Crystal Structure and Characterization of Human Heavy-Chain Only Antibodies Reveals a Novel, Stable Dimeric Structure Similar to Monoclonal Antibodies

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We report the novel crystal structure and characterization of symmetrical, homodimeric humanized heavy-chain-only antibodies or dimers (HC2s). HC2s were found to be significantly coexpressed and secreted along with mAbs from transient CHO HC/LC cotransfection, resulting in an unacceptable mAb developability attribute. Expression of full-length HC2s in the absence of LC followed by purification resulted in HC2s with high purity and thermal stability similar to conventional mAbs. The VH and CH1 portion of the heavy chain (or Fd) was also efficiently expressed and yielded a stable, covalent, and reducible dimer (Fd2). Mutagenesis of all heavy chain cysteines involved in disulfide bond formation revealed that Fd2 intermolecular disulfide formation was similar to Fabs and elucidated requirements for Fd2 folding and expression. For one HC2, we solved the crystal structure of the Fd2 domain to 2.9 Å, revealing a highly symmetrical homodimer that is structurally similar to Fabs and is mediated by conserved (CH1) and variable (VH) contacts with all CDRs positioned outward for target binding. Interfacial dimer contacts revealed by the crystal structure were mutated for two HC2s and were found to dramatically affect HC2 formation while maintaining mAb bioactivity, offering a potential means to modulate novel HC2 formation through engineering. These findings indicate that human heavy-chain dimers can be secreted efficiently in the absence of light chains, may show good physicochemical properties and stability, are structurally similar to Fabs, offer insights into their mechanism of formation, and may be amenable as a novel therapeutic modality.