywords: Dromedary; Enzyme's active region; Recombinant protein; Synthetic inhibitors; Heavy-chain antibodies; Lymphocyte

Introduction

Low-molecular-weight chemicals and proteinaceous molecules that inhibit enzymes have emerged as key medicinal agents. Recent breakthroughs in molecular biology and protein characterization, as well as the wealth of data acquired from genome sequencing studies, have resulted in the identification of an ever-increasing number of novel targets, necessitating the development of particular inhibitors quickly. The synthesis of transition-state analogs is frequently used in the development of such inhibitors. In certain circumstances, molecules are discovered following a thorough examination of a large number of chemical compounds or natural sources. An obvious alternative is the creation of antibody-based compounds. The immune system is universally acknowledged as the ideal method for producing particular binders or reporter molecules against practically all agents. Despite the antibody repertoire's omnipotence, the number of conventional antibodies that operate as competitive enzyme inhibitors (i.e. heterotetramers with two light chains and two heavy chains) is disappointingly low. The incompatible surface topology of the enzyme's active site and the antigen-binding site of conventional antibodies provides a satisfactory explanation for this rare event. According to a recent study of enzyme architectures, the active site is usually always situated in the biggest cleft on the protein surface. Similarly, depending on whether an interaction with haptens, oligopeptides, or proteins is detected, the antigen-binding surface of typical antibodies creates a cavity, a groove, or a flat surface. Convex antigen-binding surfaces are not observed in typical antibodies, which is surprising, in this regard, we have now shown that functional heavy-chain antibodies from Camelidae operate very differently than ordinary four-chain antibodies. Heavy-chain antibodies, in particular, developed the ability to identify protein cavities and, as a result, inhibit enzymes. Camelidae make a large percentage of their functional immunoglobulins as homodimers with only heavy chains and no light chains. Some protein antigens can be used to produce specific heavy-chain antibodies in a dromedary or llama. A minimum-sized antigen-binding domain is found in the N-terminal variable region of these heavy-chain antibodies (referred to as VHH). The structure of a dromedary VHH in complex with lysozyme showed the antigen-combining site of this single-domain antibody fragment's unique surface topography and the relevance of the CDR3 loop for the binding interaction. The N-terminal region of the CDR3 loop, which is 24 amino acids long, protrudes from the antigen-binding surface and penetrates deeply into the lysozyme active site. However, this single result does not support the hypothesis that the large CDR3 loop observed in dromedary heavy-chain antibodies preferentially interacts with antigen clefts and creates

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competitive inhibitors automatically. In this paper, we describe the immunization of a dromedary with extra enzymes and show that a significant part of the polyclonal heavy-chain antibodies binds to the enzymes' active region. VHHs that attach to antigens with nanomolar affinity can be easily cloned from peripheral lymphocytes. We believe that cloning and production of recombinant dromedary VHH antibody fragments in Escherichia coli is a universal and powerful technique for rapidly obtaining a novel type of potent and selective enzyme inhibitor.

Conclusion

To test the generality of creating enzyme-inhibiting dromedary heavy-chain antibodies, two enzymes were chosen: pig pancreatic -amylase and bovine erythrocyte carbonic anhydrase. Both enzymes are widely available, and inhibitors of modest molecular weight and proteinaceous nature have been identified for both, as well as simple enzymatic activity measurements. The antigen-binding site in traditional antibodies is produced by joining the variable portions of the light and heavy chains. Residues found in all six hypervariable areas (three in each domain) may play a role in the antigen's molecular interaction. They provide a suitably big surface area for protein antigens that is essentially flat. At the antigen-binding surface, large projecting flexible loops are rarely seen. These would get immobilized as a result of antigen contact, lowering the binding energy.