Biochemical and Biophysical Research Communications 422 (2012) 245–249

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The binding affinity of a soluble TCR-Fc fusion protein is significantly improved by crosslinkage with an anti-Cβ antibody

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ARTICLE INFO

Article history:
Received 23 April 2012
Available online 1 May 2012

Keywords:
Soluble form of the TCR
Affinity
Anti-Cβ antibody

ABSTRACT

The identification and cloning of tumor antigen-specific T cell receptors (TCRs) and the production of the soluble form of the TCR (sTCR) contributed to the development of diagnostic and therapeutic tools for cancer. Recently, several groups have reported the development of technologies for the production of sTCRs. The native sTCR has a very low binding affinity for the antigenic peptide/MHC (p/MHC) complex. In this study, we established a technology to produce high affinity, functional sTCRs. We generated a novel sTCR-Fc fusion protein composed of the TCR V and C regions of the TCR linked to the immunoglobulin (Ig) Fc region. A Western blot analysis revealed that the molecular weight of the fusion protein was approximately 60 kDa under reducing conditions and approximately 100–200 kDa under non-reducing conditions. ELISAs using various antibodies showed that the structure of each domain of the TCR-Fc protein was intact. The TCR-Fc protein immobilized by an anti-Cβ antibody effectively bound to a p/MHC tetramer. An SPR analysis showed that the TCR-Fc protein had a low binding affinity (*KD*; 1.1×10^{-5} M) to the p/MHC monomer. Interestingly, when the TCR-Fc protein was pre-incubated with an anti-Cβ antibody, its binding affinity for p/MHC increased by 5-fold (2.2×10^{-6} M). We demonstrated a novel method for constructing a functional soluble TCR using the Ig Fc region and showed that the binding affinity of the functional sTCR-Fc was markedly increased by an anti-Cβ antibody, which is probably due to the stabilization of the Vα/Vβ region of the TCR. These findings provide new insights into the binding of sTCRs to p/MHCs and will hopefully be instrumental in establishing functional sTCR as a diagnostic and therapeutic tool for cancer.

1. Introduction

T cell receptors (TCRs) are antigen recognition molecules that are expressed on the surface of T cells. They specifically recognize the antigenic peptide that is presented by the major histocompatibility complex (p/MHC) [1,2]. In tumor immunity, the tumor cells or their antigens are ingested by antigen presenting cells (APCs). The tumor antigens (TAs) are then processed inside the APCs, and the peptides derived from these antigens are displayed on class I MHC molecules, which are recognized by $CD8^+$ T cells. APCs express costimulatory molecules that provide the signals necessary for the differentiation of $CD8^+$ T cells into anti-tumor cytotoxic T lymphocytes (CTLs), which kill tumor cells [3].

The identification of TCRs that specifically recognize the TA-specific p/MHC complex is extremely valuable for developing tools for tumor diagnosis or TCR therapy. However, the identification of TA-specific TCRs is difficult because the establishment of TA-specific T cell clones entails large amounts of time, money, and labor for only a few clones. Nevertheless, many groups have been successful in establishing TA-specific T cell clones and analyzing the TCR genes and their properties, including their affinity and binding capacity for p/MHC.

Because the TCR is a transmembrane protein, it is necessary to generate a soluble form (sTCR) for its application as a diagnostic and therapeutic tool for cancer [4–6]. Thus, many investigators have constructed sTCRs using various methods and have characterized their various functions [7–17]. The results show that the affinity (*KD* value) of the wild-type soluble form of p/MHC is very low compared to that of antibodies, ranging from 1 to 100×10^{-6} M by SPR-based assays [1,18,19]. Specifically, the dissociation rate of TCRs is very fast (k_d, $1–10 \times 10^{-2}\ s^{-1}$) compared to antibodies (k_d, $1–100 \times 10^{-5}\ s^{-1}$), which leads to the formation of an unstable complex between the soluble TCR and the antigenic p/MHC. To increase the binding affinity, the CDR3 of the TCR V region has been optimized by amino acid replacements [20–22]. However, it is difficult to routinely perform optimal amino acid replacements.

In this study, to improve the sTCR affinity for p/MHC, we constructed a novel sTCR by removing the transmembrane and cytoplasmic domains of a TCR derived from the OT-1 TCR and

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http://dx.doi.org/10.1016/j.bbrc.2012.04.134

directly fusing the extracellular portion of the TCR with the Ig Fc region. The resulting sTCR-Fc bound to the p/MHC complex with an affinity of 10×10^{-6} M. Surprisingly, the binding affinity, which was determined by an SPR analysis, was increased by 5-fold by crosslinking the sTCR-Fc with an anti-Cβ antibody. We found that this profound increase in affinity was due to a decrease in the k_d of the sTCR. Additionally, the sTCR-Fc bound to the p/MHC tetramer in an ELISA in the presence of an anti-Cβ antibody. Our data indicate that the binding affinity of an sTCR-Fc is easily improved by adding an anti-Cβ antibody, which may promote its use as a diagnostic or therapeutic tool for cancer.

2. Material and methods

2.1. Ethics statement

The protocols for the animal experiments were approved by the Committee on Animal Experiments at the University of Toyama.

2.2. Antibodies and peptide MHC

In this study, we used an anti-mouse TCR Cβ (anti-Cβ) antibody (H57–597, eBioscience), a PE-labeled anti-Cβ antibody (eBioscience), an anti-mouse Vα2 antibody (eBioscience), a PE-labeled anti-mouse Vα2 antibody (eBioscience), an anti-human IgG Fc (anti-Fc) antibody (MP Biomedicals), a biotinylated anti-mouse Vβ5.1, 5.2 antibody (BD Bioscience), PE-labeled streptavidin (BD Bioscience), the alkaline phosphatase conjugate of the anti-IgG antibody (Sigma), the PE-labeled T-select H-2K^b OVA tetramer-SIINFEKL (OVA-p/MHC tetramer; MBL), and the biotinylated H-2K^b OVA monomer-SIINFEKL (OVA-p/MHC monomer; MBL). We also used the PE-labeled T-Select H-2K^b β-galactosidase tetramer-DAPIYTNV (β-gal-p/MHC tetramer; MBL) and the biotinylated H-2K^b β-galactosidase monomer-DAPIYTNV (β-gal-p/MHC monomer; MBL) as irrelevant controls.

2.3. Generation of OT-1 TCR-Fc constructs

OT-1 TCR transgenic mice whose transgene encoded TCR that recognized the OVA-derived peptide (SIINFEKL) in the presence of the MHC I allele H-2K^b [23] were obtained from The Jackson Laboratory. To generate OT-1 αTCR-Fc and OT-1 βTCR-Fc expression vectors, spleen cells were prepared from the OT-1 TCR transgenic mouse and the OT-1 TCR Vα/Cα and the OT-1 TCR Vβ/Cβ cDNAs were amplified by reverse transcription and polymerase chain reaction (RT-PCR) with primer sets for Vα + Cα (5′-CTCTAACGCGT CGACTCGTGATCGACCATGGACAAGATTCTGAC-3′ and 5′-ATTCTACG CGGATCCGCGCTTACTGACCAGCTTGACATCACAGG-3′) and for Vβ + Cβ (5′-CTCTAACGCGTCGACTCGTGATCGACCATGTCTAACACTG TCCT-3′ and 5′-ATTCTACGCGGATCCGCGCTTACTCACCGAGGTAAAG CCACAGT-3′). Both PCR products were digested by the restriction enzymes *Sal*I and *Bam*HI and were inserted into *Sal*I and *Bam*HI digested expression vectors that contained the human Ig Fc cDNA, including the hinge region and the CH2 and CH3 regions.

2.4. Production and purification of OT-1 TCR-Fc

We co-transfected CHO-S cells (Invitrogen) with both the OT-1 αTCR-Fc and OT-1 βTCR-Fc expression vectors using the FreeStyle™ MAX CHO Expression System (Invitrogen) for one week, collected the supernatant from the cultured cells, and then purified the OT-1 TCR-Fc using a protein G column (GE Healthcare). We purified α/β heterodimer of OT-1 TCR-Fc by Cβ and Vα2 affinity chromatography on Sepharose coupled with anti-Cβ and anti-Vα2 antibody, respectively, which were generated using NHS-activated Sepharose 4 Fast Flow (GE Healthcare) according to the manufacturer's instructions. First, we purified 1 mg of protein G column-purified OT-1 TCR-Fc protein using Cβ affinity chromatography. After elution, we purified the protein using Vα2 affinity chromatography by AKTA prime plus system (GE Healthcare) according to the manufacturer's instructions. To determine the concentration of the purified OT-1 TCR-Fc protein, the OT-1 TCR-Fc protein was captured with an anti-Fc antibody and detected with an alkaline phosphatase conjugate of an anti-IgG antibody. Concentrations of the OT-1 TCR-Fc protein were estimated based on a comparison to control IgG1 antibodies. To determine the purity of α/β heterodimer, we calculated the purity by comparing the purified protein to the control single-chain TCR protein [15] that consists of 100% Vα/Vβ/Cβ region of OT-1 TCR by sandwich ELISA with anti-Cβ and anti-Vα2 antibody.

2.5. ELISA and surface plasmon resonance (SPR) analysis

Black MaxiSorp FluoroNunc™ modules and plates (Nunc) were coated with 50 μl well^{-1} of 2.5 μg ml^{-1} of the anti-Cβ antibody or the anti-Fc antibody in phosphate-buffered saline (PBS) and then blocked with 3% bovine serum albumin in PBS. After washing, 5 nM of the OT-1 TCR-Fc was added to the plate and incubated for 1 h at room temperature. The binding of the OT-1 TCR-Fc to the coated antibody was detected using the OVA-p/MHC tetramer. As a negative control, a similar analysis was also performed using a β-gal-p/MHC tetramer. The PE fluorescence intensity was measured using an excitation wavelength of 544 nm and an emission wavelength of 590 nm with a FLUOstar OPTIMA fluorescence microplate reader (BMG LABTECH), according to the manufacturer's instructions.

Surface plasmon resonance analysis was performed with a Biacore 2000 instrument (Biacore AB, Uppsala, Sweden) in HBS-N running buffer containing 0.05% Tween 20 at 25 °C. The OVA-p/MHC monomer was immobilized to the surface of an SA sensor chip (GE Healthcare), with final immobilization levels of approximately 5000 RU, according to the manufacturer's instructions. For the kinetic measurements, 200 nM of the OT-1 TCR-Fc in the absence or presence of 33 nM of the anti-Cβ antibody was injected into the HBS-N running buffer, containing 0.05% Tween-20, at flow rates of 20 μl min^{-1} with 3 min for association and 15 min for dissociation. Similarly, 100, 50, 25, 12.5, or 0 nM of the OT-1 TCR-Fc in the absence or presence of 16.5, 8.3, 4.1, 2.1, or 0 nM of the anti-Cβ antibody was injected. As negative controls, a similar analysis was performed using the β-gal-p/MHC monomer. The binding affinity (*KD*) values were calculated after subtracting the background binding to a control flow cell using the bivalent binding model for the kinetic analysis with the BIAevaluation 4.1 software (Biacore AB). For the calculation, we used the corrected concentration of the OT-1 TCR-Fc protein according to the purity of α/β heterodimer.

3. Results

3.1. Generation and characterization of soluble TCR proteins

We designed and constructed four different types of sTCRs: (1) scFV-TCR, (2) sVC-TCR, (3) scTCR-Fc, and (4) sTCR-Fc (Supplementary Fig. 1). We found that the scFV-TCR protein was easily produced by transfecting the expression vector into *Escherichia coli* and purifying the proteins from inclusion bodies, but an SPR analysis showed no or extremely low binding affinity of this protein to the peptide/MHC (data not shown). We could not produce sufficient amounts of the sVC-TCR and scTCR-Fc protein for unknown reasons, although we tried various transfection systems (data not

shown). We were able to produce the sTCR-Fc fusion protein using CHO-S cells.

We constructed an Fc-fusion protein with an OT-1 TCR that recognizes an OVA-derived peptide (SIINFEKL) [23]. The TCR portion of this fusion protein comprises the TCR Vα/Cα region and the Vβ/Cβ region. The transmembrane and cytoplasmic domains of Cα or Cβ were removed by truncating them at the fifth amino acid residue of the TCR located downstream of the last cysteine residue and upstream of the transmembrane domain. The Cα and Cβ portions of the OT-1 TCR were linked directly to the Fc portion of a human IgG1 chain that included a hinge region. The cysteine residues in the hinge region were intact, which allowed appropriate disulfide bonding of the H chain regions, and thus the OT-1 TCR-Fc protein was produced as a dimer with a covalent linkage.

To characterize the OT-1 TCR-Fc protein, the purified protein was subjected to SDS–PAGE and Western blot analysis (Fig. 1A). Under reducing conditions, proteins of approximately 60 kDa were detected, which is consistent with the calculated molecular mass for this protein based on its predicted amino acid sequence. Under non-reducing conditions, proteins of approximately 100–200 kDa were detected. The apparent shift in molecular mass of the OT-1 TCR-Fc protein under reducing vs. non-reducing conditions indicated that there were disulfide bonds between the H chains of cysteine residues. Therefore, the OT-1 TCR-Fc proteins formed a dimeric structure.

To determine whether the structure of each domain (Vα, Vβ, or Cβ) was intact, we performed a sandwich enzyme-linked immunosorbent assay (ELISA). When the OT-1 TCR-Fc protein was immobilized by an anti-Fc antibody, antibodies specific to Vβ5.1/5.2, Cβ, and Vα2 bound to the OT-1 TCR-Fc proteins (Fig. 1B). Although the OT-1 TCR-Fc proteins were covalently linked as a dimer (Fig. 1A), dimers formed not only as α/β heterodimers, but also as α/α or β/β homodimers. To determine whether the purified OT-1 TCR-Fc protein contained the α/β heterodimer form, we pre-coated another sandwich ELISA. When the OT-1 TCR-Fc protein was immobilized by an anti-Cβ antibody, the Vα2 of the OT-1 TCR-Fc protein was detected (Fig. 1C). Similarly, when the OT-1 TCR-Fc was immobilized by an anti-Vα2 antibody, the Cβ of the OT-1 TCR-Fc protein was detected (Fig. 1C). These results suggest that the structures of each domain were intact. Next, we determined the purity of α/β heterodimer of OT-1 TCR-Fc protein using sandwich ELISA. The purity of pre- and post-purification was estimated to be approximately 15% and 45%, respectively (Supplementary Fig. 2).

3.2. p/MHC binding to the OT-1 TCR-Fc protein

The OT-1 TCR specifically recognizes the OVA (SIINFEKL)-peptide and the MHC complex (OVA-p/MHC). To determine whether the OT-1 TCR-Fc protein recognized the OVA-p/MHC, we used a pre-coated ELISA. The OT-1 TCR-Fc was incubated with an

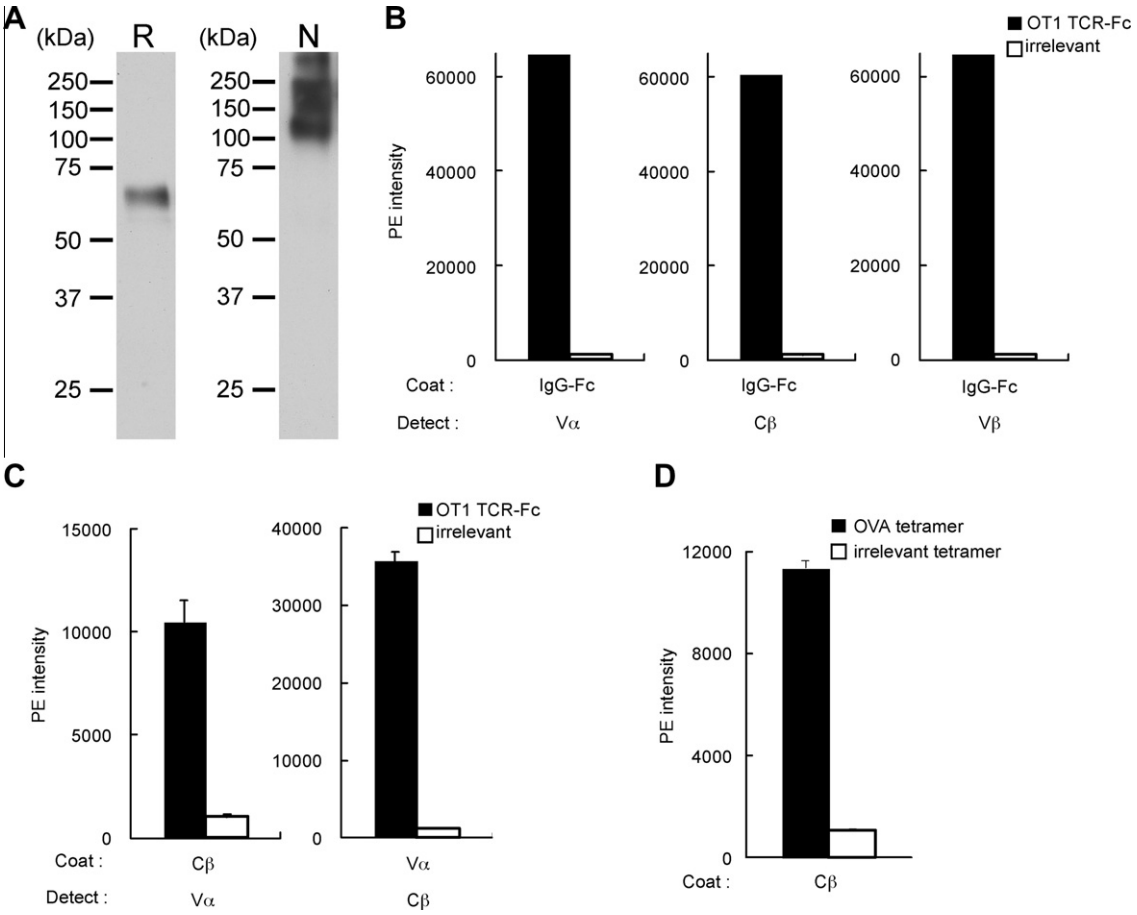

Fig. 1. Characterization of the OT-1 TCR-Fc protein. (A) The examination of the dimer form of the OT-1 TCR-Fc protein. The OT-1 TCR-Fc protein was separated by SDS–PAGE under either reducing (R) or non-reducing (N) conditions. The OT-1 TCR-Fc protein was detected by Western blotting using a horseradish peroxidase-conjugated anti-Fc antibody. (B) The examination of the OT-1 TCR-Fc domains. The OT-1 TCR-Fc protein or an IgG (control) was immobilized by an anti-Fc antibody, and the conformation of the Vα, Cβ, and Vβ domains was assessed using PE-labeled antibodies to Vα (left), Cβ (middle), or Vβ (right). (C) The examination of the α/β heterodimer form of the OT-1 TCR-Fc protein. The OT-1 TCR-Fc protein or an IgG (control) was immobilized by an anti-Cβ (left) or anti-Vα antibody (right), and Vα and Cβ were detected using a PE-labeled anti-Vα or anti-Cβ antibody, respectively. (D) The reactivity of the OT-1 TCR-Fc protein with p/MHC. The OT-1 TCR-Fc protein was immobilized by an anti-Cβ and its reactivity to the OVA-p/MHC tetramer or a β-gal-p/MHC tetramer (control) was assessed by incubation with their PE-conjugates. (B–D) The *Y*-axis shows the fluorescence intensity. All of the data represent the mean ± SD.

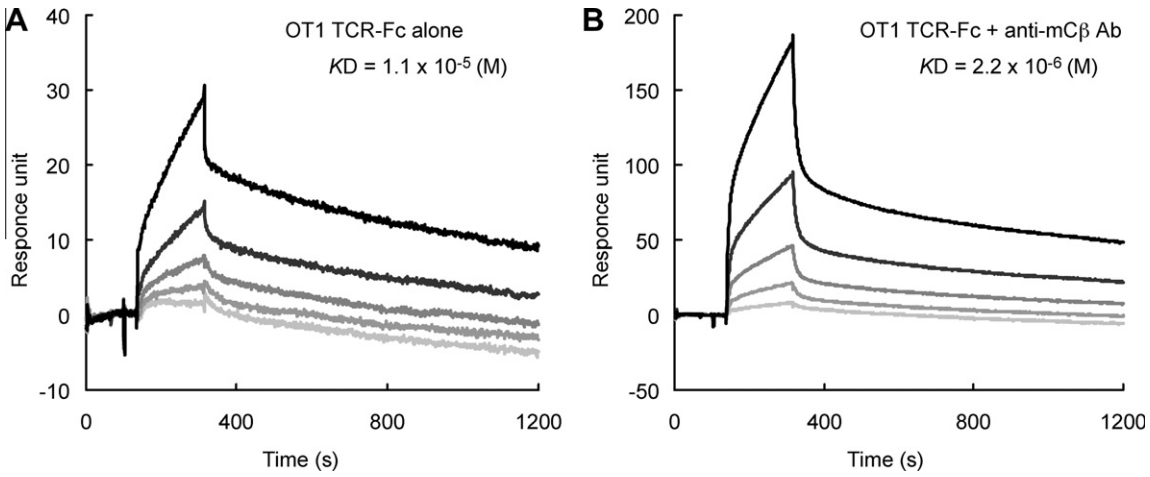

Fig. 2. Surface plasmon resonance (SPR) analysis of the OT-1 TCR-Fc protein. SPR measurements of individual analytes are shown: the OT-1 TCR-Fc protein alone (A), and the OT-1 TCR-Fc protein that was pre-incubated with an anti-Cβ antibody (B). For each sensorgram, the nonspecific responses to empty sensor chips were subtracted. Representatives of two independent experiments with similar results are shown.

anti-Cβ antibody pre-coated plate. After washing, the OT-1 TCR-Fc was incubated with OVA-p/MHC tetramers to examine whether the OT-1 TCR-Fc protein bound to the OVA-p/MHC tetramer. The OT-1 TCR-Fc protein that bound to the plate coated with the anti-Cβ antibody bound the OVA-p/MHC tetramer (Fig. 1D). The control p/MHC tetramer did not bind to the OT-1 TCR-Fc protein (Fig. 1D). These results demonstrate that the OT-1 TCR-Fc protein immobilized with an anti-Cβ antibody bound to the OVA-p/MHC tetramer.

3.3. Improvement of the binding affinity of the OT-1 TCR-Fc protein

To investigate the effect of the anti-Cβ antibody on the OT-1 TCR-Fc protein, we performed surface plasmon resonance (SPR) assays using an OVA-p/MHC monomer-bound chip. The sensorgram of the OT-1 TCR-Fc protein with the OVA-p/MHC monomer showed very low binding affinity with a very fast dissociation rate (Fig. 2A). The binding affinity (*K*D) of the OT-1 TCR-Fc protein with the OVA-p/MHC monomer was estimated to be 1.1×10^{-5} M. The *K*D value of our results using purified α/β heterodimer was 1.1×10^{-5} M, which was almost comparable (1.7-fold lower) to that of Alam et al. study (6.5×10^{-6} M) [24]. In contrast, the binding affinity of the OT-1 TCR-Fc protein that had been pre-incubated with an anti-Cβ antibody was higher (2.2×10^{-6} M) than that of the OT-1 TCR-Fc protein alone (Fig. 2B). A control p/MHC monomer did not bind to the OT-1 TCR-Fc protein (data not shown). These results indicate that the binding affinity of the OT-1 TCR-Fc protein was increased by the anti-Cβ antibody.

4. Discussion

In this study, we generated an sTCR-Fc fusion protein that was composed of the TCR-V and TCR-C regions of the OT-1-TCR and the Ig Fc region (Supplementary Fig. 1) and showed that the Vα, Vβ, and Cβ domains of the fusion protein remained intact (Fig. 1). Using sandwich ELISAs, we showed that an OT-1 TCR-Fc protein immobilized by an anti-Cβ antibody effectively bound to an OVA-p/MHC tetramer. Using an SPR assay, we also demonstrated that the binding affinity of the OT-1 TCR-Fc to the OVA p/MHC monomer was markedly increased by 5-fold when the OT-1 TCR-Fc was crosslinked by an anti-Cβ antibody (Fig. 2).

Many investigators have constructed various sTCRs, such as heterodimers of the TCR α/β chains [7,8,17], two TCR variable domains joined into a single chain (scFV-TCRs) [9–12], or a TCR that was fused to a full-length Ig molecule [13–16]. The affinity (*K*D values) of sTCRs for the p/MHC is very low (with the range of $1–100 \times 10^{-6}$ M) compared to that of antibodies [1,18,19]. The low affinity of sTCRs is one of the major hurdles to their therapeutic and diagnostic application. Thus, several investigators have attempted to improve their binding affinity with two technologies. One such technology involves optimization of the CDR3 of the TCR V region by amino acid replacement [20–22], which significantly increased the affinity of TCRs by at least $\sim 10^3$-fold. The other technique uses TCR phage display technology [25]. This technology dramatically increased the affinity of TCRs by at least $\sim 10^6$-fold [25]. However, these technologies are difficult to perform in ordinary laboratories. In the present study, we demonstrated that the binding affinity of an sTCR-Fc is easily improved by the addition of an anti-Cβ antibody.

The OT-1 TCR-Fc protein itself is monovalent, and crosslinking the OT-1 TCR-Fc protein with an anti-Cβ antibody makes the OT-1 TCR-Fc protein bivalent, resulting in an increase in the functional binding capacity (avidity) of the OT-1 TCR-Fc protein, similar to the case of bivalent antibodies [3]. However, the present study showed that an anti-Cβ antibody enhanced the binding affinity of an sTCR-Fc fusion protein to an OVA-p/MHC by approximately 5-fold (Fig. 2). What is the mechanism of the increased affinity of the sTCR by binding to the anti-Cβ antibody?

We propose a stability model in which the anti-Cβ antibody stabilizes the Vα/Vβ domains (FV) of the TCR by binding to an appropriate region of the Cβ domain. In this respect, Wang et al. showed that an anti-Cβ antibody binds to the looped-out polypeptide of the Cβ domain between the Vβ and Cβ domains, perpendicular to the Vβ/Cβ protein [26]. It is conceivable that the binding of the anti-Cβ antibody to Vβ/Cβ protein could stabilize the bent position of the Vβ and Cβ domains, which in turn could stabilize the FV conformation of the sTCR. To investigate this possibility, we are now analyzing the effect of the anti-Cβ antibody on the relative position of the Vα and Vβ domains using fluorescence resonance energy transfer (FRET) analysis.

In conclusion, we have demonstrated the utility of an anti-Cβ antibody in improving the binding affinity of an sTCR-Fc protein. Our findings may facilitate the use of soluble TCRs as a diagnostic or therapeutic tool for the treatment of cancer or infectious diseases by improving the binding affinity of soluble TCRs.

Acknowledgments

We thank Sanae Hirota for technical assistance and Kaoru Hata for administrative work. This research was supported by Grants

from the Hokuriku Innovation Cluster for the Health Science Project of the Ministry of Education, Culture, Sports, and Science of Japan, the Toyama and Ishikawa Prefectures, and Grants-in-Aid from the Ministry of Education, Culture, Sports, and Science of Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.04.134.

References

[1] M.M. Davis, J.J. Boniface, Z. Reich, D. Lyons, J. Hampl, B. Arden, Y. Chien, Ligand recognition by alpha beta T cell receptors, Annu. Rev. Immunol. 16 (1998) 523–544.
[2] K.C. Garcia, L. Teyton, I.A. Wilson, Structural basis of T cell recognition, Annu. Rev. Immunol. 17 (1999) 369–397.
[3] A.K. Abbas, A.H. Lichtman, Cellular and Molecular Immunology, fifth ed. (Updated edition)., Elsevier, Amsterdam, 2005.
[4] J.M. Boulter, B.K. Jakobsen, Stable, soluble, high-affinity, engineered T cell receptors: novel antibody-like proteins for specific targeting of peptide antigens, Clin. Exp. Immunol. 142 (2005) 454–460.
[5] P.E. Molloy, A.K. Sewell, B.K. Jakobsen, Soluble T cell receptors: novel immunotherapies, Curr. Opin. Pharmacol. 5 (2005) 438–443.
[6] S.A. Richman, D.M. Kranz, Display, engineering, and applications of antigen-specific T cell receptors, Biomol. Eng. 24 (2007) 361–373.
[7] H.C. Chang, Z. Bao, Y. Yao, A.G. Tse, E.C. Goyarts, M. Madsen, E. Kawasaki, P.P. Brauer, J.C. Sacchettini, S.G. Nathenson, et al., A general method for facilitating heterodimeric pairing between two proteins: application to expression of alpha and beta T-cell receptor extracellular segments, Proc. Natl. Acad. Sci. USA 91 (1994) 11408–11412.
[8] A. Golden, S.S. Khandekar, M.S. Osburne, E. Kawasaki, E.L. Reinherz, T.H. Grossman, High-level production of a secreted, heterodimeric alpha beta murine T-cell receptor in *Escherichia coli*, J. Immunol. Methods 206 (1997) 163–169.
[9] C. Gregoire, S.Y. Lin, G. Mazza, N. Rebai, I.F. Luescher, B. Malissen, Covalent assembly of a soluble T cell receptor–peptide-major histocompatibility class I complex, Proc. Natl. Acad. Sci. USA 93 (1996) 7184–7189.
[10] K.S. Gunnarsen, E. Lunde, P.E. Kristiansen, B. Bogen, I. Sandlie, G.A. Loset, Periplasmic expression of soluble single chain T cell receptors is rescued by the chaperone FkpA, BMC Biotechnol. 10 (2010) 8.
[11] J. Kappler, J. White, H. Kozono, J. Clements, P. Marrack, Binding of a soluble alpha beta T-cell receptor to superantigen/major histocompatibility complex ligands, Proc. Natl. Acad. Sci. USA 91 (1994) 8462–8466.
[12] D.F. Lake, M.L. Salgaller, P. van der Bruggen, R.M. Bernstein, J.J. Marchalonis, Construction and binding analysis of recombinant single-chain TCR derived from tumor-infiltrating lymphocytes and a cytotoxic T lymphocyte clone directed against MAGE-1, Int. Immunol. 11 (1999) 745–751.
[13] M.S. Lebowitz, S.M. O'Herrin, A.R. Hamad, T. Fahmy, D. Marguet, N.C. Barnes, D. Pardoll, J.G. Bieler, J.P. Schneck, Soluble, high-affinity dimers of T-cell receptors and class II major histocompatibility complexes: biochemical probes for analysis and modulation of immune responses, Cell Immunol. 192 (1999) 175–184.
[14] E. Lunde, G.A. Loset, B. Bogen, I. Sandlie, Stabilizing mutations increase secretion of functional soluble TCR-Ig fusion proteins, BMC Biotechnol. 10 (2010) 61.
[15] L.A. Mosquera, K.F. Card, S.A. Price-Schiavi, H.J. Belmont, B. Liu, J. Builes, X. Zhu, P.A. Chavaillaz, H.I. Lee, J.A. Jiao, J.L. Francis, A. Amirkhosravi, R.L. Wong, H.C. Wong, In vitro and in vivo characterization of a novel antibody-like single-chain TCR human IgG1 fusion protein, J. Immunol. 174 (2005) 4381–4388.
[16] S.M. O'Herrin, M.S. Lebowitz, J.G. Bieler, B.K. al-Ramadi, U. Utz, A.L. Bothwell, J.P. Schneck, Analysis of the expression of peptide-major histocompatibility complexes using high affinity soluble divalent T cell receptors, J. Exp. Med. 186 (1997) 1333–1345.
[17] B.E. Willcox, G.F. Gao, J.R. Wyer, C.A. O'Callaghan, J.M. Boulter, E.Y. Jones, P.A. van der Merwe, J.I. Bell, B.K. Jakobsen, Production of soluble alphabeta T-cell receptor heterodimers suitable for biophysical analysis of ligand binding, Protein Sci. 8 (1999) 2418–2423.
[18] S.M. Alam, G.M. Davies, C.M. Lin, T. Zal, W. Nasholds, S.C. Jameson, K.A. Hogquist, N.R. Gascoigne, P.J. Travers, Qualitative and quantitative differences in T cell receptor binding of agonist and antagonist ligands, Immunity 10 (1999) 227–237.
[19] J.D. Stone, A.S. Chervin, D.M. Kranz, T-cell receptor binding affinities and kinetics: impact on T-cell activity and specificity, Immunology 126 (2009) 165–176.
[20] B.J. Hare, D.F. Wyss, M.S. Osburne, P.S. Kern, E.L. Reinherz, G. Wagner, Structure, specificity and CDR mobility of a class II restricted single-chain T-cell receptor, Nat. Struct. Biol. 6 (1999) 574–581.
[21] P.D. Holler, P.O. Holman, E.V. Shusta, S. O'Herrin, K.D. Wittrup, D.M. Kranz, In vitro evolution of a T cell receptor with high affinity for peptide/MHC, Proc. Natl. Acad. Sci. USA 97 (2000) 5387–5392.
[22] K.S. Weber, D.L. Donermeyer, P.M. Allen, D.M. Kranz, Class II-restricted T cell receptor engineered in vitro for higher affinity retains peptide specificity and function, Proc. Natl. Acad. Sci. USA 102 (2005) 19033–19038.
[23] J.M. Kelly, S.J. Sterry, S. Cose, S.J. Turner, J. Fecondo, S. Rodda, P.J. Fink, F.R. Carbone, Identification of conserved T cell receptor CDR3 residues contacting known exposed peptide side chains from a major histocompatibility complex class I-bound determinant, Eur. J. Immunol. 23 (1993) 3318–3326.
[24] S.M. Alam, P.J. Travers, J.L. Wung, W. Nasholds, S. Redpath, S.C. Jameson, N.R. Gascoigne, T-cell-receptor affinity and thymocyte positive selection, Nature 381 (1996) 616–620.
[25] Y. Li, R. Moysey, P.E. Molloy, A.L. Vuidepot, T. Mahon, E. Baston, S. Dunn, N. Liddy, J. Jacob, B.K. Jakobsen, J.M. Boulter, Directed evolution of human T-cell receptors with picomolar affinities by phage display, Nat. Biotechnol. 23 (2005) 349–354.
[26] J. Wang, K. Lim, A. Smolyar, M. Teng, J. Liu, A.G. Tse, J. Liu, R.E. Hussey, Y. Chishti, C.T. Thomson, R.M. Sweet, S.G. Nathenson, H.C. Chang, J.C. Sacchettini, E.L. Reinherz, Atomic structure of an alphabeta T cell receptor (TCR) heterodimer in complex with an anti-TCR fab fragment derived from a mitogenic antibody, EMBO J. 17 (1998) 10–26.