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Production of the constant domain of murine T-cell receptor β-chain in *Escherichia coli*

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T-cell antigen receptor is a heterodimer of disulfide-linked α- and β-chains. Although the essential features of T-cell receptor seem to be rather similar to those of immunoglobulin, the amount of T-cell receptor expressed on the surface of a T-cell is not large enough to be analyzed physico-chemically. In this study, the DNA fragment encoding 120 amino acids from the 116th to the 235th of the murine β-chain which corresponds to the presumed constant domain was inserted into an expression vector in *E. coli*. A large amount of this 18 kDa protein was observed to be synthesized in *E. coli*, and might be a good source for the three dimensional analysis of the T-cell receptor molecule.

T-cell receptor; Expression vector; Structural analysis

1. INTRODUCTION

The T-cell antigen receptor is a heterodimer of disulfide-linked α- and β-chains [1]. Each chain seems to be composed of five functionally different regions: a variable domain; a constant domain; a connecting peptide; a transmembrane region; and a cytoplasmic region [2]. In contrast to Ig, TCR recognizes an antigen that is present on the cell surface in the context of molecules encoded by the MHC genes [3]. There have been few reports directly showing an interaction between TCR and an antigen associated with the MHC molecule [4]. Although the TCR is associated with the T3 molecule, the complex of a pair of α- and β-variable domains is believed to form an antigen-combining site [5]. For understanding the molecular mechanism of antigen recognition by TCR, it is necessary to examine the three dimensional structure of the TCR. The TCR gene family is part of the immunoglobulin superfamily. The extracellular portions of the molecules belonging to this superfamily consist of one or more domains homologous to each other. Each domain is approx. 110 amino acids long and has several aa conserved at similar positions (e.g., two conserved cysteines linked a 60–75 aa span). X-ray crystallographic analyses of the Ig and β_2-microglobulin showed a conserved tertiary structure named the 'antibody fold', composed of 3- to 4-stranded antiparallel β-sheet bilayers [6,7]. Computer analysis of the TCR's primary aa sequence, deduced from the nucleotide sequence, predicted essentially the same features for both the

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Abbreviations: aa, amino acid(s); bp, base pairs; Ig, immunoglobulin; MHC, major histocompatibility complex; PAGE, polyacrylamide gel electrophoresis; TCR, T-cell antigen receptor

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TCR domains and the Ig domains [8]. However, the amount of TCR expressed on the T-cell surface is not large enough to be analyzed physicochemically.

In order to obtain a larger amount of TCR, we tried to produce the TCR molecule in *E. coli*. This study reports the production of the constant domain of murine TCR β-chain in *E. coli*.

2. MATERIALS AND METHODS

E. coli W3110 was provided by T. Okazaki (Nagoya University). Plasmid 86T5 containing murine T-cell receptor C_β gene was kindly supplied by M. Davis (Stanford University). Plasmids pKYP26, containing *trp* promoter and *lpp* terminator; pTrS35, containing *trp* promoter; and pKYP200, containing double *trp* promoters, were constructed at Kyowa Hakko Kogyo Co. as described [9]. A *Bgl*II linker, T_4 DNA ligase, Klenow enzyme, and restriction enzymes were purchased from Takara Shuzo Co. The conditions of SDS-polyacrylamide gel electrophoresis (PAGE) were essentially the same as those described by Laemmli [10]. Prokaryotic DNA-directed translation kit was purchased from Amersham.

3. RESULTS AND DISCUSSION

The murine C_β is encoded by four exons [11]. In contrast with the Ig constant genes, however, these exons do not correlate with the presumed functional domains. As shown in fig.1, the first exon of the constant gene is 375 bp long. The 113 aa from Asp at the 116th to Thr at the 228th seem to correspond to the constant domain [5]. In order to produce the constant domain in *E. coli*, we constructed a recombinant plasmid as shown in fig.2. A *Bgl*II site was created by inserting the synthetic linker $\overset{\text{CAGATCTG}}{\text{GTCTAGAC}}$ into the *Eco*RV site of pBR322. The 394 bp *Sau*3A fragment which encoded the 116th to the 260th aa residues was isolated from 86T5 [2], and recloned in the *Bgl*II site of this plasmid, resulting in the plasmid pMCβA6. *Bgl*II sites were newly created at both ends of the insert. Plasmid pKYP26 including a terminator was first digested with *Kpn*I, reacted with Klenow enzyme, and finally digested with *Pst*I. The *Pst*I-*Kpn*I fragment of pKYP26 was

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        Met
        ATG                    120                                 130
Val Val Glu Asp Leu Arg Asn Val Thr Pro Pro Lys Val Ser Leu Phe Glu Pro Ser Lys Ala Glu Ile Ala Asn
GTT GTA GAG GAT CTG AGA AAT GTG ACT CCA CCC AAG GTC TCC TTG TTT GAG CCA TCA AAA GCA GAG ATT GCA AAC
           Sau3A
        140                                150                                 160
Lys Gln Lys Ala Thr Leu Val Cys Leu Ala Arg Gly Phe Phe Pro Asp His Val Glu Leu Ser Trp Trp Val Asn
AAA CAA AAG GCT ACC CTC GTG TGC TTG GCC AGG GGC TTC TTC CCT GAC CAC GTG GAG CTG AGC TGG TGG GTG AAT

                               170                                 180
Gly Lys Glu Val His Ser Gly Val Ser Thr Asp Pro Gln Ala Tyr Lys Glu Ser Asn Tyr Ser Tyr Cys Leu Ser
GGC AAG GAG GTC CAC AGT GGG GTC AGC ACG GAC CCT CAG GCC TAC AAG GAG AGC AAT TAT AGC TAC TGC CTG AGC
                                        StuI
        190                                200                                 210
Ser Arg Leu Arg Val Ser Ala Thr Phe Trp His Asn Pro Arg Asn His Phe Arg Cys Gln Val Gln Phe His Gly
AGC CGC CTG AGG GTC TCT GCT ACC TTC TGG CAC AAT CCT CGC AAC CAC TTC CGC TGC CAA GTG CAG TTC CAT GGG
                                                                        Ala Lys Ter
                               220                                 230      GCT AAG TAA
Leu Ser Glu Glu Asp Lys Trp Pro Glu Gly Ser Pro Lys Pro Val Thr Gln Asn Ile Ser Ala Glu Ala Trp Gly
CTT TCA GAG GAG GAC AAG TGG CCA GAG GGC TCA CCC AAA CCT GTC ACA CAG AAC ATC AGT GCA GAG GCC TGG GGC
                                                                  StuI
        240                                250                                 260
Arg Ala Asp Cys Gly Ile Thr Ser Ala Ser Tyr Gln Gln Gly Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu
CGA GCA GAC TGT GGG ATT ACC TCA GCA TCC TAT CAA CAA GGG GTC TTG TCT GCC ACC ATC CTC TAT GAG ATC CTG
                                                                        Sau3A
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Fig.1. Nucleotide sequence of murine T-cell receptor β-chain. The nucleotide sequence and the deduced amino acid sequence are shown as based on the published data of 86T5 [2]. The boundaries of exons are shown by vertical lines. Amino acids are numbered from the N-terminus of V domain. The polypeptide produced in *E. coli* corresponds to the region sandwiched between vertical dashed lines plus Met at the N-terminus and Ala-Lys at the C-terminus. Restriction sites used in this study for the construction of plasmids are underlined.

replaced by the *Pst*I-*Stu*I fragment of pMCβA6. In this case the *Stu*I site not at the 175th but at the 234th aa residue indicated by an arrow in fig.2A was digested under partial digestion conditions. The pMCβG11 was obtained. A *trp* promoter and two tandem *trp* promoters were inserted into this clone as follows: plasmid pTrS35 including the *trp* promoter was digested with *Sac*I, reacted with Klenow and finally digested with *Pst*I. The pMCβG11 was partially digested with *Bgl*II (in this case the *Bgl*II at the N-terminus indicated by an arrow in fig.2B was digested, but that at the terminator was not), reacted with Klenow enzyme, and finally digested with *Pst*I. The *Pst*I-*Bgl*II fragment of pMCβG11 was replaced by the above *Pst*I-*Sac*I fragment of pTrS35. pMCβJ11 was obtained. The *Pst*I-*Cla*I fragment of pKYP200 including two tandem *trp* promoters replaced the *Pst*I-*Cla*I fragment of pMCβJ11. The resulting plasmid, pMCβK8, includes two tandem *trp* promoters; a Shine-Dalgarno sequence (AAGG); a 14 nucleotides spacer; the following coding sequence: Met, 120 aa from the 116th to the 235th residue, and Lys; and a terminator. The structure was confirmed by nucleotide sequence analysis according to the M13 chain termination method [12]. *E. coli* W3110 was transfected with pMCβK8, and grown in M9 casamino acids medium. When total proteins from the cells were analyzed by SDS-PAGE, a thick band was observed at 18 kDa which was not from either W3110 or from pBR322-infected W3110 (fig.3A). To assign the 18 kDa band to $C_{\beta 1}$ polypeptide, the pMCβK8 DNA was incubated in the transcription-translation mixture in vitro [13].

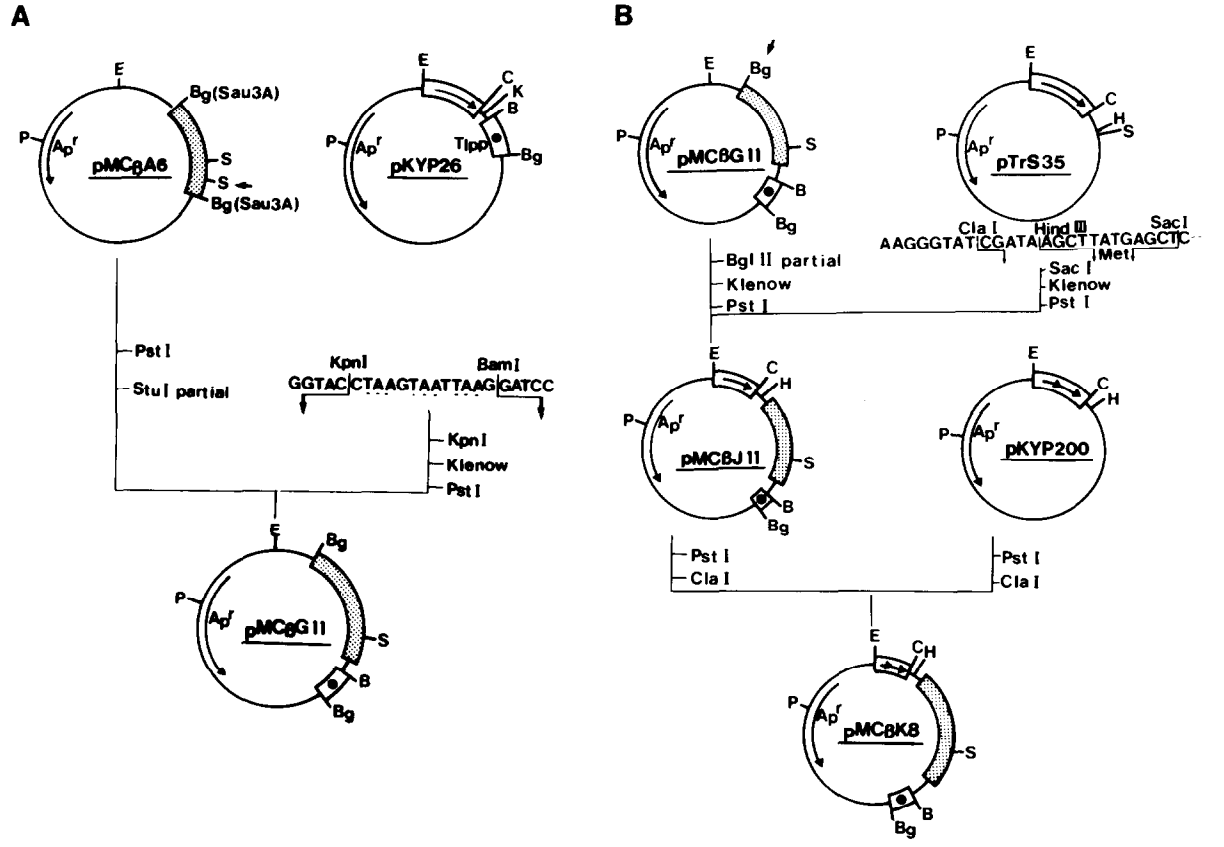

Fig.2. Construction of plasmid pMCβK8. The details are given in the text. T-cell receptor gene-derived region, *trp* promoter, double *trp* promoters and *lpp* terminator are shown by stippled boxes, open boxes with an arrow, open boxes with two arrows and open boxes with a dot, respectively. The nucleotide sequence of downstream of each promoter is shown. Abbreviations: P, *Pst*I; Bg, *Bgl*II; E, *Eco*RI; S, *Sac*I; C, *Cla*I; K, *Kpn*I; H, *Hin*dIII; Ap^r, β-lactamase gene.

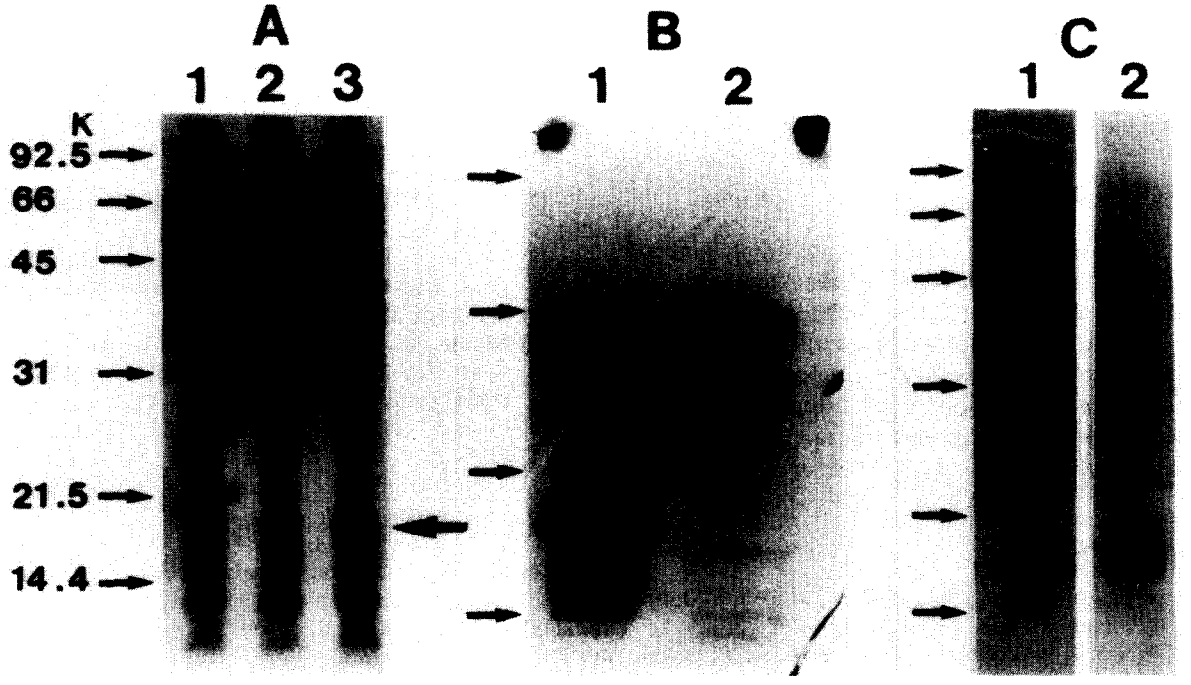

Fig.3. Production of C_β domain in *E. coli*. (A) *E. coli* W3110 (lane 1), pBR322-infected W3110 (lane 2) and pMCβK8-infected W3110 (lane 3) were grown in M9 casamino acids medium. Total proteins from the cells were analyzed by SDS-PAGE. The proteins were stained with Coomassie brilliant blue. The 18 kDa protein is indicated by a thick arrow. The position of size markers is indicated in kDa. (B) Autoradiograph of the SDS-PAGE of proteins labelled in vitro with [^{35}S]methionine. Prokaryotic DNA-directed translation kit (Amersham) was used for in vitro protein synthesis from the plasmid DNA templates. Lanes: 1, pMCβK8; 2, pKYP200. The size markers indicated by arrows correspond to 45, 31, 21.5 and 14.4 kDa. (C) SDS-PAGE of partially purified C_β protein under reduced conditions (lane 1) and non-reduced conditions (lane 2). The size markers are the same as those in A.

SDS-PAGE analysis of the products showed an intense band at 18 kDa in addition to a 30 kDa band which corresponds to β-lactamase as shown in fig.3B. This clearly indicates that the 18 kDa protein is encoded by pMCβK8.

The inclusion bodies containing the $C_{\beta 1}$ polypeptide were partially purified as follows [14]. Extracts of pMCβK8-infected W3110 cells were centrifuged in Percoll density gradient. The white thick band was recovered from the gradient, and precipitated by centrifugation at 12000 × *g* for 5 min. The precipitate was suspended in 7 M urea-containing buffer (50 mM Tris·HCl (pH 8.0), 1 mM EDTA, 50 mM NaCl, 0.1 mM phenylmethylsulphonyl fluoride) at a concentration of 400 μg proteins/ml. The suspension was dialyzed against PBS (1.15 g Na_2HPO_4, 0.2 g KH_2PO_4, 8 g NaCl, 0.2 g KCl/l), for two days and the insoluble materials were removed by centrifugation at 12000 × *g* for 5 min. The soluble fraction was analyzed by SDS-PAGE under non-reduced and reduced conditions (fig.3C). The appearance of two bands under the non-reduced conditions may reflect the existence of three cysteine residues in the $C_{\beta 1}$ domain and their illegitimate link. To examine the physico-chemical nature of the $C_{\beta 1}$ domain, it might be necessary to replace cysteine at the 185th residue with another aa by site-specific mutagenesis. In order to determine whether the *E. coli*-made $C_{\beta 1}$ polypeptide had the same epitope as that of a natural TCR, the antiserum was prepared by immunization of rabbits with the partially purified *E. coli*-made $C_{\beta 1}$ polypeptide. In preliminary results, it reacted with 90 kDa protein in a fraction of the surface molecules of murine T-cell hybridomas (F.N., unpublished). The *E. coli*-made

$C_{\beta 1}$ protein might be a good source for the three dimensional analyses of TCR. The protocol for the production of the $C_{\beta 1}$ protein described in this report should also be applicable to the synthesis of other parts of TCR molecules.

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