Major acute phase α_1-protein of the rat is homologous to bovine kininogen and contains the sequence for bradykinin: its synthesis is regulated at the mRNA level

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The complete amino acid sequence of major acute phase α_1-protein of the rat (MAP) was derived from the nucleotide sequence of cloned cDNA. Amino acid analysis and partial sequencing supported the predicted sequence. The amino acid compositions of MAP and rat low-M_r kininogen are identical within experimental variation. The sequence is homologous (60%) to that of bovine low-M_r kininogen and both proteins carry the sequence for the vasoactive nonapeptide bradykinin in their C-terminal region. The rate of synthesis of MAP and the levels of MAP mRNA change coordinately during the acute phase response to inflammation.

Major acute phase protein *Bradykinin* *Kininogen* *Protein-biosynthesis regulation* *Acute phase reaction* *Inflammation*

1. INTRODUCTION

MAP is a glycoprotein whose concentration in rat serum increases about 20-fold during acute inflammation [1–3]. This increase is due to an increase in the synthesis rate in the liver [4]. MAP inhibits proteinases with cysteine in their active centre [5]. The data reported here suggest that MAP has another important function during acute inflammation.

2. EXPERIMENTAL

The preparation of a cDNA library from rat liver mRNA in pBR322, expression screening, nucleotide and amino acid sequencing, nick translation and dot hybridization were as described elsewhere [6–10].

3. RESULTS

3.1. *Determination of the nucleotide sequence in MAP cDNA*

A clone synthesizing protein reacting with antibody against MAP was identified by expression-screening. The cDNA, excised from the plasmid with restriction endonuclease *Pst*I, had a size of about 1.4 kb. The sequencing strategy is summarized in fig.1. The nucleotide sequence and the deduced amino acid sequence are shown in fig.2. Residues 360–368 correspond to the sequence for bradykinin.

3.2. *Partial amino acid sequencing of MAP*

MAP was cleaved into fragments by treatment with cyanogen bromide [9] or proteolytic enzymes. These fragments were then sequenced as described in [10]. The reduced and carboxymethylated protein was accessible to sequencing only after treatment with pyroglutamate aminopeptidase [11], in-

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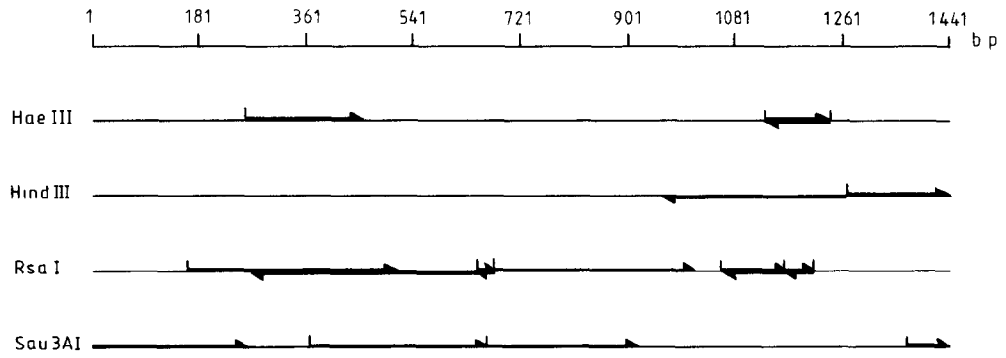

Fig.1. Sequencing strategy for analysis of MAP cDNA.

dicating that the N-terminus was glutamine. The sequences obtained, indicated in fig.2 by bold letters, agreed with those derived from the nucleotide sequence.

3.3 *Homology of MAP with low-*M_r *kininogen*

A homology of 60% was found between MAP and bovine low-M_r kininogen [12] (fig.3). In addition, the amino acid composition of rat low-M_r kininogen [13] agreed closely with that of MAP (table 1), suggesting possible identity.

3.4. *MAP mRNA levels in liver and incorporation leucine into MAP during acute inflammation*

MAP cDNA was radioactively labelled by nick translation and used for measuring MAP mRNA levels by dot hybridization [8] in cytoplasmic ex-

Table 1

Amino acid composition (mol%) of MAP and rat plasma kininogen

Amino acid	MAP (from [3])	MAP (determined, here)	MAP (calculated from sequence, here)	Kininogen fraction A (from [13])	Kininogen fraction B (from [13])
Lysine	8.1	8.6	7.5	7.4	7.7
Histidine	3.5	3.7	3.4	3.0	3.1
Arginine	3.4	3.3	3.4	3.0	3.0
Aspartic acid	10.5	10.2	10.4	10.3	10.5
Threonine	7.4	7.8	7.5	7.3	7.5
Serine	6.0	6.2	6.1	6.1	5.9
Glutamic acid	13.0	12.9	12.6	13.2	13.3
Proline	5.3	5.7	5.1	6.8	6.1
Glycine	6.7	6.6	6.3	6.3	6.7
Alanine	7.5	6.8	6.8	6.7	6.8
Half-cysteine	1.0	3.5[a]	4.1	2.7	3.0
Valine	7.1	5.8[b]	6.8	5.7	5.4
Methionine	1.4	1.1	1.2	1.2	0.6
Isoleucine	3.8	3.2[b]	4.1	3.7	3.7
Leucine	7.8	7.0	7.0	8.0	7.7
Tyrosine	3.0	3.0	3.2	4.3	4.4
Phenylalanine	4.2	4.1	3.9	4.4	4.5
Tryptophan	0.6	0.6	0.5	not given	not given

[a]Cys + carboxymethyl-cys
[b]22 h hydrolysis only

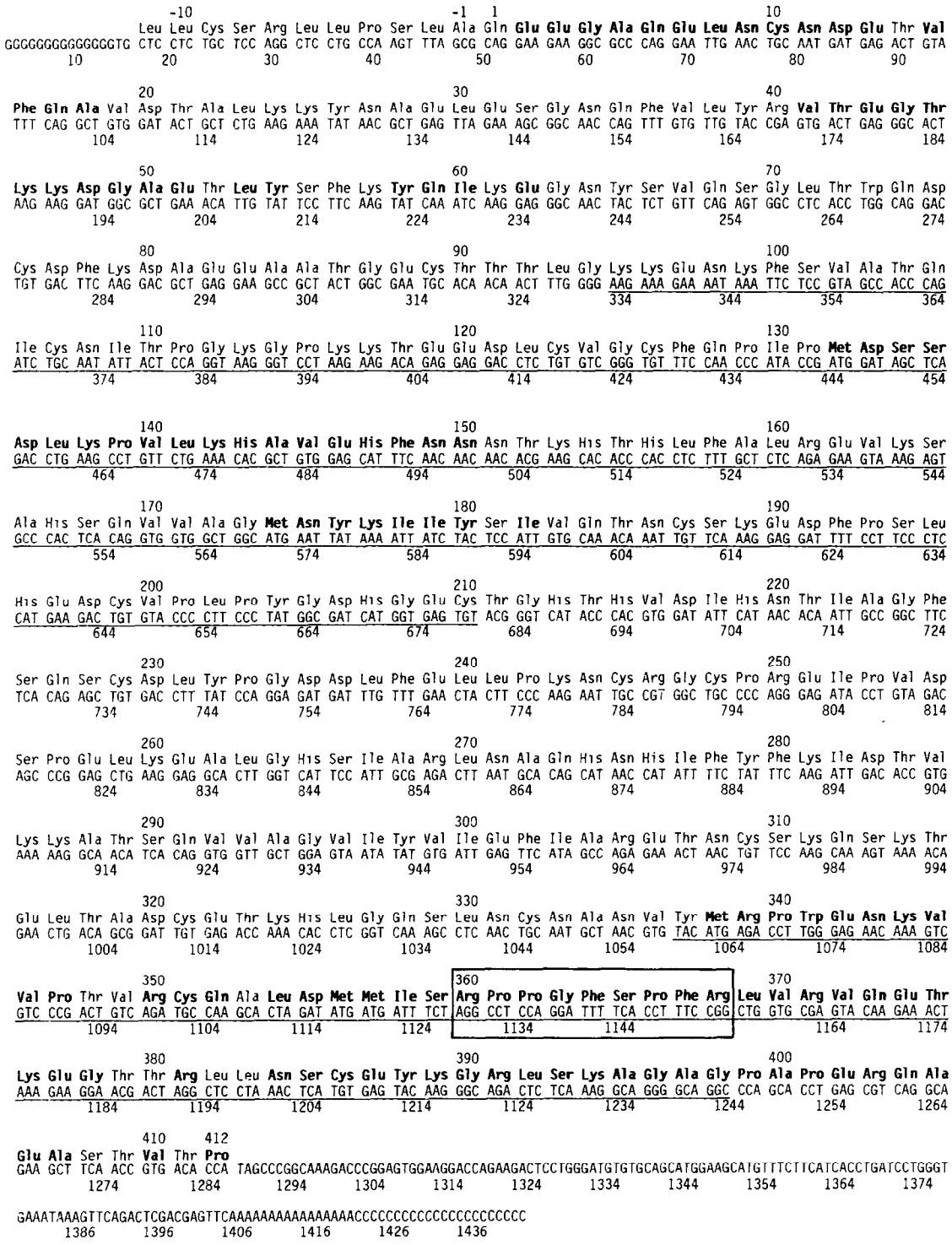

Fig.2. Nucleotide sequence of MAP cDNA and amino acid sequence of MAP. The nucleotide sequence derived from independent sequencing of both strands is indicated by underlining. Amino acid sequences derived from both nucleotide sequencing and direct analysis of MAP fragments are indicated by bold letters. Numbering of amino acids begins at the N-terminus of the mature protein. The bradykinin sequence is indicated by a box.

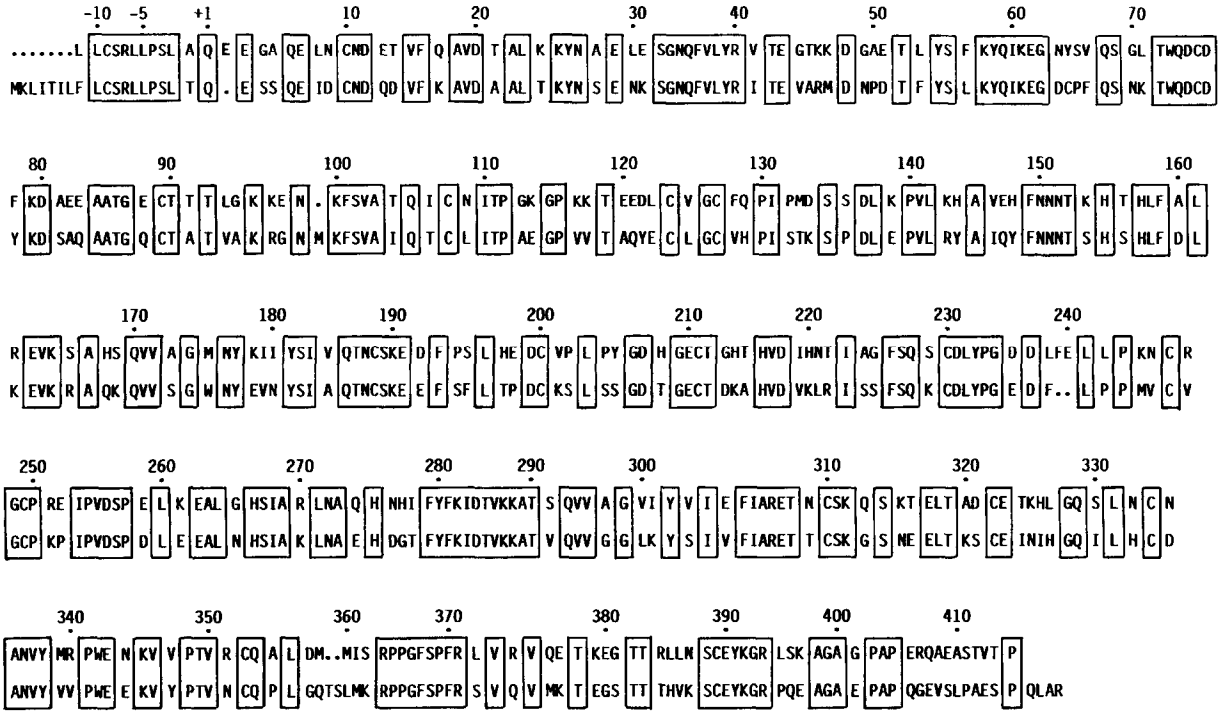

Fig.3. Comparison of amino acid sequence of MAP and bovine low-M_r kininogen. Identical sequences are indicated by boxes. The sequence for MAP is given in the upper line. Numbers refer to this sequence, including spaces (indicated by dots) which were introduced to optimize alignment.

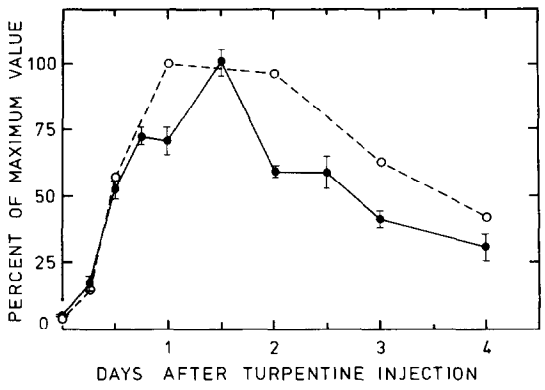

Fig.4. MAP mRNA levels in liver during acute inflammation induced by subcutaneous injection of turpentine. MAP mRNA levels were determined by cytoplasmic dot hybridization [8]. Eight animals were used per time point and analyzed individually. Bars indicate standard errors. For comparison, incorporation of [^{14}C]leucine into MAP, determined previously [3,4], is also given. MAP-mRNA levels, ●—●; [^{14}C]leucine incorporation into MAP, ○---○.

tracts prepared from livers of rats at various times after inducing inflammation. The values obtained are depicted in fig. 4 together with the rates of incorporation of [^{14}C]leucine into MAP in serum determined previously [3,4]. The shapes of the two curves are similar, suggesting that regulation of the synthesis of MAP occurs at the mRNA level.

4. DISCUSSION

The reported data add new information to the understanding of both the structure and function of MAP. The complete nucleotide sequence now presented overlaps the N-terminal sequence reported by Anderson et al. [14]. Identification of the N-terminus of the mature protein (fig.2) by removal of cyclized glutamine establishes that MAP consists of 412 amino acids and is synthesized via a precursor protein, containin a presegment of 18 amino acids ending with alanine, but no prosegment. Although MAP has been known for some time, its function is not fully understood.

Esnard and Gauthier [5] described a plasma protein (immunochemically identical with MAP [unpublished]) which inhibited proteinases with cysteine in their active centre, such as cathepsin H and L. The data reported here suggest that MAP may also be a kininogen. If so, this kininogen would be an acute-phase reactant regulated at the mRNA level (fig.4). Release of the strongly vasodilating nonapeptide bradykinin may play a role in the mechanism of the inflammatory response.

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