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Transforming growth factor β and a mesoderm inducing factor from human blood platelets are different proteins

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A mesoderm inducing factor from human blood platelets has been separated from transforming growth factor β and partially purified. The factor has similar chemical properties as mesoderm inducing factors from other sources.

Human blood platelet; Transforming growth factor β; Mesoderm inducing factor

1. INTRODUCTION

Factors which induce mesodermal tissues in the ectoderm of early amphibian embryos have been isolated from different sources [1–5]. The inducing proteins are in their chemical properties related to the transforming growth factors β (TGF-βs). It has been shown that TGF-β_1 from human blood platelets [6] and TGF-β_2 from porcine blood platelets [7,8] induce mesodermal tissues as mesothel, endothel, mesenchyme, muscle and notochord [7,8]. The concentration of TGF-βs, which is needed for mesoderm induction, is considerably higher when compared to the concentrations of the mesoderm inducing factors. The TGF-βs and the mesoderm inducing factors were isolated from different species. Species differences could therefore account for the different activities. We have tested the transforming activity and the inducing activity in chromatographically separated fractions from the same source to show whether the two activities can be separated or whether they belong to the same molecule. Transforming and inducing activities were extracted from human blood platelets which have a high content of TGF-β_1 [9] and induce also mesodermal tissues. The chemical properties of a partially purified mesoderm inducing factor were then compared with the properties of mesoderm inducing factors from other sources.

2. METHODS AND MATERIALS

2.1. *Chromatographic procedures*

Human blood platelets outdated for 1–3 days were used for the extraction of the factors. 12–15 mg of the extracted protein were applied to a 1.6 × 98 cm Bio-Gel P-60 column and eluted at +4°C with 1 M acetic acid at 5 ml/h. The molecular weight of the eluted proteins was inferred from their position in the chromatograms. The inducing fraction (≈1.0–1.5 mg protein) was applied in 0.3 mg aliquots, which were each dissolved in 0.1 ml 50% formic acid (Merck, p.A.; quartz distilled), to a 4 × 500 mm SE-HPLC column (Knauer, Berlin) filled with Polyol-Si 300 silica gel (5 μm; Serva, Heidelberg). Elution: 2 ml of 50% formic acid/min. Temperature: 20–22°C. Pressure: 12 MPa. The molecular weight of the eluted proteins was determined by a plot of the elution volume against the logarithm of the molecular weight of standard proteins [10]. The eluates were evaporated in a Speed-Vac, the residues taken up in distilled water and lyophilized. The protein eluates from five SE-chromatographies were combined for the test. Treatment with sodium thioglycolate and protein determinations [3] were carried out as described.

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2.2. *Biological tests*

The lyophilized proteins were taken up in 7 M urea. Test fractions were prepared by the addition of γ-globulin to a total amount of 2 mg protein. The material for the Bio-Gel chromatography test fractions (Table I) comes from one platelet unit, the material for the SE-HPLC test fractions (Table II) from two platelet units. After dialysis at +4°C against distilled water, the pellets for the implantation test [11,12] on *Triturus alpestris* gastrulae were prepared as described [3].

To the solution of the proteins to be tested on isolated gastrula ectoderm of *Xenopus laevis* bovine serum albumin (fraction V, Sigma 4503, 1 mg/ml final test vol.) was added, the proteins dissolved in 4 mM HCl, neutralized and prepared for the test as described [3]. The histological sections were examined.

TGF-β activity was determined by the colony forming activity of rat kidney fibroblasts of the NRK (49F) cell line in soft agar [13].

3. RESULTS AND DISCUSSION

Human blood platelets were extracted with acid/ethanol and the extracts precipitated with ethanol/ether. The protein precipitate was dissolved in 1 M acetic acid and chromatographed on Bio-Gel P-60 [9]. The eluted fractions (Fig. 1) were tested for TGF-β and for mesoderm inducing activity. TGF-β was eluted in fraction III in an amount of about 0.35 μg/platelet unit. As previously described [9] besides other proteins

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Table I

Chromatography of platelet extract on Bio-Gel P-60, tested by the implantation method

Fraction[a]	Positive (%)	Size of inductions (%)			Induced tissues (%)	
		Large	Medium	Small	Trunk/tail	Not specified
I	54	8	15	31	23	31
II	7	0	0	7	0	7
III	0	0	0	0	0	0
IV	80	14	33	33	53	27

[a]Derived from one blood platelet unit (see section 2)

Table II

SE-HPLC of Bio-Gel P-60 fraction IV

Fraction[a]	Positive (%)	Size of inductions (%)			Induced tissues (%)	
		Large	Medium	Small	Trunk/tail	Not specified
(a) Tested by the implantation method						
III	73	13	33	27	46	27

Fraction[a]	Positive (%)	Induced tissues (%)					
		Muscle		Endothel. + mesothel.	Mesenchyme	Blood cells	Blastema
		Skeletal	Heart				
(b) Tested on isolated ectoderm in solution							
III	100	20	5	100	100	50	25

[a]Derived from two blood platelet units (see section 2)

a band at about 25 kDa, the molecular mass of TGF-β [9], is shown in SDS-polyacrylamide gel electropherograms. The fraction induced colony formation in soft agar cultures of rat kidney fibroblasts indicating TGF-β activity. The amount of TGF-β in this fraction is too low to evoke mesoderm inductions [6]. Most of the mesoderm inducing activity (trunk and tail inductions with muscle and notochord) is, on the other hand, eluted in fraction IV (Table I, range of M_r 23–25 kDa). This shows that TGF-β and the mesoderm inducing factor in the platelets are not identical but can be separated by chromatography on Bio-Gel P-60. The molecular mass of the inducing factor from blood platelets is similar to that of the electrophoretically homogeneous mesoderm inducing factor from chicken embryos (≈ 25 kDa) [4] and TGF-β [9]. The separation of factors with similar molecular mass by size exclusion chromatography could depend on interactions with the Bio-Gel matrix. The amount of the inducing factor in fraction IV is 10–20 ng/platelet unit, if one assumes that the specific activity is similar to the factor from chicken embryos. The factors could not be separated by chromatography on Sephadex G-100 with 1 M formic acid/6 M urea as the eluent. The part of the mesoderm

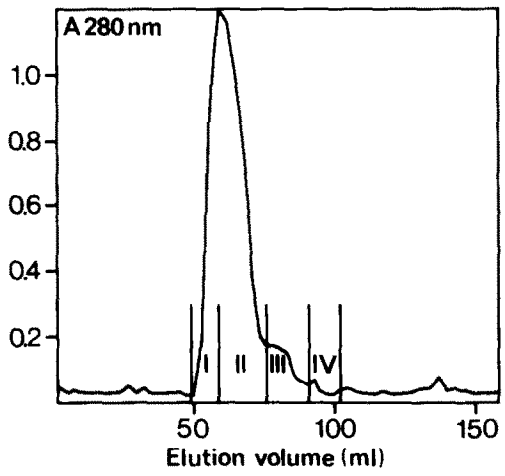

Fig. 1. Chromatography of blood platelet extracts on Bio-Gel P-60.

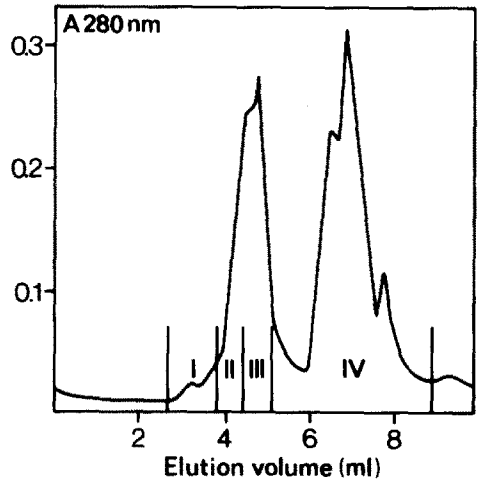

Fig. 2. SE-HPLC of fraction IV of the Bio-Gel P-60 chromatography.

inducing factor which is eluted from Bio-Gel P-60 fraction I (Fig. 1 and Table I) probably forms complexes with larger proteins. The inducing activity in fraction IV was abolished when the fraction was reduced with sodium thioglycolate probably because wrong disulfide bridges and mixed disulfides with the reducing agent are formed during reoxidation of the reduced sample at the preparation for the biological test. Mesoderm inducing factors from chicken embryos [14], calf kidney [5] and the *Xenopus* XTC-cell line [2,3] as well the TGF-βs [13] are likewise inactivated after treatment with mercaptoethanol or sodium thioglycolate. They dissociate into subunits of $M_r \approx 13$ kDa after reduction [2,4,5,13] with these agents.

Fraction IV was then subjected to size exclusion HPLC with 50% formic acid as the eluent. Only fraction III is active, whereas the other fractions did not show inducing activity (Fig. 2 and Table II). The material in fraction III comes from twice the amount of platelets as the Bio-Gel fraction IV (see section 2). This means that the activity is only partially recovered, since both fractions show nearly the same activity. Fraction III encloses proteins of 12–15 kDa (see section 2). The factor is obviously dissociated into subunits of ≈13 kDa by formic acid. The inducing factors from chicken embryos, calf kidney and the *Xenopus* XTC-cell line were under these conditions also dissociated into subunits [1,3,5].

Conformational changes caused by formic acid could dissociate the factors into subunits not covalently bound by disulfide bridges. But formic acid, a reducing agent, which is present in large excess can also reduce disulfide bridges ($HCOOH \rightleftharpoons CO_2 + 2H^+ + 2e^- \; E_o = -0.42$ V; $2\,RSH \rightleftharpoons R\text{-}S\text{-}SR + 2H^+ + 2e^- \; E_o = -0.23$ V [15]). To what extent interchain or intrachain disulfide bonds are reduced is, however, not known. The cleavage of intrachain disulfide bonds could lead to conformational changes of the chains and in turn also to their dissociation (for example see [16]). A third possibility, cleavage of acid sensitive aspartyl peptide bonds [17,18] is, under the conditions employed for the SE-HPLC, less likely.

The mesoderm inducing factor from human blood platelets has similar chemical properties as the mesoderm inducing factors, which were isolated from other sources (M_r, acid stability, composition of two subunits, hydrophobicity). The factor is not identical with TGF-β. This, however, does not exclude that other members of the TGF-β superfamily could be more closely related to the mesoderm inducing factors. Recent experiments by Asashima et al. [5,19] have shown that the EDF, which is identical to activin A [20] induces mesodermal tissues at a low concentration and that the mesoderm inducing factors isolated from chicken embryos and from calf kidney have EDF-activity, similar to recombinant EDF.

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