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RESEARCH HIGHLIGHT

Structural characterization of modification on the interface between a ligand and its receptor for biopharmaceuticals

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The binding of a protein to its target is a major mode of action for most biopharmaceutical therapies with over 70% of biopharmaceuticals involved in binding between the protein and its target. The interfaces between a biopharmaceutical and its target are key regions for its efficacy. Any modifications to the amino acids at the interfaces invariably affect interactions between the biopharmaceutical and its receptor and may result in lowering therapeutic efficacy. Degradations of biopharmaceuticals by modifications including deamidation on asparagine (Asn) and/or isomerization on aspartic acid (Asp) have been well characterized and those modifications at the interfaces have resulted in a loss of activity. To characterize modification hot-spots on the interfaces, it is necessary to identify the amino acid residues on the interfaces. We recently addressed a visualization tool for amino acids on the interfaces between a protein ligand and its receptor. This tool was applied to visualize ligand protein-receptor interaction and antigen-antibody interaction. As a model system for ligand protein-receptor interaction, erythropoietin (EPO) and its receptor were selected and amino acids on the interfaces were identified. Modifications on the interfaces were then investigated. Deamidation of Asn was identified at two amino acid residues, Asn47 and Asp147, on Interface 1 of EPO. The relative contents of deamidated residues on the interface of EPO were in the range of 3-5% of the total. As a model system for antigen-antibody interaction, Herceptin and its receptor, HER2, were chosen and amino acids on the interfaces were identified. Then modifications on the Deamidation on Asn30 of the light chain and Asn55 of the heavy chain were identified. The relative contents of the deamidated residues on the interfaces were in the range of 8-9% of the total. Along with deamidation, another modification, isomerization, was identified on the heavy chain Asp102, and the level of isomerization was 13.5% of the total. Our studies provide a targeted method focusing on the interface between a protein and its target that can be coupled with other applications, for example, identification of modified amino acids on the interfaces.

Keywords: Biopharmaceutical; Erythropoietin; Monoclonal antibody; Interface; Modification

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Biopharmaceuticals: Mode of Action

Biopharmaceuticals have been developed for human therapeutics for over a decade. Until now, over 150 biopharmaceuticals have obtained approval by European Medicines Association or US Food and Drug Administration [1]. Most biopharmaceuticals are highly complex protein molecules that also require high purity and are processed through upstream and downstream processes. Those approved biopharmaceuticals can be classified based on their

Table 1. Classification of biopharmaceuticals based on their mode of action

Mode of Action	Case	% Total
Antigen-Antibody Binding	33	21.2%
Enzyme	33	21.2%
Ligand-receptor binding	71	45.5%
Non-covalent binding to the target	11	7.1%
Others	8	5.1%
Total	156	100.0%

mode of action (Table 1) with approximately one-half of biopharmaceuticals acting through ligand-receptor binding, over 20% through antigen-antibody binding, and 7% through non-covalent binding to the target. Overall, over 70% of biopharmaceuticals are involved in binding between a protein and its target, which shows that the binding of a biopharmaceutical protein to its target is the major mode of action for most therapies.

Modification on the Binding Sites or Interfaces

The binding sites or interfaces between a biopharmaceutical and its target are key regions for the action of the biopharmaceutical and are exposed during downstream processing, which causes them to be more vulnerable to modification than other sites. Any modifications of amino acids in the binding sites or interfaces invariably affect interactions between the biopharmaceutical and its receptor and may result in lowering therapeutic efficacy [2]. From a regulatory point of view, these changes may influence the safety, purity, potency, and strength of products, which must be well-characterized, controlled, and monitored as part of the manufacturing process [2,3,4];. Physicochemical and biological analyses are required for monitoring of product quality [5,6]. Deamidation on the amino acid asparagine (Asn) and isomerization on the amino acid aspartate (Asp) have been well characterized modifications, and those modifications in the binding sites or interfaces have resulted in a loss of activity [7,8,9]. Amino acids at the binding sites or interfaces are known to be hot-spots for deamidation and isomerization [7,8,9]. Although deamidation and isomerization have also been reported on amino acids in areas other than binding sites or interfaces, the levels of modification are much lower than those found on the hot-spots [6,10].

Identification of Amino Acids on the Binding Sites or Interfaces

To characterize modification hot-spots, it is necessary to identify the amino acids on the binding sites or interfaces. This process is mainly dependent on knowledge of the three dimensional structures of proteins, but it is difficult to obtain clear information on the binding sites or interfaces for biopharmaceutical proteins even though their structures are already known. These are time-consuming and labor-intensive processes.

We recently addressed a method for identifying amino acids on the binding sites or interfaces between a ligand protein and its receptor [2]. This is a visualization tool developed using Easy Structural Biology Template Library (ESBTL) and Open Graphics Library (OpenGL). Residue information including geometry, chains, and amino acids was extracted from a PDB file loaded with the ESBTL tool. The distance between amino acids on the interface from different chains was calculated based on the x, y, and z coordinates from the geometry information extracted. The potential amino acids interacting on the interface were visualized based on the cut-off values of the calculated distance information using OpenGL.

Interface Identification between a Ligand Protein and its Receptor

As a model system for ligand protein-receptor interaction, erythropoietin (EPO) and its receptor were selected and amino acids on the interfaces were identified. The interfaces between EPO and its receptor are already characterized by structural analysis by -NMR- study [11] and X-ray study [12]. There are two interfaces between EPO and its receptor. Interface 1 of EPO is localized on the AB coil (residues 44 to 54) and helix D (residues 147 to 151) and Interface 2 is found on helices A (residues 10 to 15) and C (residues 100 to 104) [12]. Mutations on the amino acids in those interfaces of EPO prevent interaction to its receptor and finally abolish the erythropoietic activities of EPO [13,14,15]. For example, the mutant Asn147 to Lys147 of EPO showed over 100-fold reduced affinity for its receptor binding and over 200-fold in in vitro activity [14]. Thus, any chemical modifications of these residues that were generated during the manufacturing process of EPO may affect the efficacy of the final product, and any quality issues related to those modifications should be identified, well-characterized, and monitored.

Several amino acids on Interface 1 (Fig. 1) of EPO were identified, including Thr44, Val446, Asn47, Phe48, Asn147, Gly151, and Leu155 [2]. These have the potential to interact with Phe93 of the EPO receptor. Some amino acids on Interface 2 (Fig. 1) of EPO were also identified, including Arg10, Val11, and Arg14, which is an area mainly interacting with Met150 of the EPO receptor. Modifications on the interfaces were then investigated. Deamidation that converted Asn to Asp was identified at two amino acid residues, Asn47 and Asp147, on Interface 1 [2]. The relative contents of the deamidated residues on the interface were in the range of 3-5% of the total, which is larger than the

Table 2. Modifications identified on the interfaces of EPO

Amino Acid (Position)	Modification	%Modification	Location
Asn (47)	Deamidation	2.9	Interface 1
Asn (147)	Deamidation	4.8	Interface 1
Met (54)	Oxidation	3.0	Interface 1
Asp (136)	Isomerization	0.7	Not on the interfaces

Table 3 Modifications identified on the interfaces of Herceptin

Amino Acid (Position)	Modification	%Modification	Location
Asn (30)	Deamidation	8.1	Interface 1 (Light chain)
Asn (55)	Deamidation	8.6	Interface 2 (Heavy chain)
Asp (103)	Isomerization	13.5	Interface 3 (Heavy chain)

relative contents of the modified residues not on the interfaces (Table 2). Along with deamidation, another modification, oxidation, was identified at the amino acid residue Met54 on Interface 1, and the level of oxidation was 3% of the total (Table 2).

Interface Identification between antigen and antibody

The interface visualization tool we developed can be extended to identify interfaces of antigen-antibody binding. Interactions between antigen and antibody are well-defined and the majority of interactions are localized to complementary-determining regions (CDRs). There are three CDRs (CDR1, CDR2, and CDR3) on the variable regions of antibodies (Fv domain).

As a model system for antigen-antibody interaction, Herceptin and its receptor HER2 were chosen, and amino acids on the interfaces were identified. The interaction between Herceptin and its receptor HER2 is well-characterized by structural determinations using X-ray crystallography [16,17]. There are three interaction sites between Herceptin and HER2. Interface 1 is localized to the CDR1 of Herceptin and interaction is with the basal side of the loop composed of residues 593-603 of HER2, Interface 2 corresponds to CDR2 and interaction is with the loop formed by residues 557-561 of HER2, and Interface 3 is on CDR3 and interaction is with the loop formed by residues 570-573 of HER2 [17].

Using our visualization tool, amino acids on the interfaces for Herceptin were identified (Fig. 2). Asp31 and Thr31 from the heavy chain and Asp28 and Asn30 from the light chain of Herceptin were identified on Interface 1 of CDR1, Thr54 and Asn55 from the heavy chain and Ser50 and Phe53 from the

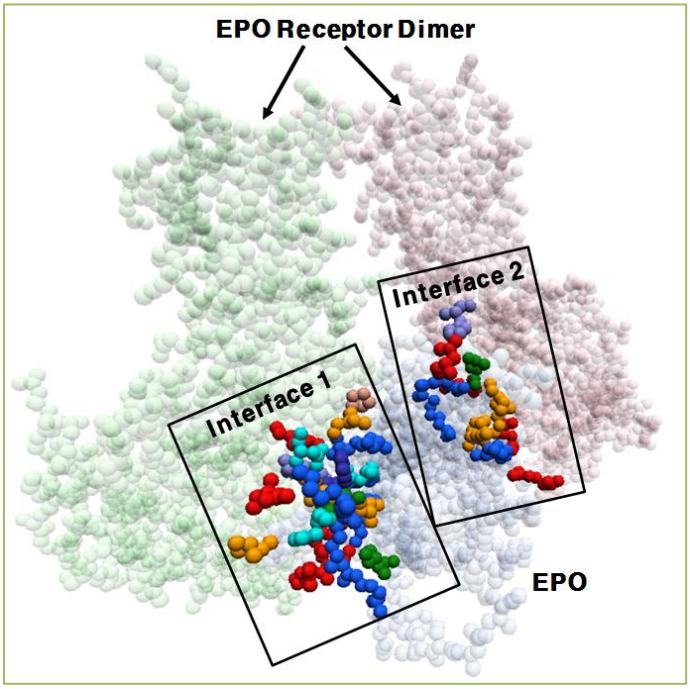

Figure 1. Interfaces visualized between EPO and its receptor.

light chain of Herceptin were identified on Interface 2 of CDR2, and Asp102 and Phe104 from the heavy chain and His91 and Tyr92 from the light chain of Herceptin were identified on Interface 3 of CDR3. Modifications on the interfaces were then assessed. Deamidations on Asn30 of the light chain and Asn55 of the heavy chain were detected. The relative contents of deamidated residues on the interfaces were in the range of 8-9% of the total (Table 3). Along with deamidation, another modification, isomerization, was identified on Asp102 of the heavy chain and the level of isomerization was 13.5% of the total (Table 3). These results provide a good match with data previously reported [6,7,8,9].

Conclusions

We recently addressed a visualization tool for amino acids on the interfaces between a protein ligand and its receptor [2]. The binding of the ligand protein to the specific receptor is a major mode of action for most biopharmaceutical therapies. Our studies provide a targeted method focusing on the interface between the ligand and its receptor that can be coupled with other applications, for example, identification of modified amino acids on the interfaces. We also show that our approach can be extended to antigen-antibody interaction. Thus, our method may contribute a novel and valuable tool for use in the identification of modification hot-spots of biopharmaceuticals.

Acknowledgements

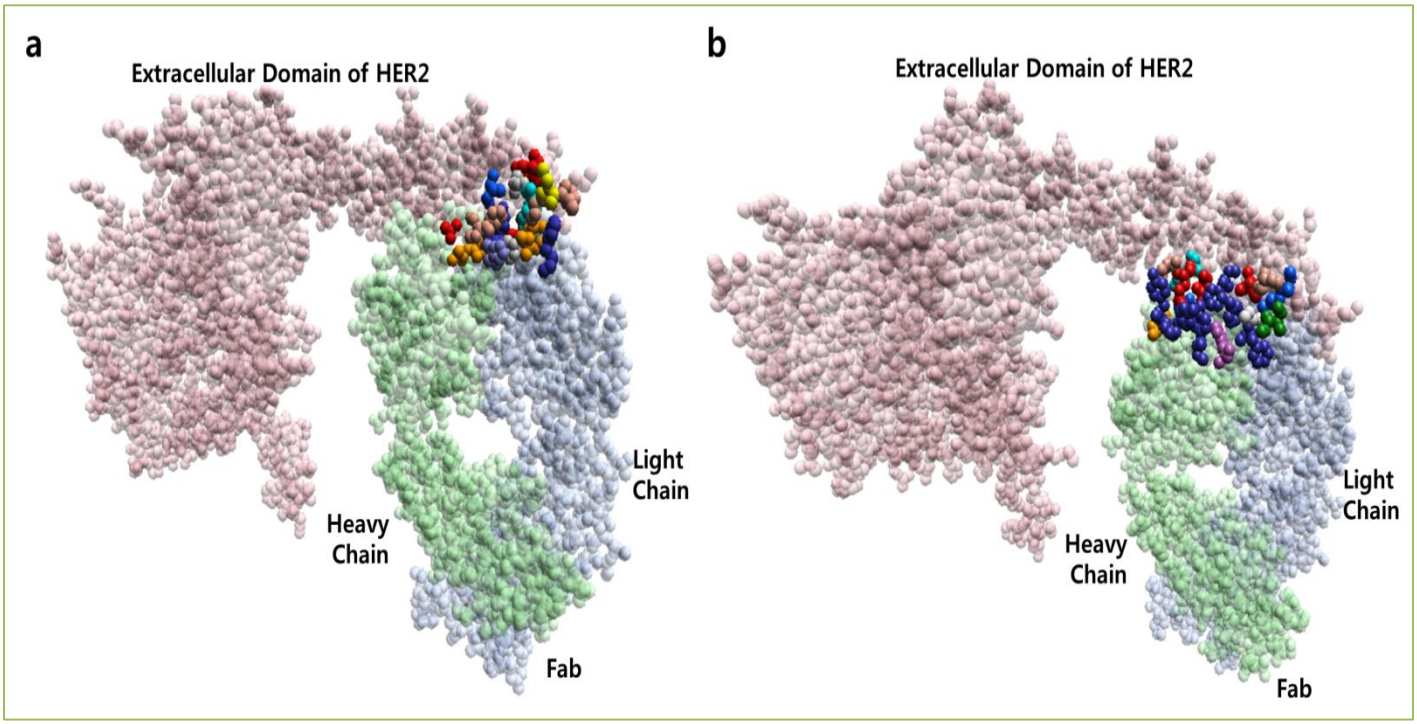

Figure 2. Interfaces visualized between Herceptin and its receptor, HER2. a, the interface was visualized between the light chain of Fab and the extracellular domain of HER2; b, the interface was visualized between the heavy chain of Fab and the extracellular domain of HER2.

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Conflicting interests

The authors have declared that no competing interests exist.

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