

# Chemical modification of $\alpha_2$ -macroglobulin to generate derivatives that bind transforming growth factor- $\beta$ with increased affinity

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**Abstract**  $\alpha_2$ -Macroglobulin ( $\alpha_2$ M) binds a number of cytokines, including transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) and TGF- $\beta$ 2. The affinity of these interactions depends on the  $\alpha_2$ M conformation. In this investigation, we treated human  $\alpha_2$ M with *cis*-dichlorodiammineplatinum (II) (*cis*-Pt), a crosslinking reagent that partially 'locks' the  $\alpha_2$ M conformation, and then with methylamine to generate a preparation ( $\alpha_2$ M-P/M) consisting of stable  $\alpha_2$ M conformational intermediates.  $\alpha_2$ M-P/M bound TGF- $\beta$ 1 and TGF- $\beta$ 2 with higher affinity than any other form of  $\alpha_2$ M studied to date. The equilibrium dissociation constants were 14 and 2 nM for TGF- $\beta$ 1 and TGF- $\beta$ 2, respectively.  $\alpha_2$ M-P/M, at 100 nM, neutralized the activity of TGF- $\beta$ 1 by about 75% in an endothelial cell proliferation assay. The equivalent concentration of native  $\alpha_2$ M or methylamine-modified  $\alpha_2$ M had no effect. These studies demonstrate that the potential of  $\alpha_2$ M as a cytokine carrier and neutralizer may not be fully realized in either the native or completely activated conformations.

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**Key words:**  $\alpha_2$ -macroglobulin; Transforming growth factor- $\beta$ ; Cytokine; Endothelium

## 1. Introduction

$\alpha_2$ -Macroglobulin ( $\alpha_2$ M) is a naturally occurring protein in the blood which functions not only as a proteinase inhibitor but also as a carrier of specific cytokines, including transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), platelet-derived growth factor-BB, and nerve growth factor- $\beta$  [1,2]. The binding affinities of  $\alpha_2$ M for different cytokines vary widely and are not always affected similarly when the  $\alpha_2$ M is modified by proteinases [1–3]. Many cytokines do not bind to  $\alpha_2$ M at all, including PDGF-AA, interferon- $\gamma$ , colony stimulating factor-1, and ciliary neurotrophic factor [1,2,4,5]. Thus,  $\alpha_2$ M may function to neutralize cytokines, with partial specificity, in normal homeostasis and in select disease states.

$\alpha_2$ M in the blood is almost entirely in the native conformation. This form of  $\alpha_2$ M is fully functional as a proteinase inhibitor but not recognized by cellular receptors [6]. When treated with small primary amines, such as methylamine, native  $\alpha_2$ M undergoes a major conformational change [7,8]. The resulting structure, which is referred to as activated  $\alpha_2$ M, retains no proteinase inhibitory activity but is recognized by  $\alpha_2$ M-specific receptors, primarily in the liver, and thus rapidly cleared from the circulation [6]. Many proteinases induce a conformational change in  $\alpha_2$ M which is equivalent to that caused by methylamine [9,10]; however, before adopting the 'fully activated' conformation, the  $\alpha_2$ M apparently transitions

through a series of variably stable structural intermediates [11–15].

Very little is known about cytokine binding to  $\alpha_2$ M conformational intermediates; however, these intermediates may be identified as a small sub-population within purified native  $\alpha_2$ M preparations [16,17]. In the present investigation, we utilized an  $\alpha_2$ M chemical modification protocol which has been previously characterized for its potential to stabilize  $\alpha_2$ M conformational intermediates [12,18]. The protocol utilizes the reagent, *cis*-dichlorodiammineplatinum (II) (*cis*-Pt), as an amino acid side-chain crosslinker to partially lock the  $\alpha_2$ M conformation prior to adding methylamine. Treatment of *cis*-Pt-modified  $\alpha_2$ M with methylamine results in only partial reorganization of the  $\alpha_2$ M structure, as determined by nondenaturing PAGE, electron microscopy, and receptor recognition experiments [12,13,18,19].

The studies presented here demonstrate that  $\alpha_2$ M, modified with *cis*-Pt and methylamine, binds TGF- $\beta$ 1 and TGF- $\beta$ 2 with higher affinity than any other  $\alpha_2$ M preparation studied to date. To confirm the results of our equilibrium cytokine-binding analyses, we performed endothelial cell proliferation assays. TGF- $\beta$ 1 inhibited endothelial cell growth as expected and  $\alpha_2$ M-P/M neutralized this activity while native  $\alpha_2$ M and  $\alpha_2$ M-methylamine, at the equivalent concentration, were ineffective.

## 2. Materials and methods

### 2.1. Materials

Porcine TGF- $\beta$ 1, which is identical in sequence to human TGF- $\beta$ 1, was from R&D Systems (Minneapolis, MN, USA). Human TGF- $\beta$ 2 was from Genzyme (Cambridge, MA, USA). TGF- $\beta$ 1 and TGF- $\beta$ 2 were radioiodinated according to the method of Ruff and Rizzino [20]. Specific activities were 100–200  $\mu$ Ci/ $\mu$ g. Methylamine-HCl, chloramine-T, bovine serum albumin (BSA) and fetal bovine serum were from Sigma (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), trypsin-EDTA, and Earle's balanced salts solution were from GIBCO BRL (Gaithersburg, MD, USA). Na<sup>125</sup>I was from Amersham (Arlington Heights, IL, USA). Acidic fibroblast growth factor and basic fibroblast growth factor were from Promega (Madison, WI, USA). Bis(sulfosuccinimidyl) suberate (BS<sup>3</sup>) and Iodo-beads were from Pierce (Rockford, IL, USA). *Cis*-Pt was from Aldrich (Milwaukee, WI, USA).

### 2.2. Preparation of $\alpha_2$ M and $\alpha_2$ M derivatives

$\alpha_2$ M was purified from human plasma by the method of Imber and Pizzo [21]. The concentration of  $\alpha_2$ M was determined by measuring the absorbance at 280 nm, using an  $A_{1\%}^{1\text{cm}}$  of 8.93 [22]. All purified native  $\alpha_2$ M preparations were screened for the presence of trace levels of partially activated forms by incubation with <sup>125</sup>I-TGF- $\beta$ 1 followed by nondenaturing PAGE, as previously described [17]. Any native  $\alpha_2$ M preparations that showed TGF- $\beta$ 1-binding to  $\alpha_2$ M species with increased mobility were discarded.

$\alpha_2$ M-MA was prepared by dialyzing native  $\alpha_2$ M against 200 mM methylamine-HCl in 50 mM Tris-HCl, pH 8.2, for 12 h at 22°C, followed by extensive dialysis against 20 mM sodium phosphate,

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150 mM NaCl, pH 7.4 (PBS) at 4°C. Complete modification of native  $\alpha_2$ M by methylamine was confirmed by loss of trypsin binding activity (>96%) [23] and by the characteristic increase in mobility by nondenaturing PAGE [24,25].

$\alpha_2$ M-P was prepared by reacting native  $\alpha_2$ M with 1.6 mM *cis*-Pt for 6 h at 37°C. Unreacted *cis*-Pt was removed by extensive dialysis against PBS.  $\alpha_2$ M-P/M was prepared by dialyzing  $\alpha_2$ M-P against 200 mM methylamine-HCl in 50 mM Tris-HCl, pH 8.2, for 12 h at 22°C, followed by extensive dialysis against PBS.

### 2.3. Determination of apparent equilibrium dissociation constants

Equilibrium dissociation ( $K_d$ ) constants were determined by the BS<sup>3</sup>-rapid crosslinking method, as has been described by our laboratory [1,2,5,26]. Cytokine-binding to  $\alpha_2$ M is modeled as a two-step reaction:



$A$  is unbound  $\alpha_2$ M,  $C$  is unbound cytokine,  $AC$  is reversibly associated (noncovalent)  $\alpha_2$ M-cytokine complex, and  $AC^*$  is irreversibly associated (covalent)  $\alpha_2$ M-cytokine complex, formed by thiol-disulfide exchange. For TGF- $\beta$ 1 and TGF- $\beta$ 2, reversible binding to  $\alpha_2$ M occurs fairly rapidly and  $k_2$  is sufficiently small so that it may be ignored in the determination of  $K_D$  values [2].

In the experiments presented here, various concentrations of  $\alpha_2$ M-P or  $\alpha_2$ M-P/M (0.002–2.5  $\mu$ M) were incubated with <sup>125</sup>I-TGF- $\beta$ 1 or <sup>125</sup>I-TGF- $\beta$ 2 (1.0 nM) in PBS with 75  $\mu$ M BSA, for 30 min at 37°C. The rapid-crosslinking agent, BS<sup>3</sup> (in H<sub>2</sub>O), was then added at a final concentration of 5 mM for 1 min. For each  $\alpha_2$ M concentration, an identical control incubation was treated with vehicle (H<sub>2</sub>O) instead of BS<sup>3</sup>. Crosslinking reactions were terminated instantaneously by acidification [4]. Samples were then denatured in 2.0% SDS for 30 min at 37°C, supplemented with Tris-HCl (100 mM) and glycerol (10%), and subjected to SDS-PAGE. The gels were sliced into 3 mm sections and the radioactivity content of each section was determined in a gamma counter. <sup>125</sup>I-Cytokine recovered in association with  $\alpha_2$ M ( $AC_e$ ) included BS<sup>3</sup>-stabilized  $AC$  and  $AC^*$ . Free cytokine (in the gels) ( $C_e$ ) included  $C$  plus  $AC$  which was not BS<sup>3</sup>-stabilized.  $AC^*$  was quantitated independently by SDS-PAGE analysis of samples that were not BS<sup>3</sup>-treated. Apparent  $K_d$  values were determined according to the following equation:

$$\frac{C_e}{AC_e} = \left( \frac{K_d}{z} \right) \left( \frac{1}{A} \right) + \left( \frac{1}{z} - 1 \right) \quad (2)$$

$z$  is the BS<sup>3</sup>-crosslinking efficiency, a constant ( $0 < z < 1$ ) for each cytokine and  $\alpha_2$ M derivative which does not vary as a function of the  $\alpha_2$ M concentration.  $AC_e$  is related to  $AC$  by the relationship:  $[AC_e] = z[AC]$ . Assumptions involved in the use of this method have been reviewed [1,2]. These include that the  $K_D$  value reflects a single cytokine-binding site per  $\alpha_2$ M and that all of the  $\alpha_2$ M in a given preparation binds cytokine with equal affinity.

The methods for preparing  $\alpha_2$ M-P and  $\alpha_2$ M-P/M involve the binding of an average of 17 mol platinum per mol of  $\alpha_2$ M [12]. Since there is almost certainly heterogeneity in the extent of platinum binding and because the amino acids modified probably vary,  $\alpha_2$ M-P and  $\alpha_2$ M-P/M must be viewed as heterogeneous preparations in which different molecules may bind <sup>125</sup>I-TGF- $\beta$  with different affinities. Each  $K_d$  value determined for  $\alpha_2$ M-P or  $\alpha_2$ M-P/M, by the BS<sup>3</sup>-rapid crosslinking method, is a preparation-averaged constant, related to the different  $K_d$  values of  $n$  different  $\alpha_2$ M species ( $A_i$ ) by the following equation:

$$\frac{1}{K_d} = \frac{1}{K_{d1}} - \frac{\sum_{i=2}^n A_i}{K_{d1} A_T} + \frac{1}{K_{d2}} - \frac{\sum_{i=1,3}^n A_i}{K_{d2} A_T} + \frac{1}{K_{d3}} - \frac{\sum_{i=1,2,4}^n A_i}{K_{d3} A_T} + \dots \quad (3)$$

$A_T$  is the total concentration of all  $\alpha_2$ M species in the preparation (sum of  $A_i$ ). As an example, if an  $\alpha_2$ M preparation consists of two species in equal proportion, which bind a given cytokine with  $K_d$  values of 100 and 500 nM, then the preparation-averaged  $K_d$  value would be 167 nM. When multiple species are present in an  $\alpha_2$ M preparation, plots of  $C_e/AC_e$  against  $1/[A_T]$  (according to Eq. 2) remain linear. Preparation-averaged  $K_d$  values are not affected by differences in the BS<sup>3</sup>-crosslinking efficiencies ( $z$  values) amongst various  $\alpha_2$ M species within a given preparation.

### 2.4. Inhibition of endothelial cell growth

FBHE cells were maintained in DMEM supplemented with 10% FBS, 20 ng/ml acidic fibroblast growth factor, and 80 ng/ml basic fibroblast growth factor. Cultures were passaged at subconfluence with trypsin-EDTA. FBHE cell proliferation assays were performed in dilute (0.2%) serum as previously described [26,27]. Briefly, FBHE cells were plated in 24-well culture plates ( $2 \times 10^4$  cells/well) and incubated in DMEM with 10% FBS for 15 h. After washing, fresh DMEM, supplemented with 0.2% FBS and TGF- $\beta$ 1, was added. Some cultures were simultaneously treated with native  $\alpha_2$ M,  $\alpha_2$ M-P,  $\alpha_2$ M-P/M, or  $\alpha_2$ M-MA. After incubation for 30 h, [<sup>3</sup>H]thymidine was added for an additional 18 h. The cells were then harvested and radioactivity incorporation was measured in a scintillation counter.

## 3. Results

### 3.1. Nondenaturing PAGE analysis of the $\alpha_2$ M derivatives

Nondenaturing PAGE is commonly used to analyze  $\alpha_2$ M conformation [24,25]. Fig. 1 shows a representative nondenaturing PAGE experiment in which the four  $\alpha_2$ M preparations were compared. Native  $\alpha_2$ M migrated in a single Coomassie-stained band. The mobility of methylamine-modified  $\alpha_2$ M was increased compared with that of native  $\alpha_2$ M reflecting the transition to the fully activated conformation. As expected, the mobility of  $\alpha_2$ M-P/M was intermediate between native  $\alpha_2$ M and  $\alpha_2$ M-MA, reflecting partial conformational change [12,18]. The low mobility bands in the  $\alpha_2$ M-P and  $\alpha_2$ M-P/M preparations suggest some intermolecular  $\alpha_2$ M-crosslinking, due to the high concentration of *cis*-Pt used.

### 3.2. Equilibrium binding of <sup>125</sup>I-cytokines to $\alpha_2$ M-P and $\alpha_2$ M-P/M

The BS<sup>3</sup>-rapid crosslinking method was used to determine  $K_d$  values for the binding of <sup>125</sup>I-TGF- $\beta$ 1 and <sup>125</sup>I-TGF- $\beta$ 2 to  $\alpha_2$ M-P and  $\alpha_2$ M-P/M. In preliminary time-course experiments, noncovalent binding of each cytokine to the modified  $\alpha_2$ M derivatives maximized within 15 min (results not shown). Fig. 2A shows a representative autoradiograph in which <sup>125</sup>I-TGF- $\beta$ 1 was incubated with various concentrations of  $\alpha_2$ M-P for 30 min, and then with BS<sup>3</sup>. The amount of <sup>125</sup>I-TGF- $\beta$ 1- $\alpha_2$ M-P complex detected was dependent on the  $\alpha_2$ M-P concentration. In the control gel, which contained samples that were treated with vehicle instead of BS<sup>3</sup>, the amount of <sup>125</sup>I-TGF- $\beta$ 1- $\alpha_2$ M-P complex was consistently less than 20% of

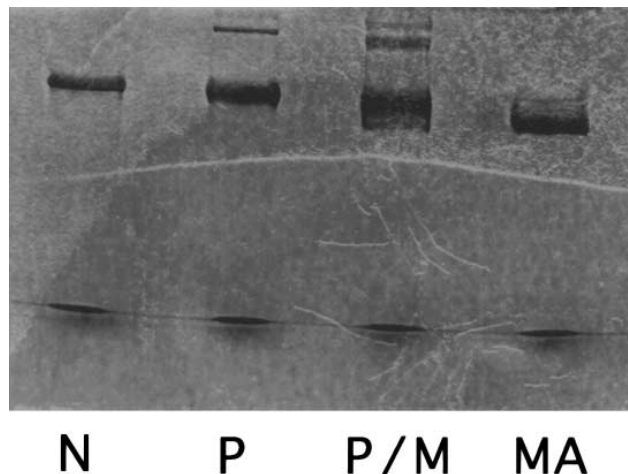


Fig. 1. Nondenaturing PAGE analysis of  $\alpha_2$ M derivatives. Native  $\alpha_2$ M (N),  $\alpha_2$ M-P (P),  $\alpha_2$ M-P/M (P/M) and  $\alpha_2$ M-MA (MA) are shown. The gel was stained with Coomassie blue R-250.

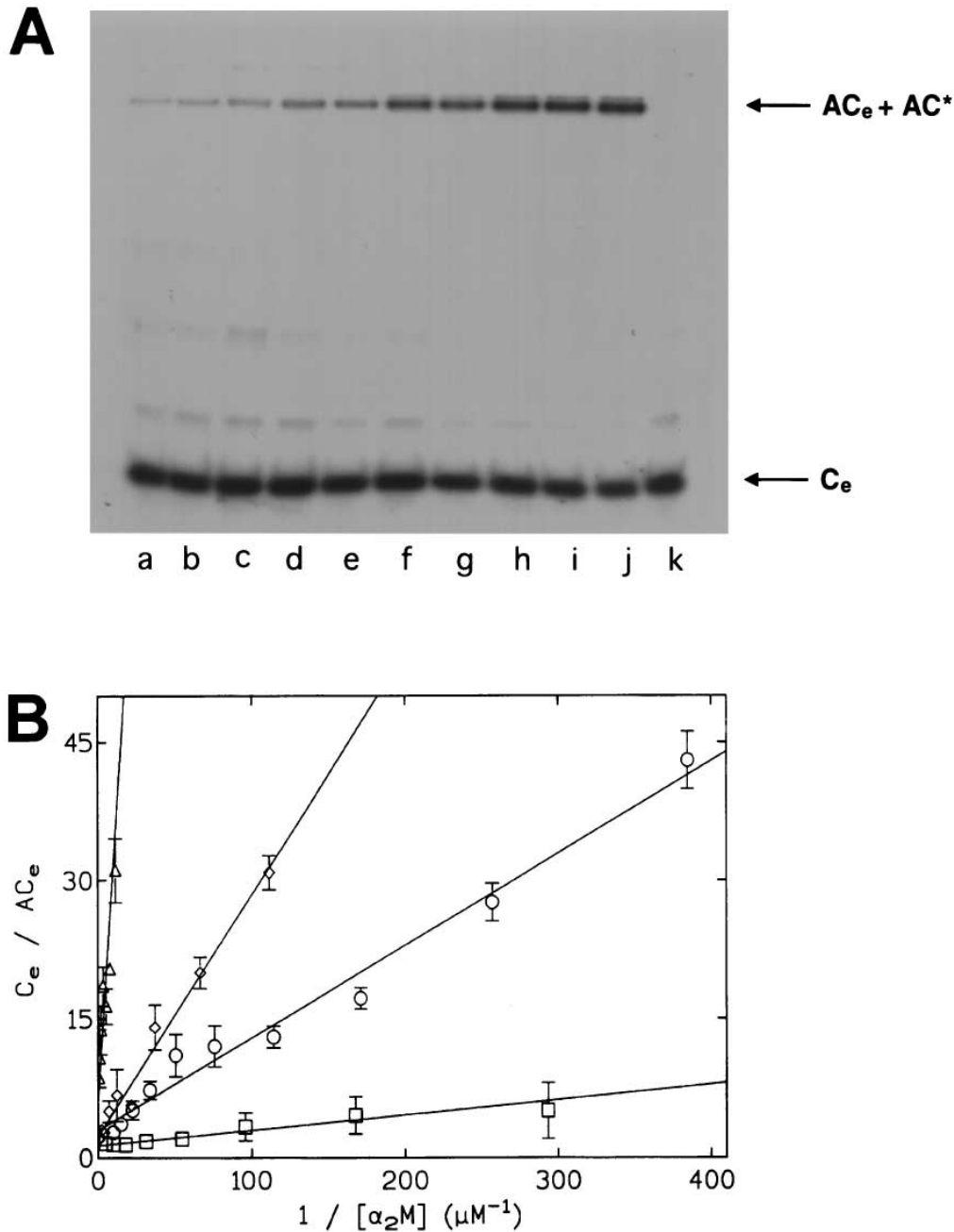


Fig. 2. Equilibrium binding of  $^{125}\text{I}$ -TGF- $\beta$ 1 to  $\alpha_2\text{M}$ -P and  $\alpha_2\text{M}$ -P/M.  $^{125}\text{I}$ -TGF- $\beta$ 1 was incubated with  $\alpha_2\text{M}$ -P for 30 min at 37°C. The samples were pulse-exposed to 5 mM BS $^3$  and subjected to SDS-PAGE and autoradiography. The autoradiograph of a representative gel is shown in (A). The concentrations of  $\alpha_2\text{M}$ -P were 2 nM (lane a), 4 nM (lane b), 6 nM (lane c), 9 nM (lane d), 13 nM (lane e), 20 nM (lane f), 30 nM (lane g), 45 nM (lane h), 67 nM (lane i), 0.1  $\mu\text{M}$  (lane j), and 0 nM (lane k). The terms,  $\text{AC}_\text{e}$ ,  $\text{AC}^*$ , and  $\text{C}_\text{e}$  are defined in the text. B: Results of BS $^3$ -rapid crosslinking studies analyzing the binding of TGF- $\beta$ 1 to native  $\alpha_2\text{M}$  ( $\Delta$ ),  $\alpha_2\text{M}$ -P ( $\circ$ ),  $\alpha_2\text{M}$ -P/M ( $\square$ ) and  $\alpha_2\text{M}$ -MA ( $\diamond$ ). The results of four separate experiments were averaged and plotted according to Eq. 2 in the text.

that detected with BS $^3$  (not shown). Thus,  $\text{AC}^*$  represented only a small fraction of the covalent  $\alpha_2\text{M}$ -P-TGF- $\beta$ 1 complex recovered after BS $^3$  treatment. Similar results were obtained when  $\alpha_2\text{M}$ -P/M was substituted for  $\alpha_2\text{M}$ -P and when TGF- $\beta$ 2 was studied.

Fig. 2B shows plots of  $\text{C}_\text{e}/\text{AC}_\text{e}$  against  $1/A_\text{T}$  for the binding of  $^{125}\text{I}$ -TGF- $\beta$ 1 to native  $\alpha_2\text{M}$ ,  $\alpha_2\text{M}$ -P,  $\alpha_2\text{M}$ -P/M and  $\alpha_2\text{M}$ -MA. The presented graphs were generated from the results of at least four separate experiments. Individual experiments, with TGF- $\beta$ 1 and TGF- $\beta$ 2, were analyzed using similar plots,

Table 1 Equilibrium dissociation constants for $^{125}\text{I}$ -TGF- $\beta$ -binding to $\alpha_2\text{M}$				
Cytokine	$\alpha_2\text{M}$ -P (nM)	$\alpha_2\text{M}$ -P/M (nM)	Native $\alpha_2\text{M}$ (nM)	$\alpha_2\text{M}$ -MA (nM)
TGF- $\beta$ 1	$36 \pm 2$	$14 \pm 4$	$320 \pm 65$	$82 \pm 6$
TGF- $\beta$ 2		$2 \pm 1$	$14 \pm 3$	$15 \pm 2$

Results were determined using the BS $^3$ -rapid crosslinking method. Each value represents the mean  $\pm$  SE ( $n=4$ ). The  $K_\text{d}$  values for the binding of each cytokine to native  $\alpha_2\text{M}$  and  $\alpha_2\text{M}$ -MA have been presented previously [1,2].

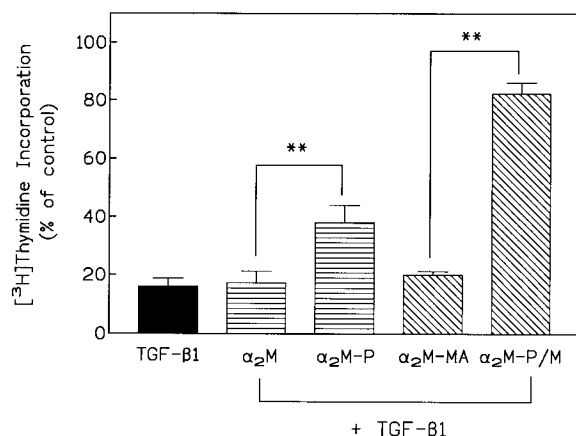


Fig. 3. Effects of  $\alpha_2$ M on TGF- $\beta$ 1 activity in an endothelial cell proliferation assay. FBHE cells were incubated with 10 pM TGF- $\beta$ 1 in the presence and absence of 100 nM native  $\alpha_2$ M,  $\alpha_2$ M-P,  $\alpha_2$ M-MA, or  $\alpha_2$ M-P/M. The culture medium contained 0.2% FBS. After 30 h, 1  $\mu$ Ci/ml [ $^3$ H]thymidine was added to the cultures for an additional 18 h. [ $^3$ H]thymidine incorporation was then determined and expressed as a percentage of that observed in control cultures, which were not exposed to  $\alpha_2$ M or TGF- $\beta$ 1. \*\*Statistically significant differences ( $P < 0.005$ ).

which were all apparently linear, as expected.  $K_d$  values from individual experiments were averaged to obtain the constants presented in Table 1. Binding constants for the interaction of TGF- $\beta$ 1 and TGF- $\beta$ 2 with native  $\alpha_2$ M and  $\alpha_2$ M-MA have been presented previously [2].

As shown in Table 1,  $\alpha_2$ M-P/M and  $\alpha_2$ M-P bound TGF- $\beta$ 1 with higher affinity than  $\alpha_2$ M-MA. The  $K_d$  for the binding of TGF- $\beta$ 1 to  $\alpha_2$ M-P/M (14 nM) was decreased by a factor of 23 compared with the  $K_d$  for TGF- $\beta$ 1 binding to native  $\alpha_2$ M (320 nM). The  $K_d$  for the binding of TGF- $\beta$ 2 to  $\alpha_2$ M-P/M (2 nM) is the lowest binding constant reported for any cytokine and any  $\alpha_2$ M derivative studied to date.

### 3.3. $\alpha_2$ M-P and $\alpha_2$ M-P/M counteract the activity of TGF- $\beta$ in cell culture

TGF- $\beta$  inhibits FBHE proliferation in cell culture and this activity is counteracted by  $\alpha_2$ M [26,27]. Experiments with a variety of human  $\alpha_2$ M derivatives and  $\alpha$ -macroglobulins from different species have shown that the fraction of TGF- $\beta$  activity neutralized directly correlates with the affinity of the  $\alpha$ -macroglobulin/TGF- $\beta$  interaction [3,26,27]. Since the  $K_d$  values determined for cytokine binding to  $\alpha_2$ M-P and  $\alpha_2$ M-P/M were preparation-averaged values, we wished to confirm that these constants accurately predict the cytokine-binding and/or -neutralizing activities of the  $\alpha_2$ M preparations. Fig. 3 shows the results of FBHE proliferation experiments in which 10 pM TGF- $\beta$ 1 inhibited [ $^3$ H]thymidine incorporation by an average of 84%. A fairly low concentration of each  $\alpha_2$ M derivative (100 nM) was added to different FBHE cultures such that high-affinity TGF- $\beta$ - $\alpha_2$ M interactions would be selectively detected. As expected, native  $\alpha_2$ M did not significantly affect [ $^3$ H]thymidine incorporation while  $\alpha_2$ M-MA had only a small effect which was not statistically significant at the  $P < 0.05$  level. By contrast,  $\alpha_2$ M-P substantially reversed the growth inhibition caused by TGF- $\beta$ 1 and  $\alpha_2$ M-P/M was even more effective. The 'double-stars' in Fig. 3 indicate statistically significant differences at the  $P < 0.005$  level. The results of these FBHE growth inhibition studies confirm that the  $K_d$  values,

determined by the BS<sup>3</sup>-rapid crosslinking method, accurately predict the TGF- $\beta$ -neutralizing activities of the modified  $\alpha_2$ M preparations, even though these preparations are heterogeneous.

## 4. Discussion

Native  $\alpha_2$ M functions as a physiologically significant carrier of TGF- $\beta$  in the blood [1]. Circulating  $\alpha_2$ M-TGF- $\beta$  complexes are mostly noncovalent and reversible [28,29]. Thus,  $\alpha_2$ M may provide a stable pool of slowly releasable cytokine activity, in the plasma, under normal homeostatic conditions. At sites of inflammation or other pathological processes that occur in tissue, concentrations of TGF- $\beta$  isoforms may be in rapid flux and  $\alpha_2$ M may serve to buffer cells against the full impact of changing TGF- $\beta$  activity. Cell culture experiments, in which cells are exposed to a bolus of cytokine or allowed to respond to autocrine-secreted cytokines, probably model the microenvironment of tissues more closely than plasma. In these *in vitro* systems,  $\alpha_2$ M has been shown to regulate cellular growth and gene expression by binding TGF- $\beta$  [26,27,30,31].

The binding affinity of  $\alpha_2$ M for TGF- $\beta$  isoforms and other cytokines is dependent on the conformational state of the  $\alpha_2$ M [1]. While most previous studies have been conducted using native  $\alpha_2$ M or fully activated, methylamine-modified  $\alpha_2$ M, the studies presented here demonstrate that neither of these well-studied  $\alpha_2$ M conformations have optimized cytokine binding activity.  $\alpha_2$ M-P and  $\alpha_2$ M-P/M bound TGF- $\beta$ 1 and TGF- $\beta$ 2 with higher affinity than previously studied forms of  $\alpha_2$ M. The same derivatives also neutralized the activity of TGF- $\beta$ 1 in FBHE proliferation assays, confirming the validity of the  $K_d$  values, determined by the BS<sup>3</sup>-rapid crosslinking method. Thus, these chemically modified  $\alpha_2$ M derivatives represent superior TGF- $\beta$ -binding agents.

Based on hydrodynamic and electron microscopy studies, we previously proposed that  $\alpha_2$ M-P/M may model intermediates which occur transiently during  $\alpha_2$ M conformational change *in vivo* [12,13,18,19]. However, electron microscopy images of  $\alpha_2$ M-P already show some changes in structure compared with native  $\alpha_2$ M [12]. Thus, the model of *cis*-Pt as an  $\alpha_2$ M 'conformational lock' may be oversimplified. The increased TGF- $\beta$ -binding affinity of  $\alpha_2$ M-P, compared with native  $\alpha_2$ M, supports the hypothesis that *cis*-Pt alone induces some changes in the structure of  $\alpha_2$ M that are yet to be defined.

In conclusion, we have shown that the TGF- $\beta$ -binding activity of  $\alpha_2$ M can be modified by reagents which alter the conformation of the molecule. The newly described derivatives may be useful as TGF- $\beta$ -activity modifiers *in vitro* and *in vivo*. Whether the same  $\alpha_2$ M modification protocol will enhance binding of other cytokines remains to be determined.

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