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Synthetic somatomedin C: Comparison with natural hormone isolated from human plasma

(solid-phase peptide synthesis/insulin-like growth factor/radioreceptor assay/monoclonal antibody/cell proliferation)

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ABSTRACT **The biological and immunological properties of a chemically synthesized preparation of somatomedin C (Sm-C) were compared with those of the natural product isolated from human plasma. The two preparations produced identical curves in the radioimmunoassay and radioreceptor assay for Sm-C and in the radioreceptor assay for insulin. They were identical in their ability to stimulate DNA synthesis in confluent BALB/c 3T3 cells previously exposed to platelet-derived growth factor, and the biological activities of both preparations were completely neutralized by a monoclonal antibody raised against native Sm-C. These studies demonstrate that the chemically synthesized product is equivalent to the native molecule in all important respects and that it can be used interchangeably with the natural product for any studies that are contemplated.**

Somatomedin C (Sm-C) and insulin-like growth factor I (IGF-I) are different names for a 70-amino-acid single-chain basic peptide that is believed to mediate most of the growth-promoting actions of somatotropin (1–3). This growth factor, Sm-C/IGF-I, which is structurally related to human proinsulin (4), stimulates DNA synthesis and cell replication in tissues of diverse origin and is one of the important mitogenic substances in serum (5). The somatomedin hypothesis of growth hormone action has recently received strong support from the demonstrations that Sm-C/IGF-I can restore growth in hypophysectomized rats (6) and that this peptide is involved in the negative feedback regulation of growth hormone secretion (7), presumably by means of its dual ability to stimulate production of somatostatin in the hypothalamus (8) and directly block the action of growth-hormone-releasing factor at the pituitary level (9).

Studies of the biological actions and physiological role of Sm-C/IGF-I have been severely hampered by the extreme scarcity of the purified peptide. Sm-C/IGF-I is not stored in any body tissue and its isolation from the myriad of other plasma proteins entails a purification of approximately 400,000-fold. Although by reprocessing chromatographic side fractions we have been able to achieve better than 5% overall recovery (10), the yield of purified peptide from 100 liters of plasma is still less than 1 mg. For these reasons, total chemical synthesis using automated solid-phase methods could offer an attractive alternative to the isolation of purified Sm-C/IGF-I from natural sources. It is of great importance, therefore, to document both qualitatively and quantitatively how various properties of the synthetic product compare with those of highly purified preparations isolated from human plasma.

To determine how closely the synthetic Sm-C (sSm-C) resembled the native product (nSm-C), a highly purified preparation of Sm-C isolated from outdated human plasma was compared with the synthetic product in three highly specific radioligand assays and in a cell growth system. The latter studies were carried out in the presence and absence of a monoclonal antibody raised against nSm-C.

MATERIALS AND METHODS

sSm-C. The methods employed in the solid-phase synthesis of Sm-C/IGF-I are reported elsewhere (11). After removal of the reduced form of the peptide from the resin by treatment with hydrofluoric acid, the crude peptide was oxidized in 2 M guanidine·HCl at pH 8.4 and purified through a succession of steps including separation of polymers from monomers by gel filtration on Sephadex G-50, chromatofocusing, and partition chromatography on Sephadex G-50. The initial isolation yielded 6.8 mg of highly purified Sm-C/IGF-I, which represented a 1% yield based on the starting load. Since only one-sixth of the starting batch was carried through the purification procedure, however, it is clearly feasible to prepare far larger quantities of Sm-C/IGF-I by this method.

nSm-C. A highly purified preparation of Sm-C was isolated from outdated human plasma by the method of Svoboda *et al.* (10). The preparation of nSm-C employed in the radioreceptor assay (RRA) and radioimmunoassay (RIA) was of sequence grade and its weight was determined by summation of amino acids in an acid hydrolysate.

RIA and RRA. RIA was performed according to the method of Furlanetto *et al.* (12) and the RRA according to the method of Marshall *et al.* (13) as modified by D'Ercole *et al.* (14).

Monoclonal Antibody to Sm-C. A monoclonal antibody to nSm-C was produced after splenocytes from a hyperimmunized mouse were boosted *in vitro* by a 5-day exposure to Sm-C in thymocyte-conditioned medium before fusion with myeloma cells.‡ The affinity of the antibody was negligible for chymotryptic fragments of Sm-C/IGF-I, various insulins, bovine, proinsulin, human growth hormone, and epidermal growth factor (EGF).

Sm-C-Dependent DNA Synthesis. Low-passage BALB/c 3T3 cells were grown to confluence in 96-well tissue culture plates (GIBCO) in culture medium consisting of equal parts of Ham's F-12 medium and Dulbecco's modified Eagle's medium supplemented with 10% calf serum (Sterile Systems, Logan, UT). The cells were exposed to platelet-derived growth factor (PDGF) (courtesy of W. J. Pledger) at 12 ng per well for 5 hr and then to serum-free medium containing 1 μCi of [*methyl*-^{3}H]thymidine (6 Ci/mmol, Schwarz/Mann; 1

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Abbreviations: Sm-C, somatomedin C; sSm-C and nSm-C, synthetic and native Sm-C; IGF-I, insulin-like growth factor I; EGF, epidermal growth factor, PDGF, platelet-derived growth factor; RIA, radioimmunoassay; RRA, radioreceptor assay.

‡Van Wyk, J. J., Underwood, L. E., Gillespie, G. Y., Svoboda, M. E. & Russell, W. E., 65th Annual Meeting of the Endocrine Society, June 8–10, 1983, San Antonio, TX.

Ci = 37 GBq). Each well contained EGF (courtesy of E. O'Keefe) at 10 ng/ml either alone or in combination with nSm-C, sSm-C, or porcine insulin (Eli Lilly) at the dosages indicated. Triplicate wells for each condition were included with and without monoclonal antibody to Sm-C (1/4000 dilution). After 28 hr, the cells were washed twice with unsupplemented culture medium, fixed with two rinses of 5% trichloroacetic acid, and solubilized in 0.1 M NaOH with 1% sodium dodecyl sulfate for determination of radioactivity.

RESULTS AND DISCUSSION

Fig. 1 compares nSm-C and sSm-C in an RIA and in an RRA for Sm-C/IGF-I. The displacement curves produced by the two preparations are indistinguishable. The difference between the native and synthetic product was likewise deemed nonsignificant in an RRA for insulin-like activity using ^{125}I-labeled insulin for binding to placental membranes (data not shown).

The biological activities of sSm-C and nSm-C were compared by assessing their ability to induce DNA synthesis in density-arrested BALB/c 3T3 cells. After exposure to PDGF, these cells require only EGF and Sm-C to traverse G_1 and enter into the S phase of the cell cycle. In this system, EGF and Sm-C act synergistically to stimulate DNA synthesis, and the response to either is highly dependent on the concentration of the other (15). At very high concentrations, insulin can act as a somatomedin surrogate by virtue of its ability to crossreact with the Sm-C/IGF-I receptor (16).

Table 1 shows the effects on thymidine incorporation of EGF alone or in combination with nSm-C, sSm-C, or insulin. To control for possible endogenous production of Sm-C, such as occurs in cultures of human fibroblasts and other cell types (17, 18), all hormonal additions were carried out in the presence and absence of a specific monoclonal antibody to Sm-C that does not crossreact with EGF or insulin.‡ In this study (Table 1), natural and synthetic Sm-C at concentrations of 16 ng/ml were indistinguishable in their stimulatory effect on DNA synthesis in the presence of EGF (10 ng/ml), and the effect of both was abolished by inclusion in the culture medium of a 1/4000 dilution of the monoclonal antibody.

Table 1. Effects of sSm-C and nSm-C on DNA synthesis in BALB/c 3T3 cells

Conditions	Antibody to nSm-C	[^{3}H]Thymidine incorporation, cpm
EGF (10 ng/ml)	−	18,681 ± 2543
	+	11,705 ± 441
EGF + insulin (1 μg/ml)	−	39,835 ± 3161
	+	39,137 ± 1442
EGF + nSm-C (16 ng/ml)	−	28,484 ± 5961
	+	12,511 ± 1369
EGF + sSm-C (16 ng/ml)	−	25,770 ± 3199
	+	14,532 ± 3751

+, Presence of monoclonal antibody to nSm-C (1:4000 dilution). Data are reported as the mean ± SD of triplicate wells.

It is of note that the antibody also inhibited the stimulatory effect of EGF alone. Since Leof *et al.* (15) have previously demonstrated that EGF will not allow the progression of BALB/c 3T3 cells through the early phases of G_1 unless low concentrations of Sm-C are simultaneously present, we postulated that the monoclonal antibody reduced the mitogenicity of EGF alone by neutralizing some endogenous Sm-C produced by the cells themselves. This was supported by the detection of low concentrations of immunoreactive Sm-C in the culture medium of density-arrested BALB/c 3T3 cells exposed to both EGF and PDGF (data not shown). This interpretation is further supported by the failure of the antibody to block the mitogenic effect of EGF in the presence of insulin (1 μg/ml).

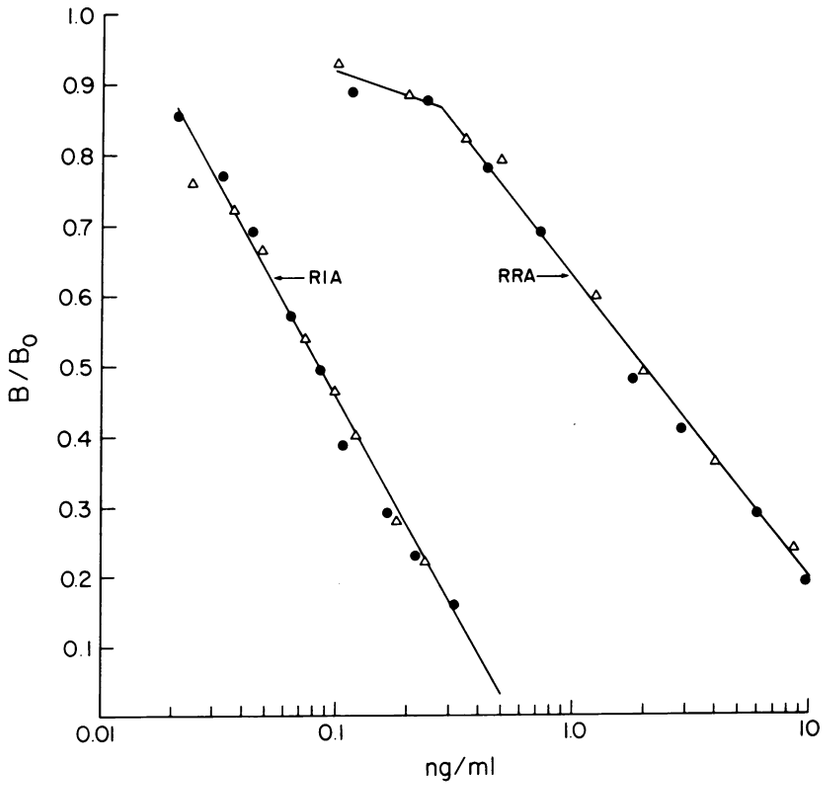

FIG. 1. Comparison of the displacement curves of sSm-C and nSm-C in RIA (left) and RRA (right). B, labeled Sm-C bound; B_0 labeled Sm-C bound in the absence of competitor. ●, nSm-C; △, sSm-C.

These studies conclusively demonstrate that chemically synthesized Sm-C is equivalent to the native molecule in all important respects and that it can be used interchangeably with the natural product for any studies that are contemplated.

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