Journal of Biochemistry and Molecular Biology, Vol. 31, No. 6, November 1998, pp. 611-614

JOURNAL OF
Biochemistry
and
Molecular Biology

Short communication

The β Subunit of CKII Interacts with the Lysosomal Protease Cathepsin L

Hyun Jae Yu, Bong-Hyun Ahn and Young-Seuk Bae*

Department of Biochemistry, College of Natural Sciences, Kyungpook National University, Taegu 702-701, Korea

Received 28 July 1998, Accepted 20 August 1998

Protein kinase CKII (CKII) is a protein Ser/Thr kinase that is ubiquitously distributed in eukaryotic cells. Although it has been suggested that CKII plays an critical role in cell growth and proliferation, its functional significance and regulation in the cells remain poorly understood. To investigate the exact biological function of CKII, we have identified proteins that interact with the subunits of CKII using the two-hybrid system. In this report, we have identified cathepsin L, a lysosomal protease, as a cellular protein capable of interacting with the β subunit of CKII. Cathepsin L does not interact with the α subunit of CKII, supporting the idea that the β subunit can mediate the interaction of CKII with target proteins. We have found that cathepsin L has several putative CKII phosphorylation sites including Thr-84, Ser-160, Ser-270, Thr-288, and Ser-301. These data suggest that CKII is a possible protein kinase for cathepsin L phosphorylation.

Keywords: Cathepsin L, Protein kinase CKII, Two-hybrid system.

Introduction

Protein kinase CKII (CKII) is a ubiquitous and highly conserved Ser/Thr kinase, which is found in all eukaryotes examined and in various subcellular compartments (Pinna, 1990; 1997; Issinger, 1993). It is a calcium-independent and cyclic nucleotide-independent enzyme which utilizes ATP as well as GTP as a phosphate donor. The minimum consensus sequence for CKII phosphorylation is Ser/Thr-X-X-Asp/Glu, but additional acidic residues are found mostly near phosphoacceptor sites. Holoenzyme CKII is a heterotetramer of two catalytic (α and/or α′) and two regulatory (β) subunits. It has been shown that the α and α′ subunits are the products of different genes and exhibit the catalytic activity of the enzyme. The β subunit is a regulatory subunit that modulates the catalytic activity of the α subunit. It mediates the tetramer formation by both the self-dimerization and the interaction of the β subunit with the α or α′ subunit (Gietz *et al.*, 1995; Kim *et al.*, 1998). In addition, the β subunit may determine its substrate specificity (Lin *et al.*, 1991; Jakobi and Traugh, 1992; Kim *et al.*, 1996). While the catalytic activity of CKII is inhibited strongly by heparin, the enzyme is stimulated by polybasic compounds (Hathaway and Traugh, 1984).

The level of CKII is greatly enhanced in proliferating cells and tumor tissues, and the α subunit causes lymphomas in transgenic mice (Issinger, 1993; Seldin and Leder, 1995). The enzyme phosphorylates a broad spectrum of substrates which are involved in cell growth, including DNA binding proteins, nuclear oncoproteins, and transcription factors (Pinna, 1990; 1997; Issinger, 1993). These observations suggest that CKII functions as a connection between growth signals and gene expressions; however, its precise physiological role and regulatory mechanism remain unknown.

In order to study the precise physiological role and regulatory mechanism of CKII, we have identified cellular proteins that interact with CKII using the yeast two-hybrid system (Fields and Song, 1989). In previous studies, we reported that the ribosomal proteins L5 and L41 were CKII-associating proteins (Kim *et al.*, 1996; Lee *et al.*, 1997). In the present study, we report that cathepsin L, one of the lysosomal proteases, interacts with the β subunit of CKII.

Materials and Methods

Yeast strain and culture condition *S. cerevisiae* HF7C (*MATα ura3-52 his3-200 ade2-101 lys2-801 trpl-901 leu2-3,112 gal4-*

* To whom correspondence should be addressed.
Tel: 82-53-950-6355; Fax: 82-53-943-2762
E-mail: ysbae@kyungpook.ac.kr

542 *gal*80-538 *LYS2*::GAL1UAS ALITATA-*HIS3 URA3*::GAL417mers(×3)-CyC1TATA-*lacZ*) (Feilotter *et al.*, 1994) was used for the two-hybrid system (Fields and Song, 1989). Yeast cultures were grown in either YPAD media (1% yeast extract, 2% peptone, 0.004% adenine sulfate, 2% glucose) or synthetic minimal media with appropriate supplements at 30°C (Rose *et al.*, 1990).

Two-hybrid screen The two-hybrid screen was performed as described previously (Kim *et al.*, 1996). Briefly, both pGBT9-CKIIβ and pGADGH-cDNA were cotransformed into *S. cerevisiae* HF7C. The tranformants were plated on synthetic media lacking tryptophan, leucine, and histidine. After 3–5 d of growth, His^+ colonies were assayed for expression of the *lacZ* reporter gene. Finally, positive clones were isolated that induced the *lacZ* reporter gene only in the presence of pGBT9-CKIIβ. DNA sequencing was carried out by the dideoxy chain termination method.

***In vivo* binding assay** The *in vivo* protein–protein interaction was monitored by the expression of the *HIS3* reporter gene using the two-hybrid system. The *S. cerevisiae* HF7C reporter strain was cotransformed with various combinations of hybrid plasmids containing a DNA binding domain or a transcriptional activation domain. Transformants were plated on synthetic media lacking tryptophan and leucine. After 4 d of growth, transformants were streaked onto selective media lacking tryptophan, leucine, and histidine, and incubated for 3 d at 30°C.

Results and Discussion

To detect proteins that interact with the β subunit of CKII, we used the two-hybrid system (Fields and Song, 1989). The DNA binding domain fusion ('bait') comprised the complete open reading frame of the β subunit of human CKII fused to the GAL4 DNA-binding domain in pGBT9, while a library of human proteins fused to the GAL4 activation domain in pGADGH represented the 'prey'. These two hybrids were co-expressed in *S. cerevisiae* HF7C which contained reporter genes *HIS3* and *lacZ*, yielding 1.3×10^6 independent transformants. From this screen, twenty colonies were finally identified that induced the expression of β-galactosidase only in the presence of pGBT9-CKIIβ. Out of the twenty clones identified, two represented the ribosomal proteins L5 and L41, respectively (Kim *et al.*, 1996; Lee *et al.*, 1997). In this paper, we describe another clone, CL10. Sequence analysis of CL10 revealed that the cDNA insert contained a reading frame of 558 nucleotides and a 3′ noncoding region of 284 nucleotides, followed by a poly(A) tract (Fig. 1). The nucleotide sequences of the insert did not differ from those of cathepsin L, a lysosomal protease, available in the GenBank database (accession number M20496). The insert encoded the C-terminal portion (amino acids 149–333) of cathepsin L.

Because CL10 was obtained from the screen using the β subunit of CKII as a bait, we tested whether cathepsin L would interact with the α subunit of CKII. The reporter

```
GAATTCGGCACGAGgacagatgttccggaaaactgggaggcttatctcactgagtgagcag      61
aatctggtagactgctctgggcctcaaggcaatgaaggctgcaatggtggcctaatggat     121
tatgctttccagtatgttcaggataatggaggcctggactctgaggaatcctatccatat     181
gaggcaacagaagaatcctgtaagtacaatcccaagtattctgttgctaatgacaccggc     241
tttgtggacatccctaagcaggagaaggccctgatgaaggcagttgcaactgtggggccc     301
atttctgttgctattgatgcaggtcatgagtccttcctgttctataaagaaggcatttat     361
tttgagccagactgtagcagtgaagacatggatcatggtgtgctggtggttggctacgga     421
tttgaaagcacagaatcagataacaataaatattggctggtgaagaacagctggggtgaa     481
gaatggggcatgggtggctacgtaaagatggccaaagaccggagaaaccattgtggaatt     541
gcctcagcagccagctaccccactgtgtgagctgtggacggtgatgaggaaggacttgac     601
tggggatggcgcatgcatgggaggaattcatcttcagtctaccagcccccgctgtgtcgg     661
atacacactcgaatcattgaagatccgagtgtgatttgaattctgtgatattttcacact     721
ggtaaatgttacctctattttaattactgctataaataggtttatattattgattcactt     781
actgactttgcattttcgtttttaaaaggatgtataaatttttacctgtttaaataaaat     841
ttaatttcaaatgtaaaaaaaaaCTCGAG                                    870
```

Fig. 1. The nucleotide sequence of CL10. The nucleotide sequence of the human cathepsin L is given in lowercase letters and those of pGADGH in capital letters. Dotted underlines represent the insertion sites, *Eco*RI and *Xho*I of cDNA into pGADGH. The adaptor sequence, used in the construction of the pGADGH cDNA library, is indicated in boldface. The stop codon (tga) and polyadenylation signal (aataaa) of the cathepsin L gene are underlined once and twice, respectively. Numbers in the right margin indicate positions of nucleotides.

strain *S. cerevisiae* was cotransformed with CL10 and either pGBT9-CKIIα or pGBT9-CKIIβ, and the protein–protein interactions were detected by the expression of the *HIS3* reporter gene. All hybrid plasmids were also tested against the empty expression vectors pGBT9 and pGADGH in order to control for autonomous activation of the hybrid proteins. In those control transformants, expression of the reporter gene was not evident. In contrast to control transformants, when cathepsin L and CKIIβ hybrid proteins were co-expressed in the reporter cells, the transformants were able to grow on selective media lacking tryptophan, leucine, and histidine. These data indicated that cathepsin L interacts specifically with the β subunit of CKII, but not with the α subunit (Fig. 2). The present study supports the idea that the β subunit of CKII can mediate the interaction of the catalytic subunit with target proteins. As a positive control, when the reporter strains were cotransformed with pGBT9-CKIIβ and pGADGH-CKIIα, transactivation of the *HIS3* gene occurred (Fig. 2).

Cathepsin L is a lysosomal cysteine protease which plays a major role in intracellular protein degradation (Barrett and Kirschke, 1981). Cathepsin L is synthesized as an inactive prepro-enzyme, which is processed to a proenzyme and targeted from the endoplasmic reticulum to the lysosome. The enzyme is further processed to the fully active mature form (Kominami *et al.*, 1988; Mason *et al.*, 1989).

CKII has been isolated from the cytoplasm, nuclei, mitochondria, and cell membrane (Pinna, 1990; 1997; Issinger, 1993). However, a recent study indicated that

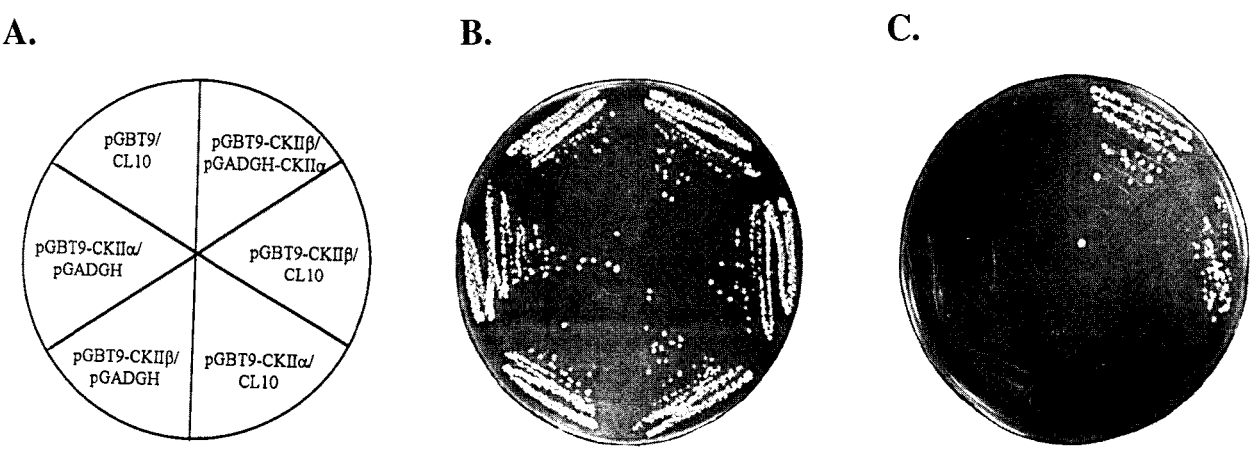

Fig. 2. Specific interaction of cathepsin L with the β subunit of CKII. The *S. cerevisiae* reporter strain was cotransformed with CL10 and each CKII subunit-fused DNA binding domain vector (A). pGBT9 and pGADGH encode the DNA binding domain and transcriptional activation domain of the GAL4 protein, respectively. pGBT9-CKIIα and pGBT9-CKIIβ are pGBT9 containing the full-length α and β subunits of human CKII, respectively (Kim *et al.*, 1996). pGADGH-CKIIα is pGADGH containing the full-length α subunit of human CKII (Kim *et al.*, 1998). Individual Trp^+ Leu^+ transformants were streaked onto selective media either lacking tryptophan and leucine (B) or lacking tryptophan, leucine, and histidine (C). The plates were incubated at 30°C for 3 d.

```
                ↓
MNPTLILAAFCLGIASATLTFDHSLEAQWTKWKAMHNRLYGMNEEGWRRAVWEKNM   56

KMIELHNQEYREGKHSFTMAMNAFGDMTSEEFRQVMNGFQNRKPRKGKVFQEPLFY  112
↓
EAPRSVDWREKGYVTPVKNQGQCGSCWAFSATGALEGQMFRKTGRLISLSEQNLVD  168

CSGPQGNEGCNGGLMDYAFQYVQDNGGLDSEESYPYEATEESCKYNPKYSVANDTG  224

FVDIPKQEKALMKAVATVGPISVAIDAGHESFLFYKEGIYFEPDCSSEDMDHGVLV  280

VGYGFESTESDNNKYWLVKNSWGEEWGMGGYVKMAKDRRNHCGIASAASYPTV]   333
```

Fig. 3. The putative CKII-phosphorylation sites within the human prepro-cathepsin L protein. The putative CKII-phosphorylation sites are underlined. Brackets indicate the region of the cathepsin L included in CL10. Four putative CKII-phosphorylation sites reside in CL10. Positions at which processing occur are indicated by arrows (Gal and Gottesman, 1988; Kominami *et al.*, 1988; Mason *et al.*, 1989). Numbers in the right margin indicate positions of amino acids.

CKII might be localized in the lumen of the endoplasmic reticulum and that it phosphorylates microsomal calcium-binding proteins 1 and 2 (Chen *et al.*, 1996). These results raise the possibility that cathepsin L may be phosphorylated by CKII in the lumen of the endoplasmic reticulum. CKII preferentially phosphorylates Ser/Thr residues followed by a stretch of acidic residues on the immediate C-terminal side. Among these acidic residues, the third position after the phosphoacceptor is the most important determinant (Pinna, 1990; 1997; Issinger, 1993). Cathepsin L has five putative CKII-phosphorylation sites, viz., Thr-84, Ser-160, Ser-270, Thr-288, and Ser-301. Among these, four putative sites reside in the clone, CL10 (Fig. 3). Whether CKII phosphorylates cathepsin L *in vitro* is under study. The ribosomal protein L5, which was identified as a CKIIβ-associating protein in the previous study (Kim *et al.*, 1996), turned out to be phosphorylated by CKII *in vitro* (Park and Bae, manuscript in preparation).

In addition to its role in protein degradation, cathepsin L is involved in a variety of disease processes including cancer metastasis. The enzyme is expressed in higher levels in malignantly transformed cells than in normal cells (Chauhan *et al.*, 1991). The expression of CKII is also enhanced in tumor tissues (Issinger, 1993). The elevated expression and their interaction of both proteins may be related to tumor growth. Further studies will be required to clarify the funtional significance of the interaction between CKII and cathepsin L.

Acknowledgments The authors wish to acknowledge the financial support of the Korea Research Foundation made in the program year of 1997.

References

Barrett, A. J. and Kirschke, H. (1981) Cathepsin B, cathepsin H, and cathepsin L. *Methods Enzymol.* **80**, 535–561.

Chauhan, S. S., Goldstein, L. J. and Gottesman, M. M. (1991) Expression of cathepsin L in human tumors. *Cancer Res.* **51**, 1478–1481.

Chen, N. Q., Davis, A. T., Canbulat, E. C., Liu, Y. X., Goueli, S., McKenzie, B. A., Eccleston, E. D., Ahmed, K. and Holtzman, J. L. (1996) Evidence that casein kinase 2 phosphorylates hepatic microsomal calcium-binding proteins 1 and 2 but not 3. *Biochemistry* **35**, 8299–8306.

Feilotter, H. E., Hannon, G. J., Ruddell, C. J. and Beach, D. (1994) Construction of an improved host strain for two hybrid screening. *Nucleic Acids Res.* **22**, 1502–1503.

Fields, S. and Song, O. (1989) A novel genetic system to detect protein-protein interaction. *Nature* **340**, 245–246.

Gal, S. and Gottesman, M. M. (1988) Isolation and sequence of a cDNA for human pro-(cathepsin L). *Biochem. J.* **253**, 303–306.

Gietz, R. D., Graham, C. and Litchfield, D. W. (1995) Interactions between the subunits of casein kinase II. *J. Biol. Chem.* **270**, 13017–13021.

Hathaway, G. M. and Traugh, J. A. (1984) Kinetics of activation of casein kinase II by polyamines and reversal of 2,3-bisphosphoglycerate inhibition. *J. Biol. Chem.* **259**, 7011–7015.

Issinger, O.-G. (1993) Casein kinases: pleiotropic mediators of cellular regulation. *Pharmacol. & Ther.* **59**, 1–30.

Jakobi, R. and Traugh, J. A. (1992) Characterization of the phosphotransferase domain of casein kinase II by site-directed mutagenesis and expression in *Escherichia coli. J. Biol. Chem.* **267**, 23894–23902.

Kim, J.-M., Cha, J.-Y., Marshak, D. R. and Bae, Y.-S. (1996) Interaction of the β subunit of casein kinase II with the ribosomal protein L5. *Biochem. Biophys. Res. Commun.* **226**, 180–186.

Kim, M.-S., Lee, Y.-T., Kim, J.-M., Cha, J.-Y. and Bae, Y.-S. (1998) Characterization of protein interaction among subunits of protein kinase CKII *in vitro* and *in vivo. Mol. Cells* **8**, 43–48.

Kominami, E., Tsukahara, T., Hara, K. and Katunuma, N. (1988) Biosynthesis and processing of lysosomal cysteine proteinases in rat macrophages. *FEBS Lett.* **231**, 225–228.

Lee, J.-H., Kim, J.-M., Kim, M.-S., Lee, Y.-T., Marshak, D. R. and Bae, Y.-S. (1997) The highly basic ribosomal protein L41 interacts with the β subunit of protein kinase CKII and stimulates phosphorylation of DNA topoisomerase IIα by CKII. *Biochem. Biophys. Res. Commun.* **238**, 462–467.

Lin, W.-J., Tuazon, P. T. and Traugh, J. A. (1991) Characterization of the catalytic subunit of casein kinase II expressed in *Escherichia coli* and regulation of activity. *J. Biol. Chem.* **266**, 5664–5669.

Mason, R. W., Wilcox, D., Wilkstrom, P. and Shaw, E. N. (1989) The identification of active forms of cystein proteinases in Kirsten-virus-transformed mouse fibroblasts by use of a specific radiolabelled inhibitor. *Biochem. J.* **257**, 125–129.

Pinna, L. A. (1990) Casein kinase-2: An eminence grise in cellular regulation? *Biochem. Biophys. Acta.* **1054**, 267–284.

Pinna, L. A. (1997) Protein kinase CK2. *Int. J. Biochem. Cell Biol.* **29**, 551–554.

Rose, M. D., Winston, F. and Hieter, P. (1990) *Laboratory Courses Manual for Methods in Yeast Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Seldin, D. C. and Leder, P. (1995) Casein kinase II transgene-induced murine lymphoma: Relation to theileriosis in cattle. *Science* **267**, 894–897.