La α_2-macroglobulina del *Ovis aries,* su purificación y la comparación con las α_2-macroglobulinas humanas

The Ovis aries α_2-macroglobulin in its purification and comparision with the human α-macroglobulins

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Resumen

Purificar la α_2-Macroglobulina del plasma del Ovis aries y compararla con las α-macroglobulinas y en particular con la α_2-Macroglobulina humana. ***Materiales y métodos****:* la α_2-Macroglobulina humana fue purificada por cromatografía de afinidad y la α_2-Macroglobulina del Ovis aries, por precipitaciones repetidas de polietilenglicol 6000, seguido de diálisis y cromatografías de cambio iónico y exclusión. Ambas proteínas fueron caracterizadas por electroforesis SDS-PAGE y nativa, dichas proteínas fueron incubadas con quimotripsina en las proporciones 1:1, 1:2, 1:4 respectivamente, las secuencias determinadas por el método de Edman. ***Resultados***: La purificación de las proteínas alcanzó una pureza mayor al 95% determinada por densitometría, en la electroforesis denaturante se demostró una sola banda de 180 kDa, para cada una de las proteínas y en el gel de electroforesis nativa, se visualizó una banda en cada proteína que migraron paralelas. En la determinación de las secuencias para las α-Macroglobulinas humanas y de Ovis aries, se encontraron varias similitudes entre ellas Tyr-Met-Val-Leu-Val, otras secuencias determinadas en las α-Macroglobulinas mostraron menor similitud. Electroforéticamente se demostró, las mismas características en ambas proteínas (humano y Ovis aries). Se demostró la forma rápida y lenta en la α_2-Macroglobulina de Ovis aries con quimotripsina, típicas formas en la reacción de la α_2-Macroglobulina humana con proteinasas. Se confirmó por determinación de secuencia, que esta proteína pertenece al grupo de las α-Macroglobulinas.

Palabras clave: *α-Macroglobulina*, *Ovis aries*, *polietilenglicol*, *tiolester*, *quimotripsina*.

Abstract

To purify the α_2-Macroglobulin from blood plasma of Ovis aries and compared it, with the human α-Macroglobulins and particularly with the human α_2-Macroglobulin. *Materials and*

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methods: The human α_2-Macroglobulin was purified by affinity chromatogrphy while the Ovis aries α_2-Macroglobulin by repeated precipitations with polyetilenglycol 6000, following by dialysis, ion-exchange and exclusión chromatographies. Both proteins were characterized by electrophoresis native and SDS-PAGE. All proteins were incubated with chymotrypsin in a molar proportion 1:1, 1:2 and 1:4 respectively. The sequencies determined according to Edmans methodology. *Results*: the purity of the proteins were determined higher than 95%, by densitometry. In denaturing electrophoresis was identified only one band of 180 kDa for each proteína and in the native one was visualized one band migrated paralell to each other. The sequencies for the α_2-Macroglobulinas, for the human and the Ovis aries, contains several similarities as Tyr-Met-Val-Leu-Val, other sequencies determined in the α-Macroglobulinas shown less similarity. Conclusions: Electrophoretic, were demontrated the same characteristics in both proteins. The rapid and slow form of the α_2-Macroglobulin of the Ovis aries with chymotrypsin were demonstrated, these forms are typically for the human α_2-Macroglobulin with proteinases. It was confirmed by sequence determination the belonging of this proteína to α-Macroglobulins group.

Key word: *α-Macroglobulin*, *Ovis aries*, *polyetilenglycol*, *thiolester*, *chymotrypsin*.

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INTRODUCTION

The family of the α-Macroglobulins (α-Ms) proteins is found in the circulatory system of vertebrates, birds' eggs and reptiles (SOTTRUP-JENSEN et al,1989a); also, they can be found in some insects (LAGUEUX et al, 2000). Belonging to this family are (i) the sub-group of the α-Ms (SOTTRUP-JENSEN et al,1989b) (ii) the proteins containing thioesters of insects in the acute phase seem to contribute to the innate immunity; thus giving the answer to this immunity. This is similar to the complement against bacterial infections (LAGUEUX et al, 2000, Levashina et al, 2001, Levy et al, 2004). The protein on the cellular surface CD 109 is found in hematopoyetic cells (LIN et al, 2002). This protein is related to cancer of the lungs (HASHIMOTO et al, 2004) and adenocarcinomas endometriales (ZHANG et al, 2005). The characteristic of this super-family is the presence of a thiolester β-cisteinil-γ-glultamil intramolecular, (Lagueux et al, 2000, Sottrup-Jensen et al, 1989b, LAW et al, 1997); also, this family can be considered as a sophisticated linking protein activated by a specific proteolysis. The α-Ms have been studied in different mammals, birds, reptiles and amphibians (SOTTRUP-JENSEN et al, 1987); the α_2-Macroglobulin (α_2-M) has been isolated and studied in rats (GAUTHIER et al, 1976, GORDON eta l, 1976) mice, (ANONICK et al, 1989), serum from horses and hamsters as a potential inhibitor of the virus causing influenza A (PRITCHETT et al, 1989); in dogs through immunity tests, an elevated plasmatic concentration has been observed of α_2-M in the acute pancreatitis (RUAUX et al, 1999); in guinea pigs it has been demonstrated that the glycoprotein Hemaglutinin inhibits the presence of the α_2-M after the infection by the virus of influenza A (RYAN et al, 1993). In the same way this has been observed in rabbits with macrophage alveolares (KAPLAN et al,1979a, KAPLAN et al,1979b). Studies made with she-goats demonstrated that uric acid affects the α_2-M, therefore lessening the physiological potential of the caprine α_2-M (KAPLAN et al,1979b, KHAN et al, 2004). The α_2-M in female bovines can have autocrine or paracrine functions in the cells of the granulose follicles; this is important for the regulation of the production of estradiol and the development of the dominant follicles (IRELAND et al, 2004). This has also been seen in other domestic animals, (Metayer et al, 2002, RYAN et al, 1991).In this study the α_2-M of the Ovis aries has been purified and compared to the α_2-M of the human being which is related to diverse pathologies; in this way the field is amplified for biological action with α_2-Ms in different species.

MATERIAL AND METHODS

Reactives

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All the reactives were in an analytical degree, such as; sodium chloride (NaCl), Sodium di-hydrogen phosphate (NaH_2PO_4), Di-sodium hydrogen phosphate (Na_2HPO_4), Polyethylenglycol-

6000 (PEG_{6000}), hydrochloric acid (HCL) and Tris were acquired from Merck, KgaA; phenylmethylsulfonylfluorur (PMSF) from Fabroquim, glycine, dimethyl-sulphoxide (DMSO), EDTA, acetic acid, sodium acetate, glycerol and methanol were obtained from Riedel de Haën; Coomassie Blue and Bromophenol Blue were obtained from Sigma. The acrylamide, N, N-methylenbisacrylamide, the Sodium dodecylsulfate (SDS), the ammonium persulfate (APS), TEMED and ß-mercaptoethanol were acquired through BioRad. The insoluble matrix were of rapid flow (Amersham Biosciences); the Type III water was used in all the solutions and was produced with Osmosis equipment Reversa Rios® (Millipore) in the laboratories of the Chemical investigative group at the University of Pamplona, N.S., Colombia, South America.

Obtaining and Processing the Samples

The sample of human plasma was gotten through the Erasmo Meoz Hospital located in the city of Cucuta, Colombia; it was analyzed and certified to be free of antigens, such as; hepatitis, HIV, Chagas, and other infectious diseases.

The sample of plasma of the Ovis aries was taken with the authorization of the ethics' committee at the University of Pamplona, approved on July 13th, 2004 and renewed on July 26th, 2011 with the Act 003. This sample was taken under the supervision of a veterinary doctor following the contemplated recommendations found in title V of the decree 08430 – this Ovis aries is a specimen found at the Experimental Farm 'Villa Marina' and is the property of the University of Pamplona. Bags especially made for blood extraction with anticoagulant solution of citrate, phosphate, dextrose and adenine (CPDA-1) made by Baxter were used. PMSF dissolved in DMSO was added to the blood obtained; this gave a final concentration of 1mM; the objective of this additive was to inhibit any activity of the serine proteases. The samples were kept under refrigeration and the plasma was separated by centrifuge at 7,000 r.p.m. for 15 mins. at 4 ºC.

Purification of the human α_2-M

250 mL of human plasma was taken which had been previously processed to remove Plasminogen (Plg) by affinity chromatography in sepharose-lysine according to the method suggested by (DEUTSCH et al, 1970); after which the plasma was dialyzed against water type III for 24 hours in a regenerated cellulose membrane MWCO 12-14 kDa and centrifuged at 7,000 r.p.m. for 10 minutes at 4 ºC; this was done to remove the precipitate of euglobulins. 200 ml of this plasma was then utilized for purification

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of the human α_2-M (α_2-MH) through affinity chromatography in sepharose-Zn++ (IMBER et al, 1981) in a chromatographic BioLogic LP from BioRad; then, this was put in a column of 2.5 x 10 cm and packed with 45 mL of the insoluble matrix. The protein was eluted with a buffer solution of 10 mM of sodium acetate, 0.15 M NaCl, pH 5.0, collecting fractions of 8 Ml. The concentration of the α_2-MH was determined through absorption at a wave longitude of 280 nm (A_{280}) using $(\varepsilon^{1\%})_{1cm}$ 8.9 (HALL et al, 1978) as an extinction coefficient. This protein was concentrated at ~ 4.0 mg / mL in an ultra-filtrated cell of AMICON from Millipore utilizing N_2 as the carrier gas**.** The procedure above-mentioned was done in the same way for the α_2-M of the Ovis aries (*α_2-MOP)*; nonetheless, no positive result was obtained.

Purification of the Ovis aries α_2-M.

A veterinary doctor took 450 ml of blood from the jugular vein of the *Ovis aries* according to the norms of bio-ethics and with the ethic committee's permission at the University of Pamplona, using Baxter® bags containing anti-coagulant trisodium citrate; the blood was kept under refrigeration until it was centrifuged at 7,000 r.p.m. at 4ºC for 15 mins. (Universal 32 R Hettich, Bäch). Then, PMSF was added to the Ovis aries plasma, for the same reason as in the experiment with human plasma.

Fractioning the plasma of the Ovine aries with PEG_{6000}.

50 ml of de-plasminogenized plasma was taken and added drop by drop 13.6% of the volume of the plasma as PEG_{6000} (50% p / v) (50 grams of PEG_{6000} dissolved in 50 ml of water type III) under constant stirring until reaching a final concentration of 6%; after 45 mins. of agitation at room temperature (18ºC), the plasma was then centrifuged at 7,000 r.p.m. for 15 mins at 4ºC. discarding the precipitate. The volume of the supernatant was again measured and PEG_{6000}, was added until a final concentration of 16% was obtained. As was explained above the plasma was centrifuged. The supernatant was discarded and the precipitate that contained the α_2-MOP, was re-dissolved in 8.6 ml of a buffer solution that contained Na_2HPO_4 30 mM, NaCl 0.15 M, EDTA 2.5 mM, PMSF 2.5 mM and 0.5 µM PTI.

Dialysis

The protein solution of the α_2-MOP was dialyzed using water type III for 40 hours in an ice bath within a regenerated cellulose membrane MWCO 12-14 kDa (Fisher Scientific) and centrifuged at 7,000 r.p.m. for 15 mins. at 4ºC to remove the euglobulins. The supernatant was again dialyzed with the buffer solution of phosphate, 20 mM Na_2HPO_4, 20 mM NaCl, pH 7.00 in the same way as described above.

Ion Exchange Chromatography

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A column of 1,5 x 10 cm (BioRad) was packed with 2 ml of Sepharose-DEAE (Amersham Biosciences) and equilibrated with the same buffer solution of the dialysis with a flow of 1.5 ml / min.,

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the dialyzed sample was applied at the same speed of flow, after which the column was washed with the same buffer solution until the A_{280} was < 0.040 U.A.; finally, the column was washed with the buffer solution Na_2HPO_4 20 mM, NaCl 60 mM pH 7.00 (buffer solution A) until the A_{280} was less than 0.008 U.A. The column was eluted by means of linear gradient from 60 to 150 mM NaCl with the buffer solution A and Na_2HPO_4 20 mM, NaCl 150 mM pH 7.00 (buffer solution B). Fractions of 3 ml were gathered; the tubes with A_{280} were collected; the protein solution was concentrated in the same way and under the same conditions as for the α_2-MH.

Exclusion Chromatography

A column of 0.5 x 120 cm was packed (BioRad) with Sephacryl S-300 HR (Pharmacia) beforehand being de-gasified and equilibrated with 3 volumes of the column with the buffer solution Na_2HPO_4 0.1 M pH 8.00 at a flow velocity of 0.3 ml / min., after applying the sample, the elution was continued with the same buffer solution and at the same velocity; fractions of 3 ml were collected and the tubes that contained (A_{280}) the α_2-MOP were also collected; the concentrated solution and the A_{280} was determined as mentioned above for the α_2-MH.

Enzymatic Hydrolization with Chymotrypsin (QT)

5 mg of QT were dissolved in 1 ml of HCl (10 mM) to obtain a final concentration of 5 mg / ml of QT. The α_2-MOP was incubated with QT in the following proportions: 1:1, 1:2, 1:4 mol / mol, respectively for 3 minutes at 37ºC; the reaction was divided into two aliquots; To each of the aliquots the same quantity was added in volumes of the electrophoresis sample buffer solution charged with the denatured as well as native buffer; additionally, 2.5 mM PMSF and 0.5 µM PTI was added to the native solution.

All the tests were either mixed with the native or denatured buffer solution; in both cases this was in a relation 1:1 (vol / vol) according to the method suggested by (LAEMMLI, 1970). None of the tests were submitted to boiling; in the case of the denaturing this was to avoid the breakage of the thiolester domain (SOTTRUP et al, 1980, HOWARD et al, 1980). In its place they were incubated for an hour at room temperature of ~ 25ºC.

The gels used were from SDS-PAGE at 7.5% for the denaturing and the native was at 5%. The electrophoretic run was done at 2 milli amper constantly for 35 mins. at a room temperature of ~ 25ºC.

Determination of the N-terminal sequence

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To determine the sequence of the N-terminals of the two □-macroglobulins the method of transference was performed in the following way: in a tube for micro-centrifuge several pieces of the membrane PVDF were taken with a size 9 that was not greater than 3 x 3 mm and were wet with methanol of 99% for 15 seconds; then they were dried.

2 µg of each proteins were taken and diluted in 2 x 500 µl of acetic acid (0.1 %); these were prepared in distilled water type I. Several membrane pieces (PVDF) were added to the diluted protein solution; this was carefully shaken for 3 mins. and kept at a temperature of 5-8 ºC; these were shaken every 8-12 hours. Then, after 2 days the membranes were taken out of each one of the tubes; these were, then, washed twice with 20% methanol in distilled water. The membranes that contained the protein were determined with the sequence of the N-terminals according to Edman`s method; these were very kindly determined for this experiment by Doctor Lars Sottrup-Jensen at the University of AAurus in Copenhagen, Denmark, Europe.

RESULTS

Purification of the human α_2-M

During the purification of the α_2-MH in the chromatography of Sepharose-Zn^{++} It was demonstrated that the majority of the proteins were not retained by the affinity chromatography of Zn^{++} (figure 1, peak 1); after being washed the column and its posterior elusion – see peak 2 – corresponded to the α_2-MH retained by the matrix of Sepharose-Zn^{++} (figure 1 – peak 2). The electrophoretic analysis of these two peaks showed that the plasma passed right through the insoluble matrix of Sepharose-Zn++ (figure 2 - lane 2) effectively; while the retained protein showed a typical band of 180 kDa of the α_2-MH (figure 2 – lane 1) which in previous studies had been identified as α_2-MH (VON SCHOULTZ et al, 1973). The percentage of purity obtained with the α_2-MH was greater than 95% according to the densitometer shown by lane 1 of figure 2 when compared with the sample that was not retained by the matrix with Sepharose-Zn++ (figure 2, lane 2); it seems that not all the α_2-MH was retained in the chromatography by affinity since the band of 180 kDa was still visualized on lane 2 of figure 2. This same method was utilized for the purification of the α_2-MOP, but no positive results were obtained in the same way as the pregnancy zone protein (PZP) belonging to the family of the □-Ms; also, it could not be purified with the same method as in the α_2-MH; for this reason the α_2-MOP was purified with some variations to the method used for the PZP done by (ARBELAEZ et al, 1997).

Purification of the Ovis aries α_2-M

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The addition of PEG_{6000} to the human or animal plasma causes saturation generating an effect of steric exclusion that precipitate proteins (DOONAN, 1996), the first precipitation was re-dissolved; the electrophoresis of this test demonstrated that the majority of the plasmatic proteins are precipitated into

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this concentration of PEG_{6000} (6%) – (figure 3, lane 3) including a part of the α_2-MOP; when comparing the two – lane 2 (plasma from Ovis aries) with lane 3, it can be fully appreciated with clarity that the precipitate obtained in the first fractioning with the organic polymer of high molecular weight, generated the same bands of normal plasma on the electrophoretic profile; thus, it can be visualized that the corresponding band to the α_2-MOP in the re-dissolved precipitate is much weaker than the plasma from α_2-MOP.It is important to bring out that in the second supernatant; the most abundant protein was the albumin indicating that the saturation at 16% with PEG_{6000}, still did not affect its solubility (figure 3, lane 4); while in the second precipitate, the α_2-MOP was found as was demonstrated when re-dissolved and in its posterior electrophoresis (figure 3, line 5). Identification can be done with the corresponding band of the α_2-MOP with a content of protein contaminates; among those found were small quantities of albumin (figure 3, lane 5). This is one of the globular proteins and the most abundant in the plasma (DOCKAL et al, 1999); it has an iso-electric point of 5 (WIIG et al, 2003), this is similar to the α_2-MH. This protein is almost always found in the procedures of purification of plasmatic proteins and in this case in particular, it is found as a contaminant of the α_2-MOP.The contaminants were reduced in the process of ion exchange chromatography as well as in the washing with 20 mM plus in the washing with 60 mM of NaCl; as can be seen in figure 3 – lanes 7 and 8 respectively, the remains are albumin as the only contaminant (figure 3 – lane 9).It was necessary to use the chromatography of exclusion to obtain a superior purity of 95% as is demonstrated in figure 4, peaks 1 and 2 which show a clear separation; this was confirmed through electrophoresis SDS-PAGE 7.5% and native PAGE at 5% (figure 5 and 6 – lanes 1 and 2) for both proteins α_2-MH and α_2-MOP respectively using the α_2-MH as the control in these electrophoresis.

Enzymatic Hydrolization with Chymotrypsin (QT)

In the enzymatic hydrolization of the α_2-Ms with the QT the molar relations were utilized as in the following: 1:1, 1:2 and 1:4 for the α_2-MOP-QT and the α_2-MH-QT. In figure 7 – lane 1 the sample of α_2-MOP is shown and in lane 2, the α_2-MOP-MA is shown; no difference

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between the two was detected nor any visible degradation of the α_2-MOP on comparing this with the corresponding human sample – band 1 of the molecular marker, α_2-MH as is shown in figure 7 – lane 7 with α_2-MH-MA. Shown in lanes 3, 4, and 5 of figure 7 the different degradations were demonstrated of the
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QT to the α_2-MOP with 1:1, 1:2, 1:4, respectively; these lanes demonstrate the typical degradation of the α_2-MH under 92 KDa as is shown here where band 2 of the molecular marker is the human Plg of 92,000 Da; these are degradations that can be proven in comparison with these of the bands of the α_2-MH on lines 9 and 10. The relation α_2-MOP with the QT enzyme – shown in figure 8 in lane 1, this shows the native α_2-MOP without the enzyme – lane 2; shows the relation α_2-MOP-QT 1:1 that is seen at this slow phase as well as in the rapid phase in equal proportions. In lane 2 the relation 1:2 shows that the slow phase disappears totally; in the same way shown in lane 3 of the 1:4 relation.

Se**quences Determination of the N-terminals**

Sequences of the α_2-Ms from both species were determined by the Edman method (EDMAN, 1970); the sequence of the α_2-MH was determined as being Ser-Val-Ser-Gly-Lys and the α_2-MOP as Asp-Ser-Val-Glu-Pro (Table 1). Only the Ser-Val showed any similarity in this small sequence between both α_2-M. In more extensive determinations the similarity improves, stemming from the a.a., 7-13 in the α_2-Ms as the sequence Tyr-Met-Val-Leu-Val-**Pro**-Ser-Leu for α_2-MH (Table 1); Tyr-Met-Val-Leu-Val-**Val**-Ser-Leu for α_2-MOP and Tyr-Met-Val-Leu-Val-**Pro**-Ser-Leu for the PZP; sequences belonging to the family of the α-Ms. In these sequences the α-MOP had a difference from the rest of the α-Ms; only in the 6th a.a. with a Val instead of a **Pro** – the human α-Ms is contained in this position.

DISCUSSION

The α_2-MOP had been identified in the plasma of the Ovis aries (BARRERA et al, 2007) but had not been able to be purified since no defined method existed and the method of purification of the α_2-MH, gave no positive results with the α_2-MOP. The method of purification of the PZP did not give any positive results either in the purification of the α_2-MOP. In this investigation and for the first time the Ovis aries α_2-MOP was purified from plasma; a protein that is in accord with all the characteristics found in it; this indicates that it is the correspondent α_2-M of the Ovis aries. One of the most important aspects in the identification of this protein was the fact that with gel of electrophoresis SDS-PAGE, a molecular weight of 180 kDa was determined; in the same way in which that of the α_2-MH in denatured conditions (SOTTRUP et al, 1984a). The native electrophoresis

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confirms the migration that is equal to these two proteins, the α_2-MH and the α_2-MOP strengthening the fact that it deals with the α_2-MOP. The reaction of this new protein with the QT gave us one of the most certain indications that it deals with the α_2-MOP where the denatured electrophoresis showed the typical degradation of this protein identified by (HOWELL et al, 1983, HARPEL, 1973), which in the same way in the native electrophoresis showed in the typical fast and slow phases of this new protein, demonstrated for the α_2-MH (ARBELAEZ et al, 1995), with the determination of the N-terminals; thus, with both proteins, this indicates that between these proteins there is a similarity existing in this short determination of sequences (4 a.a.). In the longest determination of the sequences (16 a.a.) very similar or identical sequences were shown between α_2-MOP and the human α-Ms; it is remarkable that the first a.a. of the α_2-MOP is the aspartate of this a.a; this is in harmony with other sequences of other animal proteins determined by this group of investigation, such as; the Plg of 8 animal species, among these was the Ovis aries (CAÑAS et al, 2011). All of these similarities strengthen and indicate that it is α_2-MOP, being dealt with here; its purification could not be done using the method indicated by (IMBER et al, 1981) for the α_2-MH by affinity chromatography in Sepharose-Zn^{++}. This method gave no positive results in the purification of the α_2-MOP since it was not retained by the column of Sepharose-Zn^{++}; there are clear indications that some physico-chemical characteristics vary between the α_2-MH and the α_2-MOP; this presents that difficulty in the

purification by the same chromatographic method indicated above. In the purification of the α_2-MOP a modification of the method used by (ARBELAEZ et al, 1997) for the PZP was used. Using precipitation with PEG_{6000}, chromatography by ionic exchange and exclusion, a high purity of the α_2-MOP was achieved. This analysis definitely strengthens this research dealing with an α-M; this is shown in the determination of the sequence of 16 a.a. When comparing these, not only with the α_2-MH but also with the PZP. The first a.a. of the N-terminals of these proteins are not homogeneous; some of them agree even though not in the same positions; nonetheless, with more profundity of the determination of the sequence of the a.a., 7-15, a sequence of 8 a.a. was indicated that permitted the investigation to asseverate the relationship of the family of these three proteins without any doubts; thus, indicating that this new protein is linked to the family of the α-Ms and where we can find the α_2-MH as well as the PZP (SOTTRUP et al, 1984a). This semblance in the sequence of the α_2-MOP shows a similitude more in quantity of a.a. identical to the PZP since

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this latter protein from the a.a. number 2 to 13 is identical to the α_2-MOP from a.a. 4 to 15 with the exception of the Proline that has its difference in the human α-Ms in this sequence with the α-MOP.

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Table 1. Comparison of the sequences of the human α-Ms and Ovis aries. The identical sequences between among the 3 proteins are in black; the sequences that differ in this sequence are underlined.

α_2-Ms	N-terminal sequences
α_2-MH	[1]Ser-Val-Ser-Gly-Lys-Pro-Glu-**Tyr-Met-Val-Leu-Val-**Pro**-Ser-Leu**
α_2-MOP	[1]Asp-Ser-Val-Glu-Pro-Gln-**Tyr-Met-Val-Leu-Val-**Val**-Ser-Leu**-Leu
PZP	[1]Thr-Glu-Pro-Gln-**Tyr-Met-Val-Leu-Val-**Pro**-Ser-Leu**-Leu-His-Thr

Figure 1. Elusion of the α_2-MH in the column of chromatography by affinity in Sepharose-Zn^{++}. Peak 1, human plasma that passes right through the column, peak 2, the α_2-MH, retained in the column. The red line corresponds to the conductivity and the blue line to the A_{280}.

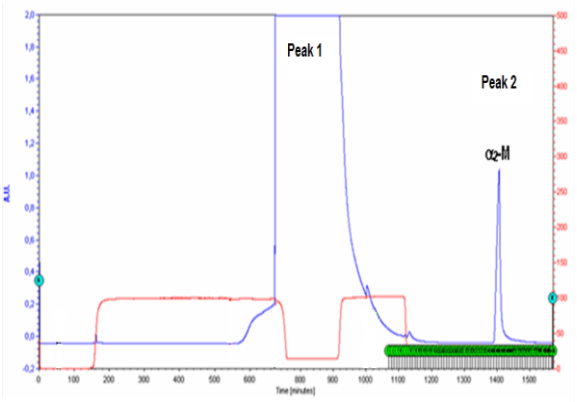


Figure 2. Electrophoresis SDS PAGE 7.5% of α_2-MH purified by chromatography by affinity in Sepharose-Zn^{++}. lane 1. α_2-MH, lane 2. Human Plasma. In the figure the position of the albumin is indicated.

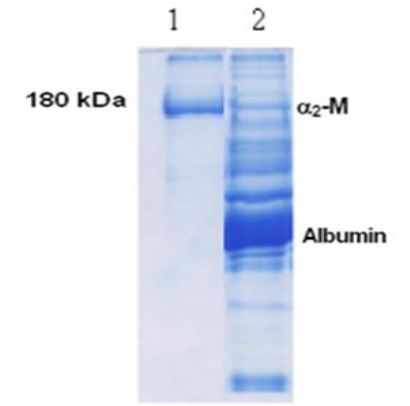
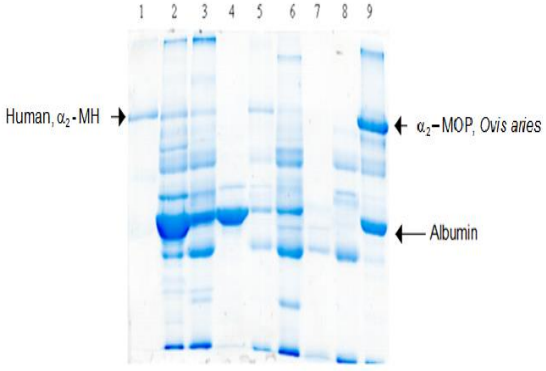


Figure 3. Electrophoresis SDS-PAGE 7.5% of the purification of α_2-MOP. lane 1; α_2-MH (control), lane 2; Plasma from Ovis aries, lane 3; the first precipitate with PEG_{6000} (re-dissolved), lane 4; Supernatant of the first precipitate with PEG_{6000}, lane 5; Second precipitate with PEG_{6000} (re-dissolved), lane 6; Precipitate from the dialysis (re-dissolved), lane 7 (ion exchange chromatography); Washed with 20 mM NaCl, lane 8 (ion exchange chromatography); Washed with 60 mM NaCl, lane 9 (ion exchange chromatography); Saline Gradient of 60 to 150 mM of NaCl.



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