

Polypeptide Composition of Human Macrophage Gelatinase

Tapio Vartio^a and Tapani Hovi^{b,c}

^aDepartment of Pathology, University of Helsinki, ^bNational Public Health Institute and ^cDepartment of Virology, University of Helsinki, SF-00290 Helsinki, Finland

Vartio, T. and Hovi, T., 1987. Polypeptide Composition of Human Macrophage Gelatinase. – *Acta Chem. Scand.*, Ser. B 41: 754–756.

The major secretory component of cultured adherent human monocyte/macrophages has been shown to be an M_r 95 000 (reduced mol. wt.) gelatin-binding protein.¹ This protein has no immunological cross-reactivity with other gelatin-binding proteins, fibronectin and an M_r 70 000 polypeptide^{1,2} also produced by cultured cells. Quite recently the M_r 95 000 protein was shown to be a gelatin-degrading proteinase (gelatinase).³ Similar gelatinase activity has also been found in granulocytes^{4–8} and has recently been shown to exist in three distinct proteolytically active forms, viz. M_r 92 000, M_r 130 000 and M_r 225 000.⁸

In this communication we report an extension of the analysis of the polypeptide composition of macrophage gelatinase and a comparison with that of human blood granulocytes. The results show that the protein has a biosynthetic precursor and partially forms a higher molecular weight polymer. All these forms of the protein are immunologically cross-reactive with each other and with the polypeptides of granulocyte gelatinase.

Materials and methods

Cell cultures and isolation of granulocytes. Human blood monocytes were isolated from the buffy coat fraction of healthy donors and were grown in a medium composed of a 1:1 mixture of RPMI-1640 and M199, supplemented with 5% newborn calf serum.^{1,9} Incubation at 37°C resulted in differentiation of most of the cells to macrophages within a week. Granulocytes were isolated from the same buffy coats as monocytes

by centrifuging the cells through a layer of Ficoll-Isopaque®.¹⁰

Isolation of the gelatin-binding proteins. Macrophages and granulocytes were extracted with 5 mM Tris-HCl, pH 7.5, containing 0.5% Triton X-100 and 1 mM phenylmethanesulfonyl fluoride. Insoluble material was sedimented, and the supernatant extract was first treated with Sepharose 4B to remove proteins binding non-specifically to plain Sepharose and then with gelatin-Sepharose (Pharmacia, Uppsala, Sweden). Gelatin-Sepharose was then washed and the bound material was eluted with Laemmli's sample buffer¹¹ for further analyses (see below).

Radioactive labelling and pulse-chase experiments on macrophage cultures. The 8-day old macrophage cultures were changed to serum-free Eagle's Minimum Essential Medium (MEM) and 10 $\mu\text{Ci ml}^{-1}$ of L-(³⁵S)-methionine (1000 Ci mmol⁻¹; Amersham International, U.K.) was added. After an incubation of 4 h the gelatin-binding proteins were isolated from the medium and eluted in Laemmli's sample buffer for analysis in SDS-PAGE. Intracellular biosynthesis of the gelatin-binding proteins was studied in macrophage cultures by pulse-chase experiments. The 8-day old cell cultures were incubated in methionine-free MEM medium for 10 min, and then ³⁵S-methionine (40 $\mu\text{Ci ml}^{-1}$) was added. After a 5 min pulse the cells were chased in unlabelled medium for periods of 0, 5, 15, 25, 40 and 60 min, extracted, and the gelatin-binding material was isolated and analysed in SDS-PAGE as above.

Electrophoretic analyses and zymography. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli,¹¹ using an acrylamide concentration of 6% in the separating gel. The samples were run non-reduced or reduced with 10% 2-mercaptoethanol. After electrophoresis, the gels were either stained with Coomassie Brilliant Blue, fluorographed,¹² or used for immunoblotting.¹³ Rabbit antiserum against the M_r 95 000 protein has been characterized previously.¹ Immunoreactions were detected by using peroxidase-coupled swine anti-rabbit IgG antiserum (Dakopatts, Glostrup, Denmark). Zymography was performed in SDS-PAGE using gel containing 2 mg ml⁻¹ of gelatin, as described previously.⁸

Results and discussion

Gelatin-binding proteins from macrophage extracts and culture medium were immunoblotted using the prepared antiserum against the macrophage gelatinase. Immunoperoxidase reactions of the non-reduced intracellular samples were seen at positions corresponding to M_r 80 000, 92 000 and 225 000, and those of the secreted material at M_r 92 000 and 225 000 (Fig. 1, lanes 1 and 3). Reactions of the reduced intracellular polypeptides were at positions corresponding to M_r 85 000 and 95 000, while the secreted material showed only the M_r 95 000 polypeptide (Fig. 1, lanes 2 and 4). Thus, the latter represents the

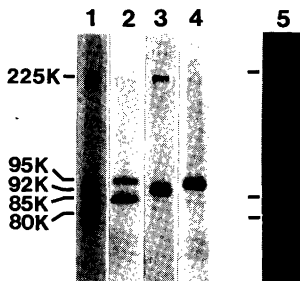


Fig. 1. Immunoblotting and zymography of the gelatin-binding proteins from macrophages. Immunoperoxidase reactions of the non-reduced gelatin-binding material from macrophage extract (lane 1); the same material reduced (lane 2); non-reduced gelatin-binding material from macrophage culture medium (lane 3); the same material reduced (lane 4). Lane 5 shows the zymography gel of the material shown in lane 1. K = kilodaltons.

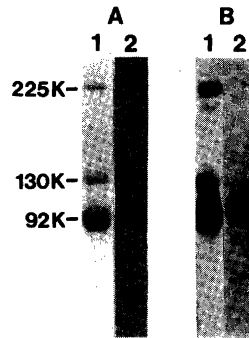


Fig. 2. Gelatin-binding proteins from granulocyte extract. Panel A, protein-stained gels and panel B, immunoblottings of similar samples under non-reducing (lane 1) or reducing (lane 2) conditions. K = kilodaltons.

reduced form of the M_r 92 000 and 225 000 polypeptides, and the M_r 85 000 that of the M_r 80 000 polypeptide. All three non-reduced polypeptide bands degraded gelatin in zymography gels, seen as negatively staining bands at M_r 80 000, 92 000 and 225 000 (Fig. 1, lane 5). The result shows that macrophage gelatinase is a polypeptide complex and resembles granulocyte gelatinase, which is composed of one polypeptide under reducing and three polypeptides under non-reducing conditions.⁸ For comparison, the gelatin-binding material was isolated from granulocyte extracts and

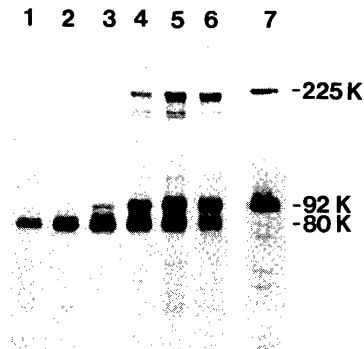


Fig. 3. Biosynthesis of the macrophage gelatinase. Fluorography of the gelatin-binding proteins from ³⁵S-methionine-labelled macrophage extracts after chase periods of 0, 5, 15, 25, 40 and 60 min (lanes 1-6, respectively). Lane 7 shows the polypeptides from the growth medium of the ³⁵S-methionine-labelled macrophages. K = kilodaltons.

immunoblotted similarly. The non-reduced material contained M_r 92 000, 130 000 and 225 000 polypeptides, while the reduced material showed only M_r 95 000 polypeptide (Fig. 2A, lanes 1 and 2; protein-stained gels). All these proteins were detected by the antiserum (Fig. 2B) and the non-reduced ones were active in zymography gels (not shown). Thus, the macrophage and granulocyte gelatinases are composed of similar but not identical polypeptides. Immunological cross-reactivity suggests, however, that in spite of the differences, these polypeptides belong to the same family of gelatinases.

In the initial stages of the pulse-chase experiments on cultured macrophages an M_r 80 000 polypeptide was seen (Fig. 3, lanes 1–3). Later, prominent M_r 92 000 and 225 000 proteins appeared (Fig. 3, lanes 4–6), suggesting that the M_r 80 000 polypeptide is their biosynthetic precursor. The culture medium from macrophages contained M_r 225 000 and M_r 92 000 proteins, but not the M_r 80 000 protein (Fig. 3, lane 7). Thus, only the former two were the secreted forms of the protein. In these experiments the M_r 80 000 polypeptide never fully disappeared in the prolonged chase samples, suggesting that a portion of it may have been retained in a precursor form.

The reason for the shift in the molecular weight of the initial precursor is not known. It may, at least partially, be associated with the carbohydrates since the protein has previously been found (by metabolic labelling of the macrophage cultures with radioactive sugars¹) to be rather heavily glycosylated. The formation of the M_r 225 000 polypeptide is still more surprising. It is possible that it is a disulfide-bonded polymer of the protein, although its electrophoretic apparent molecular weight is not compatible with a dimer or trimer of the M_r 92 000 polypeptide. This discrepancy could be due to the arrangement or amount of intramolecular disulfides, to glycosylation or to unknown polypeptide interactions which may affect the electrophoretic migration. The situation seems to be even more complicated in the case of the granulocyte gelatinase, which

has an M_r 130 000 component in addition to M_r 92 000 and 225 000 polypeptides. However, formation of the M_r 95 000 polypeptide under reducing conditions emphasizes the importance of intra- and/or intermolecular disulfide bonds in the multiple polypeptides of macrophage and granulocyte gelatinases. More extensive studies will still be needed to find out the exact reasons for the polypeptide polymorphism and for the differences between the gelatinase complexes of these phagocytic cells.

Acknowledgements. We thank Ms. Tuija Järvinen, Ms. Anne Bergholm and Ms. Mervi Eskelinen for expert technical assistance. This study was supported by the Finnish Cancer Foundation, Sigrid Jusélius Foundation and the Medical Research Council of the Academy of Finland.

References

1. Vartio, T., Hovi, T. and Vaheri, A. *J. Biol. Chem.* 257 (1982) 8862.
2. Vartio, T. and Vaheri, A. *J. Biol. Chem.* 256 (1981) 13085.
3. Vartio, T. *Eur. J. Biochem.* 152 (1985) 323.
4. Sopata, I. and Dancewicz, A. M. *Biochim. Biophys. Acta* 370 (1974) 510.
5. Murphy, G., Bretz, U., Baggiolini, M. and Reynolds, J. J. *Biochem. J.* 192 (1980) 517.
6. Dewald, B., Bretz, U. and Baggiolini, M. *J. Clin. Invest.* 70 (1982) 518.
7. Rantala-Ryhänen, S., Ryhänen, L., Nowak, F. V. and Uitto, J. *Eur. J. Biochem.* 134 (1983) 129.
8. Hibbs, M. S., Hasty, K. A., Seyer, J., Kang, A. H. and Mainardi, C. L. *J. Biol. Chem.* 260 (1985) 2493.
9. Hovi, T., Allison, A. C. and Williams, S. C. *Exp. Cell Res.* 96 (1976) 92.
10. Bøyum, A. *Scand. J. Clin. Lab. Invest.* 97 (1968) 77.
11. Laemmli, U. K. *Nature (London)* 227 (1970) 680.
12. Laskey, R. A. and Mills, A. D. *Eur. J. Biochem.* 56 (1975) 335.
13. Towbin, H., Staehelin, T. and Gordon, J. *Proc. Natl. Acad. Sci. U.S.A.* 76 (1979) 4350.

Received July 27, 1987.