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N-Terminal sequence similarities between components of the multicatalytic proteinase complex

Kathryn S. Lilley, Matthew D. Davison[1] and A. Jennifer Rivett

Department of Biochemistry, University of Leicester, Leicester, UK

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The multicatalytic proteinase complex is a high molecular weight nonlysosomal proteinase which is composed of many different types of subunit. As part of a study of the possible relationships between subunits, polypeptides derived from the multicatalytic proteinase from rat liver have been subjected to N-terminal amino acid sequence analysis. Although several of the subunits are blocked at their N-termini, sequences have been obtained for 7 of the polypeptides. Each of the 7 sequences is unique but they show considerable sequence similarity, suggesting that the proteins are encoded by members of the same gene family.

Multicatalytic proteinase; Protease; Gene family; (Rat liver)

1. INTRODUCTION

The multicatalytic proteinase complex is a major nonlysosomal proteinase which is found in a wide variety of eukaryotic cells [1–3]. It has a molecular mass of around 700 000 Da. SDS polyacrylamide gel electrophoresis of multicatalytic proteinase preparations shows a characteristic and complex pattern of bands with molecular masses in the range M_r = 22 000–35 000. These results suggest that the proteinase is composed of at least 10 different types of subunit and there is no evidence to suggest that these polypeptides are derived from a smaller number of larger subunits. Despite this apparent complexity of the subunit structure of the proteinase as judged by one and two-dimensional polyacrylamide gel electrophoresis, electron microscopy of negatively stained multicatalytic proteinase preparations shows a regular arrangement of subunits in a hollow cylindrical structure [6].

The multicatalytic proteinase complex gained its name because it shows several different types of proteolytic activity, and 3 distinct types of proteolytic site have been described, each having a different specificity [7–9]. The response of the multicatalytic proteinase to proteinase inhibitors [1] resembles those of cysteine and serine proteinases, but is not characteristic of either class. It is therefore not clear how it is related to other proteases. Also, the structure of the multicatalytic proteinase appears to be very similar to that of a number of other cylindrical particles which have been described [1,10,11] and the complex may have additional functions such as nuclease activity [12]. It also appears to be part of a larger 26 S complex which degrades proteins conjugated to ubiquitin [13].

In view of the possible multifunctional nature of the proteinase and the apparent complexity of its subunit composition, it was of interest to consider relationships between the component polypeptides. Although it is possible that some of them are related by post-translational modifications such as phosphorylation [14], glycosylation [15], or proteolysis, results of both peptide mapping [5] and immunochemical studies [4] with the proteinase from rat liver have shown that the major polypeptides are distinct. Here we present the results of N-terminal amino acid sequence analysis which suggest that several of the polypeptides are encoded by members of the same gene family.

2. MATERIALS AND METHODS

The multicatalytic proteinase was purified from frozen rat liver as described previously [8] and protein concentrations were determined by the method of Bradford [16] using bovine serum albumin as standard.

SDS polyacrylamide gel electrophoresis was carried out using the system of Laemmli [17] with a 4% stacking gel and 15% separating gel. Proteinase samples (100–200 μg/lane) were prepared by heating at 90°C for 2 min in the presence of 2% 2-mercaptoethanol and 2% SDS. Prestained molecular weight markers (Bio-Rad) were used to locate the approximate position of proteinase polypeptides during the running of the gel.

Correspondence address: A.J. Rivett, Department of Biochemistry, University of Leicester, Leicester LE1 7RH, UK

Present address: Biotechnology Department, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK

Abbreviations: PTH, phenylthiohydantoin; PVDF, polyvinylidene-difluoride; SDS, sodium dodecyl sulphate

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Proteins were electroblotted onto polyvinylidene difluoride (PVDF) membranes (Millipore) as described by Matsudaira [18] using the Bio-Rad Transblot apparatus with 48 mM Tris, 39 mM glycine, 10% methanol, transfer buffer. Protein bound to the PVDF membrane was visualized by staining with Coomassie blue prior to excision of the bands for N-terminal sequence analysis. Automated Edman degradation was carried out using an Applied Biosystems Model 470A gas-phase sequencer equipped with a Model 120A PTH amino acid analyzer. Each sequence was determined 2 or 3 times using a different proteinase preparation on each occasion. The repetitive yield, calculated over 6–11 residues for isoleucine, valine, leucine, alanine and phenylalanine residues, ranges between 87% and 96%.

3. RESULTS AND DISCUSSION

SDS polyacrylamide gel electrophoresis of multicatalytic proteinase preparations generally show 8–12 protein bands within the molecular mass range of 22 000–34 000 Da but a larger number of spots are observed on two-dimensional gels [4]. The relative amount of the component with the highest molecular weight was found to vary in different preparations and to decrease during storage for several weeks at 4°C. This subunit is also susceptible to cleavage by trypsin (A.J. Rivett, unpublished observation). An electroblot of the purified rat liver proteinase is shown in fig.1. PVDF blots used for sequencing were similar but were prepared from proteinase preparations with larger amounts of the highest molecular weight band.

Each of the 13 strips indicated in fig.1 were excised from PVDF blots for N-terminal sequence analysis. Although several of the polypeptides (those indicated but not numbered in fig. 1) were not susceptible to Edman degradation, presumably because their N-terminal residues are blocked, 7 bands did yield N-terminal sequence data which were identical for at least two different proteinase preparations. The 7 N-terminal sequences (fig.2) are unique but 5 of them show considerable sequence similarity. Sequences numbered 1 and 2 are not only closely related to each other (9 identities in 12 residues), but also share identities (33–56%) with sequences 5, 6 and 7 which are also similar to each other (9–15 identities in the first 18 residues). In most cases the non-identical residues in sequences 1 and 2 and in sequences 5, 6 and 7 involve conservative substitutions. In addition post-translational modifications such as deamidation (see residue 7 of sequences 5 and 7) may occur. The similarity of the N-terminal sequences suggests that these multicatalytic proteinase subunits are encoded by members of the same gene family. However, it is also possible that the proteins are multi-domain proteins and that the other domains bear no relationship to each other.

Sequences 3 and 4 are not obviously related to each other and show few identities (maximum 3 or 4) with the other sequences. It is possible that sequences 3 and 4 may represent N-terminal extensions and that further sequence data will permit alignment to the other sequences. However, these subunits may be unrelated to other components of the complex. Results of studies with subunit-specific polyclonal antiserum suggest that several of the major components are antigenically distinct [4]. Moreover, none of the N-terminal sequences determined (fig.2) show any similarity to the

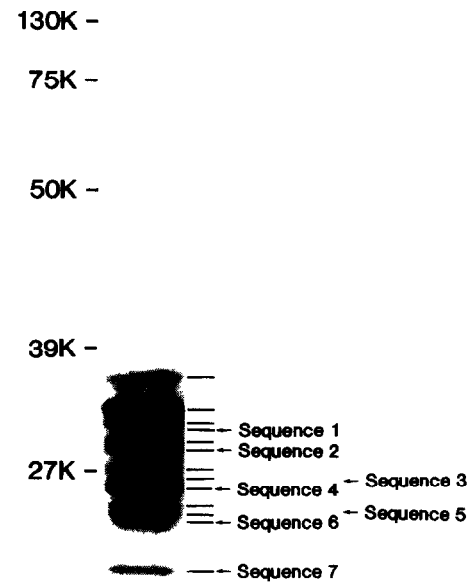

Fig.1. Electroblot of rat liver multicatalytic proteinase subunits separated by SDS polyacrylamide gel electrophoresis. The position and size of prestained molecular weight markers are as indicated on the left of the figure. Electrophoresis was carried out until the lower molecular weight marker (17 kDa) had run off the gel. Thirteen strips were cut from such blots for N-terminal sequence analysis. The centre of each strip is indicated by a bar to the right of the proteinase bands. Only those labelled sequence 1–7 gave rise to sequence data (see fig.2). Sequences 3 and 4 were derived from the same strip of PVDF for each of three sequence determinations using different proteianse preparations.

1 T T I A G V V Y K D G I _
2 T T I A G L V F K D G V I L G A D X E A T N _
3 T Q N P M V T G T S V V V A K F _
4 S F S P Y A F N G G T L L V I X E _
5 T T I M A V Q F D G G V V L G A D S _
6 T T I L A F K F Q E G V I L A X D S _
7 T T I M A V E F D G G V V V G S D S D V S _

Fig.2. N-terminal amino acid sequence data for rat liver multicatalytic proteinase. Sequences are given using the accepted one letter code for amino acids and X for those residues where the assignment was uncertain. Amino acid residues which are found at the same position in at least two sequences are printed in bold face type and sequence 4 is aligned to show similarities to the other sequences. The number of pmol of the first PTH-amino acid for each sequencer run was as follows: Sequence 1 (13 and 20 pmol); sequence 2 (13 and 15 pmol); sequence 3 (60, 78 and 36 pmol); sequence 4 (10, 27 and 14 pmol); sequence 15 (13 and 58 pmol); sequence 6 (52 and 10 pmol) and sequence 7 (34 and 6 pmol).

amino acid sequence of the largest subunit of the complex which has recently been deduced from the nucleotide sequence of cloned cDNAs of the *Drosophila* [19] and rat [20] multicatalytic proteinase. The high degree of conservation between regions of these subunits from *Drosophila* (PROS-35) and rat (C2) is interesting and consistent with earlier observations that multicatalytic proteinase complexes, even from widely different species, show some antigenic cross-reactivity on Western blots [2,10,11,21].

A search of the National Biomedical Research Foundation (NBRF, 13/3/89) and SWISSPROT (10/3/89) protein sequence data bases has revealed no obvious sequence similarity between the N-terminal sequences of multicatalytic proteinase subunits and any other proteins. Further sequence information, including identification of amino acid residues involved in catalysis will be required to establish the structural and evolutionary relationship of the multicatalytic proteinase complex to other known cysteine and serine proteinases [22,23].

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