Soft docking an L and a D peptide to an anticholera toxin antibody using internal coordinate mechanics

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Background: The tremendous increase in sequential and structural information is a challenge for computer-assisted modelling to predict the binding modes of interacting biomolecules. One important area is the structural understanding of protein–peptide interactions, information that is increasingly important for the design of biologically active compounds.

Results: We predicted the three-dimensional structure of a complex between the monoclonal antibody TE33 and its cholera-toxin-derived peptide epitope VPGSQHID. Using the internal coordinate mechanics (ICM) method of flexible docking, the bound conformation of the initially extended peptide epitope to the antibody crystal or modelled structure reproduced the known binding conformation to a root mean square deviation of between 1.9 Å and 3.1 Å. The predicted complexes are in good agreement with binding data obtained from substitutional analyses in which each epitope residue is replaced by all other amino acids. Furthermore, a *de novo* prediction of the recently discovered TE33-binding D peptide dwGsqhydp (single-letter amino acid code where D amino acids are represented by lower-case letters) explains results obtained from binding studies with 172 peptide analogues.

Conclusions: Despite the difficulties arising from the huge conformational space of a peptide, this approach allowed the prediction of the correct binding orientation and the majority of essential binding features of a peptide–antibody complex.

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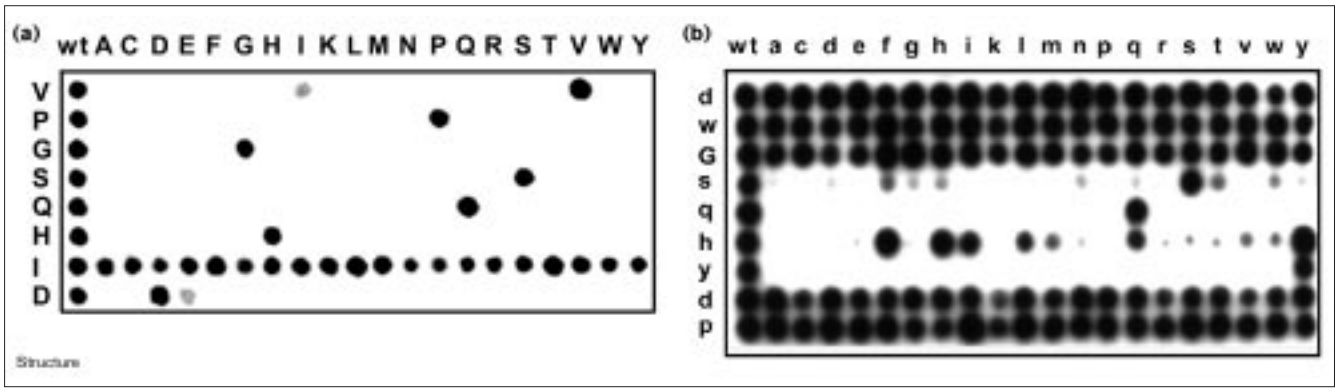
Introduction

In contrast to the abundance of docking methods for small organic ligands (reviewed by Kuntz *et al.* [1], Verlinde and Hol [2], and evaluated at the recent Critical Assessment of Protein Structure Prediction CASP2 [3]), less is known about the docking of peptides to proteins [4–7]. This is extremely challenging because peptides have a multidimensional conformational space, which is difficult to sample exhaustively. However, recent attempts to predict the binding of relatively short peptides to the SH2 (Src-homology 2) and PTB (phosphotyrosine-binding) domains using the internal coordinate mechanics (ICM) methods with biased stochastic peptide sampling in the potential field of the receptor gave encouraging results [8]. In order to evaluate the ability of the ICM docking procedure [9] to predict binding conformations of peptides that were shown to bind proteins in biochemical experiments, we investigated the flexible docking of two peptide epitopes to an antibody. The complex of the murine monoclonal IgG1 antibody TE33 [10] with the epitope peptide VPGSQHIDSQKKA derived from cholera toxin [11] served as a model system, as the three-dimensional structure of the respective complex [12] could be used for comparison. To test the ability of the method to predict previously unknown peptide-binding conformations, the recently discovered TE33-binding D peptide epitope dwGsqhydp was docked to the antibody. This D peptide analogue has been derived from the original epitope by successive substitution of L amino acids with D amino acids [13].

The docking methodology implemented in ICM [9] includes a computationally efficient optimal-bias Monte-Carlo minimization method (OBMCM), combined with pseudo-Brownian random moves [14], an extended force field including surface terms, electrostatics with the boundary element solution of the Poisson equation [15], sidechain entropy terms, and a fast algorithm for calculating molecular surfaces [16]. In order to save computer resources, the minimal epitopes were used for docking experiments. Previous binding studies with a series of truncated epitope analogues had led to the minimal sequence VPGSQHID (data not shown). The peptides were set up in their fully extended conformations. Calculations were performed using the force field ECEPP/3 [17,18] as implemented in ICM [9], pseudo-Brownian random movements of position and orientation of the peptide as well as biased-probability random steps of

Figure 1



Substitutional analysis of the peptides (a) VPGSQHID and (b) dwGsqhydp. Each residue of both peptides is either substituted by all L amino acids (rows, (a)) or D amino acids (rows, (b)) and tested for antibody binding as described [30,31]. Peptides in the left-most columns are identical wild-type peptides.

peptide-backbone torsion angles and of selected peptide-sidechain torsions and random changes of selected antibody-sidechain torsions. The docking experiments demonstrated that it is possible to reproduce the correct binding orientation and the majority of essential binding features.

Results

Binding studies using complete substitutional analyses of TE33-binding peptides

Before starting the docking experiments, binding studies with a complete set of substitution analogues of the L peptide VPGSQHID (epitope; Figure 1a) and the D peptide dwGsqhydp (Figure 1b) [12] revealed residues critical for binding. The epitope residues proline 2, glycine 3, serine 4, glutamine 5, and histidine 6 cannot be substituted at all and are thus absolutely essential for binding. Valine 1 can only be substituted by another β-branched residue, isoleucine. Aspartate 8 can only be substituted by the other negatively charged residue, glutamate. Isoleucine 7 can be substituted by all amino acids and is thus not critical for binding. The dissociation constant, determined by the method of Friguet *et al.* [19] for the peptide–antibody complex is 10^{-5} mol^{-1}.

Interestingly, although the sequence of the TE33-binding D analogue is very similar to the epitope with five identical sidechains (Gsqh and d), the binding pattern in the substitutional analysis of the D peptide is substantially different from that of the epitope. Here, glycine 3, D histidine 6, and D aspartate 8 lost their property of being key residues for binding. In this peptide, D serine 4, D glutamine 5, and D tyrosine 7 are most important for binding. The dissociation constant for this peptide–antibody complex is 2×10^{-6} mol^{-1}.

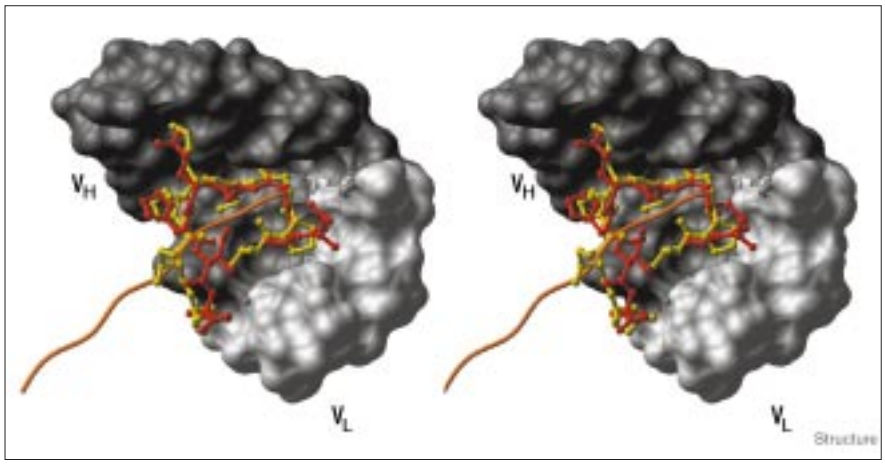
Rigid-body and flexible docking of the L peptide epitope to the X-ray structure derived antibody

In a first set of calculations, the coordinates of the antibody were taken from the crystal structure [12]. Rigid-body docking of the minimal epitope VPGSQHID with coordinates of the respective residues taken from the crystal structure revealed exact reproduction of the binding position and orientation of the peptide starting from several different peptide orientations includ-ing a reverse orientation, after 10,000 energy steps (not shown).

In the next step, flexible-docking experiments were performed with the fully extended peptide. The binding site of the peptide was found between 1,500,000 and 2,500,000 energy evaluations, independent of the starting orientation. The conformation of the peptide after docking differed slightly for various calculations. The root mean square deviation (rmsd) of the backbone atoms of the docked peptide was as low as 1.9 Å. The main difference is caused by a deviation of the backbone conformation for valine 1 and isoleucine 7. Valine now points to a hydrophobic pocket formed by residues isoleucine 94 of the light chain (L94), phenylalanine (L96), and tryptophan 50 of the heavy chain (H50) (Figure 2). The ring planes of proline 2 differ. There is slight deviation of the backbone atoms for glycine, serine, glutamine and histidine. The serine sidechain makes a hydrogen bond with serine H96 both in the crystal structure and in the modelled peptide. The sidechain orientations and interactions of glutamine and histidine, both critical for binding, are reproduced almost perfectly by the docking procedure. The same is true for the aspartate sidechain. Isoleucine 7, not critical for binding, points towards residues histidine L31, tyrosine H32 and tryptophan H100a, whereas in the crystal structure this sidechain

Figure 2

Stereoview of the complex of the X-ray derived antibody TE33 structure with the docked peptide epitope (red) compared to the peptide conformation in the crystal structure (yellow). Peptide hydrogen atoms are omitted. The N terminus of the peptide is on the right side. The antibody surface was calculated using the ICM program. The starting orientation of the peptide is shown in orange. V_H and V_L refer, respectively, to the heavy and light chains of the antibody variable domain.



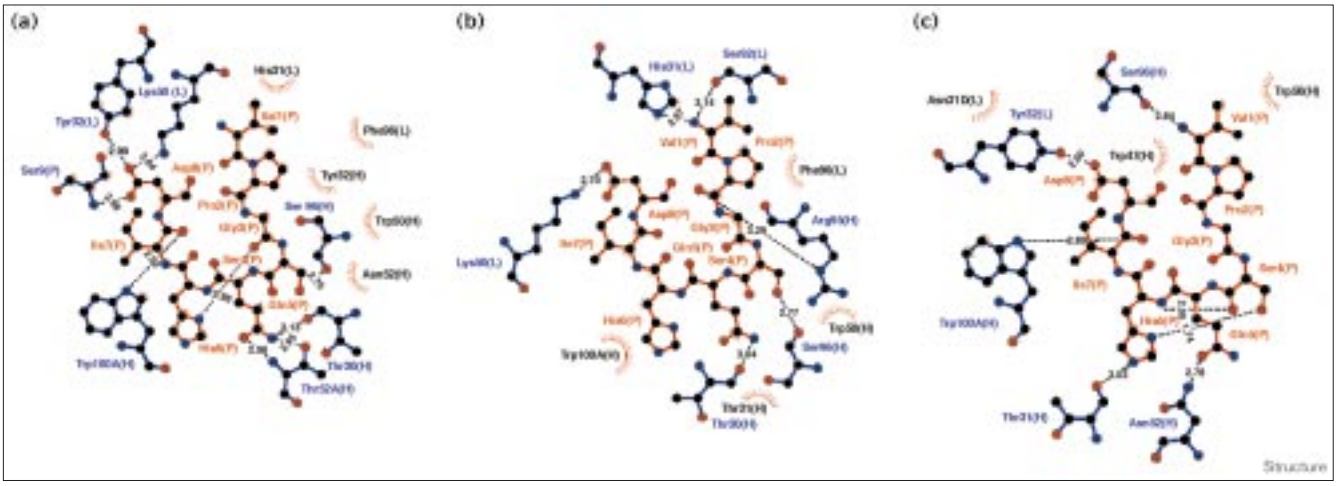
points into the solvent. All contacts of the docked peptides with the antibodies are displayed in Figures 3 and 4. The pictures were created using LIGPLOT [20]. All docking experiments were performed with starting orientations of the peptide centroids about 3 Å apart from the binding site to spare computer resources.

De novo prediction of the TE33–epitope complex

Three-dimensional coordinates of the free antibody were created by homology modelling using MODELLER (Version 3c) [21] based on two homologous murine antibodies. For the light chain the catalytic antibody 28B4 (PDB code 1KEM [22]), which has 93% sequence identity and identical lengths for all complementarity-determining regions (CDRs), was chosen. For the heavy chain the anti-steroid monoclonal antibody DB3 (PDB code 1DBA [23]; 80.3% sequence identity) was used. CDR1 and CDR2 are identical in length. CDR3 of TE33 is three amino-acids shorter in the N-terminal region, whereas the five C-terminal residues are identical.

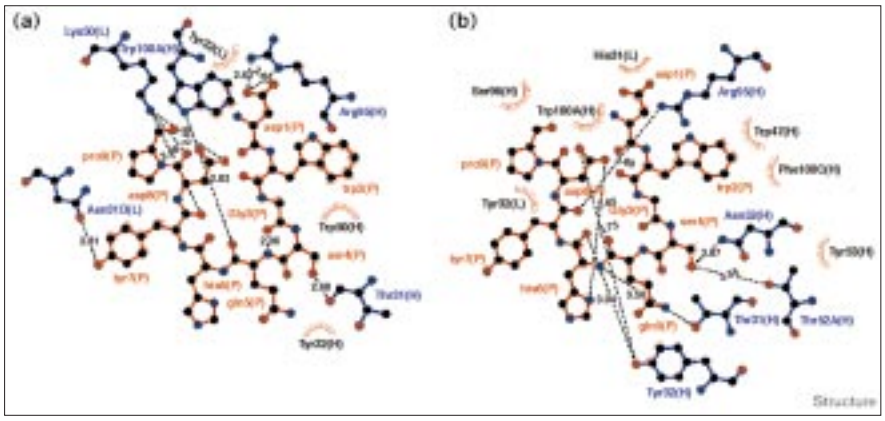
In this case, rigid-body docking experiments resulted in peptide orientations dissimilar (rmsd > 10 Å) to those in the crystal structure. This was expected because the paratope of the modelled TE33 Fv (variable) antibody fragment differs from that of the complexed antibody. Several sidechains in the CDR regions differ from those in the complexed antibody, whereas the backbone conformations

Figure 3



Plot of interactions and hydrogen bonds of the complex of the peptide epitope VPGSQHID with antibody TE33, created with the program LIGPLOT [20]. (a) The antibody–peptide complex as in the crystal structure [12]; (b) the complex of docked peptide epitope VPGSQHID and the antibody from the crystal structure; and (c) the complex of docked peptide epitope VPGSQHID and the modelled antibody. (P) indicates residues belonging to the peptide and (H) and (L) refer to residues in the heavy and light chains of the antibody, respectively.

Figure 4



Plot of interactions and hydrogen bonds of the complex of the D peptide dwGsqhydp with antibody TE33, created with LIGPLOT [20]. (a) The complex of D peptide dwGsqhydp and the antibody from the crystal structure. (b) The complex of D peptide dwGsqhydp and the modelled antibody.

only differ by an rmsd of 2 Å for the CDR regions. The overall rmsd is 1.8 Å for all atoms in the Fv fragments, and 1.0 Å for the backbone atoms. The rmsd values of the CDR regions are given in Table 1.

Flexible docking of the extended peptide to the modelled antibody now enabled the identification of the correct binding site and orientation of the ligand. A starting orientation identical to the one described above was used. In this case the rmsd of all peptide atoms was 3.8 Å and that of the backbone alone was 3.1 Å (Figure 5). The C terminus of the docked peptide accurately reproduces that of the peptide in the crystal structure, whereas the N terminus points towards the solvent. The interactions of the key residues glutamine and histidine could again be perfectly predicted, whereas serine 4 in this case makes an intramolecular hydrogen bond with histidine 6 rather than an intermolecular hydrogen bond as observed in the crystal structure (Figure 3). Glutamine 5 makes only one hydrogen bond with asparagine H52.

Table 1

Comparison of the modelled antibody TE33 created with MODELLER [21] with that from the X-ray structure of TE33.

	All atoms*	Backbone atoms*
V_L derived from antibody 28B4 [22]		
V_L	1.89	1.11
CDR1L	1.20	0.44
CDR2L	1.16	0.19
CDR3L	1.38	0.43
V_H derived from antibody DB3 [23]		
V_H	1.65	0.71
CDRH1	1.21	0.30
CDRH2	1.44	0.27
CDRH3	3.70	0.90

*Rmsd values are in Å.

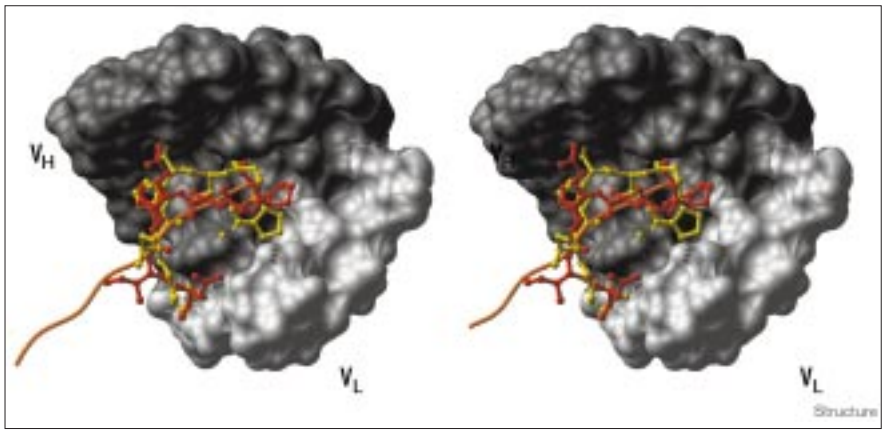
Docking of the D peptide dwGsqhydp to the X-ray structure derived and modelled antibody TE33

Flexible-docking experiments to the X-ray structure derived antibody coordinates, starting from the extended D peptide dwGsqhydp, resulted in a binding conformation that fits well into a pocket on the antibody surface (Figure 6). D serine 4, critical for binding, contacts residues threonine H31, tyrosine H32, glycine H33, and serine H96 and makes a hydrogen bond with threonine H31, which is in agreement with the results obtained from the substitutional analysis. D glutamine contacts residues serine H96 and tryptophan H100a, making two hydrogen bonds: one intramolecular bond with glycine 3 and one intermolecular bond with tryptophan H100a (Figure 4a). D tyrosine 7 makes a hydrophobic stack with tryptophan H100a and also forms hydrogen bonds with asparagine L31d and lysine L50. The C-terminal residues D aspartate and D proline are not essential for binding and point towards the solvent.

Docking experiments to the modelled antibody resulted in a binding conformation with very similar interactions observed for residues D aspartate 1, D tryptophan 2, and D tyrosine 7. A clear difference is observed for D glutamine 5, which in this case points to residues threonine H31, tyrosine H32, and tyrosine H53 (Figure 4b, Figure 6). D serine 4 contacts residues from CDRs 1 and 2 of the heavy chain and makes two hydrogen bonds with asparagine H52 and threonine H52a. D tyrosine 7, which is critical for binding, points to a hydrophobic pocket formed by residues tyrosine L54 and the hydrophobic part of arginine H95. It also forms a hydrogen bond with arginine H95. The C-terminal residues D asparagine and D proline also point towards the solvent.

Figure 5

Stereoview of the complex of the modelled antibody TE33 with the docked epitope (red) compared to the peptide conformation in the crystal structure (yellow). Peptide hydrogen atoms are omitted. The N terminus of the peptide is on the right side. The antibody surface was calculated using the ICM program. The starting orientation of the peptide is shown in orange.



Discussion

Predictions of protein–protein interactions are becoming increasingly important because of the rapidly growing numbers of detected novel proteins and their interaction partners. Stochastic global-optimization algorithms and the energy function extended with solvation and entropic effects provides the basis for conformational sampling (for a review, see [5]). We used the ICM program [9] to predict the interaction of the epitopes VPGSQHID and dwGsqhydp with the monoclonal antibody TE33, starting from the peptide sequence alone — namely, with an extended peptide conformation.

The first approach using the antibody coordinates from the complex crystal structure resulted in a prediction that reproduced the binding site and conformation of the peptide within the general accuracy range of structure determination by X-ray crystallography. Interestingly, the docking procedure produces hydrophobic interactions for the sidechain of valine 1, whereas in the crystal structure no such interactions are observed for this sidechain. The results of the substitutional analysis indicate that there might be hydrophobic interactions because valine can be substituted by isoleucine, but with lower binding affinity.

The second approach using a modelled antibody resulted in an rmsd of the backbone of 3.8 Å for all atoms and 3.1 Å for the backbone of the peptide. This deviation from the binding conformation observed in the crystal structure may well be indicative of the induced-fit mechanism [24] of the binding process. This could not be simulated because only dihedral angles of antibody sidechains were used as free variables, not allowing for any movement of the flexible CDR loop. It is still extremely computationally demanding to take these variables into account and that would have made calculations prohibitively expensive. No explicit water molecules were taken into account, as solvent interactions are implicitly included in the

Figure 6

Stereoview of the complex of the antibody TE33 with the docked D peptide dwGsqhydp (red: peptide from the complex with the X-ray-derived antibody; magenta: peptide from the complex with the modelled antibody). Peptide hydrogen atoms are omitted. The N terminus of the peptide is on the right side. The antibody surface was calculated using the ICM program. The starting orientation of the peptide is shown in orange.

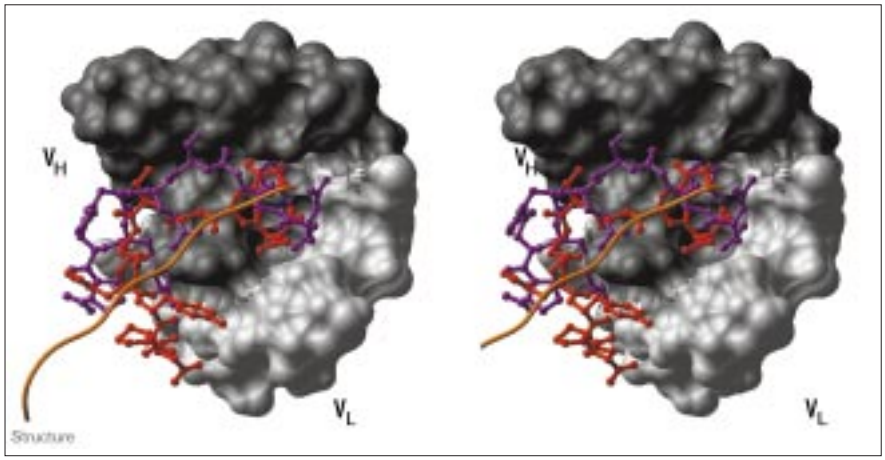
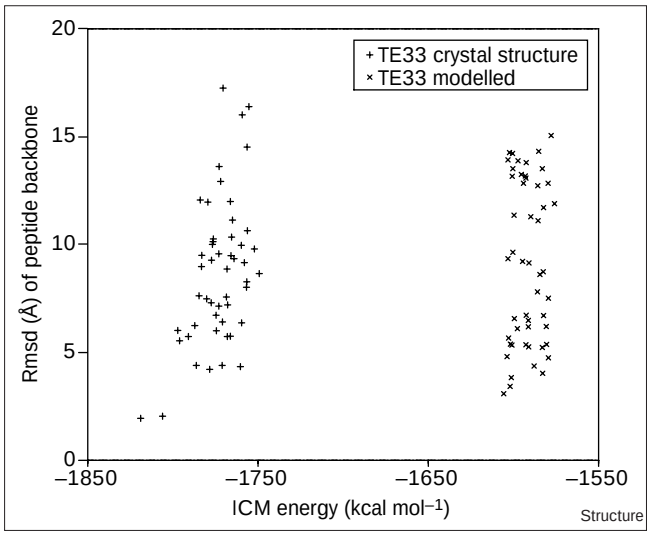


Figure 7



Plot of ICM energy versus rmsd of the peptide backbone after docking. Rmsd values of peptide docked to antibody in the TE33 crystal structure (+) and the rmsd values of peptide docked to modelled TE33 (×) are shown.

extended force field of ICM, including sidechain entropy effects and protein-surface terms. All docked peptide conformations showed negative binding energies (-6 ± 2 kcal mol^{-1} for the complex with the antibody coordinates taken from the crystal structure, and -5 ± 2 kcal mol^{-1} for the complex with the modelled antibody), indicating that a realistic binding scenario was generated. However, the crystal structure showed the lowest value (-8 ± 2 kcal mol^{-1}), which indicates that the docked conformations are not yet 'perfect'. Elongation of docking experiments to five million energy evaluations showed no improvement in energy and binding mode, however. This means that the docking procedure is 'trapped' in low-energy states, but not necessarily in the global minimum, possibly as a result of missing movements of the antibody backbone. Nevertheless, the majority of critical interactions were detected.

No crystal structure is available for the complex of TE33 with the D peptide dwGsqhydp. Therefore, no rigid-body docking was feasible. The conformations of the D peptide bound to X-ray-derived TE33 and the modelled antibody also differed. Calculated binding energies were -8 ± 2 kcal mol^{-1} for the complex of dwGsqhydp to the antibody whose coordinates were taken from the crystal structure, and -1 ± 2 kcal mol^{-1} for the complex with the modelled antibody, respectively. The binding peptide conformations, although different, fit into the same binding pocket on the antibody surface and agree well with the interaction scheme obtained from the substitutional analysis. In particular, the change of key residues' identity between histidine and isoleucine and D tyrosine is reflected well by the results of our docking experiments. The differing results with the D peptide in both experiments may result from alternative conformations of the binding site, especially the differences in sidechain conformations of the antibody. As in the case of the L peptide, we could not alter the antibody backbone conformation because of the technical reasons mentioned earlier.

In all experiments the lowest-energy conformation was considered to be a solution and in the case of the L peptide epitope this lowest-energy conformation had the lowest rmsd with respect to the crystal structure, as can be seen in Figure 7. ICM has already been successful in docking two proteins with flexible sidechains [25–27], but docking flexible peptides to proteins presents an even more demanding challenge. The results show that the methodology presented here is suitable for docking peptides to other proteins, although further improvements of the energy function as well as larger scale sampling, including sampling of the protein backbone, are necessary. In order to optimize the reliability of our docking results, we are currently working on linking biochemical information with docking techniques.

Biological implications

The interaction of proteins with linear regions of other protein-binding partners is widely observed in biological-recognition processes. A variety of protein modules involved in signal transduction, such as WW (a protein domain with two highly conserved tryptophan residues), PDZ (Post synaptic density, Disc large, Zonula occludens) or SH3 (Src-homology 3) domains interact with linear peptide epitopes. These binding peptides can be obtained by screening biologically or chemically generated libraries or by the yeast-interaction trap system. Peptide–protein interactions also play a crucial role in the regulation of the cellular and humoral immune response. Whereas major histocompatibility (MHC) molecules react exclusively with linear peptide epitopes, antibodies recognize both linear and nonlinear epitopes. It can, however, be time consuming or even impossible to analyze these interactions by X-ray crystallography or NMR. Therefore, computer-assisted modelling approaches have become increasingly important. Flexible docking of peptides is especially demanding because of their high conformational flexibility. As a lot of information is available about the interaction of antibodies with peptides, we chose the complex of the peptide epitope VPGSQHID with the anticholera toxin antibody TE33 as a model system for evaluating our docking strategy.

Materials and methods

Preparation and incubation of complete substitutional analyses have been described in detail elsewhere [13,28]. All calculations were performed on Silicon Graphics workstations equipped with 64 MB of core

memory. A typical docking run (2500,000 steps) lasted 80–90 central processing unit (CPU) hours.

In the ICM method, molecules are described by sets of internal coordinates. Relative positions of molecules in complexes and their conformations form subsets of internal coordinates and can be energy minimized locally or globally. For our docking experiments of a peptide onto an antibody the following variables were altered: six positional variables and 98 internal degrees of freedom (backbone φ and ϕ dihedral angles and all sidechain dihedrals) of the peptide and sidechain torsion angles of the binding site of the antibody (all residues that lie within 6 Å of the peptide in the crystal structure). Other variables were fixed to accelerate conformational sampling and energy calculations. We used pseudo-Brownian positional movements with an average amplitude of 4 Å for the peptide as well as random rotations around the peptide's centre of gravity by an angle close to 15°. Backbone and sidechain torsion angles of the peptide were sampled one at a time with biased-probability moves [14] with a maximal amplitude of 180°. Sidechains of the antibody were randomly changed with a maximal amplitude of 180°.

In a first set of docking experiments we used the coordinates of the bound antibody TE33 in its complex with the peptide-epitope sequence VPGSQHIDSQKK from the protein database (PDB code 1TET [12]) and an extended backbone and extended sidechains of the peptide, with the peptide being placed near to the binding site (Figure 2). Conversion of the PDB files into objects appropriate for ICM calculations was accomplished by a regularization procedure. Regularization is required because the experimentally determined PDB structures often lack hydrogen atoms and positional errors may result in the unrealistic van der Waals energy even if these structures were energetically refined (because during crystal-structure determinations hydrogen atoms are usually not resolved and different force fields are used). The following steps are required to create the regularized and energy-refined ICM model of an experimentally determined structure: an extended all-atom model of a particular protein is generated with regular geometric characteristics; the nonhydrogen atoms in the model are assigned to the equivalent atoms in the structure; the regularized structure is built from the N terminus by adding atoms one by one; methyl groups are rotated to reduce van der Waals clashes; combined geometry and energy function are optimized (100-step minimization); and polar-hydrogen positions are adjusted. This resulted in an rmsd value of 0.42 Å for the antibody backbone atoms and an rmsd of 0.57 Å for all atoms. Our docking experiments began with an extended peptide. All experiments required 2,500,000 energy evaluations to converge to the known three-dimensional structure.

In a second set of experiments the antibody TE33 was modelled by homology modelling using MODELLER [21] and QUANTA™ (Copyright MSI Inc., San Diego, CA, USA). The light chain was modelled based on a catalytic antibody (PDB code 1KEM [22]), the heavy chain was based on an anti-progesterone antibody (PDB code 1DBA [23]) keeping in mind that the antibodies optimally have highly homologous sequences with TE33 and should be unbound. The same docking procedures as with the native antibody structure were then applied.

With the L peptide epitope we performed calculations with charged N and C termini of both the antibody and the peptide, as in the crystal structure. However, this was unlike the biochemical binding experiments where, in practice, the N terminus is uncharged and the C terminus is available as an amide. Uncharged termini were then used in docking the D peptide epitope dwGsqhydp.

Each of the docking calculations consisted of the following steps: changing the relative orientation and position of the peptide and – for flexible docking – the conformation of the backbone or sidechain of the peptide and the antibody sidechain randomly; optimizing the local vacuum ECEPP/3 energy [17] with a distance-dependent electrostatic term by a conjugate-gradient algorithm; calculating surface-based solvation energy and entropic contribution from the sidechains and adding it to the total potential energy; applying the Metropolis [29] selection criterion at a temperature of 1500K; and collecting the accepted conformations and ranking them according to their potential energies.

The generated conformations, organized in a stack, were compared and the lowest-energy complex chosen. At the end, binding energies for all complexes were computed. The binding energies include an entropy term, a surface term, and an electrostatic term. The calculation of the surface term included an accurate analytical surface buildup method [16]. For the electrostatic term of the binding energy the numerical solution to the Poisson–Boltzmann equation using the boundary-element algorithm [15,25] was used. The van der Waals term was neglected because the total contribution of the difference in van der Waals energies in solvated and bound state can be comparable or less than the corresponding error. ECEPP/3 [17] atomic partial charges as implemented in ICM were used for all calculations.

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