# Human $\alpha_2$-Macroglobulin Is an Osteogenic Growth Peptide-Binding Protein

Hanna Gavish,*,‡ Itai Bab,‡ Alexander Tartakovsky,‡ Michael Chorev,|| Nora Mansur,‡ Zvi Greenberg,‡ Malka Namdar-Attar,‡ and Andras Muhlrad§

*Bone Laboratory and Department of Oral Biology, Faculty of Dental Medicine, and Department of Pharmaceutical Chemistry, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120, Israel*

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ABSTRACT: The osteogenic growth peptide (OGP) is a 14mer mitogen of osteoblastic and fibroblastic cells. Physiologically, OGP is present in high abundance in human and other mammalian sera. Most of the serum OGP is complexed noncovalently to heat sensitive, high molecular weight OGP-binding proteins (OGPBPs). Changes in serum OGP levels that follow bone marrow ablation and the low doses of exogenous OGP required for the stimulation of bone formation suggest a regulatory role for the OGPBPs. In the present work, the OGP binding activity was monitored by competitive binding to [3-$^{125}$I(Tyr$^{10}$)]-sOGP and the corresponding complexes were demonstrated on nondenaturing cathodic polyacrylamide gel electrophoresis. We show that OGP binds to both native and activated human plasma $\alpha_2$-macroglobulin ($\alpha_2$M). $\alpha_2$M was also immunoidentified in reduced and nonreduced SDS−polyacrylamide gel electrophoresis of OGP-affinity purified plasma-derived proteins. Immunoreactive OGP was detected in commercial preparations of both forms of $\alpha_2$M; OGP was purified to homogeneity from the commercial preparation of activated $\alpha_2$M. In MC3T3 E1 cells, native $\alpha_2$M, at concentrations <50 ng/mL, had a substantially increased mitogenic effect in the presence of synthetic, native-like, OGP (sOGP). Similar amounts of activated $\alpha_2$M inhibited the sOGP proliferative effect. These results suggest that the native $\alpha_2$M enhances the immediate availability of OGP to its target cells. Activated $\alpha_2$M may participate in the removal of OGP from the system.

We have recently characterized a 14-amino acid growth peptide identical to the C-terminal region of histone H4 (*1*). The peptide was initially isolated from regenerating bone marrow as an osteogenic cell growth promoting activity (*1*−*3*) and was therefore named osteogenic growth peptide (OGP).[1] *In vitro*, OGP is mitogenic to osteoblastic and fibroblastic cells (*1*, *4*, *5*). In addition, in cultures of bone marrow stromal cells it stimulates alkaline phosphatase activity and matrix mineralization (*6*). In normal and osteoporotic experimental animals OGP treatment enhances bone formation and increases trabecular bone mass (*1*). OGP also stimulates hematopoiesis, including the engraftment of bone marrow transplants (*7*, *8*).

OGP is present in the serum of human, rat, and apparently other mammalian species. Most of the serum OGP is noncovalently bound to OGP-binding proteins (OGPBPs) (*1*, *5*). Changes in the serum bound and unbound OGP that accompany the osteogenic phase of post-ablation bone marrow regeneration and associated systemic osteogenic response (*9*, *10*) as well as the low doses of exogenous OGP required for the *in vivo* stimulation of osteogenesis and hemopoiesis suggest a role for the OGPBPs in regulating the availability of OGP to its target cells.

$\alpha_2$-Macroglobulin ($\alpha_2$M) is a 720 kDa homotetramer present in high abundance in the serum (*11*). It is a multifunctional protein that serves as a broad spectrum proteinase inhibitor (*12*) and as a binding protein for several regulatory polypeptides (reviewed in ref 13). The primary circulating form of $\alpha_2$M is referred to as the "native" (*14*). It is activated by covalently entrapping proteinases, a process involving a conformational change which makes the molecule more compact and hence confers fast mobility when the molecule is subjected to nondenaturing gel electrophoresis as compared with the native or "slow" form of $\alpha_2$M (*15*). The irreversible conformational change renders the $\alpha_2$M molecule recognizable by its receptor, the $\alpha_2$M receptor/low-density lipoprotein receptor-related protein ($\alpha_2$M-R/LRP). The $\alpha_2$M-R/LRP, expressed in many cell types including fibroblasts, macrophages, adipocytes, and hepatocytes (*16*), presents the polypeptide regulators to their corresponding receptors or for degradation following internalization of the ligand−$\alpha_2$M−$\alpha_2$M-R/LRP complex (*17*).

This study demonstrates that both the native and activated forms of $\alpha_2$M derived from human plasma bind OGP noncovalently. The native form of the protein significantly and additively enhances the OGP mitogenic activity in osteoblastic MC3T3 E1 cells. This activity is inhibited by activated $\alpha_2$M.

## EXPERIMENTAL PROCEDURES

*Peptide Synthesis and Radioiodination.* The native-like synthetic OGP (sOGP), $N^{\alpha}$-Ac[Cys$^0$]sOGP, [Cys$^{15}$(*S*-NEt-

* To whom correspondence should be addressed.
‡ Bone Laboratory, Faculty of Dental Medicine.
§ Department of Oral Biology, Faculty of Dental Medicine.
|| Department of Pharmaceutical Chemistry, Faculty of Medicine.
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[1] Abbreviations: $\alpha_2$M, $\alpha_2$-macroglobulin; $\alpha_2$M-R/LRP, $\alpha_2$M receptor/low-density lipoprotein receptor-related protein; BSA, bovine serum albumin; DTT, dithiothreitol; FCS, fetal calf serum; HPLC, high-pressure liquid chromatography; irOGP, immunoreactive OGP; OGP, osteogenic growth peptide; OGPBP, osteogenic growth peptide-binding protein; PAGE, polyacrylamide gel electrophoresis; PDGF, platelet-derived growth factor; RIA, radioimmunoassay; sOGP, synthetic OGP.

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Succ)]sOGP-$NH_2$, and [3-$^{125}$I(Tyr$^{10}$)]sOGP were prepared as reported previously (*4*).

*Separation of OGPBPs from Human Plasma*. Twenty-six mL of outdated normal human plasma, with a total protein content of 65 mg/mL was obtained from the Hadassah University Hospital Blood Bank and kept frozen until use. The plasma was cleared by centrifugation at 14000*g* for 15 min at 4 °C, and the albumin was separated by precipitation of proteins with 50% ammonium sulfate. The precipitate was dissolved in PBS and dialyzed against the same solution, and proteins of molecular mass >10 kDa were concentrated in Centricon-10 microconcentrators (Amicon, Inc., Beverly, MA). Immunoglobulins were removed by adsorption on a 2 mL protein A-agarose column (Sigma Chemical Co., St. Louis, MO, catalog no. P2545). A total of 134 mg of protein was applied to the column in two batches. After each run immunoglobulins were cleared from the column by treatment with 0.1 M glycine. The absence of immunoglobulins from the flowthrough was confirmed by SDS−polyacrylamide gel electrophoresis (SDS−PAGE). Affinity purification of OGPBPs was carried out by applying 100 mg of protein present in the flowthrough of the protein A-agarose column to a 1 mL column containing 2.6 $\mu$mol of $N^{\alpha}$-Ac[Cys$^0$]OGP-maleimido conjugated to bovine serum albumin (BSA) immobilized to cyanogen bromide activated Sepharose 4B (Sigma Chemical Co., St. Louis, MO, catalog no. C9142). After successive washes of the column with 0.5 M NaCl, the bound proteins were released with 0.1 M triethylamine buffer, pH 11.5. The solution was immediately neutralized with 1 M Tris buffer, pH 6.8, and extensively dialyzed overnight against PBS.

*Binding Assay*. OGP binding activity was analyzed in a competitive binding assay (*1*, *4*). Briefly, [3-$^{125}$I(Tyr$^{10}$)]-sOGP, $10^4$ cpm, was incubated for 30 min at 37 °C with 7 $\mu$g of whole plasma proteins or OGP-affinity purified proteins or with commercially prepared $\alpha_2$M (purchased from Sigma Chemical Co., St. Louis, MO, catalog no. M7151, or Calbiochem-Novabiochem International, San Diego, CA, catalog no. 441251). The incubation was carried out in the absence or presence of unlabeled sOGP. Bound and free [3-$^{125}$I(Tyr$^{10}$)]sOGP were separated employing a nondenaturing cathodic phosphate buffered 15% PAGE, pH 6.0. This system was modified from a previously published protocol (*1*, *4*, *5*) by using a running buffer containing 8 mM sodium orthophosphate, thus enhancing the resolution to demonstrate the presence of two labeled complexes as well as the unbound radiolabeled sOGP. Gels were dried, and the position of the radiolabeled sOGP was demonstrated by autoradiography.

Some experiments were run to assess the binding of OGP to commercial preparations of $\alpha_2$M. These preparations replaced the plasma-derived material. They were considered native (Calbiochem) or activated (Sigma) on the basis of the respective manufacturer's report on the presence or absence of protease inhibitory activity, respectively.

*Immunoblot Analysis*. Proteins were separated by 3−12% SDS−PAGE (*18*) under reducing and nonreducing conditions and electroblotted onto a nitrocellulose filter (Schleicher & Schuell, Dassel, Germany, catalog no. NC 45). Nonspecific binding sites were blocked with fatty acid free BSA (Sigma Chemical Co., St. Louis, MO, catalog no. A-7030) and the filters were incubated overnight at 4 °C with polyclonal rabbit anti-human $\alpha_2$M IgG (Sigma Chemical Co., St. Louis, MO, catalog no. M1893) diluted 1:5,000. Antibody binding was demonstrated by further incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad, Hercules, CA, catalog no. 170-6515) and stained with diaminobenzadine (Sigma Chemical Co., St. Louis, MO, catalog no. D4418).

*Purification of OGP from Commercial $\alpha_2$M*. Purification of OGP from commercially prepared activated $\alpha_2$M was carried out essentially as reported recently (*5*). In short, a 3.2 mL aqueous solution containing 640 $\mu$g of $\alpha_2$M (Sigma) was boiled for 10 min to release OGP from its putative complex with $\alpha_2$M. Polypeptides of molecular mass <3 kDa were separated using Centricon-3 microconcentrators (Amicon, Inc., Beverly, MA). The filtrate, 2.64 mL, was concentrated to 110 $\mu$L using a SpeedVac apparatus (Savant Instruments Inc., Farmingdale, NY) and 100 $\mu$L was subjected to reverse phase high-pressure liquid chromatography (HPLC) on a 5-$\mu$m LiChroCART 125-4 C18 column (Merck, Darmstadt, Germany). Elution was carried out with 17−23% linear acetonitrile gradient containing 0.1% TFA, delivered at 1 mL/min. Microconcentrated preparations and chromatographic fractions were screened for the presence of immunoreactive OGP (irOGP).

*Determination of irOGP*. OGP was screened throughout the purification process with a radioimmunoassay (RIA) using anti-OGP antibody generated as described before (*1*). The reaction mixture consisted of 50 $\mu$L of the test sample and 100 $\mu$L of each of the following solutions: a 1:40 dilution of nonimmune rabbit serum and a 1:500 dilution of rabbit anti-OGP antiserum and [3-$^{125}$I(Tyr$^{10}$)]sOGP, $3 \times 10^4$ cpm. After overnight incubation at room temperature the mixture was supplemented with 1 mL of the solution of 1:50 dilution of goat anti-rabbit IgG (Sigma Chemical Co., St. Louis, MO, catalog no. R0881) and 2.5% polyethylene glycol (Sigma Chemical Co., St. Louis, MO, catalog no. P2263). This was followed by additional 2 h of shaking at 4 °C and centrifugation at 4000*g* for 15 min. The total radioactivity in the pellet was estimated in a $\gamma$ counter.

For instances in which the total irOGP (bound and free) content was determined in commercial $\alpha_2$M the RIA was preceded by incubation of this preparation with 450 nmol/mL [Cys$^{15}$(*S*-NEtSucc]sOGP-$NH_2$ for 30 min at 37 °C. This OGP analog does not react with the anti-OGP antibody but does bind to the OGPBPs, thus displacing the irOGP from the OGP−OGPBP complexes (*1*).

*Amino Acid Sequence Determination*. The irOGP peak isolated by reverse phase HPLC was subjected to automated peptide sequence analysis in an Applied Biosystems 470A sequencer. Released amino acid derivatives were identified with the aid of an on-line HPLC.

*Cell Cultures*. Osteoblastic MC3T3 E1 cell cultures were set as described previously (*1*, *4*, *5*). Cells derived from confluent maintenance cultures grown in $\alpha$ minimal essential medium supplemented with 10% fetal calf serum (FCS) were seeded in 16 mm multiwell dishes at $10^4$ cells per cm$^2$ and incubated in the same medium at 37 °C in $CO_2$−air. For the initial 46 h the cells were incubated in the same FCS-containing medium. The cultures were then washed and kept for an additional 2 h period under serum-free conditions. $\alpha_2$M alone or in the presence of $10^{-13}$ M sOGP was preincubated with 4% BSA. These preparations or BSA alone were then added to the cell cultures for a further 48 h period. Cell counts were carried out using an hemocytometer.

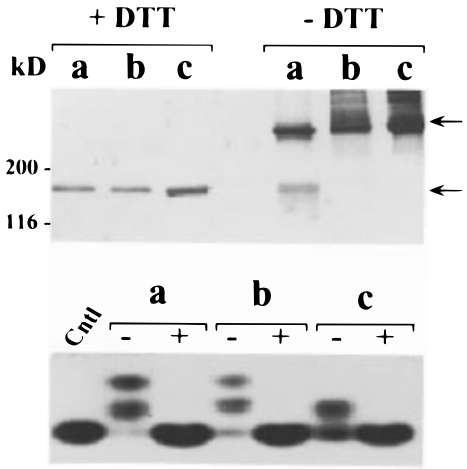


FIGURE 1: Human plasma $\alpha_2$M binds to immobilized and radiolabeled OGP. Western blot analysis of SDS−PAGE separated plasma-derived proteins using anti-human $\alpha_2$M antibody preparation (upper panel). Dithiothreitol used for reduction of disulfide bonds (DTT): whole plasma (a); OGP affinity-purified proteins (b); commercial $\alpha_2$M (Sigma) (c). Arrows indicate $\alpha_2$M bands. Autoradiograph of nondenaturing cathodic PAGE demonstrating OGP-binding activity (lower panel). Absence or presence of excess "cold" sOGP is indicated by (−) or (+), respectively. [3-$^{125}$I(Tyr$^{10}$)]sOGP alone (Cntl); [3-$^{125}$I(Tyr$^{10}$)]sOGP preincubated with whole plasma (a) or with OGP-affinity purified proteins (b) or with commercial $\alpha_2$M (Sigma) (c).

## RESULTS

$\alpha_2$M was selected as a candidate OGPBP because it is a major serum protein that binds several regulatory polypeptides (*19*−*21*). To enrich the content of OGP-binding activity, we used an approach based on affinity chromatography to immobilized OGP. The OGP-affinity purification step was preceded by the removal of albumin and immunoglobulins which bind OGP with very low affinity (data not shown). Immunoblot analysis of whole plasma and OGP-affinity purified proteins separated by SDS−PAGE showed bands with apparent molecular mass of 180 kDa under reducing conditions and >300 kDa under nonreducing conditions that reacted specifically with an anti-human $\alpha_2$M antibody preparation (Figure 1). Competitive binding of radiolabeled OGP to the putative OGPBP(s) was explored using a nondenaturing cathodic PAGE system previously employed to study the binding of OGP to the OGPBP (*1*, *4*, *5*). Minor modifications introduced to this system enabled the demonstration of two complexes formed by incubating [3-$^{125}$I(Tyr$^{10}$)]sOGP with either plasma or OGP-affinity purified proteins. The electrophoretic position of the fast migrating complex was similar to that formed between [3-$^{125}$I(Tyr$^{10}$)]sOGP and the Sigma $\alpha_2$M preparation that had been initially used in the present series of experiments. The [3-$^{125}$I(Tyr$^{10}$)]sOGP could be competed out from all the complexes by an excess of "cold" sOGP (Figures 1 and 2).

Consistent with the absence of protease inhibitory activity (1997 Sigma catalog, page 675), the Sigma $\alpha_2$M preparation exhibits a faster electrophoretic mobility on nondenaturing anodic PAGE (*22*) as compared to the preparation obtained from Calbiochem (data not shown) which retains the protease inhibitory activity (product data sheet). These properties of the Calbiochem and Sigma preparations are accordant with the native and activated conformational forms, respectively. It is demonstrated by nondenaturing cathodic PAGE that the electrophoretic mobilities of complexes formed between OGP and native and activated $\alpha_2$M migrated similar to that of the slow and fast migrating OGP−OGPBP complexes formed, respectively, by incubating [3-$^{125}$I(Tyr$^{10}$)]sOGP with plasma. In the presence of up to 4.5 nmol of "cold" sOGP, all the radioligand molecules bound to native $\alpha_2$M. Under the same tested conditions, activated $\alpha_2$M bound the radioligand for a lesser extent with partial displacement already noted in the presence of 0.045 nmol of "cold" sOGP. Even 2-fold higher concentrations of the competitor failed to displace the radioligand from its complex with native $\alpha_2$M (Figure 2).

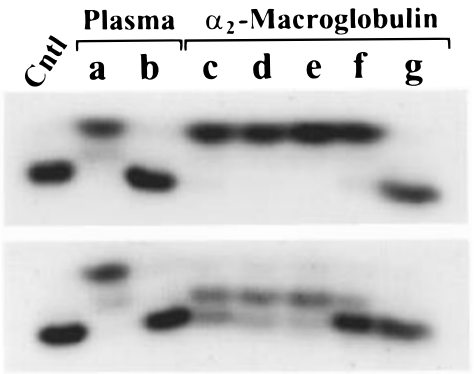


FIGURE 2: Autoradiographs of nondenaturing cathodic PAGE demonstrating OGP-binding to "native" and "activated" $\alpha_2$M. [3-$^{125}$I(Tyr$^{10}$)]sOGP was preincubated with human plasma or commercial $\alpha_2$M from Calbiochem (upper panel) or Sigma (lower panel). [3-$^{125}$I(Tyr$^{10}$)]sOGP alone (Cntl) or with the indicated preparations in the absence of competing "cold" sOGP (a, c) or in the presence of 0.045 (d), 0.45 (e), 4.5 (b, f), or 45 nmol (g) of sOGP.

To further assess the association between OGP and $\alpha_2$M, irOGP was measured in the activated $\alpha_2$M preparation. The total irOGP content of Sigma lots 025H9314 and 023H93191 was 0.1 and 0.24 nmol/mg of protein, respectively. To verify that the $\alpha_2$M-derived irOGP is indeed OGP, the peptide was purified from one of these lots (025H9314). The elution profile of the reverse phase HPLC shows that the irOGP loaded on the column recovered in a well-defined peak eluted at 21% acetonitrile, a position corresponding to that of sOGP (Figure 3). Amino acid sequence analysis revealed that the polypeptide content of the this peak consisted exclusively of OGP. The purified OGP was identical to that isolated previously from human serum, rat regenerating bone marrow, and culture medium of mouse osteoblastic cells (*1*, *4*, *5*, *23*). The difference in recovery between the filtrate obtained after boiling and the reverse phase HPLC eluate (Table 1) reflects mainly loss of material during the pre-HPLC concentration step. irOGP was also detected in heat-inactivated commercially prepared native $\alpha_2$M (data not shown).

An osteoblastic MC3T3 E1 cell proliferation assay, previously optimized to analyze the mitogenic activity of OGP (*1*, *4*, *23*), was used to assess the functional significance of $\alpha_2$M in the OGP system. Activated $\alpha_2$M added alone to the culture medium induced a moderate dose-dependent increase in cell number at 23−188 ng/mL concentration range followed by a reversal of this increase at higher doses (Figure 4). When activated $\alpha_2$M was added together with an optimal dose of sOGP (*1*, *4*, *23*), the peak increase in cell number was seen at an $\alpha_2$M concentration range lower by approximately 1 order of magnitude. This increase, however, represents a net inhibition of the OGP stimulatory effect at all doses tested except the peak. When added alone,

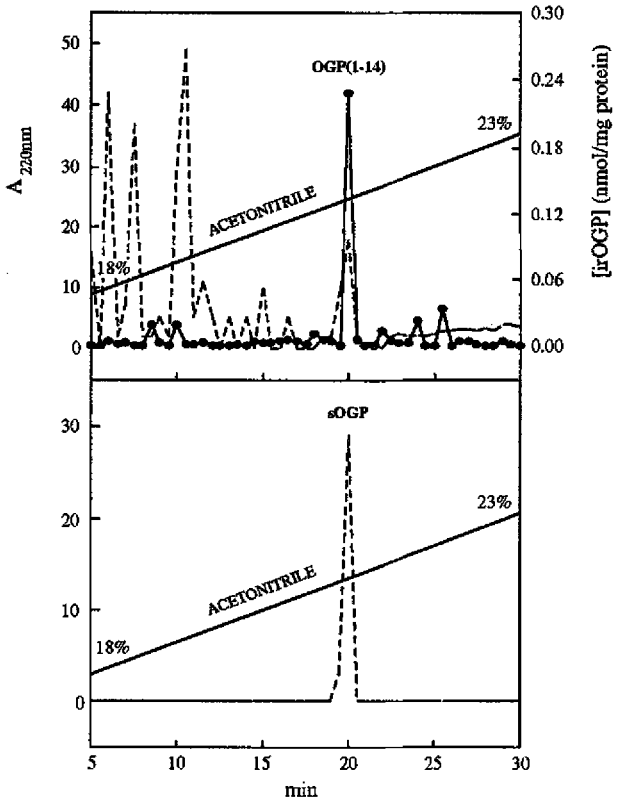


FIGURE 3: Purification of OGP from $\alpha_2$M. Reverse phase HPLC tracing of irOGP from postboiling activated $\alpha_2$M (Sigma) filtrate (upper panel). Control reverse phase HPLC of sOGP showing identity of the retention time with that of $\alpha_2$M-derived irOGP (lower panel). Profile of irOGP determined by RIA (●); UV absorbance (- - -).

Table 1: Purification of OGP from Commercial $\alpha_2$M[a]

| purification step | total irOGP (nmol) | irOGP recovery (%) | SIR[b] (nmol/mg) |
|---|---|---|---|
| crude preparation[c] | 0.080 | 100.0 | 0.100 |
| filtrate after boiling and ultrafiltration | 0.066 | 82.5 | 0.083 |
| peak HPLC fractions | 0.045 | 56.0 | 0.056 |

[a] Sigma lot 25H9314. [b] SIR, specific immunoreactivity (nmol of irOGP/mg of $\alpha_2$M). [c] Total irOGP was measured following its displacement from the OGP−$\alpha_2$M complex by [Cys[15](*S*-NEtSucc)]OGP-$NH_2$] (*1*).

native $\alpha_2$M had no effect on cell proliferation. In combination with the optimal concentration of sOGP the native $\alpha_2$M had a biphasic growth effect characterized by a dose−response amplification of the OGP-induced mitogenesis at 7−23 ng/mL $\alpha_2$M followed by inhibition of the OGP effect at higher $\alpha_2$M concentrations. This inhibition was complete at 375 ng/mL $\alpha_2$M.

## DISCUSSION

Binding proteins for soluble signaling polypeptides are emerging as important regulators of activity and tissue distribution of these potent biological mediators (*24*−*26*). Complexes formed with these proteins present in the blood and apparently other compartments of the extracellular fluid constitute large reservoirs of inactive hormones and growth factors protected from proteolytic degradation and clearance, hence providing a mechanism controlling the availability of active ligands to their target cells. Such a mechanism may

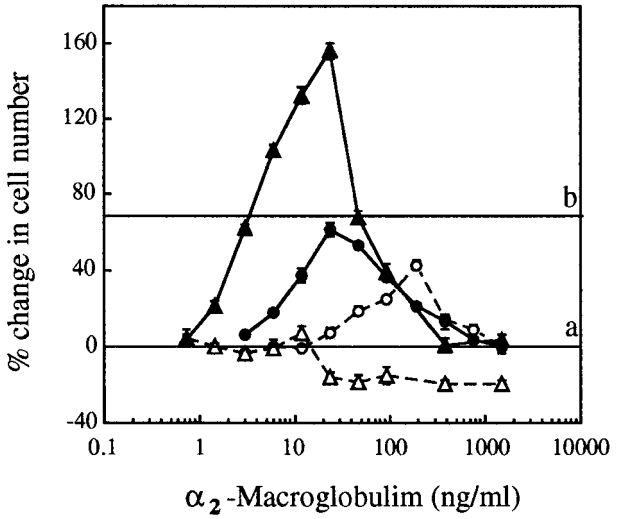


FIGURE 4: Modulation of OGP-stimulated osteoblastic MC3T3 E1 cell proliferation by human $\alpha_2$M. Growth was measured following incubation with $\alpha_2$M alone or in the presence of $10^{-13}$ M sOGP. Base line cell growth in serum-free defined medium (a); OGP-mediated growth (b). Activated $\alpha_2$M with sOGP (●); native $\alpha_2$M with sOGP (▲); activated $\alpha_2$M alone (○); native $\alpha_2$M alone (△). Data are mean ± SE obtained in three culture wells per condition.

be associated with the regulation of free ligand levels and/or the ligand transport and presentation to its specific tissues or acceptors. Another function attributed to carrier proteins is deactivation of circulating regulators by clearing them from the body (*27*, *28*).

Using a combination of affinity chromatography to immobilized OGP and binding assays we were able to demonstrate the presence of two human plasma OGP−OGPBP complexes. The binding of OGP is specific inasmuch as the [3-$^{125}$I(Tyr$^{10}$)]sOGP can be competed out from both complexes by an excess of unlabeled sOGP. These complexes are rather stable as shown by their resistance to the electrophoretic separation. Furthermore, the OGPBPs could not be dissociated from immobilized OGP using high salt concentrations (data not shown) and were released only under extreme basic conditions.

The electrophoretic mobility on SDS−PAGE of reduced and nonreduced proteins recovered from the OGP-affinity column suggested that at least one of the OGPBPs is $\alpha_2$M (data not shown). Indeed, the presence of $\alpha_2$M was confirmed by Western blot analysis. Since $\alpha_2$M does not bind to albumin (*21*) used as a spacer between OGP and the column matrix, we further studied its OGP-binding properties using commercial preparations of the native and activated protein. Both of these conformational forms formed complexes with [3-$^{125}$I(Tyr$^{10}$)]sOGP with respective slow and fast electrophoretic mobilities on nondenaturing cathodic PAGE. The rational for employing this system was its previous use for demonstrating serum OGP-binding activity concomitantly with the free radioligand (*1*, *4*, *5*). Nondenaturing anodic PAGE, widely used as reference system for studying $\alpha_2$M (*22*, *29*, *30*), was employed for analyzing the mobilities of the commercial $\alpha_2$M preparations. However, because the free positively charged radiolabeled sOGP cannot be detected in this system it was excluded from the analysis of OGP-binding activity. The present findings in the nondenaturing cathodic PAGE indicate that in plasma $\alpha_2$M is an OGPBP. Similar to platelet-derived growth peptide (PDGF), transforming growth factor-$\beta$, and nerve growth factor (*13*), OGP

binds to both conformational forms of this protein.

The interactions between $\alpha_2$M and several growth factors, cytokines and hormones were studied in recent years (*20*, *26*, *30*−*32*). Several of these studies calculated the affinity values assuming a single binding site per the $\alpha_2$M molecule (*20*, *26*) yet suggesting that more binding sites per $\alpha_2$M tetramer are possible. Attempting this approach to determine the binding parameters of OGP to $\alpha_2$M resulted in inconsistent data. Such inconsistency could have resulted from the small molecular weight of OGP and its extremely high rate of diffusion in different analytical systems. Therefore, we are able to demonstrate only qualitative data concerning the dissociation of the radioligand from the [3-$^{125}$I(Tyr$^{10}$)]-sOGP−$\alpha_2$M complexes and a comparison of the OGP-binding modes between the two conformational forms of $\alpha_2$M, suggesting that the native form possesses more available OGP-binding sites and/or a stronger binding affinity to OGP.

The association between OGP and $\alpha_2$M is further demonstrated by the purification of OGP from commercial activated $\alpha_2$M by a protocol designed previously for the isolation of OGP from blood (*5*) and by the detection of irOGP in the native $\alpha_2$M preparation. $\alpha_2$M is often purified complexed to a number of regulatory polypeptides and other proteins (*33*) and likewise, the presence of OGP in the $\alpha_2$M preparations implies the physiologic occurrence of OGP−$\alpha_2$M complexes.

In cultures of MC3T3 E1 cells, exogenously added activated $\alpha_2$M inhibited the OGP-mediated growth response. This implies that the conformationally altered $\alpha_2$M serves as a pathway for OGP clearance from the blood and extracellular fluid and its subsequent degradation via the interaction with the $\alpha_2$M-R/LRP. When added alone, activated $\alpha_2$M was mitogenic to these cells, suggesting the involvement of copurified growth factors (*33*, *34*). Alternatively, as in macrophages (*35*) and vascular smooth muscle cells (*34*), osteoblastic MC3T3 E1 cells may express a receptor-related protein that transduces a mitogenic signal in response to activated $\alpha_2$M per se.

Native $\alpha_2$M alone was not mitogenic to MC3T3 E1 cells. However, it substantially enhanced the proliferative effect of OGP. As in the case of other regulatory polypeptides (*19*, *26*, *36*, *37*), native $\alpha_2$M may increase the availability of OGP to its target cells by rendering protection against proteolytic cleavage, thus maintaining an extracellular reservoir of the OGP from which it can be controllably released over time. In concentrations higher than 50 ng/mL, native $\alpha_2$M inhibited the OGP mitogenic activity. A similar biphasic relationship was reported when native $\alpha_2$M was studied in combination with PDGF. The inhibition of PDGF activity was attributed to the conversion of native to activated $\alpha_2$M by cell-derived factors that accumulate in the culture medium during the incubation period (*36*).

In conclusion, the present study identifies human $\alpha_2$M as a plasma OGPBP. It is similar to the previously reported OGPBPs with respect to its electrophoretic mobility on nondenaturing PAGE and the release of substantial amounts of OGP by heat inactivation (*1*, *5*, *23*). *In vitro*, $\alpha_2$M appears to be an important modulator of the OGP action. *In vivo*, both forms of $\alpha_2$M bind OGP and may regulate the availability of this ligand to its tissue and cellular targets. $\alpha_2$M appears to be the major if not the sole plasma OGPBP.

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