

The β subunit of casein kinase II: cloning of cDNAs from murine and porcine origin and expression of the porcine sequence as a fusion protein

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cDNAs encoding the β subunit of pig and mouse CKII were isolated. The porcine cDNA was expressed as a fusion protein in *Escherichia coli* and used for the production of anti-CKII- β subunit specific antibodies.

CKII has been shown to possess a tetrameric structure. It has been found either as a homodimer ($\alpha_2\beta_2$) or heterotetramer ($\alpha\alpha'\beta_2$). The first report describing the primary structure of the β subunit of CKII was based on classical protein sequencing [1]. In parallel, cDNA clones encoding the α and β subunit of *Drosophila melanogaster* CKII were described [2], followed by a report where it was shown that the α/α' subunits are distinct gene products, at least in yeast [3]. Subsequently, the cloning and characterization of cDNAs encoding the subunits of human CKII were reported [4–6]. While this work was in progress, the nucleotide sequence of the mouse cDNA encoding the β subunit of CKII was reported [7]. We present here the complete mouse β -sequence of CKII and part of the porcine CKII- β subunit. In addition, we have expressed a fusion protein of the *E. coli trpE* product and the porcine CKII- β subunit and raised antibodies against it which specifically detect only the β subunit. The availability of a CKII- β -specific antibody may be helpful in determining the fate of the CKII enzyme during various stages of developmental and cellular regulatory processes.

Isolation of a cDNA from a porcine library: a cDNA clone encoding the β subunit of CKII from pig was isolated from a cDNA library made from LLC-PK₁

cells, a porcine kidney cell line (kindly provided by Dr. B. Hemmings, FM1, Ciba-Geigy, Basel). The library was screened using oligonucleotides made according to the amino acid sequence obtained after endolysine digestion of porcine enzyme and subsequent microsequencing. The sequence of the peptide was: YQ-QGDFGYCP. The corresponding oligonucleotide sequence was: TA(C/T)CAGCAGGG(C/T)GA(C/T)TTGG(C/T)TA(C/T)TG(C/T)CC.

All CKII- β cDNA clones from this library lacked the first 60 nucleotides from the coding region. The reason for this may be uncomplete methylation at *EcoRI* recognition sequences during the preparation of the cDNA library which precludes the isolation of complete CKII- β sequences when *EcoRI* is used for the construction of the clones. A similar observation was made by Jacobi et al. [6].

Preparation of a mouse cDNA library and isolation of a mouse cDNA sequence: RNA from Krebs II mouse ascites tumor cells was prepared according to Reichert and Issinger [8]. mRNA was purified using a kit from Pharmacia. A cDNA library was then constructed using the ZAP cDNA synthesis kit from Stratagene. The library was screened with the 540 bp *EcoRI/PstI* fragment of the CKII- β cDNA clone from pig. A full length cDNA clone was obtained. After in vivo subcloning it was sequenced by the nested deletion method [9].

Construction of pBB-1 for the expression of CKII- β subunit: a 540 bp *EcoRI/PstI* fragment was isolated from the porcine CKII- β clone (corresponding to amino acids 20 to 200) and subcloned into the corresponding restriction site into the pATH11 expression vector where

The sequence data in this paper have been submitted to the EMBL/Genbank Data Libraries under the accession numbers X56502, mouse mRNA CKII BETA, X56503, porcine mRNA CKII BETA.

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M  CCCTACTTCGTCTGCTGCGGCGGGTCGGCTTTTGGCGCTGTAAGTGTCTCTGCGGTTCCTTGGAAAGCACAGCTCCCTTCCCCGCCCCAGTCACAGTCCC 100
M  CGTCCGCGCCGGACATAAAGATGAGTAGCTCTGAGGAGGTGTCTGGATTTTCTGCTCTGCGGCTCCGTGGTAATGAATTTCTCTGTGAACGTGGATG 200
P                                     GAATTCCTCTGTGAAGTGGATG
M  AAGACTACATCCAGGACAAATTTAATCTTACTGGAATCAATGAGCAGGTGCCTCACTATCGACAAGCTCTGGACATGATCTTAGACCTGGAACCTGATGA 300
P  AAGACTACATCCAGGACAAATTTAATCTTACTGGAATCAATGAGCAGGTGCCTCACTATCGACAAGCTCTGGACATGATCTTAGACCTGGAACCTGATGA 300
P  R D Y I Q D R P M L Y G L N R Q V P H Y R Q A L D M I L D L R P D R 19
M  AAGCTGGAAGACAACCCCAACAGAGCGACTTGTGCAACAGGAGGTGAGATGCTTTATGGGTTGATCCAGCCGCTACATCTCCACCAACCGAGGC 400
P  CAGCTGGAAGACAACCCCAACAGAGGTGAGCTGATTGAGCAGGAGCGGAAATGCTCTATGGATTGATCCAGCCGCTATATCTCCACCAACCGTGGC 400
P  R L R D N P M Q S D L I R Q A A R N L Y G L I N A R Y I L T N R G 93
M  ATCGCAACAATGTTGAAAGATACCAAGCAGGAGACATTTGGCTACTGTCTCTGCTATATCTGTGAGAACACGCAATGCTTCTATCGGCTTTTCAAGACA 500
P  ATCGCCCCAGATGTTGAAAGATACCAAGCAGGAGACATTTGGCTACTGTCTCTGCTATATCTGTGAGAACACGCAATGCTTCTATCGGCTTTTCAAGACA 500
P  I A Q N L R E Y Q Q G D F G Y C P R V Y C R N Q P M L P I G L S D 125
M  TCCAGGCGAGGCCATGGTGAAGCTCTACTGCCCAAGTGCATGGAGGTGTACACACCAAGTCTCCAGACACCAACACAGGAGCGGCATATTTGG 600
P  TCCAGGCGAGGCCATGGTGAAGCTCTACTGCCCAAGTGCATGGAGGTGTACACACCAAGTCTCCAGACACCAACACAGGAGCGGCATATTTGG 600
P  I P G R A M V K L Y C P K C M D V Y T P R S S R H H H T D G A Y P G 159
M  CACTGGTTTCCCTCACATGCTCTTCAATGCTGATCCAGAGTACCGGCCCAAGCGAGCTGCCAACCAAGTTTGTATCCAGGCTCTATGGTTTCAAGATCCAT 700
P  CACCGGTTTCCCTCACATGCTCTTCAATGCTGATCCAGAGTACCGGCCCAAGCGAGCTGCCAACCAAGTTTGTATCCAGGCTCTATGGTTTCAAGATCCAT 700
P  T G P P N M L P M V R P E Y R P K R P A N Q P V P R L Y G F R I H 193
M  CCAATGGCTTACCAAGCTGCAGCTCCAAGCGCCAGCAACTCAAGAGCCCAAGTCAAGACTATTCGCTGATTGCCCA-CCCACCTCTCCCTCTGTCTGTGA 799
P  CCAATGGCTTACCAAGCTGCAGCTCCAAGCGCCAGCAACTCAAGAGCCCAAGTCAAGACTATTCGCTGATTGCCCAATCCACCCGCTCCCTCAGTCTTTGA 799
P  P M A T Q L Q L Q A A S M P R S P V K T I R 215
M  CACCACCATCTCTGCTGCCACCCCTTTCAGGAAG--TCTATGCTTTTATGTTAAATTAAGGAATTGTTACTGTGCTGGGAATATGAAATAAAGG--A 895
P  CACTTTCGTTCTTTCGCTGCCACCCCTTTCAGGAACCCCTATGCTTTTATGTTAAATTAAGGAGTCGTTATGCTGCTGGGAACATGAAATAAAGTGA 895
M  AGAAAAGGCCATGAAs 943
P  AGAAAAGGC----AAs

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Fig. 1. DNA sequence and deduced amino acid sequence of the CKII- β gene from mouse and pig. The DNA sequence of the mouse (M) and pig (P) CKII- β gene encoding the β subunit of mouse and pig casein kinase II is shown together with the deduced amino acid sequence of the encoded polypeptide. Differences between the porcine and murine nucleotide sequence are indicated by colons. The mouse start codon begins with nucleotide No. 122 and the stop codon is located at nucleotide No. 768. We have added spaces into the 3' noncoding region in order to obtain maximum homology.

regulatory elements and 80% of the *trpE* (anthranilate synthase) are used for the production of a fusion protein (Körner, T.J., unpublished data). The resulting construct (pBB-1) was used for transformation of *E. coli* 49 AA. Expression was carried out in *E. coli* C600. Induction was with β -indolacrylic acid (β -IAA).

Isolation of anti-CKII- β antibodies and immunodetection of the CKII- β subunit was carried out as described earlier [10] Fig. 1 shows the nucleotide sequence of the cDNA from murine and porcine origin. There are four differences in the nucleotide sequence in our mouse sequence when compared to the recently published sequence [7].

Nucleotide No. * This paper Published sequence

10	G	C
20/21	missing	T
514	C	T
598	T	C

The first two changes are located in the 5'-noncoding region, the latter changes are in the coding region, however, they do not lead to an amino acid exchange.

The degree of homology between mouse and porcine CKII at the nucleotide level is 93.2% in the coding region and about 80% in the 3'-noncoding region. The two published amino acid sequences from the human CKII- β subunit differ in one amino acid at position 194 (P/A exchange). We have isolated human cDNA clones independently and confirmed the proline residue described earlier [2].

Fig. 2 shows the Coomassie blue stained 12.5% SDS-PAGE with the bacterial extracts transformed by either pATH11 or pBB-1. Induction of the pATH11 transformed bacteria leads to the appearance of a 37 kDa protein as early as 2 h after the addition of β -IAA. In the case of the pBB-1 transformed bacteria the ap-

* The nucleotide number corresponds to our numbering system.

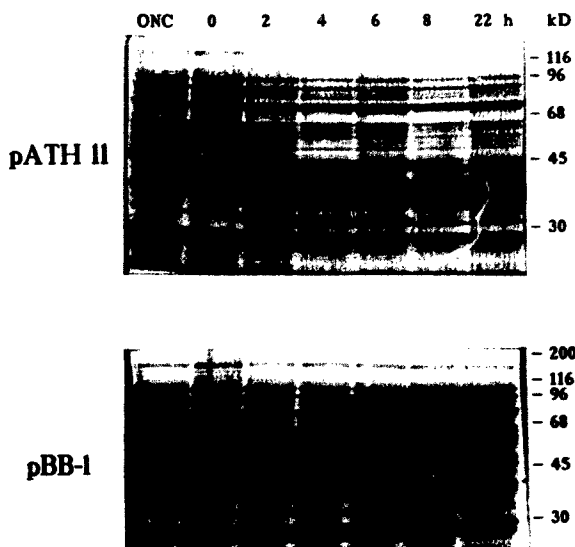


Fig. 2. β -IAA inducible proteins from pATH11 and pBB-1 transformed *E. coli* strain C600. Aliquots of the transformed bacteria were removed at the times indicated (0–8 h). The cells were frozen, sonicated and heated in SDS sample buffer. Aliquots of 50 μ g protein were applied pro slot. Separation of the proteins was carried out on 12.5% SDS-PAGE. Proteins were visualized by Coomassie blue staining. ONC, overnight culture. The molecular masses are expressed as kilodaltons on the righthand side of the gel.

pearance of a 63 kDa protein is observed after the addition of β -IAA. In this case, a fusion protein which consists of the N-terminal trpE region (37 kDa) and the C-terminal β subunit of CKII is formed. The protein is well expressed up to 8 h.

Since the first 20 amino acids did not exhibit an extraordinarily hydrophobic structure, we decided to use the CKII- β fusion protein for the isolation of specific antibodies which would specifically recognize the CKII- β subunit. The amino-terminal 75 residues carry 19 negative charges and the carboxy-terminal 69 residues carry 15 positive charges. The average hydrophobic index is slightly negative. The first 20 amino acids contain only three of the 19 negative charges located in the amino terminal 75 residues which suggested to us that the fusion protein lacking the first 20 amino acids, which form the amino terminus, would still be a good antigen for the production of anti CKII- β antibodies.

Fig. 3a, b shows immunoblots where crude Krebs II mouse ascites tumor extracts were analyzed on one-dimensional PAGE, blotted to nitrocellulose and then reacted with an antibody raised against the fusion protein of CKII- β . As one can see the crude serum also reacts with other cellular components (Fig. 3a). We have therefore affinity-purified this antibody preparation as described earlier [11]. Fig. 3b shows the immunoblot using the affinity-purified antibody directed against the recombinant fusion protein of the β subunit.

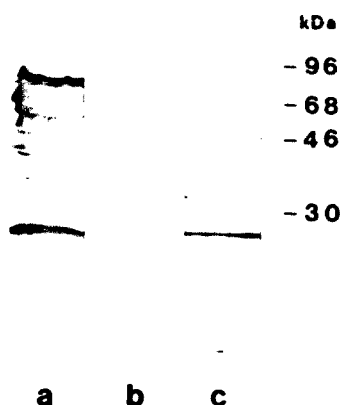


Fig. 3. Detection of CKII- β subunit by PAGE analysis and subsequent immunoblotting. Crude Krebs II mouse ascites tumor cell extract (100 μ g) was used for the detection of the CKII- β subunit reacting it with: (a) an anti-CKII- β fusion protein antibody, (b) an affinity-purified anti-CKII- β fusion protein antibody, (c) Purified CKII (1 μ g) from Krebs II mouse ascites tumor cells was reacted with the affinity-purified anti-CKII- β fusion protein antibody.

The major protein detected is the β subunit of CKII (Mr, 26000). For comparative reasons the purified holoenzyme was reacted in parallel (Fig. 3c). It is obvious that the affinity-purified anti CKII- β fusion protein antibody reacts only with the β subunit of the holoenzyme.

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