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Amino-terminal sequences of two polypeptides from human serum with nonsuppressible insulin-like and cell-growth-promoting activities: Evidence for structural homology with insulin B chain

(insulin-like growth factor/somatomedins/multiplication-stimulating activity)

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ABSTRACT **The amino-terminal sequences of two polypeptides with nonsuppressible insulin-like and cell-growth-promoting activities (NSILA I and II), isolated from human serum, were determined. Of the first 31 residues, 22 are identical in NSILA I and II. Moreover, a striking structural similarity was found between NSILA and insulin B chain: 47 and 57% of residues 1–30 in NSILA I are identical to those in insulin B chain from man and tuna fish, respectively. This high degree of sequence identity is presented as evidence for homology and thus for a common evolutionary origin of insulin and NSILA. Based on these results and on biological properties of NSILA described earlier, a new designation for NSILA is proposed: *insulin-like growth factor (IGF).***

Human serum contains an insulin-like activity not suppressible by insulin antibodies (NSILA) (1). Purified preparations of NSILA mimic most effects of insulin on adipose tissue and muscle *in vitro* and *in vivo* (2). In addition, NSILA has growth-promoting properties for cartilage and fibroblasts *in vitro* (2). Recently, two forms of NSILA (NSILA I and II) were isolated from human serum and characterized as single-chain polypeptides with an approximate molecular weight of 6,000 (3). Studies of their biological effects suggested that NSILA I and II are two forms of an insulin-like hormone whose effects on cell and tissue growth parameters predominate over those on metabolic parameters (3, 4).

This paper reports the amino acid sequence in the first 31 positions of NSILA I and II. The results document a close structural relationship of both polypeptides to insulin B chain.

MATERIALS AND METHODS

NSILA I and II were obtained from human plasma as described previously (3). The polypeptides were reduced with dithiothreitol in 6 M guanidine·HCl/0.1 M Tris·HCl at pH 9.5 for 4 hr, brought to pH 7.6 with HCl, and either *S*-pyridylethylated with 4-vinylpyridine (5) for automatic sequencing, or carboxymethylated with iodoacetate (6) for trypsin digestion, or aminoethylated with ethyleneimine (7) for chymotryptic digestion.

Automatic protein sequencing was performed with a Beckman sequencer model 890 B (updated) using the dimethylbenzylamine buffer system (8) and the Beckman peptide program (111374). The phenylthiazolinones were converted to the phenylthiohydantoins for identification by gas chromatography (8, 9) and by thin-layer chromatography (8, 10). Indirect identification of the derivatives was made by amino acid analyses of the free amino acids on a Durrum D-500 analyzer after hydrolysis with HI (11) or with 5.7 M HCl containing 0.1% $SnCl_2$ (12).

Abbreviation: NSILA, nonsuppressible insulin-like activity.

For enzymatic digestions, trypsin treated with tosylphenylalanyl chloromethyl ketone (TPCK) (Worthington), α-chymotrypsin (Worthington), and protease from *Staphylococcus aureus* V8 (Miles) were used. Peptides were fractionated on a 0.9 × 20 cm column of Beckman M-72 resin with a gradient from 0.05 M pyridine–acetate, pH 2.5, to 2.0 M pyridine–acetate, pH 5.0, at 55° (E. Rinderknecht and R. E. Humbel, in preparation).

COOH- and NH_2-terminal sequences of several peptides were determined using carboxypeptidase C and aminopeptidase M (Röhm/Roth, Darmstadt, Germany) in 0.05 M sodium citrate at pH 5.3 and 0.1 M ammonium bicarbonate at pH 8.5, respectively.

RESULTS

Automatic sequencing of NSILA I was performed three times: with 100 nmol of the *S*-aminoethylated derivative for 18 cycles and with 100 and 500 nmol of the *S*-pyridylethylated derivative for 20 and 35 cycles, respectively. All residues were identified by amino acid analyses after acid hydrolysis of the phenylthiohydantoin derivatives (Fig. 1). Where appropriate, identification was confirmed by gas chromatography and/or thin-layer chromatography. The repetitive yield was between 90 and 95%. Results were unambiguous up to residue 31.

The sequence of the first 31 amino acid residues was confirmed by analysis of corresponding peptides obtained by cleavage of reduced and alkylated NSILA I by trypsin, chymotrypsin, and *S. aureus* protease. The sequences of these peptides were determined by digestion with carboxypeptidase C, aminopeptidase M, and automatic sequencing (E. Rinderknecht and R. E. Humbel, in preparation).

The sequence of NSILA II was determined twice, once with 100 nmol for 16 cycles and once with 500 nmol for 35 cycles of *S*-pyridylethylated NSILA (Fig. 1). The results were unequivocal, although each degradation step liberated two amino acid derivatives, indicating the presence of two components in the ratio of 3:1. The minor component lacks NH_2-terminal alanine, but is otherwise of identical sequence. Repetitive yields were again between 90 and 95%.

DISCUSSION

Comparison of the first 30 amino acid residues reveals a high degree of sequence identity between NSILA I and II. When the sequences of NSILA I and II are aligned as indicated in Fig. 2, 73% of all residues in position 1 through 30 are identical.

The finding in human sera of two variants, NSILA I and II, with more than two amino acid substitutions suggests the presence of different gene loci rather than that of different alleles for NSILA. However, the latter possibility has not yet been excluded, since NSILA I and II have been isolated from

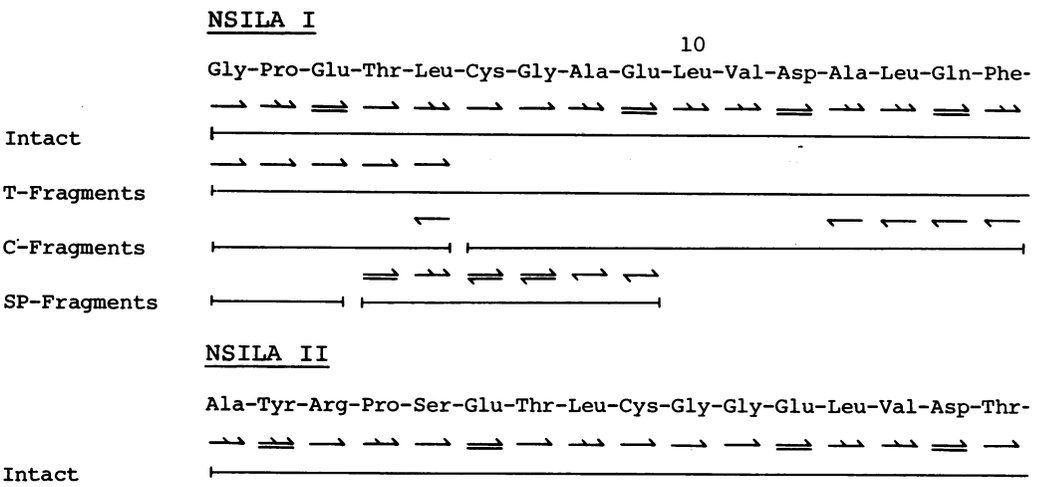


Fig. 1. Summary of the amino acid sequence data on the NH_2-terminal parts of NSILA I and II. T-Fragments, fragments obtained with trypsin; C-Fragments, fragments obtained with chymotrypsin; SP-Fragments, fragments obtained with staphylococcal protease. Each X indicates an unidentified amino acid. Residues were identified by: —, automatic Edman degradation and amino acid analysis; —, automatic Edman degradation

large pools of human serum (3). Likewise, the heterogeneity of NSILA II has not been examined in individuals. Here, the heterogeneity might be simply due to the influence of a peptidase during preparation or, alternatively, the molecule with the NH_2-terminal alanine might be an intermediate form in the conversion of a hypothetical precursor to NSILA.

The most remarkable feature of the NH_2-terminal sequence of NSILA is doubtless the close structural relationship to insulin B chain (Fig. 2). In NSILA I and human insulin B chain, 47% of all residues are identical. In a search of all known sequences of insulin B chains from different species (14), that of tuna fish was found to show the highest degree of sequence identity, 57%. The demonstrated sequence similarity of NSILA and of insulin B chain is taken as evidence for a common ancestor molecule in the evolution of insulin and NSILA. The postulated homology is further corroborated by the fact that of the 10 invariant residues in all known sequences of insulin B chains (14), only one, Tyr 16, is substituted in NSILA.

Several other substitutions are of particular interest. NSILA I and II lack His 10, the zinc-binding residue in all insulins except those of hagfish, guinea pig, and coypu, which do not form hexamers containing zinc atoms. Ala 14, one of the contact residues in insulin hexamer formation, is replaced in NSILA II by Thr as it is in guinea pig and coypu. His 5, present in all insulins except that of coypu, is replaced in NSILA I and II by Thr. Indeed, the degree of similarity between insulin B chains of man and coypu is the same as that between coypu insulin B chain and NSILA II, i.e., 50% of identical residues. NSILA may thus resemble an ancestral insulin which has not yet evolved to a more complex structure able to aggregate.

In view of the homology of NSILA to insulin B chain, it appears fruitful to search the sequence beyond residue 31 for homology with proinsulin. A feature which seems to be a prerequisite for cleavage of prohormones into active hormones is the presence of a set of two basic residues (15). The lack of such a set at position 33–36 (E. Rinderknecht, unpublished results) is in line with the fact that NSILA is a single-chain molecule. A single arginine residue seems to be at position 37 (residue 36 of Fig. 1). The remainder of the basic residues (one lysine, three arginines) must be located beyond position 37. We suggest that the structure of proinsulin, which can be cleaved to yield a two-chain molecule, has evolved through a gene duplication out of an ancestral single-chain molecule whose essential structural features are preserved in NSILA. Additional gene duplications have been postulated to account for the distant structural relationship of proinsulin to nerve growth factor (16).

The physiological role of NSILA is not entirely clear. NSILA I and II have been shown previously to have about equal insulin-like activities in adipose tissue *in vitro*, with specific ac-

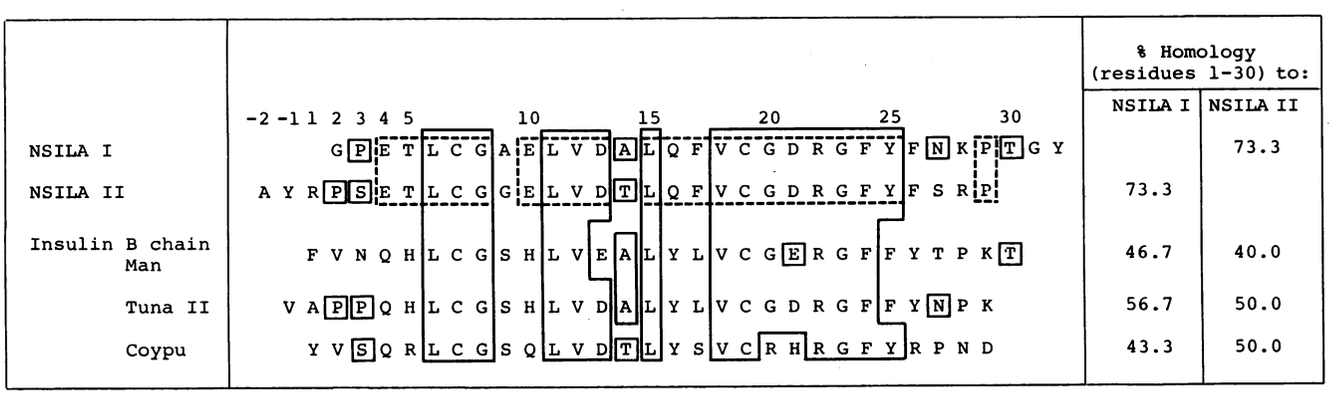
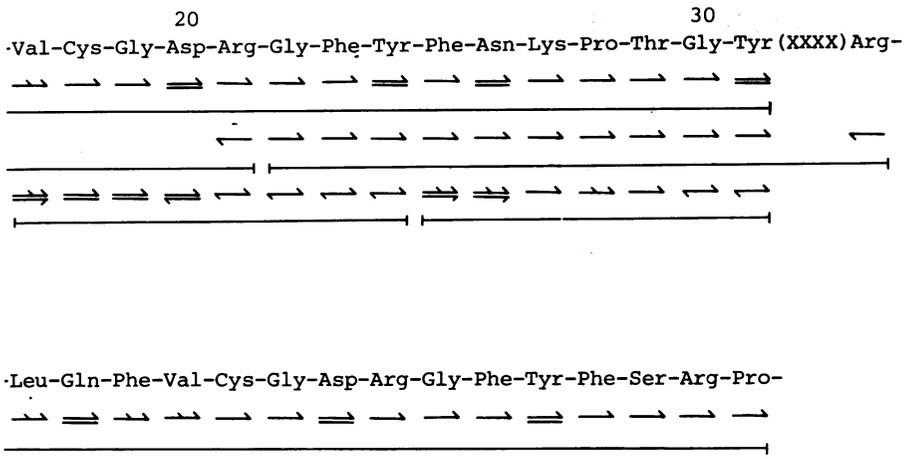


FIG. 2. Alignments of amino acid sequences of NSILA I, NSILA II, and insulin B chains from man, tuna fish, and coypu. One-letter symbols for amino acid residues are as recommended in ref. 13. The numbering of residues of NSILA was chosen to correspond to conventional numbering of insulin B chains. Boxes in broken lines indicate residues identical in NSILA I and II. Boxes in solid lines indicate residues identical in NSILA and insulin B chains.



and gas chromatography; ═, automatic Edman degradation and thin-layer chromatography; —, digestion with aminopeptidase M and amino acid analysis; ←, digestion with carboxypeptidase C and amino acid analysis.

tivities 60 times lower than that of insulin (3). Furthermore, effects of NSILA on growth parameters *in vitro* like synthesis of DNA, RNA, and protein, growth rate of fibroblasts, and activity of ornithine decarboxylase (2–4) have been shown at concentrations occurring in human serum and with a specific activity 50 to 100 times greater than that of insulin.

These results suggested that the physiological function of NSILA is that of a growth factor. Moreover, there is evidence that NSILA is a growth factor dependent on growth hormone. Subnormal levels of NSILA were found in hypopituitary dwarfs and supranormal levels in acromegalics (17). Additionally, NSILA complements serum from hypophysectomized animals to yield a normal stimulation of sulfate incorporation into chick cartilage (4). Thus, NSILA may qualify as a somatomedin (18).

These functional and the structural properties of NSILA as presented here lead us to suggest a new designation for NSILA. We propose to replace the too-restrictive operational term *nonsuppressible insulin-like activity* by the more general term *insulin-like growth factor* or *IGF*. Considering the functional similarities of IGF with somatomedin A and C (19) and with multiplication-stimulating activity (20), the latter substances may eventually turn out to be members of a family of insulin-like growth factors.

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