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STUDY OF A POSSIBLE STRUCTURAL RELATIONSHIP BETWEEN THYMOPOIETIN AND THE FACTEUR THYMIQUE SERIQUE

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SUMMARY

Background peptide chemistry, and the known 49-amino acid sequence of thymopoietin and the known 9-amino acid sequence of the facteur thymique serique (FTS) allowed the concept that Arg^{49} of thymopoietin might be linked to Gln^{1} of FTS in a new 58-amino acid peptide in tissue. Cleavage between Arg^{49} and Gln^{50} adjacent to the unique Lys^{48}-Arg^{49} moiety could liberate thymopoietin and the [H-Gln^{1}]-FTS which could cyclize to FTS by the known reaction. In support of, rather than negating, this concept, synthetic FTS and the new dodecapeptide consisting of Val-Lys-Arg linked to the N-terminal of [H-Gln^{1}]-FTS showed comparable immune stimulating activity, in vivo; both peptides appeared more active than synthetic thymopoietin II.

INTRODUCTION

Two closely related polypeptides (thymin I and II) were isolated from bovine thymus as reported by G. Goldstein in 1974 (1). Both of these polypeptides contain 49 amino acids, and were renamed thymopoietin I and II. Thymopoietin II has Ser^{1}, Glu^{2}, and Thr^{43}; thymopoietin I has Gly^{1}, Gln^{2}, and His^{43}; otherwise, these two sequences are identical, according to Schlesinger and G. Goldstein in 1975 (2).

In 1977, Bach et al. (3) described the organic chemical and biochemical characterization of their facteur thymique serique (FTS), which exists in the serum of various mammalian species, including swine. They considered it important that the presence of this nonapeptide in serum of several animals species is thymus dependent.

FTS is a nonapeptide having the sequence: Glx-Ala-Lys-Ser-Glx-Gly-Gly-Ser-Asn. Presumably Glx^{1} is <Glu ("rather natively pyroglutamic acid or transformed in vitro"). The amino acid in position 5 was presumed to be Gln, because of the isoelectric point of the peptide. The synthetic peptide, < Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH (Hirschmann et al. of Merck Sharp and Dohme Research Labs., West Point, PA) having this sequence behaved chemically and biologically like the isolated FTS.

We considered the possibility of a structural relationship between thymopoietin (II) of 49 amino acids and FTS of 9 amino acids. The sequence of thymopoietin at the C-terminal end has Lys^{48}-Arg^{49}-OH. The N-terminal

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end of FTS is <Glu[1]. It is known that H-Gln in position 1 of peptides is chemically unstable, and readily cyclizes to <Glu. For example, H-Gln-His-Pro-NH_2 readily cyclizes to TRH (4), and [H-Gln[1]]-neurotensin (13 amino acids) cyclizes to neurotensin (5). It seemed clear that FTS in the serum could be a fragment of a larger peptide which had been cleaved, probably enzymatically, to yield the [H-Gln[1]]-nonapeptide which then could non-enzymatically cyclize to the [<Glu[1]]-nonapeptide corresponding to the isolated nonapeptide (FTS).

It is known that certain peptides are cleaved, presumably enzymatically, at either side of a Lys-Arg moiety, and presumably because of the chemical uniqueness of this "double basic" moiety in a sequence. Pro-glucagon having 37 amino acids cleaves between Thr^{29} and Lys^{30} of the moiety Thr^{29}-Lys^{30}-Arg^{31} to give glucagon having 29 amino acids (6). Bovine proinsulin which cleaves between Arg^{60} and Gly^{61} of the moiety Lys^{59}-Arg^{60}-Gly^{61} gives bovine insulin (7). Other "double basic" moieties are Arg-Arg and Lys-Lys which, as received by Steiner (8) may participate in such cleavages of peptide hormone precursors. "Large gastrin" has been presumed to be cleaved between Lys and Gln of a Lys-Lys-Gln moiety to give [Gln[1]]-gastrin which cyclized to [<Glu[1]]-gastrin.

Consequently, it is conceivable that in the thymus, serum, or in other tissue, that there is a peptide of 58 amino acids which has the moiety Lys^{48}-Arg^{49}-Gln^{50} and which is similarly cleaved enzymatically between Arg^{49} and Gln^{50} to give thymopoietin terminating in Arg^{49}-OH and the nonapeptide, FTS, with a temporary H-Gln[1] moiety which then cyclizes to give the [<Glu[1]]-nonapeptide as isolated by Bach et al.

RESULTS AND DISCUSSION

As one approach to test this concept of the existence of such a thymic peptide having 58 amino acids (which in itself might also be a fragment of a larger peptide), we have synthesized not only the nonapeptide, FTS, but also a related dodecapeptide. This dodecapeptide is H-Val-Lys-Arg-Gln-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH, which contains the critical Lys-Arg moiety and which we thought was more important than initially adding Ala^{46}, Thr^{45}, etc., to lengthen the peptide beyond 12 amino acids.

From a viewpoint of function, the addition of the two basic amino acids, Lys-Arg- to FTS may be considered a substantial chemical change, and which could significantly alter the biological activity of FTS, particularly to decrease activity.

In a cooperative study with Dr. Emile G. Bliznakov, the synthetic nonapeptide and dodecapeptide were compared for reactivation of impaired immune

competence in aged mice. The results by Bliznakov et al. (9)clearly showed the profound impairment of the humoral, hemolytic, primary, immune response in aged mice (22 months) as compared with this response in young mice (10 weeks). A partial but significant reactivation of the age-determined impairment of the immunological responsiveness resulted from the subcutaneous administration of these two synthetic peptides. The nonapeptide and dodecapeptide showed comparable activity at levels of 10 μg/mouse/injection.

These biological data in vivo show that the addition of the tripeptide moiety, Val-Lys-Arg- to the N-terminal end of the nonapeptide (FTS) maintained the immune stimulating activity rather than having significantly decreased it.

We have also synthesized thymopoietin II (10) as Fujino et al. (11). Our specimen of thymopoietin II, at the same dosage of 10 μg, was also active, but the nona- and dodecapeptides appeared to be more active than thymopoietin II. Dr. Bliznakov and we are now endeavoring to optimize the experimental conditions of the bioassays, and recognize that the short half-lives of these peptides in the circulation necessitates different schedules of administration.

Schlesinger et al. (12) described a synthetic tridecapeptide, H-Gly-Glu-Gln-Arg-Lys-Asp-Val-Tyr-Val-Gly-Leu-Tyr-Leu-OH, which corresponds to positions 29-41 of thymopoietin II, and showed that it had the same selective T-cell differentiating activity as thymopoietin II, but only 3% of the potency of thymopoietin. They concluded that the key residues involved in the active site of thymopoietin are present in this fragment. However, this conclusion seems premature since it is based upon only one fragment of 13 amino acids of the entire polypeptide of 49 amino acids, and this fragment has only 3% activity. In our assays, FTS and the corresponding dodecapeptide appeared more, rather than less, active than thymopoietin.

It is chemically interesting that the C-terminal tripeptide end of the 74-amino acid ubiquitin, (Schlesinger et al. (13)), is Arg^{72}-Leu^{73}-Arg^{74}-OH. Ubiquitin "mimics" thymopoietin-induced differentiation, but has other activities. A. Goldstein et al. (14) described, in 1977, the sequence of another peptide from calf thymus, termed thymosin $_{\alpha 1}$, and which has 28 amino acids. No sequence data are yet available from the isolates of Trainin et al. (15).

Our results may support, rather than negate, the concept that Arg^{49} of thymopoietin might be linked to Gln^{1} of the facteur thymique serique in a new peptide of 58 amino acids. Bach and Carnand (16) in 1976, meaningfully considered the main questions on whether the several peptides being studied by different groups are related, and whether there is one or several thymic hormones.

METHODS

Thymopoietin II, facteur thymique serique (FTS), <Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH, and its dodecapeptide analog, H-Val-Lys-Arg-Gln-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH, were synthesized by the solid-phase methodology on a Beckman 990 Peptide Synthesizer. Amino acid derivatives were purchased from Peninsula Laboratories, San Carlos, CA., and Beckman Instruments, Inc., Palo Alto, CA. α-Amino functions were protected by the tert-Boc group. Side-chain functionalities were protected as benzyl (Ser, Thr, Asp, and Glu), o-chlorobenzyloxycarbonyl (Lys), o-bromobenzyloxycarbonly (Tyr), and Tos (Arg). <Glu was incorporated as <GluOH. Coupling reactions, with a three-fold excess of amino acid derivatives and DCC, were generally performed in CH_2Cl_2 except for Boc-Arg(Tos) which was coupled in a mixture of CH_2Cl_2 and DMF. Gln and Asn were incorporated as their p-nitrophenyl esters by an active ester coupling procedure. Double coupling procedures were used, and coupling reactions were monitored by the Ninhydrin test (17).

The peptide chains were constructed on the modified Merrifield resin of Mitchell et al. (18). The protected peptides were deblocked and cleaved from the resins by treatment of anhydrous liquid HF in the presence of anisole. Thymopoietin II was purified by gel-filtration over Sephadex G-50 followed by Sephadex LH-20. FTS and the dodecapeptide analog were purified over Sephadex G-25. Thymopoietin II gave a major spot on tlc, R_f=0.66 (1:1:1:1, EtOAc:HOAc:n-BuOH:H_2O) which is compatible to R_f=0.60, reported by Fujino et al.(11). FTS exhibited a single spot on tlc, R_f=0.22 (1:1:1, HOAc:n-BuOH:H_2O). The dodecapeptide analog did not migrate (R_f=0) in several solvent systems, but its purification on Sephadex G-25 was apparently satisfactory.

The extensive details of synthesis, purification, purity and analyses of the three peptides will be published separately.

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