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Amino Acid Sequence of Rabbit Factor H of Complement. Purification of Peptides Produced by Cyanogen Bromide Cleavage

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Rabbit factor H, a regulatory protein of complement, has a blocked N-terminus. After treatment with pyroglutamyl aminopeptidase, its N-terminus could be determined. The C-terminus of rabbit factor H was determined to be tyrosine by carboxypeptidase A treatment. The peptides produced by cyanogen bromide cleavage were purified by gel chromatography, followed by high-performance liquid chromatography. The N-terminal sequences of eight peptides thus purified were analyzed. When the sequence determined in this study was compared with that of mouse factor H derived from the complementary deoxyribonucleic acid (cDNA) sequence, which has recently been reported by Kristensen and Tack, 100 of the 167 residues were identical with those of mouse factor H (about 60% homology).

Keywords——amino acid sequence; rabbit factor H; complement; cyanogen bromide cleavage; peptide analysis

Introduction

Factor H is a regulatory protein of the alternative pathway of complement.[1] The protein not only inhibits the formation of the alternative pathway C3 convertase through competition with factor B, but also serves as a cofactor for the cleavage of C3b to iC3b by factor I.[2] Factor H is a single-chain glycoprotein with a molecular weight of 150000.[3] Recently the complete amino acid sequence of mouse factor H has been deduced from the nucleotide sequence of its complementary deoxyribonucleic acid (cDNA).[4] Mouse factor H was composed of 1216 amino acid residues, and consisted of 20 repetitive units. The internal repetitive units were composed of about 60 amino acids and had a characteristic framework of highly conserved residues.

Our interest in factor H arose because factor H plays an important role in the recognition function of the alternative complement pathway.[5] Structural comparison between mouse and rabbit factors H may give valuable information on the domain exhibiting the recognition function. Hence, we purified the peptides released by cyanogen bromide cleavage of rabbit factor H, and subjected those peptides to sequence analysis.

Experimental

Cyanogen Bromide Cleavage of Rabbit Factor H——Rabbit factor H was purified as described previously.[5a] Rabbit factor H (20 mg) was reduced and carboxymethylated with iodoacetate by the method of Sim and Discipio.[3] The carboxymethylated factor H was dissolved in 5.25 ml of 70% (v/v) formic acid and cleaved with 80 mg of cyanogen bromide at room temperture for 17 h. The peptides produced were fractionated by gel chromatography on a Sephadex G75 column, and separated into eleven pools, I—XI. Each pool was applied to a reverse-phase column (7.8 × 300 mm) of TSK gel ODS-120T (5 μm) equilibrated with 0.1% trifluoroacetic acid, and was eluted with a gradient of 0—70% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1.0 ml/min.

Amino Acid Sequence Analyses——The carboxymethylated rabbit factor H was treated with pyroglutamyl

aminopeptidase prior to N-terminal sequence determination.[6] N-Terminal sequences of peptides were determined by automated Edman degradation using an Applied Biosystems 470A gas-phase protein sequencer. Phenylthiohydrantoin (PTH)-amino acids were identified by high-performance liquid chromatography (HPLC) on a Senshupak SEQ-4 column (4.6 × 300 mm) using a gradient system of acetonitrile/40 mM acetate (pH 5.2)/distilled water. C-Terminal residues of peptides were determined by digestion with carboxypeptidase A. Amino acids released were analyzed with a Hitachi 835 amino acid analyzer.

Results

N-Terminal Sequence and C-Terminal Residue of Rabbit Factor H

Rabbit factor H revealed no N-terminal residue when it was analyzed by the protein sequencer. Its N-terminal residue was determined to be Asp in the yield of 26% after digestion with pyroglutamyl aminopeptidase. Thus the N-terminus of native rabbit factor H was a pyroglutamyl residue. When the N-terminal sequence of rabbit factor H was compared with those of human and mouse factors H, only 7 and 10 residues of the 17 and 18 residues were identical, respectively (Fig. 1). The C-terminus of rabbit factor H was determined to be tyrosine by carboxypeptidase A digestion (data not shown).

Purification of the Peptides Produced by Cyanogen Bromide Cleavage

The peptides which were produced from the carboxymethylated factor H with cyanogen

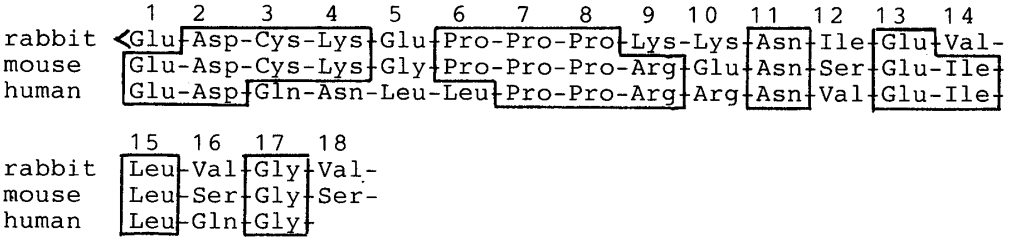

Fig. 1. Comparison of the N-Terminal Sequence of Rabbit, Mouse and Human Factors H

Boxes enclose the identical residues in the sequences.

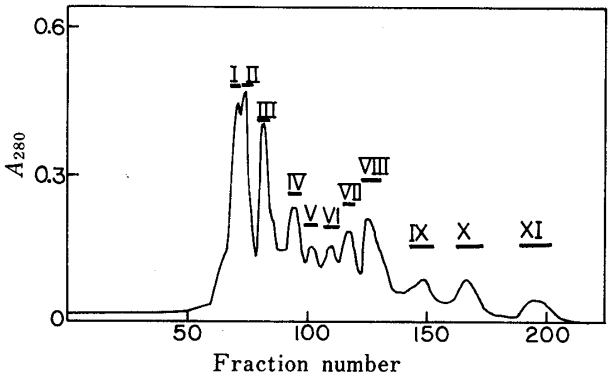

Fig. 2. Fractionation of Cyanogen Bromide Peptides of Rabbit Factor H by Gel Chromatography on Sephadex G-75

The sample was dissolved in 50% formic acid (3 ml) and applied to a column (2.5 × 100 cm) of Sephadex G-75 which was equilibrated with 10% formic acid and run at a flow rate of 10 ml/h. Fractions of 2.5 ml were collected and analyzed for absorbance at 280 nm.

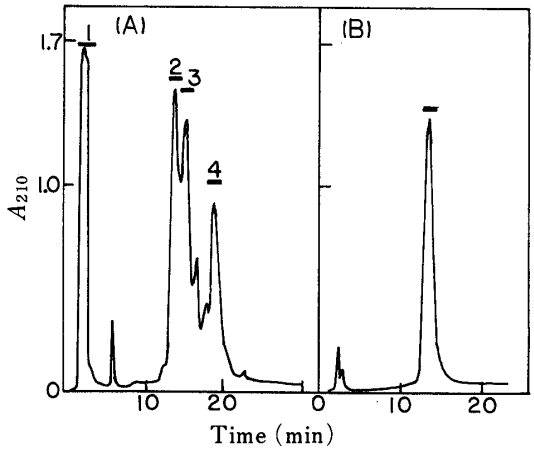

Fig. 3. Purification of C-Terminal Peptide of Rabbit Factors H

The sample was dissolved in 0.1% trifluoroacetic acid. The conditions for HPLC are described in Materials and Methods. (A) Pool VIII of Fig. 2 was applied to the column. (B) Peak 2 of Fig. 3A was applied to the column. The single peak eluted was designated peptide VIII-2.

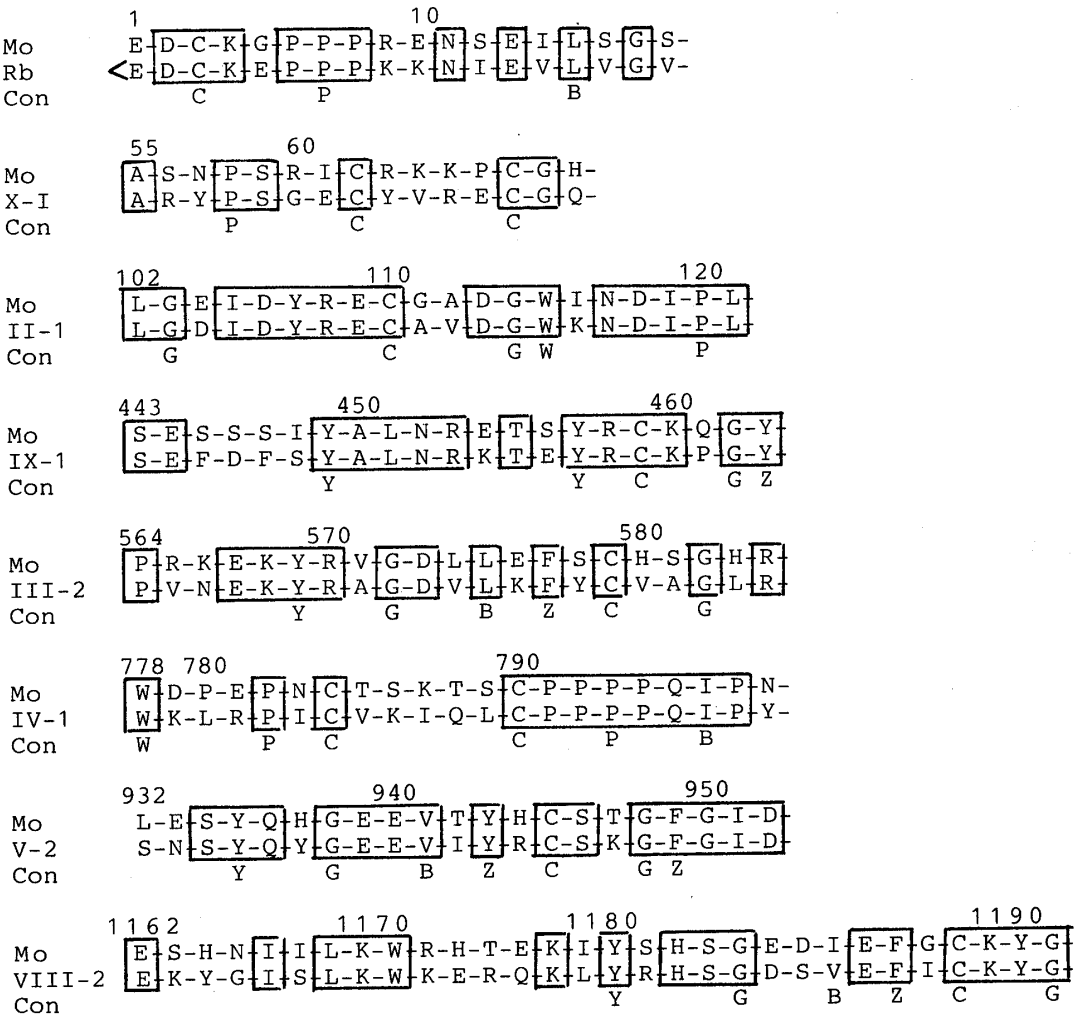

Fig. 4. Comparison of Amino Acid Sequences between Rabbit and Mouse Factors H

The amino acid residues are expressed by one letter abbreviations. The conserved residues of the repetitive units found in mouse factor H are shown on the third line as Con.[6]

The conserved residues are as follows; -x-x-x-x-C-x-x-P-x-x-B-x-N-G-x-x-B-x-x-x-x-x-x-x-Y-x-x-G-x-x-B-x-Z-x-C-x-x-G-Z-x-x-x-x-G-x-x-x-x-x-x-x-C-x-x-x-G-x-W-x-x-x-P-x-C-x-. B indicates a hydrophobic amino acid and Z is phenylalanine or tyrosine. The residual numbers are those of the amino acid sequence of mouse factor H.

bromide were fractionated into 11 pools, I—XI (Fig. 2). The peptide representing the C-terminal portion was detected in pool VIII by the quantitative determination of tyrosine released by carboxypeptidase A treatment. Pool VIII was further fractionated by HPLC (Fig. 3A). Peak VIII-2 had tyrosine as the carboxyl terminus. Peak VIII-2 showed a single peak when the sample was rechromatographed on the same column (Fig. 3B). All of the other pools were also fractionated by HPLC on the same column (data not shown). Eight peptides were purified so that their amino acid sequences could be determined.

N-Terminal Sequences of the Cyanogen Bromide-Cleaved Peptides

The N-terminal sequences of purified peptides were determined for 15—30 residues (Fig. 4). Peptide III-2 and IV-1 contained significant amounts of contaminating peptides, but their sequences were determined by quantitative determination of PTH-amino acids. Peptide V-2 and VI-1 had the same N-terminal sequence, but showed a different chromatographic character. It is well-known that a peptide produced by cyanogen bromide cleavage gives two peaks on HPLC using reverse phase columns; each of the two peaks has either homoserine or

homoserine lactone at the C-terminus. It is likely that peptides V-2 and VI-1 are of this type. None of the purified peptides had a blocked N-terminus that represented the N-terminal portion of rabbit factor H. The sum of amino acid sequences determined here accounted for 167 residues, 14% of the total amino acid composition of rabbit factor H.

Discussion

Factor H is composed of about 1200 amino acid residues. Since the content of methionine of rabbit factor H is 1.2%, it is expected to produce about fifteen peptides on cyanogen bromide treatment. We isolated eight peptides, each of which had a different N-terminal sequence except for peptides V-2 and VI-1. Thus about a half of the peptides remain to be isolated. The amino acid sequence of rabbit factor H was compared with that of mouse factor H which has been deduced from the nucleotide sequence of a cDNA clone.[4] The amino acid sequence of mouse factor H which revealed the highest homology to each of the eight peptides of rabbit factor H in the molecule of mouse factor H was analyzed by the homology matrix method.[6] Mouse factor H was constructed of 20 repetitive units composed of 61 amino acids. The conserved residues in the repetitive units are also shown in Fig. 4. Those conserved residues were well retained in rabbit factor H. Homology between rabbit and mouse factors H was about 60% (100 of 167 residues). Although the amino acid sequence of human factor H has been determined for 365 residues,[7] the data were insufficient for homology analysis with rabbit factor H. There was 79% or 78% homology in amino acid sequence between rabbit and human or mouse C3, the major component of complement, respectively.[8] Therefore, homology between rabbit and mouse factors H is significantly lower than that between rabbit and mouse C3.

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