New Approach to Peptide Ligand Design Based on Atom-by-atom *de novo* Design†

Yoshihiko Nishibata, Chiaki Fukushima, Momi Murase and Toshikazu Miyagishima

Lead Optimization Research Laboratory, Tanabe Seiyaku Co., Ltd.
2-2-50, Kawagishi, Toda, Saitama, 335, Japan

De novo ligand design is a key developing technology for the computer-aided design of enzyme inhibitors. By this method, one can design a number of novel inhibitor candidates based on the 3D structure of the target enzyme [1-6]. There are two different ways to design ligands *de novo*. One is so called atom by atom, and the other is fragment-based.

Nonpeptide inhibitors are more desirable than peptides as drugs because of stability, selectivity and possibility of oral administration. Therefore, most of *de novo* methods are also focused on designing nonpeptide inhibitors. However, peptide inhibitors are easier to synthesize and play an important role in the lead generation stage in many cases.

Moon and Howe [3] have reported the *de novo* peptide design method GROW. It designs peptides that fit the binding site of the enzyme by connecting amino acids. Caflisch *et al.* [5] show HIV-1 protease inhibitors can be assembled from several type of small molecules such as N-methylacetamide, acetamide, methanol and so on.

We have shown the usefulness of atom by atom *de novo* design method [2]. The method is suited to generate a wide variety of chemical structures. Conversely, it is difficult to limit the output to particular type of backbone. So, there are no way to design inhibitor peptides by atom-based method so far.

We report a new approach to design inhibitor peptides using LEGEND [1,2], an atom by atom *de novo* ligand design system. In our method, a known peptide inhibitor is used as a template. The 3D structure of the peptide is extracted from the crystallographic structure of the enzyme-inhibitor complex. All the atoms of side chains are removed, and the main chain portion of the peptide is used as a starting structure for

LEGEND. LEGEND attempts to fill the remaining valences of the starting structure with appropriate atoms. Therefore, in this case, it generates ligand candidates by adding various groups to C_α atoms of the main chain.

We applied this method to α-lytic protease to evaluate our method. Bone *et al.* have reported the crystallographic results of series of protein-inhibitor complexes including binding pocket mutants [7,8]. We used the crystallographic coordinates of α-lytic protease (mutant with Met 192 replaced by Ala) complexed with methoxysuccinyl-Ala-Ala-Pro-alanine boronic acid as a template.

The coordinates of the main chain atoms and C_β to C_δ of Pro were extracted and used as a starting structure. From 100 generated structures, 58 are selected using the energy criteria. All the generated structures are of tetrapeptide. The result shows this approach can design peptides using the atom by atom method. It suggests Ala can be replaced by a larger amino acid and Pro can be changed to various substituted prolines. No D amino acid is found in the generated structures. It means that a peptide including D amino acid cannot bind to the enzyme with same conformation as original ligand. More detailed evaluation is now undertaken and will be discussed.

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References

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