Published in final edited form as:

J Sep Sci. 2009 May ; 32(10): 1686–1695. doi:10.1002/jssc.200800694.

Computational structural analysis of an anti-L-amino acid antibody and inversion of its stereoselectivity

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Abstract

The binding site of a monoclonal anti-L-amino acid antibody was modeled using the program SWISS-MODEL. Docking experiments with the enantiomers of phenylalanine revealed that the antibody interacts with L-phenylalanine *via* hydrogen bonds and hydrophobic contacts, whereas the D-enantiomer is rejected due to steric hindrance. Comparison of the sequences of this antibody and an anti-D-amino acid antibody indicates that both immunoglobulins derived from the same germline progenitor. Substitution of four amino acids residues, three in the framework and one in the complementarity determining regions, allowed *in silico* conversion of the anti-L-amino acid antibody into an antibody that stereoselectively binds D-phenylalanine.

Keywords

Amino acid; Antibody; Computer-modeling; Inverted stereoselectivity; Ligand-docking

1 Introduction

Over billions of years, nature has developed an intricate network of specific interactions between biomolecules that enable not only targeted communication of information at the cellular and subcellular level but also controlled conversion of substrates to desired products. Noncovalent forces such as ionic, hydrophobic, and hydrogen bonds, as well as shape complementarity between, e.g., proteins and their macromolecular or low-molecular weight binding partners, result in the formation of transient complexes of distinctive stability. Only if there is a certain balance between complex association and dissociation, an organism's healthy state can be maintained. Disease may result when biomolecules lose their ability to properly interact with each other; just as important as their aptitude to recognize appropriate binding partners, however, is their capacity to reject other, sometimes closely related molecules.

Among the most astounding examples of biodiscrimination ranks the ability of proteins to distinguish compounds that differ solely in their stereochemistry. As early as in 1894, Emil Fischer reported on the effect of a substrate's configuration on enzymatic activity [1]. This was followed by descriptions of stereoselective binding properties of hormone receptors in 1904 [2] and antibodies in 1928 [3]. Since then, many more examples of protein stereo- and enantioselectivity, respectively, have been found [4–6]. Interestingly, many scientists consider stereoselectivity to be an inherent property of proteins. However, if, or to what extent a protein actually discriminates between different stereoisomeric forms of a ligand depends on a variety of factors. Antibodies represent a particularly good model system to

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study protein stereoselectivity as they can be raised against virtually any molecule. As Landsteiner's classic antibody studies showed [7], a protein is likely to display stereoselective properties if it interacts with atoms in the ligand structure that are linked to a stereogenic center. In general, only such moieties that come in close enough contact with amino acid residues on the protein surface will be involved in complex formation. The overall strength of interaction (i.e., affinity) of a protein-ligand-complex, though, is determined by the sum of attractive and repulsive forces between the two [8,9]; if the latter prevail, the ligand is rejected.

Despite the importance for areas such as rational drug design, factors governing chiral biodiscrimination remain rather poorly understood. This is also obvious from the fact that the historic three-point attachment model by Easson and Stedman [10], notwithstanding its shortcomings [11,12], is still the most widely used working model for rationalizing chiral discrimination.

Advances in X-ray crystallography and NMR spectroscopy have resulted in thousands of protein structures that have been deposited in the Protein Databank (PDB) [13] and can be utilized for homology-based modeling of yet unsolved structures. These analytical tools, in conjunction with molecular docking and molecular dynamics simulations, have shed more light on structural elements involved in protein-ligand interactions and chiral biodiscrimination, respectively [14–21].

We have recently used homology-based computer modeling and molecular docking for investigating the structure and binding properties of an anti-D-amino acid antibody [22]. The objective of the current study was to analyze the structure of an antibody that recognizes L-amino acids and to identify residues that allow an inversion of its stereoselectivity. Both antibodies belong to the class of proteins known as immunoglobulins G (IgGs). IgGs comprise two identical light and two identical heavy chains, each of which is composed of one variable domain and one and three, respectively, constant domains. The variable domain can be subdivided into relatively conserved framework regions (FRs) and regions with a higher degree of variability known as complementarity determining regions (CDRs); these sequences are flanked by highly conserved residues that denote their beginning and ending [23–27]. The three CDRs in each the variable light chain (VL) and the variable heavy chain (VH) form a total of six loops through which an antibody can interact with a target molecule, called antigen. Homology-based computer modeling of immunoglobulins is greatly facilitated by the low variability of the FRs and the fact that five of the six CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, and CDR-H2) assume only a limited number of possible loop conformations known as canonical classes [28]. CDR-H3 has a higher degree of variability with regard to length and residue composition; it is the only non-canonical loop and, therefore, presents a particular challenge for molecular modeling.

2 Materials and methods

2.1 Antibody production and characterization

The monoclonal anti-L-amino acid antibody 80.1 (anti-L-AA 80.1) was produced with permission of the Institutional Animal Care and Use Committee at Northern Illinois University (ORC #292) following previously published procedures [29,30].

In brief, eight-week-old BALB/c mice were immunized three times, at intervals of two weeks, with 50 µg of a conjugate of keyhole limpet hemocyanin (KLH; Sigma, Rehovot, Israel) and *p*-amino-L-phenylalanine (Sigma, Deisenhofen, Germany), prepared by diazotization. Four and three days prior to fusion, final boosts were given intraperitoneally. Splenocytes of mice showing strong immune responses were fused with P3X63-AG8.653

myeloma cells using polyethylene glycol (MW 3,500, Sigma, St. Louis, MO). Hybridomas were selected in hypoxanthine/aminopterin/thymidine medium (Sigma, St. Louis, MO), and supernatants were screened by enzyme-linked immunosorbent assay (ELISA) as described below. Hybridomas producing stereoselective antibodies were cloned at least twice by limiting dilution.

Antibody stereoselectivity was first verified in noncompetitive ELISAs on 96-well microtiter plates (Nunc, Rochester, NY) using conjugates of bovine serum albumin (BSA) and *p*-amino-D- or -L-phenylalanine, prepared by diazotization, or underivatized BSA, as solid-phase coatings (100 µl/well; 1 µg/ml in 50 mM carbonate buffer, pH 9.6; 14 h at 4°C). Unoccupied adsorption sites in microtiter wells were blocked with 1% gelatin in phosphate buffered saline (PBS) containing 0.05% Tween 20 (250 µl/well; 2 h at 37°C). Antibody samples (50 µl) in varying dilutions were incubated for 2 h at 37°C. Bound antibody was detected with horseradish peroxidase-conjugated anti-mouse antibody (Jackson, West Grove, PA; 1/10,000 in PBS; 100 µl/well; 2 h at 37°C) utilizing the enzymatic conversion of *o*-phenylenediamine (Sigma, St. Louis, MO). Absorbance was read in a FLUOstar plate reader (BMG Labtechnologies, Offenburg, Germany) at 492 nm following acidification with 1 N sulfuric acid.

In competitive ELISA procedures, antibody (50 µl/well) at a fixed concentration was incubated together with varying concentrations of inhibitor (50 µl/well). All other steps were analogous to the noncompetitive test. Absorbance values were converted to % inhibition using the equation: % inhibition = $[1 - (A/A_0)] \times 100$, where A represents values in the presence of competitor, while A_0 is the absorbance without competitor.

2.2 Sequence analysis

The mRNA of 10⁸ hybridoma cells producing anti-L-AA 80.1 was isolated on an oligo(dT) spin column (Qiagen, Valencia, CA) and converted into cDNA in a first-strand reaction with reverse transcriptase. Using the degenerate primers described by Wang et al. [31], the antibody heavy (~340 bp) and light (~325 bp) chain sequences were amplified from the cDNA template by PCR. The two sequences were then ligated separately into the plasmid pCR2.1 for transfection into *E. coli* Top 10 (Invitrogen, Carlsbad, CA). DNA sequencing was performed by the NIU Plant Molecular Biology Laboratory at Northern Illinois University (DeKalb, IL). The obtained nucleotide sequences were analyzed and translated into the corresponding amino acid sequence using CodonCode Aligner (CodonCode Corporation, Dedham, MA). The online program Basic Local Alignment Search Tool (BLAST; <http://www.ncbi.nlm.nih.gov/BLAST/>) was utilized to verify the origin of the derived sequences by comparing them to those deposited in the National Center for Biotechnology Information's (NCBI) nonredundant (nr) database. The three-dimensional structures of the most homologous antibodies were retrieved from the PDB and used for computer-modeling of the anti-L-AA 80.1 structure.

2.3 Antibody modeling

The variable domains of anti-L-AA 80.1 were modeled utilizing the Project Mode on the SWISS-MODEL website (<http://swissmodel.expasy.org/>) [32]. The template file for the alignment of anti-L-AA 80.1 incorporated two variable heavy and light chains from four highly homologous antibodies. In particular, the B chain of PDB 1BLN and the H chain of PDB 2DQU were used for the heavy chain, whereas the A chain of PDB 1ORQ and the L chain of PDB 2BRR were used for the light chain [33–36]. DeepView (<http://www.usm.maine.edu/spdbv/>) was employed for aligning the anti-L-AA 80.1 sequences with the template structure. Since the CDR-H3 sequence of anti-L-AA 80.1 differs in length from the corresponding sequences of PDB 1BLN and PDB 2DQU, respectively,

multiple alignments were created, and the modeled structures were scored using ANOLEA (<http://protein.bio.puc.cl/cardex/servers/anolea/index.html>) [37], GROMOS [38], and Verify-3D (http://nihserver.mbi.ucla.edu/Verify_3D/) [39]. The structure with the overall best scores was used as model in subsequent studies.

2.4 Docking studies

Docking studies were performed with the program Discovery Studios Modeling-SBD 1.2 (DSM 1.2; Accelrys, Burlington, MA). The three-dimensional structures of the enantiomers of phenylalanine, which were used as ligands, were obtained from Klotho: Biochemical Compounds Declarative Database (<http://www.biocheminfo.org/klotho/>). The antibody's binding site was identified using LigandFit and the binding site volume was varied between 200 Å³ to 678 Å³. A torsional step size of 30° was employed when carrying out Monte-Carlo based docking of a non-flexible ligand. A maximum of ten ligand poses were retained with threshold requirements of 1.5 Å root mean square deviation (RMSD) and an energy difference of 20.0 kcal/mol. Pose optimization of the ligand comprised ten iterations of rigid body steepest descent minimization in conjunction with one hundred iterations of Broyden-Fletcher-Goldfarb-Shanno (BFGS) minimization. Shape fitting utilized an RMSD threshold for the ligand-site match of 2.0 Å. Clustering of the ligand poses was ignored and no poses were rejected based on the DockScore. The following scoring functions were used to score ligand poses: LigScore [40], PLP1 [41], PLP2 [42], Jain [43], PMF [44], and Ludi3 [45,46]. All resulting ligand poses were inspected with DSM 1.2 for clashes and hydrogen bonding between the ligand and protein structures. The interactions between protein and ligand were further investigated with Ligplot [47].

2.5 Molecular dynamics simulations

The effect of amino acid substitutions on the structure and binding properties of anti-*L*-AA 80.1 was investigated with molecular dynamics (MD) simulations. All calculations were performed on a dual processor quad-core Linux PC using the MD program GROMACS [48], version 3.3.1, with each simulation completing in about four days. The MD simulations of the modeled anti-*L*-AA 80.1 contained both the variable heavy and variable light chains. Mutations to anti-*L*-AA 80.1 were performed using the mutagenesis function of Pymol (DeLano Scientific, Palo Alto, CA). The mutated structures were subsequently solvated in a dodecahedral periodic box, where the distance between the box and the closest protein atom was 10 Å. The close contacts created through solvating the structures were minimized using 200 steps of steepest descent energy minimization. Then, preliminary MD simulations were performed, in which water molecules were allowed to equilibrate around the restrained protein structures for 5 ps. These resulting structures were used as starting points for 10 ns MD simulations in explicit water. Since previous MD simulations had shown excessive movement of the N-terminal residues, which result from a lack of interactions between constant domain and variable domain, the first N-terminal residue was restrained in both chains during the 10 ns MD simulations. The protein structures produced during the 10 ns simulation were used in the investigation of the effects of amino acid substitutions on the stereoselective binding properties of anti-*L*-AA 80.1.

Further MD simulations performed with the liganded structures of anti-*L*-AA 80.1 (with *L*-phenylalanine), anti-*D*-AA 80.1 and anti-*D*-AA 67.36 (both with *D*-phenylalanine) confirmed the stability of the docked complexes.

3 Results and discussion

3.1 Production, sequence analysis, and computer-modeling of anti-L-AA 80.1

The murine monoclonal antibody anti-L-AA 80.1 was produced by classical hybridoma technology using a conjugate of the hapten *p*-amino-L-phenylalanine and the protein KLH in the immunization step [29,30]. The immunogen had been synthesized in such a way that the stereogenic center of the hapten was exposed on its surface, which resulted in the production of an antibody that stereoselectively binds to L-amino acids but not to the corresponding D-enantiomers. The high degree of stereoselectivity of anti-L-AA 80.1 was verified by noncompetitive and competitive ELISA using various free amino acids, such as phenylalanine (Fig. 1), as inhibitors.

In order to determine the primary structure of anti-L-AA 80.1, the mRNA of clones producing this antibody was isolated and transcribed into the corresponding cDNA. Following amplification by PCR using degenerate primers [31] and ligation into a plasmid, the nucleotide sequences encoding the variable domains of the heavy (VH) and light chain (VL), respectively, were determined and converted into the corresponding amino acid sequence. A BLAST search verified that the sequences represent murine immunoglobulin heavy and light chains, respectively, and yielded four highly homologous sequences, namely 1BLN (*E* value 2×10^{-49} , bit score 189) and 2QHR (*E* value 5×10^{-49} , bit score 187) for VH, and 2BRR (*E* value 3×10^{-44} , bit score 172) and 1OB1 (*E* value 1×10^{-38} , bit score 153) for VL. The crystal structures of these four antibodies have been published previously [33–36] and were retrieved from the PDB. The sequences were numbered according to the AHO numbering scheme [27] and used to create a template for homology-based modeling of the three-dimensional structure of anti-L-AA 80.1.

The structure obtained from SWISS-MODEL is shown in a surface contour representation in Figure 2a. The binding site forms a small pocket, whose area and volume were calculated to be $420.6 \text{ \AA}^2$ and $518.6 \text{ \AA}^3$, respectively, using CASTp [49]. The six CDRs of the antibody are depicted in Figure 2b in a ribbon diagram. They fall into the following canonical classes: L1 is class 7; L2, L3, H1, and H2 fall into class 1. The CDR-H3 is non-canonical and contains 13 residues, which form an S-shaped flat loop.

3.2 Docking studies with anti-L-AA 80.1

The three-dimensional structure of anti-L-AA 80.1 obtained by homology-based modeling using SWISS-MODEL served as a starting point for subsequent ligand docking studies. However, it had been demonstrated previously that addition of localized side-chain flexibility within the binding site leads to more realistic docking results with regard to binding specificity and affinity [22]. Therefore, Pymol's rotamer and mutagenesis function was utilized to create side chain rotamers of the binding site residues Asp40H, Ser42H, Ser57H, Ser59H, Tyr67H, Tyr69H, Tyr113H, Thr114H, and Trp134H; only those rotamer conformations that resulted in significant side chain clashes were omitted in subsequent docking studies.

Using the program DSM 1.2, multiple docking experiments were then performed with the various structures of anti-L-AA 80.1 and the enantiomers of phenylalanine as ligands. The structure that produced the best docking results (designated anti-L-AA 80.1R) contained rotamers of residues Ser42H, Ser57H, Tyr113H, and Thr114H. DSM 1.2 identified a binding site with a volume of $75.50 \text{ \AA}^3$ that is formed by the six CDRs. It was found that this site stereoselectively docks L-phenylalanine (Fig. 3), but not the corresponding D-enantiomer, which is in agreement with experimental results.

DSM 1.2 identified four hydrogen bonds between the antibody's binding site and L-phenylalanine (Fig. 4a): One between the backbone carbonyl of Asp112H and the ligand's α -amino group (H-donor-acceptor distance 2.13 Å), two hydrogen bonds between the backbone carbonyl of Tyr113H and the ligand's α -amino group (H-donor-acceptor distances 2.28 Å and 2.03 Å, respectively), and one more between the side chain carboxyl group of Asp40H and the α -amino group of the ligand (H-donor-acceptor distance 2.54 Å). In addition to the four hydrogen bonds, Ligplot suggests that five binding site residues form hydrophobic contacts to the ligand (Fig. 4b). These residues are Asp40H, Ser57H, Tyr69H, Thr114H and Trp134H. The resulting LUDI score [45,46] for the interaction between anti-L-AA 80.1R and L-phenylalanine is 377, which corresponds to an equilibrium dissociation constant of 169 μ M.

In an attempt to explain the astounding, experimentally observed capability of anti-L-AA 80.1 to discriminate between the enantiomers of phenylalanine, the structure of the D-enantiomer, which could not be docked, was manually overlaid onto the docked structure of L-phenylalanine using Pymol. It was found that clashes occur between the phenylring of D-phenylalanine and the indol ring of Trp134H, and between the hydrogens linked to the ligand's β -carbon and those hydrogens at the β -carbon of Asp40H (Fig. 5). These types of steric interference appear to prevent D-phenylalanine from accessing the binding site.

3.3 Comparison between anti-L-AA 80.1 and anti-D-AA 67.36, a D-specific antibody

We have previously shown that the murine monoclonal antibody anti-D-AA 67.36 stereoselectively recognizes D-amino acids, while L-amino acids are not bound [22]. The two antibodies, anti-D-AA 67.36 and anti-L-AA 80.1, thus, possess opposing stereoselectivities. However, a comparison of the amino acid sequences of their variable domains revealed a surprising degree of similarity; only a total of twelve residues are different, six in each the VH and VL. While VL of anti-D-AA 67.36 contains two threonine residues at positions seven and eight, anti-L-AA 80.1 has a serine and a proline at those positions. Position sixteen in anti-D-AA 67.36 contains a tryptophan residue, whereas there is a glycine in anti-L-AA 80.1. All other differences were found at the C-terminus of the VL; anti-D-AA 67.36 contains a leucine, lysine, and arginine at positions 147, 148, and 149, whereas anti-L-AA 80.1 only has an arginine and an asparagine at positions 147 and 148. It should be noted that all differences in the VL sequences of the two antibodies were localized in the FRs, and that no differences within the CDRs were found.

A comparison of the VH sequences of anti-L-AA 80.1 and anti-D-AA 67.36 showed that they differ in five amino acid residues within the FRs and one within the CDRs. At positions five and six within FR1, anti-D-AA 67.36 contains two glutamate residues, while at position 29 there is a phenylalanine; anti-L-AA 80.1 has two glutamine residues and a leucine at these positions. In FR3, position 85 is a valine in anti-D-AA 67.36 but an alanine in anti-L-AA 80.1. Position 145 of FR4 is threonine in anti-D-AA 67.36 and isoleucine in anti-L-AA 80.1. The last difference is at position 132 within CDR3, which is a serine in anti-D-AA 67.36 and a glycine in anti-L-AA 80.1.

The overall high degree of similarity between the two sequences suggests that both antibodies, anti-D-AA 67.36 and anti-L-AA 80.1, have the same germline progenitor, which differentiated by affinity maturation into two antibodies of opposing stereoselectivity. In order to test this hypothesis, the nucleotide sequences of anti-D-AA 67.36 and anti-L-AA 80.1 were compared with germline sequences using the IMGT/V-QUEST tool of the International ImmunoGeneTics Information System (IMGT) [50,51]. A common germline sequence was derived through identification of the V and J gene segments of the light chains, and the V, D, and J gene segments of the heavy chains, which were found to be the same for both antibodies. The light chain V-gene was identified as IGKV4-57-1*01 with

scores of 1351 and 1324, and identities of 97.87% and 96.81% for anti-L-AA 80.1 and anti-D-AA 67.36, respectively. The light chain J-gene was identified as IGKJ5*01 (anti-L-AA 80.1: 129, 87.88%; anti-D-AA 67.36: 156, 96.97%). The following genes were identified for the heavy chain V-, D-, and J-genes: IGHV5-9*02 (anti-L-AA 80.1: 1213, 91.93%; anti-D-AA 67.36: 1267, 94.04%), IGHD1-1*01 (anti-L-AA 80.1: 55, 75%; anti-D-AA 67.36: 64, 80%), and IGHJ3*01 (both antibodies: 211, 97.73%). Following conversion of the resulting germline sequence into the corresponding amino acid sequence, a comparison with the primary structures of anti-L-AA 80.1 and anti-D-AA 67.36 revealed that there is only one position that is different for all three sequences, namely position 5 in FR1 of the heavy chain; this residue is a valine in the germline, a glutamate in anti-D-AA 67.36, and a glutamine in anti-L-AA 80.1 (Fig. 6). Based on the observed high degree of sequence similarity it can be assumed that indeed both anti-L-AA 80.1 and anti-D-AA 67.36 derived from the same progenitor.

3.4 *In silico* inversion of the stereoselectivity of anti-L-AA 80.1

Since the primary structures of anti-L-AA 80.1 and anti-D-AA 67.36 differ only by a few residues, it seemed to be feasible to identify those positions that are decisive for determining whether the antibodies' binding sites recognize either L- or D-amino acid ligands. A comparison of the three-dimensional structures of the two antibodies showed that their light chains are virtually identical, which is also reflected in a very small RMSD of 0.033 Å. Assessment of the heavy chains revealed that three substitutions, namely Gln5/Glu5, Gln6/Glu6, and Leu29/Phe29, are spatially close to each other, i.e., are found in the same region in both antibodies. These substitutions in addition to the change of a glycine to a serine at position 132 appeared to cause a rearrangement of the CDR-H3, which affects the overall shape of the binding site.

Interestingly, both sequences contain a proline residue within CDR-H3, although this amino acid has been found to occur with a frequency of only 2.5 % in murine CDR-H3s [52]. Due to its unique structural properties, proline is the only standard amino acid that can form peptide bonds of either *cis* or *trans* configuration (all other peptide bonds are *trans*). It has been shown that the configuration of this residue can have a great effect not only on the local structure of a particular amino acid sequence but also on, e.g., the surface contour of a protein, especially in flexible loop regions [53–55].

In an attempt to convert the L-amino acid specific antibody anti-L-AA 80.1 into an antibody that stereoselectively binds to D-amino acids, ten nanosecond MD simulations were performed with the anti-L-AA 80.1R structure where the following heavy chain substitutions were performed either individually or in combination: Gln5Glu, Gln6Glu, Leu29Phe, Ala85Val, Gly132Ser, and Ile146Thr. In addition to these substitutions both the *cis* and *trans* configurations of Pro133 were investigated. As previously mentioned, the amino acid substitutions in the VL have virtually no effect on the three-dimensional structure. Therefore, these residues were not considered in the MD simulations.

The structures obtained in the MD simulations were employed in docking experiments using the enantiomers of phenylalanine as ligand. It was found that the modeled structure that contained Pro133 in *cis* configuration and Gln5Glu, Gln6Glu, Leu29Phe, and Gly132Ser substitutions stereoselectively binds D-phenylalanine but no longer the L-enantiomer.

An overlay of this new structure, which was named anti-D-AA 80.1, with the structure of anti-D-AA 67.36 showed that both proteins, including their binding sites, are very similar (RMSD=1.399 Å), with the largest difference being a slight change in the orientation of CDR-H3 (Fig. 7). A plot of the RMSD as a function of time confirmed the stability of the anti-D-AA 80.1 structure (Fig. 8).

DSM 1.2 identified four hydrogen bonds between anti-D-AA 80.1 and D-phenylalanine; one between an imidazole nitrogen of His42L and the α -amino group of the ligand (H-donor-acceptor distance 2.17 Å), another one between the backbone nitrogen of Trp134H and the carboxyl group of D-phenylalanine (H-donor-acceptor distance 2.26 Å), and two hydrogen bonds between the Gln107L side chain amide nitrogen and the α -carboxyl group of D-phenylalanine (H-donor-acceptor distances 1.85 Å and 2.43 Å, respectively; Fig. 9a). Ligplot indicated hydrophobic contacts between the ligand and Tyr40L, Tyr109L, Thr114H, Pro133H, and Trp134H (Fig. 9b); π -stacking interactions occur between the phenyl ring of D-phenylalanine and the side chain of Trp134H, which align in an off-centered parallel orientation [56], as well as between the ligand and the light chain residues Tyr40L and Tyr109L, respectively, through the formation of T-shaped structures [56]. The resulting LUDI score was determined to be 497, which corresponds to an equilibrium dissociation constant of 11 μ M. It is noteworthy that this value is in the same range as the value for the interaction between anti-D-AA 67.36 and D-phenylalanine as determined experimentally (1.4 μ M) and by docking studies (9 μ M) [22].

A comparison of anti-D-AA 67.36 and anti-D-AA 80.1 shows that both antibodies use the same residues Tyr40L, Tyr109L, and Trp134H to make hydrophobic contacts with the ligand D-phenylalanine. In addition to these interactions, anti-D-AA 80.1 makes further hydrophobic contacts with the ligand through residues Thr114H and Pro133H, and forms three hydrogen bonds utilizing His42L, Trp134H, and Gln107L. Anti-D-AA 67.36 uses His42L as an additional hydrophobic contact, while the side chain hydroxyl group of Ser132H forms a hydrogen bond with the α -amino group of D-phenylalanine.

An overlay of the ligand in the two binding sites, furthermore, shows that both position and orientation of D-phenylalanine are almost identical (Fig. 10). However, the ligand is situated a little bit higher in anti-D-AA 67.36, namely about 2.28 Å (measured as the distance between the α -carbons). The RMSD of the overlaid ligand structures is only 1.53 Å. This difference can be considered insignificant bearing in mind that acceptable ligand poses obtained from docking studies may deviate by as much as 2.0 Å from the crystallographically determined position of a ligand in a binding site [57,58].

4 Concluding remarks

The structure and binding properties of the anti-L-amino acid antibody anti-L-AA 80.1 were investigated using homology-based modeling and molecular docking. Four hydrogen bonds and five hydrophobic contacts between the antibody and L-phenylalanine result in a calculated equilibrium dissociation constant of 169 μ M. Manually overlaying D-phenylalanine onto the binding site revealed severe clashes with a tryptophan and an aspartate residue, which prevent this enantiomer from entering the binding pocket. Comparison of the nucleotide and resulting amino acid sequences of anti-L-AA 80.1 and anti-D-AA 67.36 showed few differences, which made possible the inversion of the stereoselectivity of anti-L-AA 80.1 by substituting selected amino acid residues. A structure obtained in 10 ns molecular dynamics simulations, called anti-D-AA 80.1, that contained four amino acid substitutions (Gln5Glu, Gln6Glu, Leu29Phe, and Gly132Ser) as well as a *cis* proline (Pro133) was shown to stereoselectively bind D-phenylalanine. Four hydrogen bonds and five hydrophobic contacts result in an equilibrium dissociation constant of 11 μ M, which is in the same range as the previously determined affinity for the interaction between anti-D-AA 67.36 and D-phenylalanine.

Abbreviations

Anti-D-AA	anti-D-amino acid antibody
Anti-L-AA	anti-L-amino acid antibody
BLAST	Basic Local Alignment Search Tool
BSA	bovine serum albumin
CDR	complementarity determining region
ELISA	enzyme-linked immunosorbent assay
FR	framework region
KLH	keyhole limpet hemocyanin
MD	molecular dynamics
PBS	phosphate buffered saline
PDB	Protein Databank
VH	variable heavy chain
VL	variable light chain
RMSD	root mean square deviation

Acknowledgments

We would like to thank Dr. James Horn for his help with the MD simulations and Dr. Scott Grayburn for useful discussions concerning DNA sequencing. This work was supported by the National Institutes of Health (1 R15 GM076000-01).

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Figure 1.

The monoclonal anti-L-amino acid antibody anti-L-AA 80.1 binds L-phenylalanine (○) but not D-phenylalanine (●) as determined by noncompetitive ELISA. Data points represent means of three independent assays. Missing error bars are obscured by symbols. Experimental details are given in the section “Materials and methods.”

Figure 2.

a) Surface contour representation of anti-L-AA 80.1R as obtained by homology-based computer modeling using the program SWISS-MODEL. The location of the binding site pocket is indicated by an arrow. b) Ribbon diagram illustrating the orientation of the CDR loops of the binding site.

Figure 3. Closeup of the binding pocket of anti-L-AA 80.1R with the docked ligand L-phenylalanine (black) shown in a stick (a) and a spherical representation (b).

Figure 4.

Interactions between anti-*L*-AA 80.1R and *L*-phenylalanine (shown in black) as predicted by DSM 1.2 and Ligplot. a) Hydrogen bonds between the ligand and the binding site residues (gray) Asp40H, Asp112H, and Tyr113H. b) Binding site residues (gray) participating in hydrophobic contacts.

Figure 5.

A spherical representation of D-phenylalanine (black) within the binding site of anti-L-AA 80.1R shows severe clashes between the ligand and the antibody residues Asp40H and Trp134H (both gray). It should be noted that D-phenylalanine could not be docked and that this figure was produced by manually placing the ligand in the binding site using PyMol.

Figure 6.
Amino acid sequence alignments of the heavy- and light-chain variable domains of anti-D-AA 67.36, anti-L-AA 80.1, and the germline progenitor according to the AHO numbering scheme.

Figure 7.
Overlay of the CDR loops of anti-D-AA 67.36 (black) and anti-D-AA 80.1 (gray).

Figure 8.
RMSD as a function of time for the 10 ns MD simulation of anti-D-AA 80.1.

Figure 9.

Interactions between anti-D-AA 80.1 and D-phenylalanine (shown in black) as predicted by DSM 1.2 and Ligplot. a) Hydrogen bonds between the ligand and the binding site residues (gray) Trp134H, His42L, and Gln107L. b) Binding site residues (gray) participating in hydrophobic contacts.

Figure 10.

Position of D -phenylalanine in the binding site of anti- D -AA 67.36 (a) and anti- D -AA 80.1 (b) relative to amino acid residues participating in complex formation. An overlay structure of the ligand in both binding sites omitting antibody residues (c) shows its almost identical position and orientation. Note that D -phenylalanine in the binding site of anti- D -AA 67.36 is shown in black and in gray in the binding site of anti- D -AA 80.1.