J. Biochem. Mol. Biol. Vol. 28, No. 4 pp. 353~358 July 31, 1995

Epitope Tagging with a Peptide Derived from the preS2 Region of Hepatitis B Virus Surface Antigen

Hyun Ah Kang, Gwan-Su Yi[1] and Myeong-Hee Yu*

Korea Research Institute of Bioscience and Biotechnology, KIST, Taejon 305-333, Korea

(Received February 24, 1995)

Abstract: Epitope tagging is the process of fusing a set of amino acid residues that are recognized as an antigenic determinant to a protein of interest. Tagging a protein with an epitope facilitates various immunochemical analyses of the tagged protein with a specific monoclonal antibody. The monoclonal antibody H8 has subtype specificity for an epitope derived from the preS2 region of hepatitis B virus surface antigen. Previous studies on serial deletions of the preS2 region indicated that the preS2 epitope was located in amino acid residues 130~142. To test whether the amino acid sequence in this interval is sufficient to confer on proteins the antigenicity recognizable by the antibody H8, the set of amino acid residues in the interval was tagged to the amino terminal of β-galactosidase and to the carboxyl terminal of the truncated $p56^{lck}$ fragment. The tagged β-galactosidase, expressed in *Escherichia coli*, maintained the enzymatic activity and was immunoprecipitated efficiently with H8. The tagged $p56^{lck}$ fragment, synthesized in an *in vitro* translation system, was also immunoprecipitated specifically with H8. These results demonstrate that the amino acid sequence of the preS2 region can be used efficiently for the epitope tagging approach.

Key words: epitope tagging, monoclonal antibody H8, preS2 epitope.

The interaction of an antibody with an antigen forms the basis of all immunochemical techniques. The region of an antigen that is recognized as an antigenic determinant by an antibody is defined as an epitope. The epitope is identified as either continuous, comprised of a short linear sequence, or discontinuous, comprised of residues separated in the primary amino acid sequence. Epitope tagging is the process of fusing a set of amino acid residues that are recognized as an antigenic determinant to a protein of interest. By introducing an immunological marker into a tagged protein, the protein of interest, tagged with a well-characterized epitope, facilitates various immunochemical analyses using a specific monoclonal antibody. The usefulness of monoclonal antibodies stems from their specificity of binding, their homogeneity, and their ability to be produced in unlimited quantities. The epitope tagging approach using the monoclonal antibody that recognizes an epitope derived from the influenza hemagglutinin, or the antibody for an epitope from the human myci protein has been applied in several studies, such as localization and association with other molecules of a protein of interest (Kolodziej and Young, 1991).

The monoclonal antibody H8 (mAb H8) was generated by immunizing mice with the hepatitis B virus (HBV) surface antigen isolated from a Korean hepatitis patient (Chung and Kim, 1987). The antibody H8 shows subtype specificity for the preS2 region of the HBV surface antigen; it interacts specifically with the preS2 region of the adri subtype but has little cross-reactivity with the adwi subtype (Lee *et al.*, 1990). Although the preS2 region of HBV surface antigen is a minor component of the virus particle, it has been known to carry an epitope which is immunodominant (Neurath, 1986) and elicits protective immunity in animals by inducing neutralizing antibodies (Budkowska *et al.*, 1986; Itoh *et al.*, 1986). The preS2 epitope has been considered to be a conformation-independent determinant which is made up of amino acids within residues 120~145 (Neurath *et al.*, 1985). Serial deletion analyses of the preS2 region has indicated that the amino acid residues 130~142 are required to represent the antigenicity of preS2 to the mAb H8 (Kwon *et al.*, 1990).

The objectives of the present study are to investigate whether the amino acid sequence in the suggested interval alone is sufficient to be an epitope recognized specifically by the mAb H8, and to examine the immu-

[1] Present address: Magnetic Resonance Lab, Korea Basic Science Center, Taejon, Korea

*To whom correspondence should be addressed.
Tel: 82-42-860-4140, Fax: 82-42-860-4593

noprecipitation capability of the mAb H8 with the epitope-tagged proteins. We fused the set of amino acid residues in the interval to the amino terminal of β-galactosidase and to the carboxyl terminal of truncated p56 ^{lck} fragment and then tested the antigenicity of the tagged proteins with the mAb H8. We report here that the preS2-sequence and the antibody H8 can be used efficiently for epitope tagging.

Materials and Methods

Reagents and enzymes

Restriction enzymes, T4 DNA ligase and T4 polynucleotide kinase were purchased from New England Biolabs (Gaitherburg, USA) and Gibco.BRL (Gaithersburg, USA). [α^{32} S]dATP, [^{35}S]methionine and DNA sequencing kit were from Amersham (Buckinghamshire, UK). Conditions for enzyme reactions were set following the manufacturer instruction.

Bacterial strains and plasmids

As the bacterial host for subcloning plasmids, *E. coli* strain DH5α (*supE*44 Δ*lac*U169 [φ80 *lacZ* ΔM15] *hsdR*17 *recA*1 *endA*1 *gyrA*96 *thi*-1 *relA*) was used and for expressing the β-galactosidase fusion proteins, *E. coli* JM109 (*recA*1 *endA*1 *gyrA*96 *hsdR*17 *supE*44 *relA*1 *thi*Δ(*lac-proAB*) F'[*traD*36 *proAB*$^+$ *lacI*^{q} *lacZ*ΔM 15]) was used. Plasmid pTSZ and pTSZ(F141T), as described previously (Kwon *et al.*, 1990: Moon *et al.*, 1994), encode hybrid β-galactosidase proteins fused with either the wild or the mutant *adr* type of the preS2 fragment (Fig. 1A). Plasmid LCK/pBSKS$^+$, which contains cDNA of mouse p56^{lck}, was kindly provided by Dr. M. Y. Han (Korea Reserch Institute of Bioscience and Biotechnology). Plasmid pF(BGL)AT contains cDNA of human α_1-antitrypsin connected downstream of the T7 promoter and the 5′ untranslated region of rabbit β-globin gene (Lee *et al.*, 1993).

Construction of the expression vector for preS2 epitope-tagged *lacZ* protein

The plasmid pETSZ expressing the preS2 epitope-tagged β-galactosidase was constructed by replacing the DNA fragment coding for the whole preS2 region in the plasmid pTSZ with the synthetic oligonucleotide cassette coding for the amino acid residues 130~143 in the preS2 region (Fig. 1B). Detailed construction is as follows. The plasmid pTSZ was digested with *Eco*RI and *Bam*HI to remove the DNA fragment containing the tac promoter and the sequence encoding the preS2 region. The *Bam*HI/*Eco*RI fragment of pTSZ carrying the *lacZ* gene was ligated with the epitope-coding cassette in frame with the *lacZ* gene. Then, the *Eco*RI fragment of the tac promoter was inserted into the *Eco*RI site of the ligated plasmid, generating an expression vector, pETSZ, which carries the *lacZ* gene that is fused with the DNA sequence of the preS2 epitope under the control of the taci promoter.

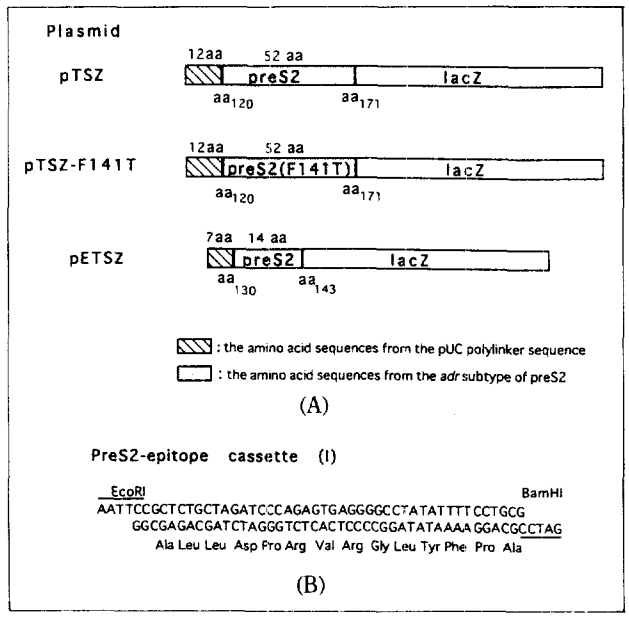

Fig. 1. A. Schematic representation of the preS2-lacZ fusion proteins. Plasmid pTSZ encodes the fusion protein in which the adr subtype preS2 sequence from amino acid residue 120 to residue 171 was fused in frame to 5′ end of B-galactosidase. The preS2(F 141T)-lacZ fusion protein, encoded by pTSZ(F141T), has the substitution of threonine for phenylalanine at residue 141 of the preS2 sequence. pETSZ encodes the fusion protein where the sequence expected as a preS2 epitope (residues 130~142) was tagged to 5′ end of β-galactosidase. The hatched box represents the amino acid sequences from the pUC 7 polylinker sequence. The amino acid sequence derived from the adri subtype of preS2 region of HBV surface antigen is indicated as the dotted box. B. The synthetic oligonucleotide cassette used to tag β-galactosidase with a preS2 epitope. The sequence encodes amino acid residue 130~143 of the adri preS2 sequence. The cassette was generated by annealing of two single oligonucleotides synthesized with an automated DNA synthesizer. To facilitate subcloning process, an *Eco*RI site and a *Bam*HI are included at 5′ end and 3′ end of the oligonucleotide cassette, respectively.

Construction of the expression vector for preS2-epitope tagged p56^{lck}

To express the N-terminal truncated p56^{lck} fragment containing amino acid residues 1-150 under the control of the T7 promoter, the 0.45 kb *Pst*I/*Bam*HI fragment of cDNA encoding mouse p56^{lck} was obtained from the plasmid LCK/pBSKS$^+$ and ligated with the *Bsa*HI-*Pst*I synthetic oligonucleotide linker coding for the first four amino acids of p56^{lck} together with the 3.2 kb partially digested *Bsa*HI/*Bam*HI fragment of pF(BGL) AT, generating a plasmid, pF(BGL)LCK. To tag the lck

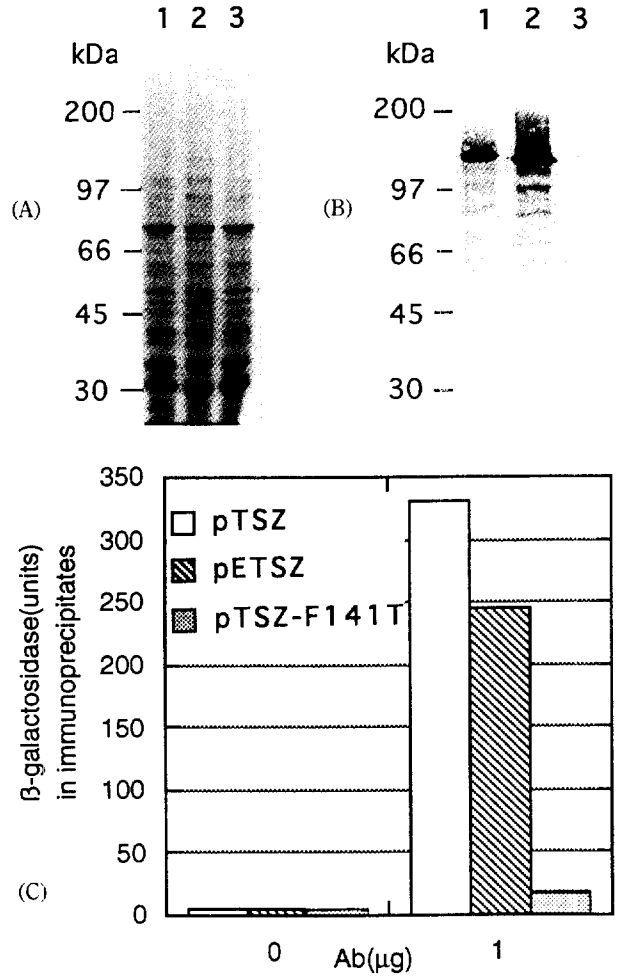

Fig. 2. Analysis of preS2 epitope-tagged β-galactosidase with the antibody H8A. Recombinant *E. coli* JM109 cells harboring plasmid pTSZ (lane 1), pETSZ (lane 2), and pTSZ-F141T (lane 3) were grown at 37℃ and the expression of preS2-β-galactosidase fusion protein was induced by adding 2 mM IPTG. Cells were suspended in cell lysis buffer (20 mM HEPES, 100 mM DTT, pH 7.5, 1 mM PMSF, 10 μg·ml^{-1} aprotinin, 10 μg·ml^{-1} leupetin, 10 μg·ml^{-1} pepstain and sonicated. Cell extracts containing eqivalent amounts of total protein were analyzed on 8% SDS/polyacrylamide gel (Laemmli, 1970). Proteins were stained with Coomasie Brilliant Blue. Molecular weight markers are indicated in kilodaltons on the left. B. Western blot analysis of cell extracts in panel A was performed with the mAb H8 and peroxidase-conjugated anti-mouse IgG antibody as previously described (Lee *et al.*, 1990). C. Immunoprecipitation of the cell extracts containing the same levels of β-galactosidase activity was carried out with 1 μg antibody H8 as described in Materials and Methods. Immunoprecipitates were resuspended in β-galactosidase assay buffer and then incubated with ONPG (o-nitrophenyl-β-D galactopyranoside). The activity of β-galactosidase in immunoprecipitates is expressed as Miller units (Miller, 1972).

fragment with the preS2 epitope, the synthetic oligonucleotide cassette encoding the amino acid sequence of preS2 (Fig. 3B) was inserted into the *Bam*HI and *Eco*RV sites of pF(BGL)LCK. The resulting plasmid, pF(BGL)LCK-preS2, contains the sequence coding for the *lck* fragment fused at its C-terminal with the preS2

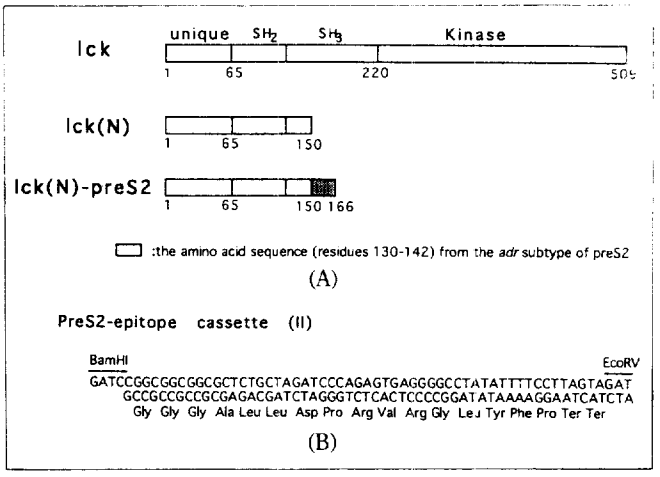

Fig. 3. A. Schematic representation of p56^{lck} proteins. lck; the full length lck protein is represented on the basis of the domain structure characteristic of src family members. lck(N); *in vitro* synthesized N-terminal truncated p56^{lck} fragments containing amino acid residues 1~150. lck(N)-preS2; the lck fragment tagged with the preS2 epitope that is presented as block box. B. The synthetic oligonucleotide cassette to tag the lck(N) fragment with preS2 epitope. The oligonucleotide contains the preS2 epitope sequence derived from amino acid residue 130 to residue 142, followed by two stop codons. Three codons for glycine are placed at the junction of the lck fragment and the epitope because the flexibility provided by glycine (Kamtekar *et al.*, 1993) might be advantageous for the exposure of the epitope sequence recognized by the antibody H8. To facilitate subcloning process, a *Bam*HI site and an *Eco*RV site are included at 5′ end and 3′ end of the oligonucleotide cassette, respectively.

epitope. To verify the construction of pF(BGL)LCK-preS2, DNA sequencing was carried out by the dideoxy chain termination method (Sanger *et al.*, 1977) using double-strand plasmid DNA as a template.

In vitro translation of cloned genes

In vitro synthesized mRNAs encoding the lck(N) fragment or the lck(N)-preS2 were generated by T7 polymerase using *Eco*RV-linearized pF(BGL)LCK or *Eco*47III-linearized pF(BGL)LCK-preS2 as templates, respectively. The reaction was performed with the SP6/T7 transcription system (Boehringer-Mannheim, Mannheim, Germany) at 37℃ for 60 min. *In vitro* translation was carried out at 30℃ for 60 min with the rabbit reticulocyte lysate system which was pre-treated with micrococcal nuclease (Promega, Madison USA).

Immunoprecipitation

A portion of cell extracts or [^{35}S]methionine-labelled rabbit reticulocyte lysate translation products were diluted in 250 μl ice-cold HND buffer (20 mM HEPES, 100 mM KCl, 10% glycerol, 0.4% NP-40, 5 mM EGTA, 100 μg·ml^{-1} bovine serum albumin, 1 mM dithiothreitol, 1 mM phenylmethylsulfonylfluoride, 10 μg·ml^{-1} aprotinin, 10 μg·ml^{-1} leupeptin, 10 μg·ml^{-1} pepstatin,

pH 7.5). To remove proteins that bind to protein A-Sepharose beads, 30 μl of protein A-Sepharose CL-4B (Sigma, MO, U.S.A.) saturated with HND buffer was added and incubated for 30 min at 4℃. After pelleting the protein A-Sepharose beads, the supernatant was incubated with the mAb H8 for 2 h at 4℃, then centrifuged for 10 min. The supernatant was then incubated further with protein A-Sepharose for 1 h, followed by centrifugation. The protein A-Sepharose pellets were washed three times with HND buffer, and then analyzed by the assay of β-galactosidase activity or by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE).

Results and Discussion

PreS2 epitope-tagging of β-galactosidase

Our previous study showed that deletions of the preS 2 antigen from the amino and carboxyl termini up to residue 130~132 and 140~143 did not affect its antigenicity to the mAb H8 (Kwon *et al.*, 1990). The region indicated for the antigenicity (residues 130~143) is long enough to carry a continuous epitope which is considered to reside within four to seven residues (Goodman, 1989). Thus, in order to investigate whether the suggested amino acid sequence alone is sufficient in representing the antigenicity to the mAb H8, we fused the amino acid sequence from residue 130 to 143 of the preS2 region to the 5′ end of β-galactosidase (Fig. 1) and tested the antigenecity of the tagged protein. As a positive control, the lysate of cells harboring the plasmid pTSZ, which expresses the β-galactosidase hybrid protein fused with the whole preS2 sequence (Fig. 1A), was included in the analysis. The cell lysate containing the mutant preS2(F141T)-*lacZ* fusion protein, expressed from the plasmid pTSZ-F141T (Fig. 1A) was used as a negative control. The pTSZ-F141T plasmid has an amino acid substitution (Phe141 →Thr) in the preS2 sequence which causes the loss of antigenicity of the sequence to the mAb H8 (Moon *et al.*, 1994).

Cell extract was prepared from the *E. coli* strain harboring the plasmid pETSZ that expresses the preS2 epitope-tagged β-galactosidase and analyzed by Western blot with the mAb H8. Fig. 2B shows that the epitope-tagged β-galactosidase having the expected molecular weight was detected with the preS2 specific mAb H8 (lane 2), demonstrating that the 14 amino acid residues derived from the preS2 region were sufficient to confer on the tagged protein the antigenicity recognized by the mAb H8. Fig. 2B also shows that the mAb H8 recognized specifically the hybrid lacZ protein fused with the wild type preS2 sequence (lane 1), but not the protein fused with the mutant preS2 sequence (lane 3). Both of the preS2 and preS2(F141T)-β-galactosidase fusion proteins seemed to be expressed at the same level since both of the cell lysates showed similar levels of activity in the assay of β-galactosidase. The protein level of epitope-tagged-β-galactosidase expressed from pETSZ appeared to be twice as high as those of the fusion proteins in that the cell lysate harboring pETSZ showed a two-fold higher activity of β-galactosidase (data not shown) and stronger intensity of signal in Western blot analysis (Fig. 2B). This could be due to either increased mRNA stability or decreased protease-attack of the epitope-tagged β-galactosidase.

The major advantages of using monoclonal antibody over polyclonal antibody in immunochemical assays are its unique specificity of binding and its homogeniety, thus reducing nonspecific background. However, a problem encountered when using monoclonal antibodies is their affinity. For example, monoclonal antibodies with affinities lower than about 10^7 M^{-1} are difficult to use in immunoprecipitation (Harlow and Lane, 1988). The affinity of monoclonal antibody H8 for the synthetic peptide containing amino acid residues from 120 to 145 was reported as approximately 2.5×10^8 M^{-1} (Jin *et al.*, 1993), which is high enough to form a stable antibody-antigen complex detectable in various immunochemical techniques. To further evaluate the utility of the preS2 epitope and mAb H8 as immunological tools, we tested the capability of the antibody H8 to immunoprecipitate the preS2-β-galactosidase fusion proteins. Cell lysates containing equivalent amounts of the β-galactosidase were immunoprecipitated with the antibody H8 and the β-galactosidase activity in immune complexes were measured. The activities in the precipitates (Fig. 2C) showed that the β-galactosidase fused with the wild type preS2 sequence was efficiently immunoprecipitated. Analysis of the β-galactosidase activities remaining in the supernatant after immunoprecipitation indicated that more than 90% of the preS2-β-galactosidase was precipitated as immunocomplex. The epitope-tagged β-galactosidase was also immunoprecipitated at about 70% of the efficiency of the β-galactosidase fused with the wild type whole preS2 sequence, while the β-galactosidase fused with the mutant preS2 (F141T) sequence was not immunoprecipitated. The results demonstrate that the epitope-tagged protein can be efficiently immunoprecipitated using the mAb H8, which is highly specific to the tagged sequence.

PreS2 epitope-tagging of $p56^{lck}$

The above results showed that the peptide derived from the preS2 region of HBV can be used as a portable epitope to tag proteins with an immunological mark-

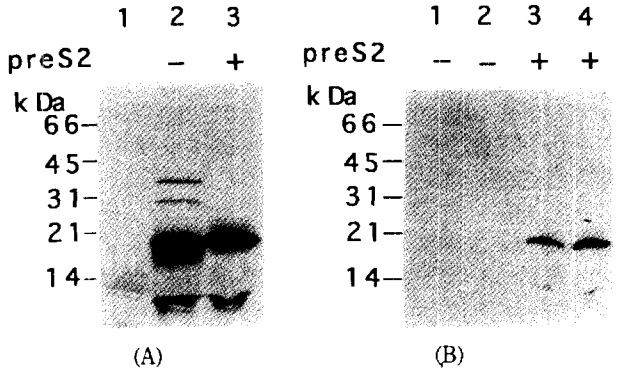

Fig. 4. Immunoprecipitation of the preS2-tagged p56^{lck} fragment with the mAb H8. A. *In vitro* synthesized mRNAs coding for the lck(N) fragment or for the lck(N)-preS2 fragment were generated by T7 polymerase. One μg of each mRNA was translated in the translation reaction mixture (final volume of 25 μl) containing 17.5 μl rabbit reticulocyte lysates and 20 μCi [^{35}S] methionine. An aliquot (1 μl) of each *in vitro* translation reaction mixture was analyzed in 15% SDS/PAGE. Lane 1 : *in vitro* translation without any added mRNA; Lane 2 : *in vitro* synthesized lck(N) fragment; lane 3 : *in vitro* synthesized lck(N)-preS2. Molecular weight markers are indicated in kilodaltons on the left. B. 7 μl (lane 1 and 3) or 14 μl (lane 2 and 4) of *in vitro* translation reaction mixture was immunoprecipitated with 2 μg mAb H8 as described in Materials and Methods. Immunoprecipitates were heated at 95℃ for 5 min in SDS sample buffer and analyzed by 15% SDS/PAGE. Lane 1 and 2 : the lck(N) fragment; Lane 3 and 4 : the lck(N)-preS2, the lck fragment tagged with the preS2 epitope. Gels were fixed, amplified, and fluorographed with EN^{3}HANCE (Dupont, MA, U.S.A.).

er that is recognized by a monoclonal antibody. The epitope tagging approach using a well-characterized monoclonal antibody is particularly useful in the study of protein-protein interaction; proteins that associate with a tagged protein can be easily identified by co-immunoprecipitation and such associated proteins will be absent in immunoprecipitates from the lysates containing the untagged protein. Therefore, we applied the preS2 epitope tagging approach in setting up an *in vitro* system to study p56^{lck}, a T-cell specific protein tyrosine kinase. p56^{lck} is a member of the srci family of non-receptor protein tyrosin kinases and displays structural features which are common to all Src-like kinases, such as SH2, SH3, and kinase domains (Turner *et al.*, 1990, Fig. 3A, top). The important role(s) of p56^{lck} in T-cell signaling has been demonstrated in recent studies that show the interaction of p56^{lck} with CD4 and CD8 (Turner *et al.*, 1990), and 4-1BB, a novel inducible T-cell antigen (Kim *et al.*, 1993). The association was shown to involve the unique N-terminal region of p56^{lck}. As an initial step to establish an *in vitro* system employed to define the role of the N-terminal region of p56^{lck} in protein-protein interaction, we expressed *in vitro* the N-terminal truncated fragment of p56^{lck}, lck(N), which contains amino acid residues 1~150 (Fig. 3A, middle). To provide the lck(N) fragment with an immunological marker that can be monitored by a monoclonal antibody, the amino acid sequence derived from the preS2 region in HBV was tagged at ged at the C-terminus of the lck(N) fragment (Fig. 3A, bottom). Since residue 143 in the preS2 region was suggested to be supplementary for the antigenicity to the mAb H8 (Kwon *et al.*, 1990), the sequence from residue 130 to 142 of preS2 was fused with the lck(N) fragment (Fig. 3B). The epitope-tagged fragment, lck(N)-preS2, exhibited slightly lower mobility on 15% SDS /polyacrylamide gels than its untagged counterpart of ca. 17 kDa (Fig. 4A). Immunoprecipitation assay of the *in vitro* translation products with the antibody H8 showed that the epitope-tagged lck(N) fragment was precipitated as immunocomplex with the antibody, whereas no precipitation of the untagged lck fragment was observed (Fig. 4B). The result demonstrates that the sequence within amino acid residues 130~142 of preS2 region in HBV contains an antigenic determinant that is sufficient to form a stable complex with the mAb H8.

The precipitation efficiency of the preS2 epitope-tagged lck(N) fragment with the mAb H8 was about 5%, which is about 10-fold lower than the value obtained from the immunoprecipitation of preS2 epitope-tagged β-galactosidase. It was reported that differences in the extent of immunoprecipitation could be due to several factors, such as the intrinsic affinity of the antibody for the epitope, the valency of the antibody and antigen, and the geometric arrangement of the interacting components that govern the overall strength of an antibody and antigen, the avidity (Harlow and Lane, 1988). The high efficiency of precipitation in the case of the tagged β-galactosidase could be explained primarily by the fact that β-galactosidase is a homotetramer. Four copies of the preS2 sequence in the tagged protein present identical, repeating epitopes that encourage bivalent binding of antibody, which eventually increases the overall stability of the complex between the antibody and the tagged protein. In addition, the residue 143 in the preS2 sequence used for tagging β-galactosidase might contribute to the stabilization of the interaction between the tagged protein and the antibody as implicated in the previous deletion analyses (Kwon *et al.*, 1990).

The present study showed that the amino acid sequence of residues 130~142 derived from the adri subtype preS2 region of HBV can confer the antigenicity recognized by the mAb H8 on proteins tagged with the peptide. The mAb H8 showed avidity high enough for immunoprecipitation of the preS2 epitope-

tagged proteins and showed little cross reactivity with the untagged proteins. Thus, the preS2-epitope and monoclonal antibody H8 can be a useful tool for the epitope tagging approach. The approach has been widely applied to monitor the cellular location and post-translational modification of the tagged protein, and to purify the protein in the absence of a functional assay. Especially, it has been a popular approach for studying protein-protein interaction since co-immunoprecipitation is one of the easiest methods for identifying potential interactions between the antigen and other proteins. The *in vitro* system established in the present study for the preS2-epitope tagged lck(N) fragment can be explored as a means of characterizing the interaction between $p56^{lck}$ and other proteins involved in T-cell transduction, via co-immunoprecipitation assay using the mAb H8.

Acknowledgement

We are grateful to Dr. M.Y. Han for providing plasmid LCK /pBSKS$^+$ and thankful to Dr. H. J. Hong for generous gift of the purified antibody H8.

References

Budkowska, A., Riotot, M.-M., Dubreuil, P., Lazizi, Y., Brechot, C., Sobczak, E., Petit, M.-A. and Pillot, J. (1986) *J. Med. Viol,* **20**, 111.

Chung, H. K. and Kim, S. R. (1987) *Recent progress in molecular biology and genetic engineering in Korea,* **1**, 165.

Goodman, J. W. (1989) in *Antigenic determinants and immune regulation* (Sercarz, E. ed), Basel, Karger.

Harlow, E. and Lane, D. (1988) in *Antibodies*, pp. 23-35, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Itoh, Y., Takai, E., Ohnuma, H., Kitijima, K., Tsuda, F., Machida, A., Mishiro, S., Nakamura, T., Miyakawa, Y. and Mayumi, M. (1986) *Proc. Natl. Acad. Sci. USA.* **84**, 9174.

Jin, B. R., Ryu, C. J., Park, S. S., Uk, N., Hong, H. J. and Han, M. H. (1993) *Molec. Immunol.* **30**, 1647.

Kamtekar, S., Schiffer, J. M., Xiong, H., Babik, J. M. and Hecht, M. H. (1993) *Science* **262**, 1680.

Kim, Y-J., Pollk, K. E., Zhou, Z., Shaw, A., Bohlen, J. B., Fraser, M. and Kwon, B. S. (1993) *J. Immunol.* **151**, 12 55.

Kolodziej, P. A. and Young, R. A. (1991) *Meth. Enzym.* **194**, 508.

Kwon, K-S., Kim, C. S., Park, J. S., Han, M. H. and Yu, M.-H. (1990) *Kor. J. Microbiol.* **28**, 13.

Lee, K. N., Lee, S. C., Kwon, K.-S., Chung, H. K. and Yu, M.-H. (1990) *Kor. J. Microbiol.* **28**, 6.

Lee, K. N., Shin, H. S., Kwon, K.-S., Park, S. D. And Yu, M.-H. (1993) *Mol. Cells,* **3**, 71.

Laemmli, U. K. (1970) *Nature* **227**, 680.

Miller, J. H. (1972) in *Experiments in molecular genetics*, pp. 352-355, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Moon, A., Kwon, K.-S. and Yu, M.-H. (1994) *Korean Biochem. J.* **27**, 60.

Neurath, A. R., Kent, S. B. H., Strick, N., Taylor, P. and Stevens, C. E. (1985) *Nature* **315**, 154.

Neurath, A. R., Kent, S. B. H., Parker, K. M. Prince, Strick, N., Brotman, B. and Sproul, P. (1986) *Vaccine* **4**, 35.

Sanger, F., Nicklen, S. and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA.* **74**, 5463.

Turner, J. M., Brodsky, M. H., Irving, B. A., Levin. S. D., Perlmutter, R. M. and Littman, D. R. (1990) *Cell* **60**, 755.