BOVINE PLASMA HIGH MOLECULAR WEIGHT KININOGEN: THE AMINO ACID SEQUENCE OF FRAGMENT 1 (GLYCOPEPTIDE) RELEASED BY THE ACTION OF PLASMA KALLIKREIN AND ITS LOCATION IN THE PRECURSOR PROTEIN

Yong Nam HAN, Hisao KATO, Sadaaki IWANAGA and Tomoji SUZUKI
Division of Plasma Proteins, Institute for Protein Research, Osaka University, Suita, Osaka-565, Japan

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1. Introduction

Bovine high molecular weight (HMW) kininogen is a monomeric plasma glycoprotein having a mol. wt. of about 76 000 [1]. It consists of a single chain polypeptide with a masked N-terminal residue and C-terminal leucine and carries the vasoactive peptide, bradykinin, in the intrachain disulfide loop [2]. On incubation of this kininogen with plasma kallikrein, two major peptide fragments, in addition to the kinin, are liberated [3]. One of these fragments, fragment 2 named tentatively histidine-rich peptide, has a biological activity which strongly inhibits the activation of Hageman Factor (Factor XII) by glass or kaolin [4,5]. Its amino acid sequence consisting of the total 41 residues has been established [6]. The other peptide, named fragment 1, has been characterized to be a glycopeptide containing also high level of histidine [7]. During these studies, we found that above two fragments are not directly produced from the precursor protein but a large fragment, named fragment 1·2 is first liberated in parallel with the formation of bradykinin and subsequently cleaved by plasma kallikrein into fragment 1 (glycopeptide) and fragment 2 [10]. We describe here the complete amino acid sequence of fragment 1 and its location in HMW kininogen.

2. Materials and methods

HMW kininogen and prekallikrein were highly purified, respectively, from a fresh bovine plasma by the previous methods [1,8], and the latter was activated by purified prekallikrein activator (Factor XIIa) [9]. The specific activity of the kallikrein was 23.5 TAME units per mg protein. A large fragment, fragment 1·2, was prepared according to the previous method [10]. This large fragment (41 mg) was further incubated at 37°C for 80 min with 0.66 mg of the purified kallikrein in 8.0 ml of 0.2 M NH_4HCO_3, pH 8.0, and the resulting digest was separated on a column of Sephadex G-75 (3.0 × 142 cm) in 0.2 M (NH_4) HCO_3, pH 8.0. Two peptide fragments, fragment 1 (28 mg) and fragment 2 (8.0 mg) were obtained. The sequence studies including tryptic and α-chymotryptic digestions, and peptide isolation, analysis and sequence determination were carried out as described previously [6].

3. Results and discussion

Fig. 1 shows the complete amino acid sequence of fragment 1 isolated from HMW kininogen. For the sequence analysis, direct Edman degradation was first performed and the partial N-terminal sequence up to 12 residues was established. A large CNBr fragment isolated from whole fragment 1 was also used to determine the sequence of serine-21 to serine-30. Fragment 1 (1.6–2 μmole) was then digested, respectively, with TPCK-treated trypsin and α-chymotrypsin, and the resulting peptides were fractionated and purified. Much of sequence work was based on the eight major tryptic peptides, and the order of these and other aspects of the sequence were estab-

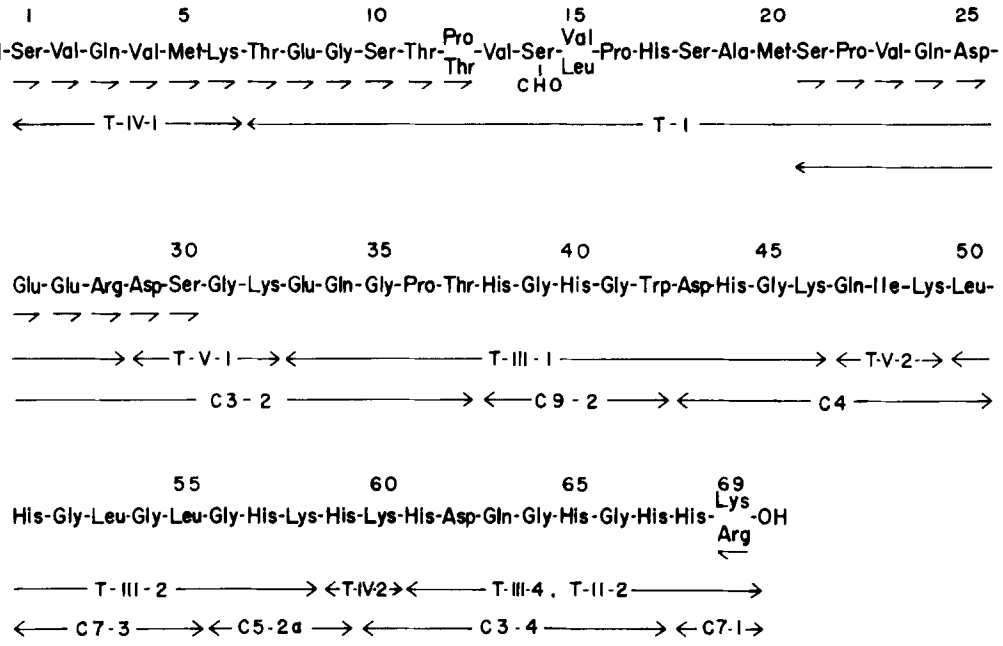

Fig.1. Complete amino acid sequence of fragment 1 (glycopeptide) derived from bovine HMW kininogen. The peptides from digests with trypsin (T) and α-chymotrypsin (C) are shown by arrows. Horizontal arrows pointing to the right and left below the amino acid residues denote that the sequences were determined by direct Edman degradation or the dansyl-Edman method [6] and selective tritium labeling, respectively [15].

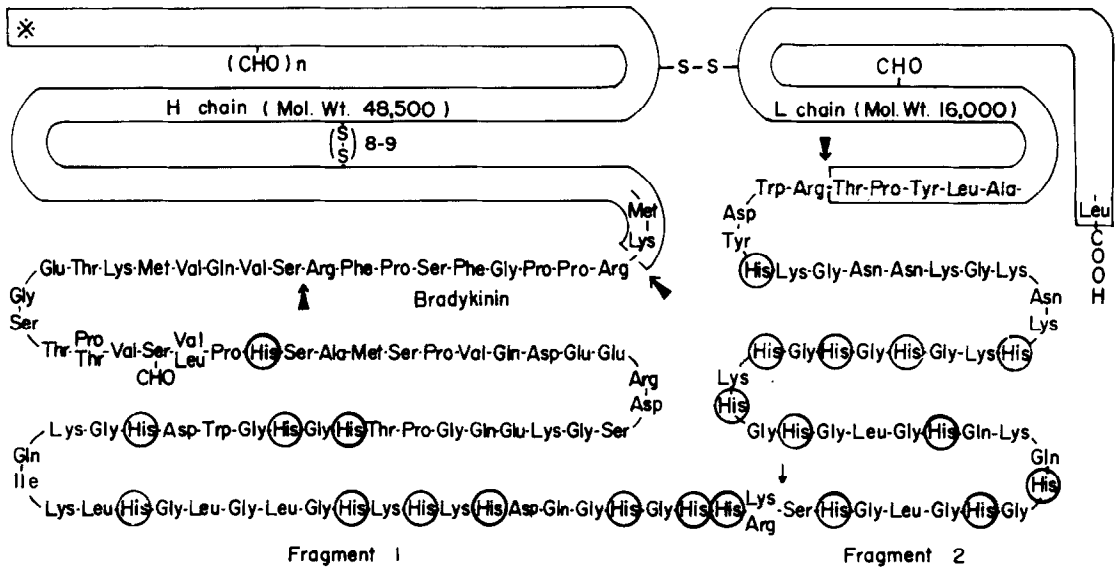

Fig.2. Amino acid sequence of the C-terminal large portion following bradykinin moiety and the locations of fragment 1 and fragment 2 in bovine HMW kininogen. CHO represents a carbohydrate chain. Plasma kallikrein-sensitive bonds are shown by arrows.

lished by seven major chymotryptic peptides and three CNBr peptides. Fragment 1 consisted of a total of 69 amino acid residues with N-terminal serine and C-terminal arginine (and lysine). It contained a single oligosaccharide chain consisting of galactosamine, galactose and sialic acid, which is probably linked with a serine residue at No. 14. Moreover, fragment 1 contained a genetic variant, as indicated with the findings of amino acid substitutions at the positions of Thr-12 (Pro), Leu-15 (Val) and Arg-69 (Lys). The other interesting finding was that repeating sequence with the type of His–Gly–X appears five times in the C-terminal half of the fragment. This type of repeated structure had also been found in fragment 2 (histidine-rich peptide) [6].

Fig.2 shows a most probable linear sequence following the C-terminus of bradykinin moiety along the polypeptide chain of HMW kininogen. As previously reported [7], the kinin-free kininogen, which consists of heavy and light chains bridged by a single disulfide bond and is devoid of bradykinin, fragment 1 and fragment 2, constitutes the N- and C-terminal large portions of whole HMW kininogen. Thus, the three peptide fragments could be located in the portion between the heavy and the light chains. The order of these fragments along the polypeptide chain seems to be aligned as shown in Fig.2, because the N-terminal Ser–Val–Gln–Val–Met sequence of fragment 1 was the same as the C-terminal sequence of the CNBr-fragment (Lys–Arg–Pro–Gly–Phe–Ser–Pro–Phe–Arg–Ser–Val–Gln–Val–Met) containing kallidin [2] and because fragment 1·2 with the N-terminal sequence of Ser–Val–Gln was an intermediate which is subsequently cleaved into fragment 1 and fragment 2 [10]. The interesting features of the partial primary structure of HMW kininogen are that an unusual histidine-rich region, which contains approx. 70% out of the total histidine residues of the kininogen, is found in the sequences of fragment 1 and fragment 2, and that a repeating sequence with the type of His–Gly–X or Gly–His–X appears many times in such region. Further, the region contains a total of thirteen lysine and three arginine residues. Thus, the C-terminal portion following kinin moiety appears to be extremely basic and hydrophilic.

Quite recently, a new function of HMW kininogen has been suggested by findings of Flaujeac [11], Fitzgerald [12] and Williams [13] traits with a deficiency of the kininogen. The deficient plasma does not release kinin upon incubation of kallikrein and also has a prolonged-activated partial thromboplastin time and inability to form plasmin. Of these kininogen deficient traits, bovine HMW kininogen used here corrects a prolonged-activated partial thromboplastin time on the deficient plasma from Flaujeac trait but the kinin-free HMW kininogen is only 0.6% as effective as native HMW kininogen [14]. These observations strongly suggest that the function of HMW kininogen is dependent upon the unusual histidine-rich polypeptide region with the total 110 amino acid residues containing fragment 1 and fragment 2. Thus, some participation of the kallikrein fragments in the intrinsic blood coagulation and fibrinolysis, in addition to the kinin-forming system is now being suggested.

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