Volume 219, number 1, 37–39 FEB 04861 July 1987

# Evidence of an $\alpha_2$-macroglobulin-like molecule in plasma of *Salamandra salamandra*

## Structural and functional similarity with human $\alpha_2$-macroglobulin

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Received 5 May 1987

A high-$M_r$ ($M_r$ 750 000) $\alpha_1$-macroglobulin, obtained from *Salamandra salamandra*, is described. Salamander $\alpha_1$-macroglobulin is composed of two monomers of equal $M_r$, which are composed of two polypeptide chains, each of $M_r$ 180 000, linked by disulfide bonds. The molecular parameters of this protein, its binding to trypsin and inactivation by methylamine suggest that salamander $\alpha_1$-macroglobulin is closely related to human $\alpha_2$-macroglobulin and to other related proteins described in the animal kingdom.

$\alpha_1$-Macroglobulin; $\alpha_2$-Macroglobulin; Trypsin protecting activity; Antiprotease; (Salamander, Urodele)

## 1. INTRODUCTION

$\alpha_2$-Macroglobulin ($\alpha$2M) is a major protease inhibitor of human serum [1]. This protein has an $M_r$ of 720000 and is composed of two monomers of $M_r$ 360000, non-covalently linked [2]. Four thiol ester bonds have been described, which can be cleaved by small amines, like methylamine [3–6]. $\alpha$2M inhibits the four classes of endoproteases by a unique mechanism of steric hindrance, not involving the active site [7–9].

Analogs of human $\alpha$2M have been found in the sera and plasma of other mammalian species, i.e. in rats, rabbits and dogs [10–13]. In those species, $\alpha_1$- and $\alpha_2$-macroglobulins can be observed depending on the physiological conditions (normal or acute-phase) [14–16]. Macroglobulins have also been described for the bird [17,18], reptile [17], frog [17–19] and fish [20,21] as well as for the horseshoe crab *Limulus limulus* [17,22,23]. The molecular properties of a urodele salamander antiprotease ($\alpha$1M) are described here [24].

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*Abbreviations:* $\alpha$2M, $\alpha_2$-macroglobulin; $\alpha$1M, $\alpha_1$-macroglobulin; STI, soybean trypsin inhibitor; BSA, bovine serum albumin; TAME, *N*-$\alpha$-*p*-toluenesulfonyl-L-arginine methyl ester; 2ME, 2-mercaptoethanol; PAGE, polyacrylamide gel electrophoresis

## 2. MATERIALS AND METHODS

Salamander antiprotease $\alpha$1M (S-$\alpha$1M) was isolated from plasma of 20 male salamanders as in [24], its native $M_r$ of 750000 being determined as in [25], and stored at −80°C in 0.01 M phosphate buffer 0.15 M NaCl, pH 7.2 (buffer A). Human $\alpha$2M (H-$\alpha$2M) was prepared from plasma of seven donors according to Barrett et al. [2], except that the last stage of immuno-adsorption was omitted. Storage conditions were as for S-$\alpha$1M. Porcine trypsin (Sigma) was dissolved in 1 mM HCl to a concentration of $6.6 \times 10^{-7}$ M and was 60% active [26].

Protein concentration was determined by the

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00145793/87/$3.50 

technique of Lowry et al. [27], using BSA as reference. Esterase activity of $\alpha$M-trypsin complexes was studied in buffer B consisting of 46 mM Tris-HCl, 11 mM $CaCl_2$, pH 8.1, with 10 mM TAME as substrate. Buffer C consisted of 20 mM Tris-HCl, pH 8.0. STI (Sigma) was $6.6 \times 10^{-6}$ M in buffer A. Stock solution of methylamine (Sigma) was 200 mM in buffer C. Electrophoresis was performed as described [24], except that samples to be denatured were incubated for 45 min at 37°C to prevent autolysis of $\alpha$2M or $\alpha$1M [2].

## 3. RESULTS

The $M_r$ values of S-$\alpha$1M and H-$\alpha$2M subunits were determined by PAGE (fig.1). The results demonstrate that the monomers of both molecules behave similarly, i.e. giving one band of $M_r$ 360000. Under reducing conditions, the polypeptide chains of S-$\alpha$1M and H-$\alpha$2M had nearly the same $M_r$ ($M_r$ 180000 for S-$\alpha$1M and 170000 for H-$\alpha$2M). These data are in good agreement with those given in the literature [2]: the half-molecule of H-$\alpha$2M ($M_r$ 360000) is composed of two polypeptide chains ($M_r$ 180000). The capacity of S-$\alpha$1M and H-$\alpha$2M to complex trypsin without affecting its esterolytic activity was investigated using the technique of Ganrot [9] (fig.2). In the absence of methylamine, both molecules exhibited the same binding to trypsin, as demonstrated by their protection of trypsin from inhibition by STI. In contrast, such protection was not found after treatment with methylamine, thus indicating that the amine prevented the formation of the complexes between the macroglobulins and trypsin, presumably by hydrolysing the thiol ester (fig.2). Thus, S-$\alpha$1M appears, both structurally and functionally, as a homologue of H-$\alpha$2M.

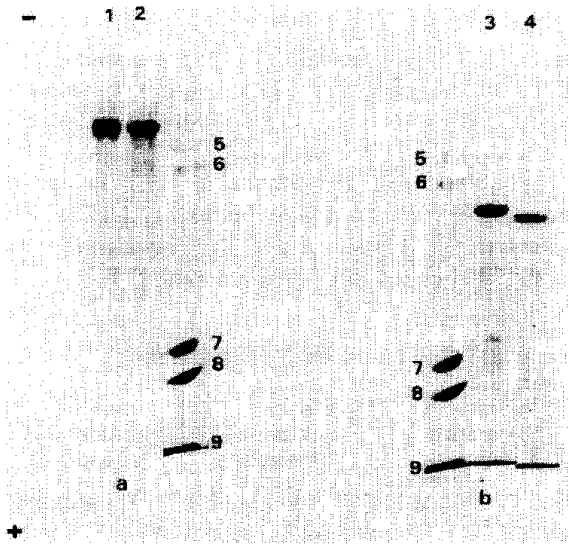


Fig.1. Polyacrylamide (7%) gel electrophoresis of salamander $\alpha$1M and human $\alpha$2M, in the presence of 2% SDS (a) and 2% SDS + 5% 2ME (b). Stacking gel: 5% in acrylamide. Tracks 1,3: salamander $\alpha$1M (30 $\mu$g); tracks 2,4: human $\alpha$2M (30 $\mu$g). Voltage, 150 V. Time of migration, 5 h. Kit migrating position: (5) thyroglobulin (330 kDa); (6) ferritin (220 kDa); (7) albumin (67 kDa); (8) catalase (60 kDa); (9) lactate dehydrogenase (36 kDa).

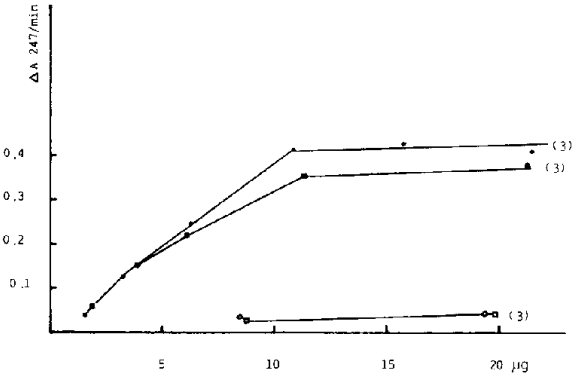


Fig.2. Esterolytic activity of S-$\alpha$1M or H-$\alpha$2M in the presence or absence of 0.2 M methylamine, followed by addition of STI. For experiments performed in the absence of methylamine, 0.45 $\mu$g active trypsin was mixed with increasing amounts (from 0 to 30 $\mu$g) of $\alpha$1M (●) or $\alpha$2M (■). After incubation for 15 min at 37°C, 1.5 $\mu$g STI (10 $\mu$l) was added. After a further incubation for 10 min at 37°C, this mixture was added to a cuvette containing 1300 $\mu$l buffer B and 150 $\mu$l TAME (10 mM in distilled water). The increase in absorbance at 247 nm was recorded for 4 min at 25°C. For experiments performed in the presence of methylamine, 8.1 and 19.3 $\mu$g $\alpha$1M (○) or 8.5 and 19.9 $\mu$g $\alpha$2M (□), diluted in buffer C, were incubated for 180 min at room temperature with 100 $\mu$l of 200 mM methylamine in buffer C. The mixture was then added to 0.45 $\mu$g trypsin as described before. The rest of the manipulation was as described above. The points on each line represent the mean of 3 experiments.

## 4. DISCUSSION

$\alpha$-Macroglobulins have been described in all the classes of vertebrates [17] and even in the invertebrate *L. limulus* [17,22,23]. Feldman and Pizzo [19] have shown that frog $\alpha$1M has the same $M_r$ ($M_r$ 730000) as human $\alpha$2M and that it is composed of four polypeptide chains of $M_r$ 180000, associated in disulfide-bonded pairs. Salamander is a Urodele amphibian, more primitive than frog on the phylogenic scale, and one of the earliest tetrapods to demonstrate viviparity [24]. Our results allow one to suggest that S-$\alpha$1M has the same molecular characteristics as those in anurans such as frog [19], and different from those in fish [17]. S-$\alpha$1M is composed of four polypeptide chains of $M_r$ 180000, as described in vertebrate classes, except in fish where $\alpha$Mh is composed of two chains of $M_r$ 105000 and 90000 [20].

The reaction of trypsin with S-$\alpha$1M and H-$\alpha$2M could not be inhibited by STI, while a major activity against TAME was maintained. The stoichiometric ratio between trypsin and S-$\alpha$1M was similar to that given by Feldman and Pizzo [19] for frog. In contrast, trypsin treated with methylamine–S-$\alpha$1M complex remained highly sensitive to STI. As expected [28], the same behaviour was observed with the human control $\alpha$2M. Thus, these data are consistent with the hypothesis that *Salamandra salamandra* $\alpha$1M, a protein of $M_r$ 750000 is the homologue of human $\alpha$2M and of similar proteins in other animal species.

## ACKNOWLEDGEMENTS

Human plasma was obtained from the CDTS of Orléans, through the courtesy of Dr P. Conte and J. Lasserre. We very much appreciated the reviewing of this manuscript by Dr M. Steinbuch (CNTS, Orsay) and Dr A.P. Ryle (Department of Biochemistry, Edinburgh). We would like to express our gratitude to Mrs M.L. Sallenave for typing this manuscript.

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