doi:10.1016/j.jmb.2006.01.023 J. Mol. Biol. (2006) 357, 1566–1574

JMB

Available online at www.sciencedirect.com

SCIENCE DIRECT®

ELSEVIER

Antibody Elbow Angles are Influenced by their Light Chain Class

Robyn L. Stanfield[1]*, Adam Zemla[2], Ian A. Wilson[1,3] and Bernhard Rupp[2,4]*

[1]*Department of Molecular Biology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037 USA*

[2]*Lawrence Livermore National Laboratory, 7000 East Avenue Livermore, CA 94551, USA*

[3]*Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037 USA*

[4]*q.e.d. Life Science Discoveries 6487 Half Dome Court Livermore, CA 94551, USA*

**Corresponding authors*

We have examined the elbow angles for 365 different Fab fragments, and observe that Fabs with λ light chains have adopted a wider range of elbow angles than their κ chain counterparts, and that the λ light chain Fabs are frequently found with very large (>195°) elbow angles. This apparent hyperflexibility of λ chain Fabs may be due to an insertion in their switch region, which is one residue longer than in κ chains, with glycine occurring most frequently at the insertion position. A new, web-based computer program that was used to calculate the Fab elbow angles is described.

Keywords: antibody; elbow angle; lambda; kappa; computer program

Introduction

Antibodies are composed of two light (L; ~25,000 Da) and two heavy (H; ~50,000 Da) polypeptide chains that combine to form one Fc and two Fab modules that can be isolated as functional fragments by proteolytic cleavage of the intact immunoglobulin. Within each Fab fragment are two types of distinct structural domains termed variable (V_L,V_H) and constant (C_L,C_H1), with the amino acid residues linking V_L to C_L and V_H to C_H1 called switch residues. Since the early 1970s, when Fab and light-chain dimer structures first became available, it was noted that these fragments displayed a variability in the angle between their variable and constant domains,[1] referred to as the elbow bend or elbow angle and defined as the angle between the pseudo-2-fold axes relating V_L to V_H, and C_L to C_H1 (Figure 1). While these early antibody structures sparked speculation that the elbow angle might change in response to ligand binding,[2] no convincing data have since been found to support this theory. Rather, it appears that the elbow angle may simply serve to increase Fab flexibility, thus enhancing the ability of an antibody to bind bivalently to ligands arranged on a pathogen surface, such as a virus or bacteria.

The Fab elbow angle (Figure 1) is a useful descriptor of the overall topology of the Fab fragment, serving as a measure for the relative disposition of the variable *versus* the constant domains. The elbow angle is almost always cited in Fab structure reports that include comparison of liganded *versus* unliganded Fab structures, and assessment of Fab switch region flexibility. In order to simplify the elbow angle calculation, we have developed a web-based program to more readily calculate the elbow angle for RCSB Protein DataBank (PDB) formatted Fab coordinates. We have tested this method by calculating elbow angles for 536 Fab fragments from the PDB (of which 365 are non-redundant). The elbow angle calculations are consistent with previous compilations but now clearly demonstrate that the propensity for λ light chains to assume elbow angles beyond 195° is significant compared to κ light chains.

Abbreviations used: V_L, variable light; V_H, variable heavy; C_L, constant light; C_H1, constant heavy; POB, protein Data Bank.

E-mail addresses of the corresponding authors: robyn@scripps.edu; bernhardrupp@sbcglobal.net

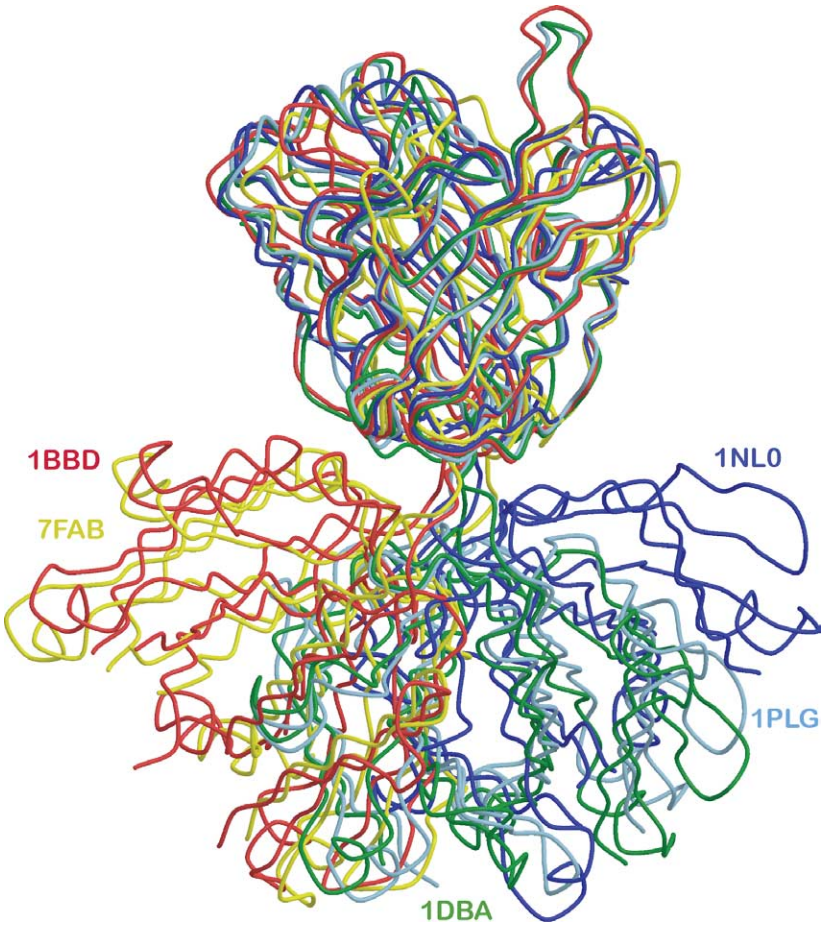

Figure 1. Superposition of a variety of Fabs with different elbow angles. Fabs from PDB files 1bbd (red), 7fab (yellow), 1dba (green), 1plg (cyan), and 1nl0 (blue) have been superimposed on their variable light chain regions. The range of elbow angles is shown from small (1bbd, 127°; 7fab, 132°) to around 180° (1dba, 183°) to large (1plg, 190°; 1nl0, 220°).

Procedures

Calculation of elbow angles

The elbow angle of a Fab antibody fragment is defined as the angle between the two, not necessarily intersecting, pseudo-dyad axes relating the light (V_L) and heavy (V_H) chain variable domains, and the light (C_L) and heavy (C_H1) chain constant domains. Small deviations in the exact locations of the pseudo-dyad axes arise depending on which residues are used for their calculation; however, the resultant deviations for the values of the elbow angles are usually limited to only a few degrees. It is standard practice to use only structurally conserved residues from the antibody framework region for this calculation to eliminate errors due to differences in conformations of the complementarity determining regions. If the Fab coordinates used for the calculation have been numbered in a consistent way (such as the Kabat & Wu convention[3]), these structurally equivalent residues are easily defined. The calculation is then easy; however, most of the Fab structures deposited in the RCSB Protein DataBank[4] are not numbered or labeled consistently, making such comparisons more difficult. The program we use for the V_L-V_H and C_L-C_H1 superposition (LGA[5]) also refines the sequence alignment between the domains and, thus the superposition geometry does not depend critically on the numbering system used for the Fab.

However, additional fine points in the elbow angle calculation need to be addressed. The elbow angle is calculated as the dot product of the V_L-V_H and C_L-C_H1 pseudo-dyad angle, and always computes between 0° and 180°. Although one could readily determine the absolute value in mathematical terms through the sign of the determinant of the basis matrix formed by the two pseudo-dyad vectors and their cross-product vector, this approach does not overcome the problem. Due to the reduction of the information to a single scalar angle value, the relative orientation of the axes is lost, and the solutions become degenerate (imaginable as located on a cone) between 90° and 180°. To regain the domain orientation on an absolute scale and to solve the complement ambiguity, one needs to compare each Fab to a "standard" Fab with a defined elbow angle. In this case, we use unliganded Fab 8F5 (PDB code 1bbd) as the standard, with an elbow angle of 127°. The Fab to be tested is first superimposed *via* its variable domain (V_L-V_H) onto the variable domain of the standard Fab. From this V-aligned orientation, the constant domain (C_L-C_H1) of the test Fab is aligned with the constant domain of the standard Fab, yielding the rotation relative to 1bbd. The sum of the standard Fab's elbow angle (127°) plus the θ_3