IDENTIFICATION OF TWO SOMATOMEDIN A ACTIVE POLYPEPTIDES AND IN VIVO EFFECTS OF A SOMATOMEDIN A CONCENTRATE

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Summary

The first chemical characterization of two polypeptides from human serum which stimulate the in vitro incorporation of ^{35}S sulfate into chick cartilage is described. These two polypeptides, designated Somatomedin A_1 and A_2 have a molecular weight of approximately 7000. Although each peptide contains 1 cysteine residue and has asparagine as amino terminal residue, there are apparent differences in the amino acid composition. Administration of a Somatomedin A concentrate to hypophysectomized rats gave an increase in tibial width similar to that obtained with 20 µg human growth hormone.

Introduction

In their now classical study Salmon and Daughaday [1] found that the in vitro stimulatory effect of serum on the incorporation of labelled sulphate into proteoglycans of cartilage was due to an intermediary, growth hormone dependent factor which they named the sulphation factor. Since then several anabolic effects obtained in vitro have been ascribed to the sulphation factor activity in serum or serum fractions [2]. As a result of these multiple effects the name somatomedin was introduced as a more general term for the sulphation factor [3]. Hall [4] reported in 1972 on the biological activity and purification of a human somatomedin. In 1973 Uthne [5] defined the somatomedin activity measured by the chicken cartilage assay of Hall [6] as the somatomedin A. At the same time it was demonstrated that serum contains at least two different somatomedins, since stimulatory effects on various types of cells appeared to be due to a factor different from somatomedin A. This second factor was tentatively defined as somatomedin B [5]. Subsequently van Wyk et al [2] isolated a somatomedin C, characterized by its activity in the cartilage segments of hypophysectomized mice.

In the present communication we want to report on the isolation and the first chemical characterization of two polypeptides having somatomedin A properties e.g. stimulating the uptake of labelled sulphate into chicken cartilage [6]. We also want to give some preliminary data on in vivo action of a somatomedin A concentrate.

Materials and Methods

The purification of the concentrate used in the studies in rats and the isolation of the two somatomedin A active peptides have been carried out by the procedure described in the companion paper by Fryklund et al [7]. Thus, an acid ethanol extract of Cohn fraction IV obtained from human plasma was chromatographed over Sephadex G-75 and Sephadex G-50 yielding the somatomedin A concentrate. This was partially used in the studies in rats, the remaining material being further purified by zone electrophoresis at pH 7.5 and pH 5.0 [7]. Gel filtration on Sephadex G-50 (fine) was used as a final step. End group analysis and amino acid analysis were performed as described [7].

Hypophysectomized female rats (Sprague-Dawley) weighing 60 - 75 g at the time of ectomy were used in the *in vivo* study. The rats were purchased from the Hormone Assay Company, Chicago, Ill. and kept on a standard rat chow diet with free access to drinking water. During the first week after ectomy, 5 % glucose and terramycin were added to the drinking water to prevent hypoglycemia and infections. The antibiotic was discontinued 5 days prior to the experiment. The animals were divided into groups of 6 rats. Subcutaneous injections were given daily for 5 consecutive days. The control animals were treated with 0.5 ml of a medium consisting of 2.5 g human albumin plus 0.9 g NaCl per 100 ml. Human growth hormone (Crescormon^{R}, AB Kabi, Stockholm, Sweden) and the somatomedin A preparation was dissolved in the same medium. The rats were weighed daily and sacrificed 24 h after the last injection. Tibial width was determined according to the method of Greenspan et al [8]. The somatomedin A preparation was studied at a dosage of 15 U per rat per day. One unit of somatomedin A is arbitrarily defined as the biological activity of one ml of a normal human reference serum determined by the chick embryo assay of Hall [6]. Crescormon^{R} was tested at dosages of 20 and 40 µg per animal and day.

Results and Discussion

The first gel filtration on Sephadex G-50 gave the chromatographic pattern shown in Fig. 1. The major part of the somatomedin A activity was found in fraction 1, while fraction 2 contained most of the somatomedin B activity [5,7]. A part of the material of fraction G-50-12-1 was used in the studies in rats while the remaining part was further purified.

The results of the effects on total body weight and tibial widht are shown in Figures 2 and 3. There was no significant effect on total body weight following treatment with the somatomedin A preparation whereas 20 µg of human

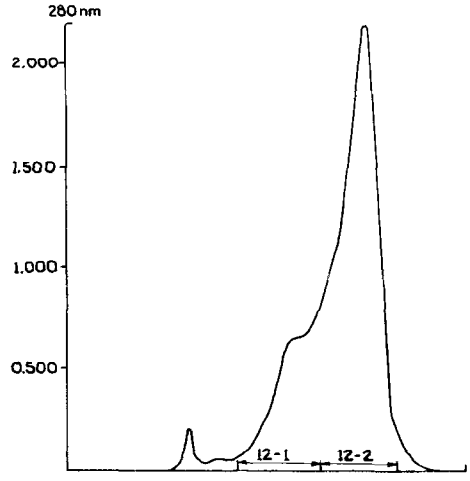


Figure 1

Gel chromatography on Sephadex G-50 medium (2.5 x 100 cm) in 1 % formic acid. Flow rate 25 ml/h, fraction size 6 ml. Fraction G-50-12-1 contained most of the SM-A active material [6].

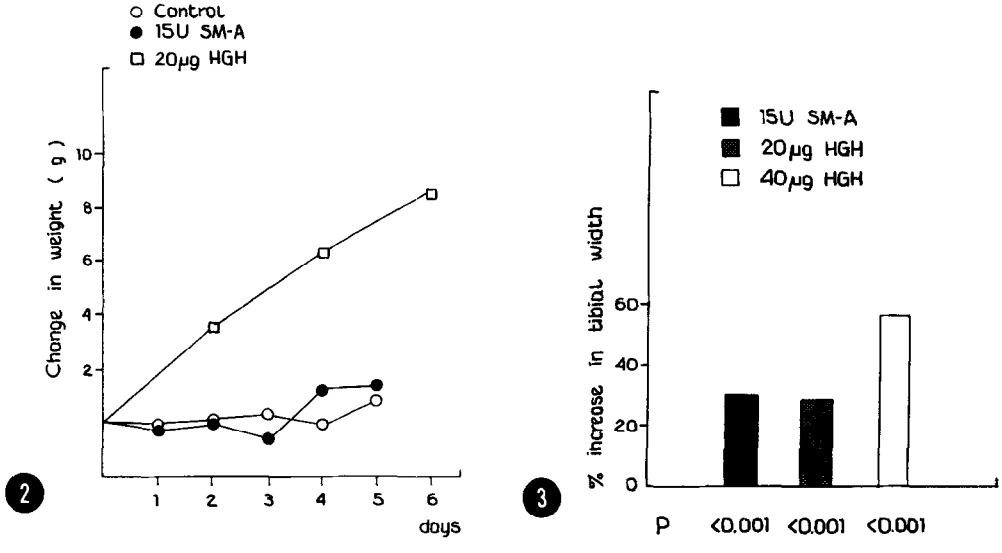
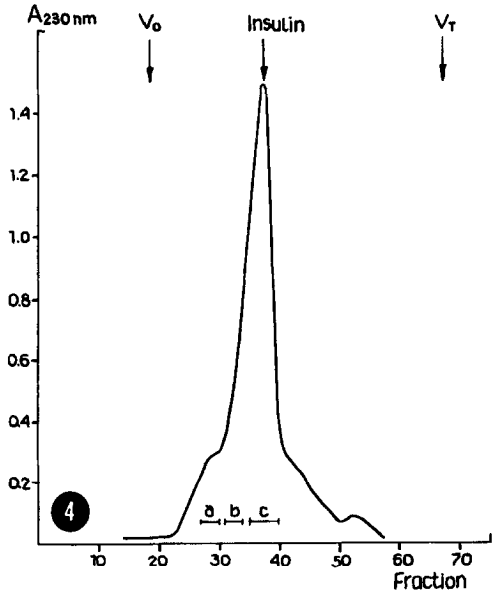


Figure 2

Effect on body weight of hypophysectomized rats treated with HGH and the somatomedin A concentrate (G-50-12-1).

Figure 3

Effect on the tibial width of hypophysectomized rats treated with HGH and the somatomedin A concentrate (G-50-12-1).



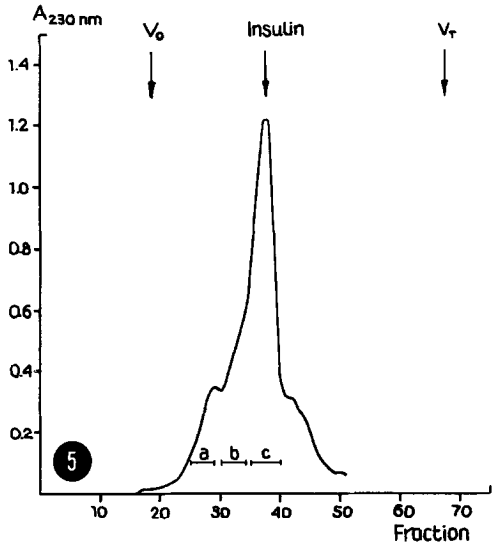


Figure 4

Gel chromatography of somatomedin A_1 on Sephadex G-50, fine, (1.5 x 88 cm) in 0.02 N HCl. Flow rate 10 ml/h, fraction size 2.3 ml. Fraction C increased sulphate uptake in vitro in chick cartilage [6].

Figure 5

Gel chromatography of somatomedin A_2. Experimental conditions see Figure 5. Fraction C increased sulphate uptake in vitro in chick cartilage [6].

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growth hormone caused a normal response (Fig. 2). However, tibial width was significantly affected by the administration of the somatomedin A concentrate, a 30 % increase being observed. This corresponds to the effect of 20 µg of human growth hormone (Fig. 3). The somatomedin A preparation used in the rats contains the major portion of the somatomedin A activity following chromatography on Sephadex G-50. However, the preparation is not homogeneous and consists of a mixture of peptides of similar molecular size. It can therefore not be excluded that the stimulatory effect on the tibia might be attributed to a combined effect of several factors, some of them not necessarily carrying somatomedin A activity. This question can only be answered when sufficient amounts of highly purified somatomedin A becomes available.

When the somatomedin A active region from the filtration on Sephadex G-50, (G-50-12-1, Fig. 1), was subjected to electrophoresis at pH 7.5 most of

Table I. Amino acid composition of two peptides with somatomedin A activity

	Somatomedin A_1	Somatomedin A_2
Aspartic acid	3.95 (4)	4.75 (5)
Threonine	4.08 (4)	1.85 (2)
Serine	7.46 (7-8)	3.50 (3-4)
Glutamic acid	4.84 (4)	4.65 (5)
Proline	7.79 (8)	7.30 (7)
Glycine	9.72 (10)	7.10 (7)
Alanine	4.63 (5)	7.00 (7)
Cysteine	* 1.00 (1)	** 1.00 (1)
Valine	3.81 (4)	4.90 (5)
Methionine	1.00 (1)	1.00 (1)
Isoleucine	1.28 (1)	0.81 (1)
Leucine	2.00 (2)	3.30 (3)
Tyrosine	0.53 (1)	0.97 (1)
Phenylalanine	1.04 (1)	2.20 (2)
Histidine	1.38 (1)	3.75 (4)
Lysine	2.65 (2-3)	2.50 (2-3)
Arginine	3.82 (4)	4.65 (5)
NH_2-terminal	Asn	Asn

* determined as S-carboxymethyl cysteine

** determined as Cysteic acid

the activity was found in the neutral region. This neutral material was further resolved on electrophoresis at pH 5.0, giving two adjacent but separated peaks (peak 2 and 3) which stimulated sulphate uptake. Each peak was further gel filtered on a small column of Sephadex G-50 (fine) in 0.02 N HCl. Peak 2 gave the pattern shown in Fig. 4, fraction C corresponding to somatomedin A_1. Fig. 5 shows the Sephadex G-50 purification of peak 3, fraction C corresponding to somatomedin A_2.

End group analysis yielded asparagine as the amino terminal residue in somatomedin A_1 as well as in somatomedin A_2. However, in SM-A_1 traces of glycine (< 5 %) and in SM-A_2 traces of threonine (< 10 %) were also identi-

fied. The amino acid analysis also indicates that the two peptides are not entirely homogeneous (Table 1). Based on the amino acid composition together with the elution volume from Sephadex G-50 the molecular weight of the two peptides is approximately 7000. Both substances increased the incorporation of labelled sulphate into proteoglycans of chicken cartilage [6]. However, a dose response curve parallel to that of the arbitrary reference serum used as a standard was not obtained. The chemical similarities between the two peptides are obvious and most striking is the presence of only one cysteine residue in each peptide. The most apparent differences are the serine and the histidine contents. Although we have apparently chemically separated two somatomedin A active peptides, we cannot exclude the possibility that one peptide might have been converted into the other due to enzymatic or chemical alterations and that the small differences in the amino acid compositions are partially due to peptide impurities. We have also considered the possibility that the two peptides may derive from a common "pro-somatomedin" yet to be discovered.

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Literature

1. Salmon W.D. jr and Daughaday W.H. (1957) J. Lab. Clin. 49, 825-836.
2. van Wyk J.J., Underwood L.E., Lister R.C. and Marshall R.N. (1973) Am. J. Dis. Child. 126, 705-711.
3. Daughaday W.H., Hall K., Raben M.S., Salmon W.D. jr, van den Brande J.L. and van Wyk J.J. (1972) Nature 235, 107.
4. Hall K. (1972) Acta Endocrinol Suppl 163, 1-52.
5. Uthne K. (1973) Acta Endocrinol Suppl 175, 1-35.
6. Hall K. (1970) Acta Endocrinol 63, 338-350.
7. Fryklund L., Uthne K., Sievertsson H. and Westermark B. (1974) Biochem. Biophys. Res. Commun (Companion paper).
8. Greenspan F.S., Li C.H., Simpson M.E. and Evans H.M. (1949), Endocrinology 45, 455-463.