Correction. In the article "Kinetic evidence for hapten-induced conformational transition in immunoglobulin MOPC 460" by D. Lancet and I. Pecht, which appeared in the October 1976 issue of Proc. Natl. Acad. Sci. USA 73, 3549–3553, the authors have requested the following changes. On p. 3550, right-hand column, second line from bottom, and p. 3551, left-hand column, fourth line from the top, "Fig. 2" should be "Fig. 1A." In the legend of Table 2, third line, note (f) should read "AG = -RTlnK." On p. 3553, left-hand column, third paragraph, fifth line, "ko" should be replaced by "Ko."

Correction. In the membership list of the National Academy of Sciences that appeared in the October 1976 issue of Proc. Natl. Acad. Sci. USA 73, 3750–3781, please note the following corrections: H. E. Carter, Britton Chance, Seymour S. Cohen, E. A. Doisy, Gerald M. Edelman, and John T. Edsall are affiliated with the Section of Biochemistry (21), not the Section of Botany (25).

Correction. In the Author Index to Volume 73, January–December 1976, which appeared in the December 1976 issue of Proc. Natl. Acad. Sci. USA 73, 4781–4788, the limitations of computer alphabetization resulted in the listing of one person as the author of another’s paper. On p. 4786, it should indicate that James Christopher Phillips had an article beginning on p. 128, and James Charles Phillips had an article beginning on p. 3820.

Correction. In the article "Studies of human myasthenia gravis: Electrophysiological and ultrastructural evidence compatible with antibody attachment to acetylcholine receptor complex" by J. E. Rash, E. X. Albuquerque, C. S. Hudson, R. F. Mayer, and J. R. Satterfield, which appeared in the December 1976 issue of Proc. Natl. Acad. Sci. USA 73, 4584–4588, a printer’s error deleted the first author of ref. 1. The correct reference is:

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)

ERNST RINDERKNECHT AND RENÉ E. HUMBEL
Biochemisches Institut der Universität Zürich, CH-8028 Zürich, Switzerland

Communicated by Donald F. Steiner, September 9, 1976

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 vivo and in vitro (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 purified by di-thiothreitol 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 aminooethylated with ethylenemine (7) for chymotryptic digestion.

Automatic protein sequencing was performed with a Beckman sequencer model 890 B (updated) using the dimethyl-benzylamine buffer system (8) and the Beckman peptide program (11374). The phenylthiohydantoins were converted to the phenylthiohydantoin derivatives 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% SnCl4 (12).

For enzymatic digestions, trypsin treated with tosylphenylalaninyl 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°C (E. Rinderknecht and R. E. Humbel, in preparation).

COOH- and NH2-terminal sequences of several peptides were determined using carboxypeptidase C and aminopeptidase M (Röhm/Boehr, 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 NH2-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...
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 peptide during preparation or, alternatively, the molecule with the NH2-terminal alanine might be an intermediate form in the conversion of a hypothetical precursor to NSILA.

The most remarkable feature of the NH2-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. 1. Summary of the amino acid sequence data on the NH2-terminal part 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](image)

![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.](image)
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 acromegals (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.

We thank Edwin Schwander for technical assistance and Dr. Kenneth J. Wilson for helpful suggestions. This work was supported in part by Schweizerischer Nationalfonds, Grants 3.275.74 and 3.409.74.