Original Article

Prediction of Contact Residues in Anti-HIV Neutralizing Antibody by Deep Learning

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SUMMARY: The monoclonal antibody 1C10 targets the V3 loop of HIV-1 and neutralizes a broad range of clade B viruses. However, the mode of interaction between 1C10 and the V3 loop remains unclear because crystallization of 1C10 and the V3 peptide was unsuccessful due to the flexible regions present in both 1C10 and the V3 peptide. In this study, we predicted the 1C10 amino acid residues that make contact with the V3 loop using a deep learning (DL)-based method. Inputs from ROSIE for docking simulation and FastContact, Naccess, and PDBtools, to approximate interactions were processed by Chainer for DL, and outputs were obtained as probabilities of contact residues. Using this DL algorithm, D95, D97, P100a, and D100b of CDRH3; D53, and D56 of CDRH2; and D61 of FR3 were highly ranked as contact residues of 1C10. Substitution of these residues with alanine significantly decreased the affinity of 1C10 to the V3 peptide. Moreover, the higher the rank of the residue, the more the binding activity diminished. This study demonstrates that the prediction of contact residues using a DL-based approach is a precise and useful tool for the analysis of antibody-antigen interactions.

INTRODUCTION

The neutralizing antibody (nAb) against human immunodeficiency virus type 1 (HIV-1) is key to controlling HIV-1 infection (1–3). The V3 loop of HIV-1 is a target for nAbs. Anti-V3 nAbs can be classified into 2 groups; ladle and cradle types, which differ based on their mode of binding to V3 peptides (1,2). Ladle nAbs, represented by 447-52D, require the conserved sequence, GPGR/Q, at the crown of the V3 loop for contact. On the other hand, cradle nAbs, which possess unique features including the usage of the same immunoglobulin heavy chain (HC) gene (IGHV5-51), have V3 crown-independent binding (4–6). The anti-V3 nAb, 1C10, was isolated from an elite controller who maintained their viral load under detectable levels without anti-retrovirus therapy for more than 20 years (7). Although 1C10 is more potent against most clade B viruses, including Tier 2 viruses, compared with other anti-V3 nAbs, the mechanism of broad neutralization by 1C10 remains unclear (7).

Elucidating the antibody-antigen structure is essential to improving our understanding of the interaction

between an antibody and an antigen. However, some proteins (e.g. membrane proteins) are difficult to crystallize while bound to their antigens (8). Flexibility is the major obstacle in crystalizing antibody-antigen complexes. In our previous study, KD-247, a humanized version of a mouse monoclonal nAb, which targets the V3 loop (3,9), was successfully crystallized. Unfortunately, we were unable to generate a crystal structure of the KD-247-V3 peptide complex (10). Crystallization of 1C10 and the V3 peptide was also unsuccessful, and the mode of binding involved in broad neutralization still remains unclear. Docking simulation is a useful alternative to analyze antibody-antigen interactions. However, the prediction of contact residues by this method is not always considered and is elusive in docking simulation (11–13). In addition, although simulation does not always require a crystal structure, the success rate of the docking is highly dependent on the source of the complex components to be simulated, especially backbones which have been constructed by homology modeling (14,15).

Deep learning (DL) has been applied to biological research and accumulating evidence has proven its prediction capability for elucidating the binding mode of anti-HIV nAbs (16–18). In the present study, we predicted the contact residues of anti-V3 nAb 1C10 using the DL-based method to reveal residues that are important for binding to the V3 loop.

MATERIALS AND METHODS

Docking simulation of crystal structure and homology models by Rosetta Online Server that

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Fig. 1. (Color Online) Approach for the prediction of contact residues in anti-V3 nAb. Docking simulation was performed by ROSIE, and 1,000 docked poses were generated. Local energy and AA positions were calculated by FastContact, Naccess, and PDBtools. Parameters from these analyses were normalized and used as inputs for DL on Chainer. Multi-layer perceptron (MLP) was constructed by an input layer including 43 nodes, 2 hidden layers including 10 nodes per layer, and 1 output layer including 2 nodes. The output of this MLP was either “true contact residue” or “false contact residue.”

Includes Everyone (ROSIE): ROSIE contains a protein-protein docking simulation based on RosettaDock, which is freely available at (https://rosie.graylab.jhu.edu/ligand_docking) as reported by Lykocov et al. (19). We chose the Docking2 protocol on ROSIE to output 1,000 decoys each using the crystal structure of anti-V3 nAbs including 447-52D (Protein Data Bank [PDB] ID: 4M1D) (20), 1334 (PDB ID: 6DB7) (5), F425 B4e8 (PDB ID: 2QSC) (21), and 311-11D (PDB ID: 6DB6) (5), of which, the HC, light chain (LC), and V3 loop were separately used as templates for the homology modeling of 1C10 by Swiss-model (22) followed by combining an alignment with the original crystal structure on Pymol (23). The crystal structures and the homology models were uploaded to the ROSIE server to generate a docking score (24–29).

Estimation of the credibility of contact residues in docking poses: To identify the amino acids (AAs) involved in the interaction in the 1,000 docked poses from ROSIE, additional analyses were performed. The interaction between the HC and the V3 loop was analyzed using FastContact (30) and Naccess (31). Residues in the range of 5 Å from Arg315 of the V3 loop were independently determined by contact.py in PDBtools (32). The unbound antibody structure was extracted using BioPython (33). Character and structural positions on the V3 loop (34) or on the variable region of the HC were also used as parameters. Comparison of these parameters was performed using the mean and *P* values of the data, which were calculated using the Welch’s *t*-test of each value obtained by Python (35). Boxplots are generated in Matplotlib (36).

Deep learning by Chainer: To standardize the input data, input parameters were converted to a range from 0 to 1 for continuous variants and 0 or 1 for objective variants. Blank data were filled by the mean value of

the 1,000 poses in respective categories. A multi-layer perceptron (MLP) was composed by 1 input layer including 43 nodes, 2 hidden layers including 10 nodes per layer, and 1 output layer including 2 nodes (Fig. 1). DL was performed on Chainer (37) using rectified linear unit as an activation function, batch size = 5, and epoch = 20. The results obtained from 3,000 simulations using the crystal structures of 1334, F425 B4e8, and 311-11D were randomly separated to a teacher model (30%) and a student model (70%). After training with the teacher model, the model was applied to the output of ROSIE simulating the 1C10 homologous model to 1334. Relative frequency selected as a contact residue in the prediction was calculated by the number selected as a contact residue at each residue divided by the total number selected as a contact residue at all the residues.

Analysis of binding activity of 1C10 mutants to the V3 peptide: The 7 predicted contact residues of 1C10 were substituted with an alanine by PCR mutagenesis (7,36). The resultant 1C10 HC mutants, 1C10drCDR2-m (D53A, D56A, and D61A), 1C10drCDR3-m (D95A, D97A, D100aA, and P100bA), dr95-m (D95A), dr97-m (D97A), dr100a-m (D100aA), and dr100b-m (P100bA) were transfected into HEK293 cells with a 1C10 LC expressing vector using the FectoPro Expression System (PolyPlus, Illkirch, France). The binding activity of the culture supernatants of transfected cells was analyzed by enzyme-linked immunosorbent assay (ELISA) to the V3 peptide of JRFL (NNT20; NNTRKSIHIGPGRAFYTTGE), as previously described (7). The AA positions in 1C10 are shown according to the Kabat numbering scheme.

RESULTS

1C10 homology modeling using anti-V3

	1334	F425 B4e8	311-11D	447-52D
Docking simulation of 1C10 homology model				
ROSIE docking score	-195.30	-186.58	-121.67	-35.887
Original crystal structure				
ROSIE docking score	-221.41	-227.70	-223.47	-223.84
Contact residues on HC	W50, N52 G52a, D53, T57, Y97, D99, P100i	N100d	T31, R50, N52, G97, D98, Y100, I100c, W100i	W33, D95, D100f, Y100g, Y100h, Y100j
CDRH3 RMSD (Å)	-	3.43	3.38	1.45
CDRH3 length	22	14	14	20
V3 loop RMSD (Å)	-	2.25	0.68	3.69
V3 sequence	RKRIHIGPGRAFY	RKRIHIGPGRAYTT	RKRIHIGPGRAYT	CRKRIHIGPGRAYTC

Fig. 2. Comparison of docking scores of cognate and homology models among 4 anti-V3 antibody crystal structures. The best docking scores obtained from ROSIE docking simulation of 1C10 models based on 1334, F425 B4e8, 311-11D, and 447-52D structures are shown. The redocking simulation of 4 anti-V3 antibodies was also performed using their intact structures on PDB. Root mean square distance (RMSD) of the top 10 output complexes was calculated by PyMol.

antibody-V3 structures: Homology models of anti-V3 nAb 1C10 were analyzed by docking simulation using ROSIE based on the structures of the anti-V3 antibodies, 447-52D, 1334, F425 B4e8, and 311-11D. Thousands of poses were generated through the low-resolution stage followed by the high-resolution stage, and 1,000 poses were selected from the lowest total energy score. ROSIE also calculated values categorized as energy fractions including *fa_atr*, *fa_dun*, *fa_elec*, *fa_pair*, *fa_rep*, *fa_sol*, *l_sc*, and total score, and the location of a pose containing *rms*, *lrms*, *cen_rms*, and *Fnat*.

The 1C10 homology model with a typical ladle-type antibody, 447-52D, resulted in a significantly higher docking score (−35.887) compared to the 447-52D crystal structure (Fig. 2). The 1C10 homology model using 311-11D showed a moderate score (−121.67). The models using 1334 and F425 B4e8 showed low docking scores (−195.30 and −186.58, respectively) which were close to the scores of the original crystal structures. The binding mode of F425 B4e8 is unique and uses only 1 AA on the HC to make contact with the V3 loop, because the LC harbors the main points of contact with the V3 loop. However, 1334 and 311-11D used closely-related AAs of complementarity-determining region 3 of heavy chain (CDRH3) for binding to the V3 loop (Fig. 2). Therefore, we selected 3 binding models with the lowest docking scores using 1334, F425 B4e8, and 311-11D, but excluded the model that used 447-52D, for further analysis. We focused on the interaction with HC for 2 reasons. Firstly because the dominance of CDRH3 in the anti-V3 antibody-V3 contacts, as is commonly observed for antigen-recognition by general immunoglobulin. Secondly, because this allows for faster calculation for the detection of contact residues in the analyses.

Analysis of contact residues in docking poses: Of the residues identified as contact residues from existing crystal structures, N100d in F425 B4e8 was the top-ranked contact residue by homology modeling. However, the top-ranked contact residues in the 1334 and 311-11D models were not the contact residues in the crystal structure (Fig. 2 and Table 1). The actual binders

identified by the crystal structure were not always as highly ranked as the contact residues in the redocking poses from ROSIE, although they were listed in the top 20 ranks (Table 1).

To obtain data for contact residues, each AA residue from the 1,000 poses generated by ROSIE was analyzed by Fastcontact, Naccess, and PDBtools (Fig. 1). FastContact ranked the interaction between residues on the HC and V3 peptide by electrostatic force and free energy and provided energetic values containing the epitope positions on the V3 loop involved in the HC-related interactions. Naccess analyzed the type and/or polarity of AA branches engaged in the interaction by comparing the solvent-accessible surface area of a receptor to a receptor-ligand complex. PDBtools was used as a pickup tool for uncommon attractive forces such as salt bridge or cation- π interaction with a distance of 5 Å from R315 on the V3 loop, rather than hydrogen bonds or van der Waals forces. The parameters used to predict contact residues included: (i) appearance frequency as a contact residue in the 1,000 simulated poses from ROSIE; (ii) total energy evaluation in ROSIE output including energetic and locational scores; (iii) energy at the binding sites including electrostatic force and free energy calculated by Fastcontact and interaction energy at AAs on the accessible surface determined by Naccess; and (iv) interaction with epitopes on the V3 loop by Fastcontact, especially with Arg315 which is critical for typical anti-V3 abs-V3 loop interactions, using PDBtools.

Prediction of contact residues of 1C10 by MLP neural network algorithm: MLP was developed to predict contact residues in anti-V3 antibody-V3 peptide complexes by DL (Fig. 2). To avoid overfitting to the 1334 model, we used parameters obtained from 1,000 docking poses of each of the 3 homology models using 1334, F425 B4e8, and 311-11D. In addition to using output values from ROSIE, FastContact, Naccess, and PDBtools for DL, we used AA characteristics, such as polarity, charge, hydrophobicity, and location of the immunoglobulin variable region. Thirty percent of data sets were used as a teacher model to train the DL algorithm by Chainer, and the remaining 70% was

Table 1. Appearance frequency¹⁾ as contact residues in docking poses

Rank	1334				F425B4e8				311-11D			
	AA	freq	actual	pred	AA	freq	actual	pred	AA	freq	actual	pred
1	N100f	18.36	-	18.12	N100d	13.89	+	99.86	Y99	17.78	-	0.00
2	D100e	13.84	-	0.74	D95	9.44	-	0.00	D98	12.92	+	38.89
3	N100d	13.52	-	0.00	N61	8.40	-	0.00	E100b	9.18	-	0.00
4	P100g	9.95	-	0.00	D53	6.93	-	0.00	E95	6.99	-	0.00
5	T100b	7.36	-	0.00	T57	6.32	-	0.00	N52	6.78	+	20.73
6	D99	5.81	+	42.03	E55	6.22	-	0.00	Y100	6.51	+	19.91
7	G100c	4.11	-	0.00	T56	6.20	-	0.00	S100a	6.27	-	1.12
8	E58	4.08	-	0.00	E99	5.29	-	0.00	N54	5.65	-	2.53
9	P98	3.50	-	0.00	G10b	3.97	-	0.00	D56	5.41	-	0.00
10	A100h	3.18	-	0.00	Y58	3.83	-	0.00	S100d	3.31	-	0.03
11	V100a	3.12	-	0.00	S52	3.59	-	0.00	S96	2.36	-	0.01
12	D54	2.39	-	0.15	H59	3.57	-	0.00	T31	2.21	+	6.77
13	N52	1.98	+	14.01	S100c	2.67	-	0.00	R58	2.02	-	0.00
14	Y97	1.14	+	8.91	S100a	2.42	-	0.00	I100c	1.38	+	4.14
15	P100i	0.96	+	5.30	Y97	2.07	-	0.00	T57	1.34	-	0.00
16	D53	0.83	+	6.29	L64	1.61	-	0.00	N28	1.32	-	0.00
17	T57	0.83	+	0.00	S49	1.57	-	0.00	G97	1.20	+	3.65
18	P100j	0.76	-	1.44	Y98	1.39	-	0.00	Y32	1.14	-	0.00
19	L56	0.61	-	0.00	S52a	1.25	-	0.00	T30	1.06	-	0.00
20	W50	0.55	+	0.00	N73	1.07	-	0.00	R50	0.92	+	1.21
Total		96.88		96.99		91.70		99.86		95.75		98.9

¹⁾: Relative frequencies (%) identified as a contact residue (freq) in the ROSIE-docking poses and a predicted contact residue by DL (pred) are shown. These frequencies were calculated by the number selected as a contact residue at each residue divided by a total number selected as a contact residue at all the residues. Contact residues in the crystal structure (actual) are shown as (+).

used as a student model to check the accuracy of the algorithm for the untrained model as well as to avoid overfitting to the teacher model. During training on the teacher model, the accuracy reached more than 95% for the teacher and the student model (Fig. 3). The MLP algorithm successfully identified contact residues in models 1334, F425 B4e8, and 311-11D, irrespective of their appearance rate in the docking simulation (Table 1). Most actual contact residues in the crystal structures were frequently selected as contact residues in the prediction of all 3 models by DL, although T57 and W50 in 1334 were not predicted as contact residues. In contrast, the residues that were highly ranked in the docking simulation, but were not actual binders, were rarely selected as contact residues by DL.

This MLP algorithm was applied to predict the 1C10 contact residues. DL was performed using 1,000 docking poses of the 1C10 homology model, which was based on the 1334-V3 structure, because of the lowest docking score among anti-V3 antibodies (Fig. 2). The AA residues predicted as contact residues were mainly in the CDRH3 and CDRH2 (Fig. 4A and Table 2). The top 10-ranked AA residues were 5 residues in the CDRH3, 3 residues in the CDRH2, 1 residue in the CDRH1, and 1 residue in FR3.

Importance of the predicted AA residues on 1C10 binding activity: To verify the contact residues

Fig. 3. (Color Online) Learning to improve accuracy on deep-learning models. During 20 epochs containing a forward and a backward pass per an epoch, the accuracy on the 30% of the total data as a teacher model reached at more than 97% and on the 70% of the total data as a student model reached at approximately 95%.

predicted by this DL-based method, 1C10 mutants with substitutions in the predicted residues were constructed, and their affinity for the V3 peptide was studied. The mutant 1C10drCDR3-m, in which high-ranked residues

Fig. 4. (Color Online: B and C) Binding affinity of 1C10 mutants to the V3 peptide. Effect of alanine substitution at AA residues predicted in the top 7 contact residues of 1C10 HC by DL was examined by ELISA using the V3 peptide, JRFL NNT20. (A) The location of the top 7 AA residues in 1C10 are shown schematically. The AA residues in CDRH2 and FR3 were substituted to alanine in the 1C10 mutant, 1C10drCDR2-m. The AA residues in CDRH3 were substituted to alanine in the mutant, 1C10drCDR3-m. The mutant, 1C10drCDR2&3-m, contained all 7 substitutions. Point mutants with D95A, D97A, P100aA, and D100bA were also constructed, and designated as dr95-m, dr97-m, dr100a-m, and dr100b-m, respectively. (B) Binding activities of 1C10 mutants, 1C10drCDR2-m, 1C10drCDR3-m, and 1C10drCDR2&3-m, were compared. (C) Binding activities of 1C10 point mutants, dr95-m, dr97-m, dr100a-m, and dr100b-m, were compared.

in the CDRH3, D95, D97, P100a, and D100b, were mutated to an alanine, did not bind to the V3 peptide at all (Fig. 4A and B). The mutant 1C10drCDR2-m, in which D53 and D56 in the CDRH2 and D61 in FR3 were mutated to an alanine, showed significantly reduced binding to the V3 peptide. The complete loss of 1C10 binding activity by CDRH3 mutations was further analyzed using point mutants (Fig. 4C). The substitution D95 to an alanine, which was the most frequently predicted contact residue (Fig. 4A and Table 2), abolished 1C10 binding activity. Substitution at D100b, the third ranked residue markedly decreased 1C10 binding activity. Substitution at P100a, the fifth ranked residue, and D97, the seventh ranked residue, moderately decreased binding activity. These results suggest that the contact residues predicted by the DL-based method are important for 1C10-V3 binding, and that ranking of residues by DL-based prediction is linked to their contribution to binding.

DISCUSSION

We have developed an MLP algorithm to predict the contact residues in an anti-V3 antibody-V3 complex in silico. Furthermore, we have shown that this DL-based method can precisely predict residues which are important for in vitro binding. The precise prediction of contact residues demonstrates the usefulness of the DL-based method for understand antibody-antigen interactions, especially for complexes that are difficult to crystallize. Moreover, this study suggests that the prediction of contact residues using this DL-based

method with various parameters from multiple analyses is much better than using a simple docking simulation. Importantly, the biological data, which were obtained by neutralization assay of mutant antibodies, support our computational analyses. For the first time, we demonstrate that DL can be used to predict which AA residues are involved in the interaction between the anti-V3 antibody and the V3 loop of HIV-1.

Although homologous modeling using the ladle-type antibody, 447-52D, was not readily adaptable to 1C10 (Fig. 2) (1,20), homology modeling of 1C10 using other types of anti-V3 antibodies, 1334, F425 B4e8, and 311-11D, was preferable. Two antibodies, 1334 and 311-11D, showed binding to the V3 loop mainly by HC, whereas F425 B4e8 bound mainly to the LC, which was in contrast to the HC-dependent binding exhibited by other anti-V3 antibodies. We selected 1334, F425 B4e8, and 311-11D for 1C10 homology modeling by ROSIE docking scores instead of binding modes. Although contact residues were variable among these 3 antibodies in crystal structures, the contact residues predicted by the DL-based method were consistent with the actual contact residues (Table 1).

Most binding residues identified in crystal structure analysis were ranked in the top 20 frequent binders in the docking simulation by ROSIE (Table 1). However, the rank of binders in the simulation did not correspond to the binders in the crystal structure. Although N100d of F425 B4e8, which is the only actual contact residue in the HC of this antibody, was most frequently identified as a contact residue, the rankings of the actual contact residues in 1334 and 311-11D were not high

Table 2. Contact residues of 1C10 predicted by DL-based method

Region	HC ¹⁾ position	HC AA	predict ²⁾	ranking ³⁾
CDR3	95	ASP	26.23	1
	100b	ASP	17.37	3
	100a	PRO	6.42	5
	97	ASP	4.94	7
	100f	PRO	2.4	8
	100	ILE	0.35	11
	104	GLU	0.22	14
	100e	ALA	0.09	17
	100c	LEU	0.09	17
CDR2	56	ASP	23.63	2
	53	ASP	9.14	4
	52a	ASN	0.85	10
	54	GLY	0.28	12
	50	ILE	0.22	14
	51	ILE	0.03	19
	52	SER	0.03	19
	31	ASN	1.93	9
CDR1	28	MET	0.06	19
	33	ALA	0.03	19
	61	ASP	5.22	6
FR3	58	TYR	0.25	13
	65	GLN	0.13	16
	75	ASN	0.03	19
	4	GLU	0.03	19

¹⁾: Heavy chain.

²⁾: Relative frequency selected as a contact residue in the prediction.

³⁾: Ranking of frequency predicted as a contact residue by DL-based method.

in the redocking simulation. This is partially due to the calculation of energy by ROSIE, which does not consider the component amino acids involved in the interaction (24–27). Analysis by Fastcontact, Naccess, and PDBtools contributed to the precise prediction of contact residues by the addition of local energy and locational parameters (30–32). DL is suitable for the comprehensive analysis of various parameters obtained from multiple analyses (16–18). The precise prediction of contact residues in this study demonstrates the usefulness of the DL-based method using various parameters from multiple analyses to reveal antibody-antigen interactions.

In summary, with supporting data of local energy on the contact sites, our DL algorithm predicted the contact residues of the anti-V3 nAb, 1C10, for the V3 loop. The contact residues predicted by the DL-based method were shown to be important for binding to the V3 loop. We focused on the identification of contact residues in the paratope of 1C10 in this study. Prediction of contact residues in the epitope in the V3 loop using this DL algorithm will be useful to further elucidate the interaction between anti-V3 nAbs and the V3 loop. Ascertaining both the epitope and paratope contact residues will lead help us understand the mechanism by

which 1C10 is able to neutralize a broad range of clade B viruses.

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Conflict of interest None to declare.

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