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Conserved aromatic residues as determinants in the folding and assembly of immunoglobulin variable domains

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ABSTRACT

Detailed analysis of amino acid distribution, focusing on the "framework" regions of both heavy- and light-chain variable immunoglobulin (Ig) domains, distinguished those conserved sequence elements shared by both heavy-chain (VH) and light-chain (VL) domains from those conserved determinants unique to either VH or VL domains alone. Mapping of conserved chemical functionality onto characterized PDB structures showed the analogous placement and utilization of shared determinants in VH and VL structures that are generally similar. Identical Arginine–Aspartic acid ion-pairs located symmetrically on the lateral surfaces of VH and VL domains, respectively, as well as paired glutamine residues that constitute a central contact site between VH and VL domains represent clearly shared molecular features. Three sites of shared aromaticity were found localized to symmetrical sites lining the inaccessible interface of the VH–VL duplex, suggesting an expanded role for strategically conserved aromatic residues from a postulated determinant of individual Ig domain folding to now implicate conserved aromatic sites in the subsequent multi-subunit assembly of native antibody superstructure. Differential domain-specific conservation, representing evolutionary diversification and molecular asymmetry between heavy- and light-chain variable domains was limited, but included amino acids from each functional class and must be evaluated with regard to their possible involvement in heterologous aspects of IgV protein structure–function.

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1. Introduction

The immunoglobulin variable domain (IgV) family is comprised of nine-stranded protein structures exhibiting classic "β-sandwich" architecture, with a characteristic pattern of antiparallel hydrogen-bonded β-strands, stabilized by a single intra-domain disulfide bond. The best recognized role of the IgV type domains is in the primary components of the adaptive immune system, including B-cell and T-cell receptors and all soluble antibody isotypes.

These heterodimeric proteins recognize and bind diverse biological constituents through defined molecular interactions complementary to the surface chemistry of their binding targets. The documentation of amino acid hypervariability in three defined loop regions within the variable domains of both heavy and light chain Ig proteins established the location and extreme genetic diversity of these antigen-specific complementarity-determining regions (CDRs) characteristic of functional immunoglobulins (Wu and Kabat, 1970). The stable association of heavy and light chain proteins, in the formation of functional antibody or receptor structure, results in the configuration of heavy and light chain CDRs into a combined antigen recognition/binding site at the accessible terminal interface of Ig protein superstructures.

The successful folding, alignment and stable association of heavy and light-chain proteins, critical to the progressive formation of native Ig superstructure, must ultimately be defined and guided by complementary intra- and inter-domain interaction of a subset of more conserved "framework" residues which are the subject of this analysis. The antibodies and associated immunological receptors precisely formed by these interactions not only bind antigen with high affinity and specificity, but then effectively signal binding events through sensitive conformational changes that promote interactions leading to activation of pathways in both the innate and adaptive immune response.

Separate genes affect independent biosynthesis and translocation of antibody heavy-chain IgH and light-chain IgL peptides into the endoplasmic reticulum (ER) where individual Ig domains are folded into their disulfide-stabilized Ig structure and subsequently assembled into their composite IgH_2–IgL_2 superstructure.

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Substantial effort has been directed into monitoring the folding and assembly of immunoglobulins occurring in the ER (Askonas and Williamson, 1968; Schubert, 1968; Baumal et al., 1971; Stott, 1972; Leitzgen et al., 1997; Feige et al., 2009), where they are subject to interaction with resident chaperone proteins which appear to monitor and assist not only the sequential folding and disulfide pairing of individual Ig domains but also the correct compositional pairing and assembly of each multi-domain antibody superstructure (Haas and Wabl, 1983; Munro and Pelham, 1986; Kassenbrock et al., 1988; Haas, 1991, 1994; Knittler and Haas, 1992; Melnick et al., 1992, 1994; Knittler et al., 1995; Hochstenbach et al., 1992; Reddy et al., 1996; Skowronek et al., 1998; Lee et al., 1999).

Recent recognition of the extreme cysteine-associated conservation of aromatic residues in several major modular protein families, including the epidermal growth factor (EGF), Laminin, complement-like Sushi, Fibronectin type III (FnIII) and IgV-related Immunoglobulin constant (IgC) domain families (Campion et al., 2013; Hoxha and Campion, 2014), as well as the apparent targeting of aromatic residues present in characterized substrates of both ER-resident Protein Disulfide Isomerase (PDI) (Ruddock et al., 2000; Klappa et al., 2001) and immunoglobulin-binding chaperone BiP have suggested a role for strategically placed aromaticity in protein folding and/or quality control. A few studies have directly employed Ig fragments and synthetic peptides to characterize aromatic determinants of BiP folding substrates in the ER (Blond-Elguindi et al., 1993a,b; Knarr et al., 1995).

Genomic "germline" IgH and IgL loci experience extensive genetic recombination, where combinatorial assembly along with somatic hypermutation contribute to the considerable degree of immunological diversity observed in antigen recognition and binding. Characterization of both genomic and functional transcript sequence has provided a wealth of Ig amino acid sequence data useful for "mining" those conserved elements of IgV protein structure and function that have survived both the segregation of separate VH and VL genes as well as the extensive amplification and diversification that has occurred within constituent variable (V), diversity (D) and junction (J) gene segment clusters (Matsuda et al., 1998).

Previous studies focusing on IgV amino acid distribution sought to identify "core residues" that participate in intra-domain packing of the β-strands in common Ig structures (Padlan, 1994; Chothia et al., 1998), as well as to establish contact sites in the VH-VL interface and assess their impact on the conformation of IgV hypervariable binding structures (Chothia et al., 1985; Vargas-Madrazo and Paz-García, 2003). This study focuses on comparing VH and VL consensus sequence and structure to identify conserved molecular determinants both unique and shared by these related family members to assess chemical functionalities that participate in both shared and differential elements of IgV protein structure-function.

2. Methods

Over five hundred heavy-chain and six hundred light-chain sequences from human, mouse, bovine, rabbit, frog, chicken and zebrafish genome/proteome reference files were downloaded from respected databases (www.ncbi.nlm.nih.gov) (NCBI Resource Coordinators, 2015) and IMGT/GENE-DB www.imgt.org (Giudicelli et al., 2005) to generate separate heavy-chain (VH) and light-chain (VL) datasets. Each data file was "filtered" to eliminate duplicate polypeptide sequences and to include as many VH and VL polymorphisms as possible. The presence of multiple sites of amino acid conservation in both datasets made alignment of Ig framework residues relatively uncomplicated (see Supplemental data). Numerous immunoglobulins whose IgV structures have been determined were down-loaded from the Protein Data Bank (www.rcsb.org) and examined using DSViewerPro5.0 (Accelrys Inc.) Amino acids representing VH- or VL-specific or shared elements of Ig sequence and structure are illustrated as CPK atomic structures relative to a minimized protein backbone.

3. Results

Heavy-chain variable (VH) and light-chain variable (VL) domain-specific sequence files were examined and compared in an effort to identify and locate sites of amino acid conservation both unique to or shared by VH and VL domains. Amino acid data from separate VH and VL datasets was compiled and tabulated to demonstrate these similarities and differences in both plots and sequence alignments showing the distribution of separate amino acid functional classes within the framework regions that constitute the primary protein scaffold.

3.1. Ionic acid/base residues

Numerous sites exhibiting ionic acid/base amino acid chemistry were observed in both VH and VL sequence files, with basic residues generally occurring more frequently (Fig. 1A). While acidic residues Aspartic acid (Asp) and Glutamic acid (Glu) and basic residues Lysine (Lys) and Arginine (Arg) were observed at numerous locations distributed over the solvent accessible surface of the native VH–VL duplex, two conserved acidic sites, one dominated by Glutamic acid (Glu), the other dominated by Aspartic acid (Asp), and a single conserved basic site dominated by Arginine (Arg) were found to be shared by both VH and VL domains in framework region-3 (FR-3). The shared Asp and Arg residues combine to create duplicate intra-domain ion-paired partners comprising a shared Asp-Arg "salt-bridge" appearing on opposite lateral surfaces of the VH–VL duplex (Fig. 2A). A unique VH-specific "salt-bridge" formed by conserved Glu and Arg in VH framework region-2 (FR-2) was found to be replaced by paired Lys and Gln in VL domains. These and only a few additional domain-specific sites of ionic residue conservation were identified in FR-1 and FR-3 (Fig. 4A and C).

3.2. Polar residues

Both VH and VL domains possess a high frequency of polar residues, predominately Serine (Ser) and Threonine (Thr) having a polar (—OH) side-chain functional group (Fig. 1B). Employed primarily in solvent-exposed hydrophilic regions, the generally shared Ser/Thr sites were observed at analogous locations on the exterior of their respective VH or VL structures (Fig. 3A). Far fewer polar residues exhibited the amide or imidazole side-chains of Asparagine (Asn), Glutamine (Gln), or Histidine (His), with the exception of a Gln site shared with near absolute conservation in FR-2 of both VH and VL domains (Fig. 1B). Part of a highly conserved -Gln-X-Pro-Gly- sequence element shared both VH and VL domains, the Gln side-chains were found to mirror each other in the VH–VL duplex, demonstrating close contact and strong amide–amide inter-domain H-bond potential (Fig. 2A).

3.3. Aliphatic residues

The non-polar residues Alanine (Ala), Valine (Val), Leucine (Leu), Isoleucine (Ile) and Methionine (Met) together were observed to represent a substantial portion of the amino acids with shared conservation in both VH and VL domains (Fig. 1C). Along with polar Ser and Thr, these aliphatic residues were found distributed through all three framework regions, often in alternating patterns of polar and nonpolar functionality. The bulk of shared aliphatic sites were found to be similarly localized and oriented to the solvent-inaccessible interior of both VH and VL domains or in the VH–VL duplex (Fig. 3B). A limited number of differential VH and

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