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The design for therapeutic agents of Leucine Rich Repeat protein using bioinformatics

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by progressive joint deterioration; Furthermore, RA can also affect body tissues, including the skin, eyes, lungs, heart and blood vessels. The early stages of RA can be difficult to diagnose because the signs and symptoms mimic those of many other diseases. It is not known exactly what triggers the onset of RA and how to cure the disease. But recent discoveries indicate that remission of symptoms is more likely when treatment begins early with strong medications known as disease-modifying anti-rheumatic drugs (DMARDs).

Tumor necrosis factor (TNF) inhibitors are typical examples of biotherapies that have been developed for RA. The substances may occur naturally in the body or may be made in the laboratory. Other biological therapies care biological response modifiers (BRMs) such as monoclonal antibodies, interferon, interleukin-2 (IL-2) and a protein binder using repeat units. These substances play significant anti-inflammatory roles.

Proteins with recurrent, conserved amino acid stretches mediate interactions among proteins for essential biological functions; for example, ankyrin (ANK), Heat repeat protein (HEAT), armadillo repeat protein (ARM) and tetratricopeptide repeats (TPR). Here, we describe Leucine rich repeats (LRR) that ideally fold together to form a solenoid protein domain and is more applicable to our current study than the previously mentioned examples. Although BRMs have limitations in terms of immunogenicity and effector functions, among other factors, in the context therapeutic use and for proteomics research, We has become clear that repeat-unit-derived binding proteins will increasingly be used in biotechnology and medicine.

Keywords: *Rheumatoid arthritis (RA), Biological response modifiers (BRMs), Leucine rich repeat (LRR), Hybrid LRR technique*

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1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, heterogeneous autoimmune disease, primarily and variably affecting the small joints of the hands and feet of patients. Although distinct advances has been made in the care of patients with RA during the last twenty-years, RA still remains a chronic inflammatory form of arthritis that can also effect on osteoporosis, cardiovascular events, and risk of cancer. Clinical experience clearly shows that acting early is the best way to control the disease and induce remission. Furthermore, disease-modifying anti-rheumatic drugs (DMARDs) are currently used to control symptoms during all stages of RA. It has been shown to be more effective, including anti-TNF agents and methotrexate (MTX), and may be more effective in patients with RA than starting with monotherapy at all stages of RA in patients with low disease activity. Some biotherapies stimulate or suppress the immune system to help the body fight cancer, infection, and other diseases[1-2].

Therefore, we propose an alternative RA treatment using repeat proteins based on their unique structural modules. Several classes of repeat proteins have been found and developed to obtain reagents with specificities and affinities in a range that was previously considered unique to antibodies.

The importance of shape-complementarity to understand biological function resides not only in their high frequency among known sequences, but also in their abilities to confer multiple binding and structural properties on proteins. These properties are suitable for protein-protein interactions, mediating many important biological functions, including cell adhesion, signaling process, neural development, bacterial pathogenicity, extracellular matrix assembly, and the immune response. The repeat scaffold proteins are ubiquitous and non-globular molecules and their tandem arrays have attracted much attention as alternative binding scaffolds to antibodies. These are characterized by consecutive homologous structural motifs, or repeats, which stack together to form elongated structures. For examples, the extracellular domains of Toll-like receptors (TLRs) are formed into a Leucine-rich repeat (LRR), which is a shared, typical structural motif (LxxLxLxxN) and this family comprises approximately 6000 proteins in the Pfam database (http://pfam.sanger.ac.uk)[3-5].

The modular architecture of repeat proteins has evolved to be suitable for many critical biological functions of proteins by evolutionally accepting their point mutations, insertion, deletion, or rearrangement of the repeat unit. Recently, with the growth of general computational analysis for organizing the repeats, the relationship between the amino acid sequence and the three-dimensional structure of proteins with internal repeats have been discussed. Although repeat proteins consist of adjacent series of usually non-identical repeated amino acid sequences, there are similarities, including the formation of short-ranged intra-repeat and inter-repeat interactions. These interactions can be formed by hydrogen bonds between hydrophobic and aromatic amino acid residues. Major repeat protein families include LRRs, Ankyrin (ANK), Armadillo repeat protein arm (ARM) and Heat repeat protein (HEAT) and Tetratricopeptide repeat (TPR). Their respective motifs comprises hairpins with two elements of secondary structure such as α helix/α helix, β sheet/β sheet, or α helix/β sheet units[6-9].

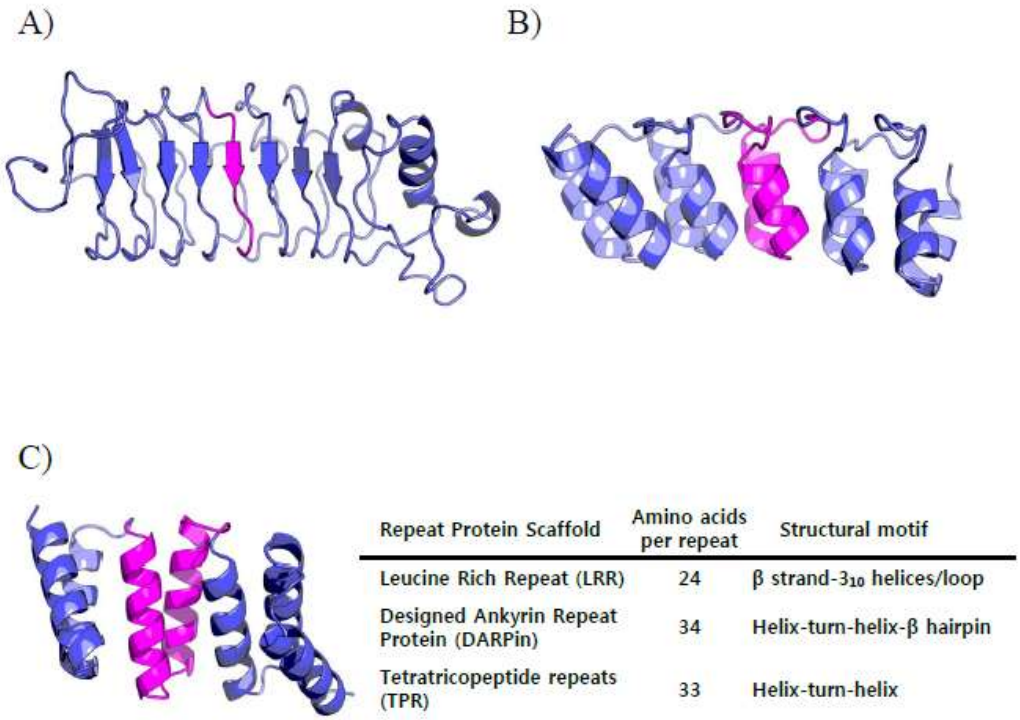
To find the most specific, high-affinity binding molecules, artificial antibodies or antibody fragments have typically been designed by generating libraries of constructs randomly mutagenized at the loop regions or at permissible surface areas; however increasing use of these products in research, biotechnology, and as medical treatments, have revealed that antibodies suffer from some fundamental disadvantages. Therefore, there has been a growing interest in replacing engineered immunoglobulins with artificial repeat protein scaffolds because of an enhanced potential in using the structural features of these molecules. Also, because the short-range and regularized interactions of artificial proteins are the dominant features, the molecules have the potential to be developed as alternative scaffolds for use as therapeutic proteins, biosensors, and so on. Therefore, we describe our current knowledge concerning the nature of motifs, folding, structures, and functions from different repeat classes as well as the scope of potential applications[6],[10-11].

2. THEORY

Leucine Rich Repeat (LRR) scaffold

At that time, which was first known the modeling of LRR structure from a ribonuclease inhibitor (RI), the LRRs correspond to structural units consisting of a β-strands and a α-helix. These units are arranged so that all the β-strands and the α-helices are parallel to a common axis, resulting in a non-globular, horseshoe-shaped molecule with a curved parallel β-sheet lining the inner circumference of the horseshoe and the helices flanking its outer circumference. Since that time, new structural information on proteins with LRRs has rapidly accrued and been classified in different LRR subfamilies and in proteins with diverse functions.

Figure 1.



Repeat Protein Scaffold	Amino acids per repeat	Structural motif
Leucine Rich Repeat (LRR)	24	β strand-3_{10} helices/loop
Designed Ankyrin Repeat Protein (DARPin)	34	Helix-turn-helix-β hairpin
Tetratricopeptide repeats (TPR)	33	Helix-turn-helix

Figure 1. The protein structure with repeat module. A) the crystal structure of human Slitrk1 LRR1 (PDB ID : 4RCW)[4], B) the crystal structure of E3_19 a designed ankyrin repeat protein (PDB ID : 2BKG)[21], C) Design of stable alpha-helical arrays from an idealized TPR motif (PDB ID : 1NA0)[22], The structures with Leucine Rich Repeat (LRR), Ankyrin, TPR moduleAre shown in blue and magenta, which is colored amino acids per repeat unit.

Several subfamilies may be distinguished based on the three-dimensional structure, due to different lengths (about 20 ~ 30 amino acid residues) and consensus sequences of the LRR repeat. The former structure influences twist, tilt, rotation angles, and radii of the LRR module while the latter is comprised of a concave region, forming a β-strand by a typical structural motif (LxxLxLxxN), and a consecutive convex region which is contained α-helix or 3_{10} helices and loops by variable residues. Generally, seven classes of the LRRs have been proposed that are classified as “typical”, “ribonuclease inhibitor-like (RI-like)”, “SDS22^{+} protein-like (SDS22-like)”, “plant-specific (PS)”, “bacterial”, “cysteine-containing (CC)” and “Treponema pallidum LRR (TpLRR). These classes show distinct, regular protein conformations: 1) leucines point inwards into the protein and are involved in forming the hydrophobic core. They are also sometimes substituted by other hydrophobic

amino acids such as valines, isoleucine, and phenylalanine; 2) asparagines are important in maintaining the overall shape of the protein because they form a continuous hydrogen-bonded network with carbonyl oxygens in the backbone of neighboring LRR modules. They can be replaced by other amino acids capable of donating hydrogens such as threonine, serine, and cysteine and ; 3) the variable "x" residues in the motif are exposed to the solvent, and some of them play important roles in ligand interactions. The grouping scheme had been applicable to proteins containing only a single module based on their sequences and structural patterns before being known to the atypical structures of the TLR series[12-13].

To date, the structures of six of the ten human TLRs in complex with their physiological or synthetic ligands have been reported in the literature. Among the TLR structures, the β-sheet conformation of TLR4 as well as that of TLR1, TLR2 and TLR6 deviates substantially from the consensus LRR structures due to splitting into a single module. They can be divided into N-, central, and C-terminal domains and undergo sharp structural transitions at the domain boundaries. The structural discontinuities found in these TLRs seem to be caused by irregular LRR sequences concentrated in the central domain, which lack aspargine ladders that stabilize the overall horseshoe-like structure by forming a continuous hydrogen-bond network. The broken asparagine ladders in the central domains may allow the unusual distortion found in the structures. Whereas the N- and C- terminal domains agree well with consensus structure of the typical subfamily, the lengths of LRR modules vary little around a value of 24 amino acid residues, and the structurally important asparagine ladder and phenylalanine spine are conserved.

For the first time, the structures of TLR1/2, 4, 2/6, and 5 were determined by using a "Hybrid LRR technique". To overcome the difficulties in producing target proteins in soluble form and in crystallizing them, the TLR and the VLR (Hagfish variable lymphocyte receptor) fragments were fused at their conserved motifs so that local structural incompatibility could be minimized. As the hybrid technique, TLR and VLR fragments are at the most conserved "LxxLxLxxN" sites because they are easy to be identified from amino acid sequences and because they always form β-strands that can be superimposed among different LRR structures[14],[15],[16].

Therefore, structural studies of these LRR proteins using the "Hybrid LRR Technique" will make significant contribute to their biological and medical research; for example, TOY (TLR4 decoy receptor) was designed and generated soluble fusion proteins capable of binding MD-2. When administered in either preventive or therapeutic manner, it resulted in a favorable pharmacokinetic profile in vivo. Also, the trial of hybrid protein has facilitated a general computational method for building idealized repeats that integrates available family sequences and structural information[17-18].

This computational prediction of LRR proteins as well as repeat proteins is more reliable than a prediction of globular proteins and these proteins have increasingly attracted much attention due to their unique structural features. Design of these proteins with high affinity and specificity for a target ligand is usually engineered by structure-based mutagenesis, which is focused at a permissible surface area, and by selection of variants against a given target via phase display or related techniques. The one example is that a TLR4 decoy receptor composed of hybrid LRR modules was used and its binding affinity for MD-2 was improved by replacing one or more amino acids of interaction interface. The other is known to "repebody", which is redesigned by using internalin-B cap in substitution for the N-terminal domain of LRR module. It has been recently developed a high-affinity protein binder, which suppresses non-small cell lung cancer in vivo by blocking the IL-6/STAT3 signaling[19-20].

Figure 2.

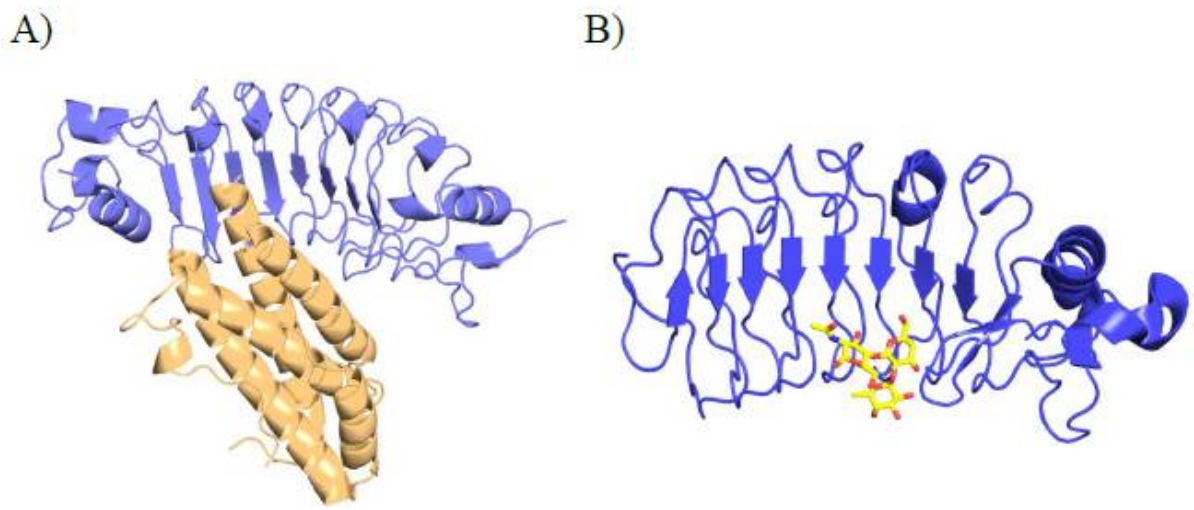


Figure 2. The structures of protein binder containing Leucine Rich Repeat A) Crystal structure of r-D3E8(repebody)/human IL-6 complex (PDB ID : 4J4L)[20]. r-D3E8 and IL-6 are shown in blue and wheat, respectively. B) Crystal structure of Variable Lymphocyte Receptor (VLR) RBC36 in Complex with H-trisaccharide (PDB ID : 3E6J)[23]. VLR and RBC36 are colored in blue and yellow, respectively.

If the above-mentioned method based on computational analysis can be said to be the first generation, it is truly inventive that the transformation of the LRR scaffold's curvature allows for the new proteins with custom-designed shapes for a specific application. Using Rosetta repeat-protein-idealization method, these are generated by appropriately combining idealized building-block, which has an LRR module with 22 or 24 residues and a constant curvature, and junction modules, contained irregular LRR or wedge, to produce a local change in the protein curvature. This approach is confirmed by protein structural analysis and by energy-driven design calculations to arrive at the idealized building-block and junction modules. Also, the computational analysis is convenient to achieve high-accuracy models of the complex repeat proteins generated by the module assembly process and thus increase the potential of their uses as biosensors, in proteomics research, and potentially also as therapeutic proteins.

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REFERENCES

[1] P. Miossec "Introduction: 'Why is there persistent disease despite aggressive therapy of rheumatoid arthritis?'" Arthritis Res Ther. Vol.16, No.3 pp. 113, 2014. doi:10.1186/ar4592

[2] E.G. Favalli, M.G. Raimondo, A. Becciolini, C. Crotti, M. Biggioggero, R. Caporali "The management of first-line biologic therapy failures in rheumatoid arthritis: Current practice and future perspectives.", Autoimmun Rev. Vol.16, No.12, pp.1185-1195, 2017. doi: 10.1016/j.autrev

[3] Y. Lee, J.J. Lee, S. Kim, S.C. Lee, J. Han, W. Heu, K. Park, H.J. Kim, H.K. Cheong, D. Kim, H.S. Kim, K.W. Lee "Dissecting the critical factors for thermodynamic stability of modular proteins using molecular modeling approach." PLoS One. Vol.9, No.5, pp: e98243, 2014. doi: 10.1371/journal.pone.0098243

[4] J.W. Um, K.H. Kim, B.S. Park, Y. Choi, D. Kim, C.Y. Kim, S.J. Kim, M. Kim, J.S. Ko, S.G. Lee, G. Choii, J. Nam, W.D. Heo, E. Kim, J.O. Lee, J. Ko, H.M. Kim "Structural basis for LAR-RPTP/Slitrk complex-mediated synaptic adhesion." Nat Commun. Vol.14, No.5, pp: 5423, 2014. doi: 10.1038/ncomms6423

[5] M.A. Andrade, C. Perez-Iratxeta, C.P. Ponting "Protein repeats: structures, functions, and evolution." J Struct Biol. Vo1.134, No.2-3, pp: 117-131, 2001. doi: 10.1006/jsbi.2001.4392

[6] E.R. Main, A.R. Lowe, S.G. Mochrie, S.E. Jackson, L. Regan "A recurring theme in protein engineering: the design, stability and folding of repeat proteins." Curr Opin Struct Biol.Vol.5, No.4, pp:464-471, 2005. doi:10.1016/j.sbi.2005.07.003

[7] B. Kobe, A.V. Kajava "When protein folding is simplified to protein coiling: the continuum of solenoid protein structures." Trends Biochem Sci. Vol.25, No.10, pp:509-515, 2000. doi:10.1016/s0968-0004(00)01667-4

[8] F. Parmeggiani, R. Pellarin, A.P. Larsen, G. Varadamsetty, M.T. Stumpp, O. Zerbe, A. Caflisch, A. Plückthun "Designed armadillo repeat proteins as general peptide-binding scaffolds: consensus design and computational optimization of the hydrophobic core." J Mol Biol. Vol.7, No.376, pp:1282-1304, 2008. doi: 10.1016/j.jmb.2007.12.014

[9] E.R. Main, S.E. Jackson, L. Regan "The folding and design of repeat proteins: reaching a consensus." Curr Opin Struct Biol.Vol.18, No.4, pp:482-489, 2003. doi:10.1016/s0959-440x(03)00105-2

[10] A. Skerra "Alternative non-antibody scaffolds for molecular recognition." Curr Opin Biotechnol. Vol.18, No.4, pp:295-304, 2007. doi:10.1016/j.copbio.2007.04.010

[11] B. Kobe, A.V. Kajava "The leucine-rich repeat as a protein recognition motif." Curr Opin Struct Biol. Vol.11, No.6, pp:725-732, 2001. doi:10.1016/s0959-440x(01)00266-4

[12] J.Y. Kang, X. Nan, M.S. Jin, S.J. Youn, Y.H. Ryu, S. Mah, S.H. Han, H. Lee, S.G. Paik, L.O. Lee "Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer." Immunity. Vol.31, No.6, pp:873-884, 2009. doi: 10.1016/j.immuni.2009.09.018

[13] B.S. Park, D.H. Song, H.M. Kim, B.S. Choi, H. Lee, J.O. Lee "The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex." Nature. Vol.458, No.7242, pp:1191-1195, 2009. doi: 10.1038/nature07830

[14] H.M. Kim, B.S. Park, J.I. Kim, S.E. Kim, J. Lee, S.C. Oh, P. Enkhbayar, N. Matsushima, H. Lee, O.J. Yoo, J.O. Lee "Crystal structure of the TLR4-MD-2 complex with bound endotoxin antagonist Eritoran." Cell, Vol.130, No.5, pp: 906-917, 2007. doi:10.1016/j.cell.2007.08.002

[15] M.S. Jin, J.O. Lee "Structures of the toll-like receptor family and its ligand complexes." Immunity, Vol.29, No.2, pp:182-191, 2008. doi: 10.1016/j.immuni.2008.07.007

[16] M.S. Jin, J.O. Lee "Application of hybrid LRR technique to protein crystallization." BMB Rep, Vol.41, No.5, pp:353-357, 2008. doi:10.5483/bmbrep.2008.41.5.353

[17] K. Jung, J.E. Lee, H.Z. Kim, H.M. Kim, B.S. Park, S.I. Hwang, J.O. Lee, S.C. Kim, G.Y. Koh "Toll-like receptor 4 decoy, TOY, attenuates gram-negative bacterial sepsis." PLoS One. Vol.4, No.10, pp:e7403, 2009. doi: 10.1371/journal.pone.0007403

[18] S.C. Lee, K. Park, J. Han, J.J. Lee, H.J. Kim, S. Hong, W. Heu, Y.J. Kim, J.S. Ha, S.G. Lee, H.K. Cheong, Y.H. Jeon, D. Kim, H.S. Kim "Design of a binding scaffold based on variable lymphocyte receptors of jawless vertebrates by module engineering." Proc Natl Acad Sci U S A., Vol.109, No.9, pp:3299-3304, 2012. doi: 10.1073/pnas.1113193109

[19] J.J. Lee, H.J. Kim, C.S. Yang, H.H. Kyeong, J.M. Choi, D.E. Hwang, J.M. Yuk, K. Park, Y.J. Kim, S.G. Lee, D. Kim, E.K. Jo, H.K. Cheong, H.S. Kim "A high-affinity protein binder that blocks the IL-6/STAT3 signaling pathway effectively suppresses non-small cell lung cancer." Mol Ther. Vol.22, No.7, pp:1254-1265, 2014. doi: 10.1038/mt.2014.59

[20] H.K. Binz, A. Kohl, A. Plückthun, M.G. Grütter "Crystal structure of a consensus-designed ankyrin repeat protein: implications for stability" Proteins, Vol.65, No.2, pp:280-284, 2006. doi:10.1002/prot.20930
[21] E.R. Main, Y. Xiong, M.J. Cocco, L. D'Andrea, L. Regan "Design of stable alpha-helical arrays from an idealized TPR motif." Structure, Vol.11, No.5, pp:497-508, 2003. doi:10.1016/s0969-2126(03)00076-5
[22] B.W. Han, B.R. Herrin, M.D. Cooper, I.A. Wilson "Antigen recognition by variable lymphocyte receptors." Science, Vol.321, No.5897, pp:1834-1837, 2008. doi: 10.1126/science.1162484
[23] H.K. Kim "Effects of Korean Radish on DSS-Induced Ulcerative Colitis in Mice" IJACT, Vol.6, No.4, 97-108, 2018. doi https://doi.org /10.17703//IJACT2018.6.4.97
[24] H.K. Kim, J.H. Lee "Anticardiovascular Diseases Effects of Fermented Garlic and Fermented Chitosan" IJACT, Vol.6, No.4, 109-115, 2018. doi https://doi.org /10.17703//IJACT2018.6.4.109