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PURIFICATION OF A HIGH MOLECULAR WEIGHT SOMATOMEDIN BINDING PROTEIN FROM HUMAN PLASMA

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SUMMARY: The somatomedins insulin-like growth factor I and II (1,2) are in serum bound to high-molecular weight binding proteins (6,7,8). By use of a four step chromatographic procedure a somatomedin binding protein was isolated from outdated human plasma. Exclusion chromatography on Sephadex G-200 disclosed a molecular weight of 150 kDa. After lyophilization however, the binding activity was found in a lower molecular weight range of 35-45 kDa. A partial amino acid sequence analysis of the lyophilized material revealed a possible N-terminal sequence of Ala-Pro-Trp. This sequence is identical to the N-terminal sequence of the 35 kDa somatomedin binding protein previously isolated from human amniotic fluid (16).

Two main forms of somatomedins exist in serum, termed insulin-like growth factor I and II (IGF-I and IGF-II) (1,2). Somatomedin C and somatomedin A are homologues to IGF-I, while multiplication stimulating activity (MSA III-2) is the rat homologue to human IGF-II (3,4,5). Unlike insulin and other circulating polypeptide hormones, the somatomedins have the unique property of being bound to binding proteins (6,7,8). In normal and acromegalic sera a 150 kDa somatomedin binding protein (SMBP) is more abundant, while in serum from GH-deficient patients a 40-60 kDa binding protein predominates (9,10,11,12).

The 150 kDa SMBP in serum is stable under neutral conditions, while the acid extraction procedure generally used for the isolation of somatomedins, converts the 150 kDa SMBP to a lower molecular weight form of 40-60 kDa (13,14). Attempts to recombine the 150 kDa complex after acid dissociation or other purification procedures, have been met with limited success (15). The reason for this lability of the high molecular weight form is not known. In the present work a new approach for purifying the 150 kDa SMBP under neutral conditions is described.

Abbreviations: IGF-I, insulin-like growth factor I; IGF-II insulin-like growth factor II; SmC, Somatomedin C; MSA, multiplication stimulating activity; SMBP, somatomedin binding protein; HPLC, high performance liquid chromatography; FPLC, fast protein liquid chromatography; RIA, radioimmunoassay.

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MATERIAL and METHODS

Material. Outdated human plasma was used as starting material. IGF-I and IGF-II was purified as previously described (4). CM-Affigel Blue and the HPLC hydroxy apatite (HPHT) column were purchased from BioRad, Richmond, CA, USA, the Ultrogel hydroxy apatite from LKB, Bromma, Sweden and the FPLC Superose SR-12 column from Pharmacia, Uppsala, Sweden. All other reagents were of analytical laboratory grade.

Affinity chromatography. To a column of CM-Affigel Blue (13x7 cm) 200-250 ml of serum diluted 4:1 with 0.05 M potassium phosphate was applied. The elution was performed with a stepwise sodium chloride gradient between 0.05 to 1.4 M, pH 7.3. The fraction containing somatomedin binding activity was adsorbed by a Ultrogel hydroxy apatite column (5x4 cm) at 4^{o} C. When fractions derived from 2-3 l of serum had been adsorbed, the hydroxy apatite column was eluted by a stepwise potassium phosphate gradient, pH 7.3 in room temperature at a flow of 1.5 ml/min. Elution of the HPLC hydroxy apatite column (7.8x100 mm) was performed in room temperature by a linear potassium phosphate gradient between 0.05-0.5 M, pH 7.0 at a flow of 0.35 ml/min, during 90 min.

Exclusion chromatography. The Sephadex G-200 column (1.6x51 cm) was eluted at room temperature with phosphate buffered saline at a flow of 0.19 ml/min. Sample volume applied was 0.5 ml.

FPLC gel permeation chromatography was performed by use of a SR-12 column (1x30 cm) eluted with 0.05 M potassium phosphate, pH 7.1, at a flow of 0.4 ml/min, in room temperature.

Electrophoresis. The polyacrylamide gel electrophoresis was performed under non-denaturating conditions in 0.05 M Tris-borate, pH 8.35. The samples were electrophoresed at 20 mA for 1.5 h and stained with 0.25 % Coomassie brilliant blue.

Ultrafiltration. Ultrafiltration was performed with Ultra-Thimbles (Schleicher & Schüll, West Germany), molecular weight cut-off 10 kDa.

RESULTS

The majority of somatomedins and somatomedin binding proteins present in serum was adsorbed to the CM-Affigel blue column at neutral pH, whereas approximately 2/3 of the protein content passed through. The adsorbed material was eluted by a stepwise sodium chloride gradient as shown in Fig. 1. The fractions eluted with 0.2 M sodium chloride containing a high molecular weight binding protein (150 kDa SMBP), were applied to a hydroxy apatite column. When eluate from the Affigel column derived from 2-3 l of serum had been adsorbed, the hydroxy apatite column was eluted by a stepwise potassium phosphate gradient (Fig 2). The fraction eluted with 0.16 M potassium phosphate contained more binding activity than the other eluates. This fraction was further purified by FPLC gelpermeation chromatography, as shown in Fig 3. After separation on the SR-12 column the 150 kDa SMBP fractions (from 10-15 separations) were pooled and applied to a HPLC hydroxy apatite column. Two major fractions were eluted by a linear potassium phosphate gradient. The first fraction containing the 150 kDa SMBP was concentrated ten times by ultrafiltration and then subjected to a non-denaturating gel electrophoresis. Only one band was weakly discernable after staining with 0.25% Coomassie blue. The amount of [^{125}I]-IGF-II bound to acromegalic serum or the 150 kDa SMBP was analysed by exclusion chromatography on

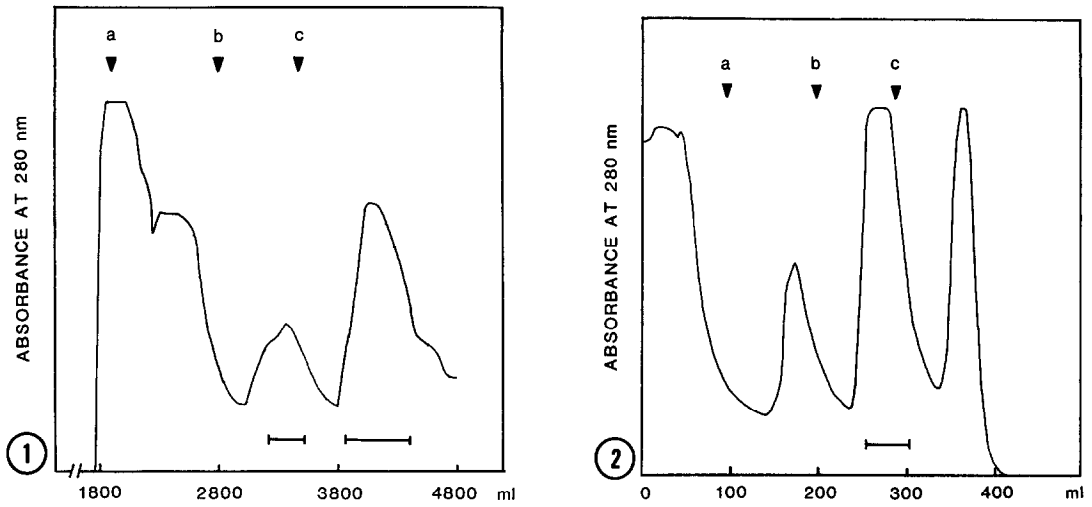

Fig. 1. Elution profile of serum applied to the CM-Affigel blue column eluted with a stepwise gradient of a) 0.1 M, b) 0.2 M, and c) 1.4 M sodium chloride, respectively. Fractions containing the 150 kDa SMBP (left bar), and the somatomedin activity (right bar) were pooled separately.

Fig. 2. Elution profile of the hydroxy apatite column (5x4 cm) eluted with a stepwise gradient of a) 0.08 M, b) 0.16 M , and c) 0.5 M potassium phosphate, respectively. Bar indicates fractions containing the 150 kDa SMBP.

Sephadex G-200. As demonstrated in Fig. 4, the elution profiles of [^{125}I]-IGF-II were similar for both samples. Cold IGF-II (50 ng/ml), but not insulin (4000 ng/ml), displaced [^{125}I]-IGF-II from the 150 kDa SMBP (Fig. 4). However, this pattern was changed after desalting and lyophilization of the 150 kDa SMBP. The bound [^{125}I]-IGF-II was now eluted at a molecular weight range of 35-45 kDa (data not shown). A partial amino acid sequence analysis of the lyophilized 150 kDa SMBP revealed a possible N-terminal sequence of Ala-Pro-Trp for the major components.

DISCUSSION

Several decades ago it was realized that factors with different biological activities, existed in serum as high molecular weight complexes (6,7,8). These factors, later known as somatomedins or insulin-like growth factors, could be dissociated from a high molecular weight binding protein by exposure to acidic conditions. However, this treatment converts the 150 kDa SMBP to an acid stable part with an estimated molecular weight of 40-60 kDa, which retains its binding capacity, while the remainder (the acid labile part) is destroyed. Thus, after exposure to acidic conditions it is not possible to regain the original 150 kDa SMBP by recombination of the smaller subunits.

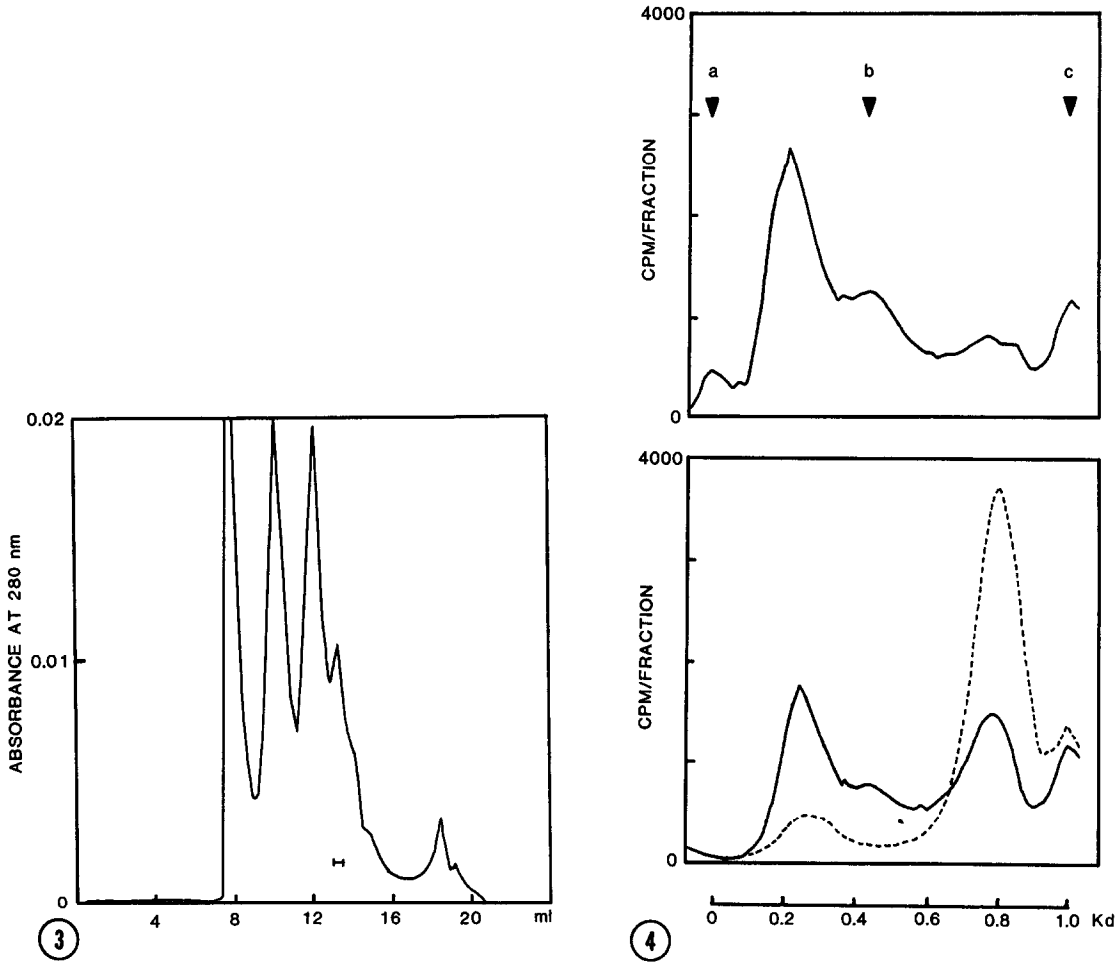

Fig. 3. Gel permeation chromatography with a FPLC SR-12 column. Elution was performed with 0.05 M potassium phosphate, pH 7.1, at flow of 0.4 ml/min. Bar indicates fraction containing the 150 kDa SMBP.

Fig. 4. Exclusion chromatography on Sephadex G-200 (1.6x51 cm). Solid line indicates elution profiles of [^{125}I]-IGF-II incubated with acromegalic serum (upper panel) or 150 kDa SMBP (lower panel). Dashed line indicates incubation performed in the presence of cold IGF-II (50 ng/ml). Elution position of Blue dextran, human serum albumin and sodium chloride denoted by a, b, and c, respectively.

As shown by the present results it is possible to develop a purification procedure for the 150 kDa SMBP, by which the molecular size is kept intact. However, for unknown reasons the purified 150 kDa SMBP decomposed after lyophilization. The structural analysis of this material disclosed an N-terminal sequence of Ala-Pro-Trp for the major components. This sequence is identical to the N-terminal sequence of the 35 kDa SMBP isolated from human amniotic fluid (16). This finding indicates that the 35 kDa SMBP could be a subunit of the 150 kDa SMBP, or that they both contain a similar amino acid sequence. Wilkins and

D'Ercole (17) found several forms of SMBPs after affinity labelling of human serum with SmC/IGF-I, and they proposed that the 150 kDa SMBP might be an oligomer of smaller SMBPs. In this connection it is of interest that the intact 150 kDa SMBP does not crossreact in the RIA for the 35 kDa SMBP (18), and that the 35 kDa SMBP is not detectable in a RIA for the 150 kDa SMBP (19).

Further chemical characterization of the 150 kDa SMBP is necessary in order to elucidate its relation to the 35 kDa SMBP. The present purification procedure makes it possible to isolate the 150 kDa SMBP in its intact form.

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