Volume 239, number 2, 219–222 FEB 06448 November 1988

Expression of human cathepsin B protein in *Escherichia coli*

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Received 22 August 1988

A cDNA fragment containing the coding sequence for the mature enzyme of human lysosomal proteinase cathepsin B was inserted in the pET plasmid expression vectors, so that it was placed under the control of transcription and translation signals from bacteriophage T_7. Upon induction, cathepsin B antigen was detected by in situ immunoscreening of lysed *E. coli* and by Western blot analysis of bacterial lysates. To our knowledge this is the first report of abundant synthesis of cloned cathepsin B in any expression system. Subfragments of cathepsin B can also be generated by this technique and will be used to study cathepsin B structure and function.

Gene expression system; Bacteriophage T_7; Expression vector; recombinant DNA; (Human)

1. INTRODUCTION

Cellular proteinases have distinct specificities and occupy various subcellular localizations [1]. Among these enzymes, the cysteine proteinases must serve important cellular functions because they are found from protozoans to mammals [2]. The lysosomal acid hydrolase cathepsin B is one member of the cysteine proteinases. It is involved in intracellular protein degradation, but has also been implicated in several diseases such as tumor metastasis [3]. We have been interested in the biochemistry and molecular biology of cathepsin B. Towards this goal we have characterized pCB-1, a plasmid cDNA clone for human cathepsin B, and also several λgt11 clones from both plasmid and phage human liver cDNA libraries [4,5]. Independently, λ clones from human hepatoma and kidney cDNA libraries have been characterized by Dr D.F. Steiner and associates [6].

Cathepsin B is normally purified from mammalian tissues such as spleen and liver but is difficult to obtain in sufficient quantity. In this communication, we reported our success with the synthesis of human cathepsin B protein in a prokaryotic expression system developed by Dr William Studier and associates [7]. Human cathepsin B cDNA was placed under the control of transcription and translation signals of bacteriophage T_7, and expressed in *E. coli* after induction of the T_7 RNA polymerase previously cloned into the host bacterial strain.

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2. MATERIALS AND METHODS

2.1. Construction of expression vectors

A flow chart for the construction of cathepsin B expression vector is shown in fig.1. The λC8 clone was screened from a human liver λgt11 cDNA library using an insert from our partial cathepsin B clone pCB-1. The library was a generous gift from Dr Vincent Kidd, University of Alabama at Birmingham [8]. After subcloning at the *Eco*RI restriction site into M13mp19 for DNA sequencing, we found the clone had the complete sequence for the mature enzyme, the whole 3′-untranslated region including poly(A) tail, but only a limited pro-sequence [5]. We then generated a single base pair mutation using the M13-C8 no.5 clone and a 19-mer oligonucleotide (synthesized at the University of Alabama at Birmingham) by the in vitro mutagenesis method of Nakamaye and Eckstein [9] (reagents from Amersham). This created a new *Pvu*II restriction site at the first codon of the mature enzyme. The resulting *Pvu*II-*Eco*RI fragment, containing 5′-cathepsin B coding sequence (for codons 1–250), was ligated to the 3′-*Eco*RI-*Pvu*II

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00145793/88/$3.50

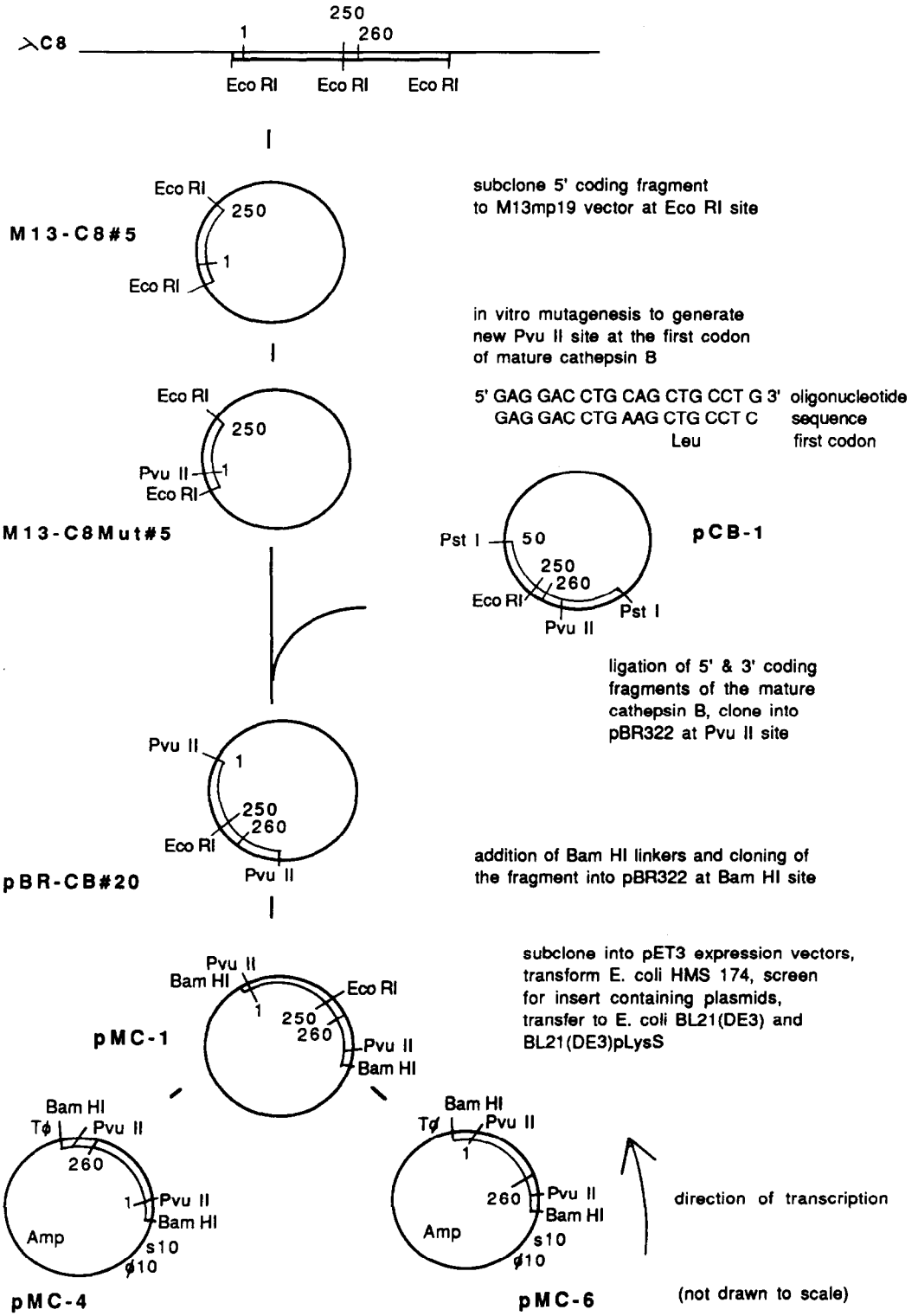

Fig.1. Construction of plasmid vectors pMC-4 and pMC-6 containing human cathepsin B cDNA.

fragment, containing the remaining codon sequence and some 3′-untranslated segment, and cloned into pBR322 (pBR-CB no.20). *Bam*HI linkers (New England BioLab) were added and again recloned into pBR322 (pMC-1). Human cathepsin B cDNA containing *Bam*HI linkers were cloned into pET3 expression vectors and these were transformed into appropriate *E. coli* strains for gene expression analysis. These latter vectors and hosts were kindly provided by Dr William Studier, Brookhaven National Laboratory. Standard molecular cloning techniques were done according to Maniatis et al. [10].

2.2. *In situ immunoscreening*

E. coli strains BL21(DE3) and BL21(DE3)pLysS, transformed with the pET3 plasmids containing human cathepsin B cDNA, were screened for gene expression after IPTG (isopropyl-β-D-thiogalactoside) induction. The screening method of Helfman and Hughes [11] was followed with these modifications: we used horseradish peroxidase-conjugated sheep anti-human cathepsin B (Serotec) and diaminobenzidine as coloring reagent.

2.3. *Western blot analysis*

Prestained molecular mass standards (Amersham) and total bacterial lysates were separated by SDS-polyacrylamide gel (10%) electrophoresis and the protein blotted onto nitrocellulose filter using a Hoefer apparatus. The dye Ponceau S was used for total protein staining [12]. For antibody staining, the filter was pretreated with borate saline-bovine serum albumin and with normal goat serum; and the peroxidase-conjugated anti-human cathepsin B (Serotec) was pre-treated with bacterial lysate (without cathepsin B plasmid) for background reduction.

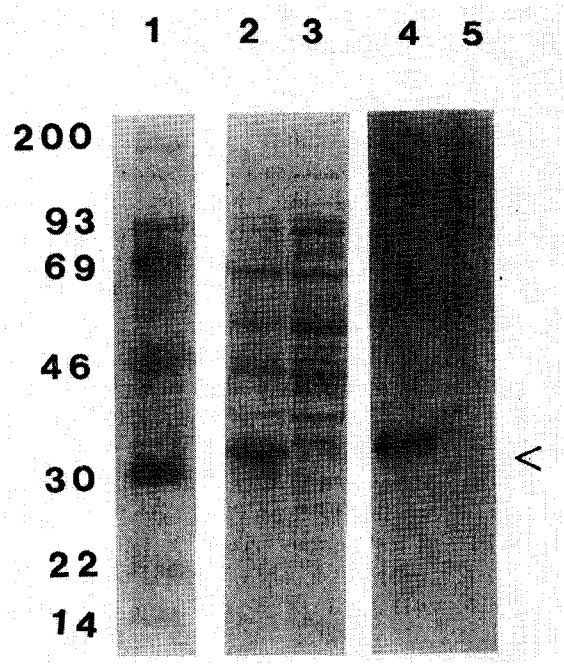

Fig.2. Western blot of bacterial lysates to anti-human cathepsin B. Lanes: (1) molecular mass standards; (2) pMC-4 lysate, protein stain; (3) pMC-6 lysate, protein stain; (4) pMC-4 lysate, antibody binding; (5) pMC-6 lysate, antibody binding.

3. RESULTS

3.1. *Screening for human cathepsin B expression in* E. coli

Human cathepsin B cDNA containing *Bam*HI linkers was cloned into the *Bam*HI site of pET3 expression vectors. The ligated plasmids were first used to transform *E. coli* HMS174, and those containing cathepsin B inserts were then used to transform other host strains for immunoscreening. After IPTG induction, bacterial clones producing immunoreactive cathepsin were detected as dark brown spots on nitrocellulose filters (not shown). By restriction mapping of plasmid DNA, we isolated pMC-4, a clone with the correct orientation for transcription, and pMC-6, one with the opposite orientation (fig.1).

3.2. *Characterization of human cathepsin B produced in bacteria*

Bacterial gene products were analyzed by protein blotting. Upon IPTG induction, one prominent protein band at approximately 33 kDa was observed (fig.2, lane 2). The band was absent in lysate containing the opposite-orientation plasmid (fig.2, lane 3); and the band was immunoreactive to human cathepsin B antiserum (fig.2, lane 4). Thus a 33 kDa cathepsin B antigen was generated by the pET expression vector system. We have also found that the expression was stable in the host BL21(DE3)pLysS but not in BL21(DE3). The former bacterial host has an additional plasmid coding for the T_7 lysozyme gene that improves on the T_7 gene expression system [13].

4. DISCUSSION

Maximizing gene expression in bacteria involves a variety of conditions [14]. For the expression of cloned human cathepsin B cDNA, we were unsuccessful in our initial attempts with plasmid vectors including pUC-18 and pKK233-2 ([15], not shown), but we achieved cathepsin B synthesis with the pET3 vectors that contain the strong bacteriophage T_7 $\phi 10$ promoter. To our

knowledge, this is the first demonstration of abundant protein synthesis from cloned cathepsin B cDNA in any gene expression system.

The 33 kDa cathepsin B protein can thus be expressed in sufficient quantity for our next goal: to study cathepsin B structure and function. Overlapping cathepsin B peptide fragments can be produced by the same technique, with the construction of a series of deletion mutants from the pMC-4 plasmid containing the cathepsin B cDNA. Bacterially expressed protein and peptide fragments will be readily available sources of materials for generating monoclonal and polyclonal antibodies, which will be used to assess the relevance of different regions of cathepsin B for the expression of its biological activities. That this approach is feasible with the T_7 expression system has been demonstrated for human factor IX [16].

Normal human cathepsin B has 252 amino acids [17]. Our bacterially expressed 33 kDa product actually is a fusion protein. It contains the first 11 amino acids of the T_7 gene 10 protein, the inframe *Bam*HI linker, the 254 amino acids (252 amino acids plus a 2 amino acid linkage between light and heavy chain) and a 6 amino acid carboxyl-terminal extension peptide of cathepsin B. Enzyme activity has not been detected. Unlike normal cathepsin B, the bacterially expressed product is not glycosylated. In the normal 252 amino acid cathepsin B there is a further cleavage into a light chain and a heavy chain [17]. Processing of cathepsin B by the aspartic proteinases pepsin and cathepsin D has been documented [18–20]. Our bacterially derived cathepsin B may thus also be used as substrate for the study of cathepsin B processing enzymes.

Acknowledgements: We thank Dr Bonnie Sloane for a poster presentation of these data at the 7th International Symposium on Intracellular Protein Catabolism in Japan, May 1988. We are grateful to Drs John Bird, Vincent Kidd and William Studier for their help, Misses Mei-feng Cui and Kathleen Shelton for technical assistance, and Ms Lynn Weingart for manuscript preparation. M.M.C. is a Busch Postdoctoral Fellow. This work was supported by National Science Foundation Grant DCB 88-96114 to D.F.

REFERENCES

[1] Bond, J.S. and Butler, P.E. (1987) Annu. Rev. Biochem. 56, 333–364.
[2] Turk, V. (1986) Cysteine Proteinases and Their Inhibitors, Walter de Gruyter, Berlin.
[3] Sloane, B.F., Rozhin, J., Hatfield, J.S., Crissman, J.D. and Honn, K.V. (1987) Exp. Cell Biol. 55, 209–224.
[4] Fong, D., Calhoun, D.H., Hsieh, W.T., Lee, B. and Wells, R.D. (1986) Proc. Natl. Acad. Sci. USA 83, 2909–2913.
[5] Fong, D. (1987) Fed. Proc. 46, 491.
[6] Chan, S.J., San Segundo, B., McCormick, M.B. and Steiner, D.F. (1986) Proc. Natl. Acad. Sci. USA 83, 7721–7725.
[7] Rosenberg, A.H., Lade, B.N., Chui, D.S., Lin, S.W., Dunn, J.J. and Studier, F.W. (1987) Gene 56, 125–135.
[8] Humphreys-Beher, M.G., Bunnell, B., VanTuinen, P., Ledbetter, D.H. and Kidd, V.J. (1986) Proc. Natl. Acad. Sci. USA 83, 8918–8922.
[9] Nakamaye, K.L. and Eckstein, F. (1986) Nucleic Acids Res. 14, 9679–9698.
[10] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[11] Helfman, D.A. and Hughes, S.H. (1987) Methods Enzymol. 152, 451–457.
[12] Salinovich, O. and Montelaro, R.C. (1986) Anal. Biochem. 156, 341–347.
[13] Moffatt, B.A. and Studier, F.W. (1987) Cell 49, 221–227.
[14] Reznikoff, W.S. and Gold, L. (1986) Maximizing Gene Expression, Butterworths, Stoneham, MA.
[15] Amann, E. and Brosius, J. (1985) Gene 40, 183–190.
[16] Lin, S.W., Dunn, J.J., Studier, F.W. and Stafford, D.W. (1987) Biochemistry 26, 5267–5274.
[17] Ritonja, A., Popovic, T., Turk, V., Wiedenmann, K. and Machleidt, W. (1985) FEBS Lett. 181, 169–172.
[18] Mort, J.S. and Recklies, A.D. (1986) Biochem. J. 233, 57–63.
[19] Nishimura, Y. and Kato, K. (1987) Biochem. Biophys. Res. Commun. 148, 254–259.
[20] Nishimura, Y., Kawabata, T. and Kato, K. (1988) Arch. Biochem. Biophys. 261, 64–71.