Biochimie 92 (2010) 1568–1579

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Review

Kininogens: More than cysteine protease inhibitors and kinin precursors

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ARTICLE INFO

Article history:
Received 25 January 2010
Accepted 10 March 2010
Available online 24 March 2010

Keywords:
Aging
Angiogenesis
Cathepsin
Cystatin
Cysteine protease
Kininogen

ABSTRACT

Two kininogens are found in mammalian sera: HK (high molecular weight kininogen) and LK (low molecular weight kininogen) with the exception of the rat which encompasses a third kininogen, T-Kininogen (TK). Kininogens are multifunctional glycosylated molecules related to cystatins (clan IH, family I25). They harbor three cystatin domains but only two of them are tight-binding inhibitors of cysteine cathepsins. HK and LK, but not TK, are precursors of potent peptide hormones, the kinins, which are released proteolytically by tissue and plasma kallikreins. Besides these classical features novel functions of kininogens have been recently discovered; they are described in the second part of this review. HKa, which corresponds to the kinin-free two-chain HK and its isolated domain D5 (kininostatin), possesses angiostatic and pro-apoptotic properties, inhibits the proliferation of endothelial cells and participates in the regulation of angiogenesis. Moreover, some HK-derived peptides display potent and broad-spectrum microbicidal properties against both Gram-positive and Gram-negative bacteria, and thus may offer a promising alternative to conventional antibiotic therapy. Of seminal interest, a kininogen-derived peptide inhibits activation of the contact phase system of coagulation and protects mice with invasive Streptococcus pyogenes infection from pulmonary lesions. On the other hand, TK is a biomarker of aging at the end of lifespan of elderly rats. However, although TK has been initially identified as an acute phase reactant, and earlier known as alpha-l-acute phase globulin, the increase of TK in liver and plasma is not known to relate to any inflammatory event during the senescence process.

1. A brief aide-memoire

1.1. Kininogens as kinin precursors

Kininogens are multifunctional and multidomain glycoproteins related to cystatins (family 1: stefins; family 2: cystatins; family 3: kininogens) [1,2]. A previously proposed evolutionary scheme assumed a near simultaneous origin and diversification of stefins, cystatins and kininogens, occurring approximately one billion years ago. However, only two ancestral lineages, stefins and cystatins, are present throughout the eukaryotes, while kininogens are much younger and restricted to the vertebrates and therefore originated at most 650 million years ago [3]. In contrast to stefins and cystatins, three-dimensional structures based on X-ray crystallographic or NMR data have not been solved so far for kininogens. Following a preliminary note by Abelous & Bardier in 1909 [4], between 1926 and 1939 Werle and colleagues described in their pioneering studies the nature and functional relationships of the basic components of the kallikrein–kinin system (KKS): (tissue) kallikreins, kinins, kininogens and kininases and particularly numerous pharmacological effects of peptide hormone kinins [5]. Kininogens were primarily identified as substrates from which kinins were released by kallikrein enzymes, opening a boulevard for extensive investigations of the KKS (for a didactic and historic summary see: The E.K. Frey–E. Werle Foundation of the Henning L. Voigt Family: http://www.frey-werle-stiftung.de/).

The first chemically characterized kinin was the nonapeptide bradykinin (Arg^1–Pro^2–Pro^3–Gly^4–Phe^5–Ser^6–Pro^7–Phe^8–Arg^9). Both kallidin (Lys-BK) and bradykinin are potent vasodilators and smooth muscle stimulators released from blood plasma by the venom of *Bothrops jararaca* [6]. They both cause the isolated guinea pig ileum to contract slowly, compared to the rapid contraction observed in the

Abbreviations: ACE, angiotensin-I-converting enzyme; ADAM, a disintegrin and metalloproteinase; ADAMT, a disintegrin and metalloproteinase with thrombospondin motifs; AMP, Antimicrobial peptide; BN-Ka rat, Brown Norway-Katholiek rat; FGF, fibroblast growth factor; HK, high molecular weight kininogen; KKS, kallikrein–kinin system; LK, low molecular weight kininogen; MMP, matrix metalloproteinase; TGF-β, transforming growth factor β; TK, T-kininogen (thiostatin); VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell.

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doi:10.1016/j.biochi.2010.03.011

presence of histamine or acetylcholine. Major mammalian kinins are besides BK and Lys-BK some biologically active metabolites, including des-Arg^9-BK (Arg–Pro–Pro–Gly–Phe–Ser–Pro–Phe) and des-Arg^{10}-kallidin. When injected into humans or animals, BK and Lys-BK reproduce archetypal signs of inflammation: redness, local heat, swelling and pain. Although kinins are short-lived mediators, they are implicated in numerous physiological and pathophysiological processes. They activate endothelial cells, induce vasodilatation and contraction of smooth muscles, enhance microvascular permeability, stimulate the production of superoxide radicals and nitric oxide, modulate the mobilization and release of histamine, arachidonic acid, prostaglandin E2, pro-inflammatory Interleukin-1 and TNF-alpha. Kinins are also involved in cardiac homeostasis, in the regulation of renal function and blood pressure, and in various immune and inflammatory disorders [2,7–9]. The kinin moiety of kininogen may be submitted to post-translational modifications, such as the hydroxylation of the third proline of BK, without impairing its biological activities [10]. Supplementary kinins were found specifically in rats: T-kinin (Ile-Ser-BK) and Met-T-kinin [11]. Kinins are rapidly hydrolyzed in sera and other biological fluids by kininases (BK half-life: 27±10 s [12]) including angiotensin-I-converting enzyme, neutral endopeptidase 24.11 (neprilysin), aminopeptidase P, or carboxypeptidase N [2,13,14].

The precursors of kinins in mammals are HK (apparent molecular mass of 90–120 kDa) and LK (50–68 kDa). Mammalian kininogens are glycoproteins synthetized in liver that occur predominantly in blood plasma at respective concentrations of 170/220 μg/ml for LK and of 70/90 μg/ml for HK [15], but are also found in other body fluids and organs such as kidney and in cells such as neutrophils [16–18]. Early purification and partial characterization of human kininogens were initiated during the 1960s and 1970s [19–21]. Both native forms have an identical N-terminal heavy chain (domains D1, D2, and D3), a short D4 domain corresponding to the BK moiety, and because of an alternative splicing of the gene transcript, two different C-terminal light chains: LK discloses a single D5 domain (D5L), while HK shares a D5 domain (D5H) along with a D6 domain [22,23]. Indeed HK and LK are coded by a single gene (also referred as K gene) located on chromosome 3 that consists of eleven exons and ten introns. The first nine exons code for the heavy chain of both kininogens, exon 10 encodes a mutual sequence for both kininogens (bradykinin and a 12-mer peptidyl moiety downstream to BK) plus the HK light chain, whereas exon 11 codes for the LK light chain (4–5 kDa) [22]. D2 and D3 domains are cystatin-like domains that act as tight-binding inhibitors of cysteine cathepsins [24–27], while the D1 domain, which has lost its inhibitory properties, may operate as a calcium transporter [28]. These three cystatin-like domains have arisen from an ancestral gene by two successive gene duplications [1,29]. According to a recent phylogenetic study two paralogous kininogen genes were initially present in vertebrates, but during evolution one original gene was saved with frequent multiplication in amphibians, yet lost in mammals [30]. In addition to its inhibitory properties, the D3 domain binds to platelets and endothelial cells (for review: [1]). It has been shown recently that a 26-mer fragment within D3 possesses a potent antibacterial activity [31]. D5 domain of HK (also called kininostatin) has cell binding sites, anti-angiogenic properties, attachement sites for heparin binding [32,33] and may be an effector of the innate immunity due to its potency to kill bacteria [34]. Such bactericidal properties are described later in this review (see section II). HK binds to negatively charged surfaces through a cluster of His residues located in its D5 domain (D5H). The D6 domain has prekallikrein- and factor XI-binding sites [1]. In contrast to HK, LK light chain (D5L) lacks prekallikrein-binding sites and hence contact activation capability; its function remains unclear. While a reduced circulating level of both kininogens has been reported under pathological conditions such as cirrhosis [35], surprisingly kininogen deficiency in humans is apparently asymptomatic [36].

Both rat LK and HK also arise from a single gene (K gene) by alternative splicing. However, they encompass some peculiar features, since Arg-bradykinin is found instead of Lys-bradykinin, and tissue kallikrein releases BK and not kallidin [37]. Moreover, rat plasma contains a third type of kininogen, so-called T-kininogen (TK) (for review see [38]). This additional kininogen (formely known as alpha-l-acute phase globulin) is the only mammalian kininogen described to date whose concentration increases dramatically during inflammation [39]. TK was first identified as an acute phase reactant in rat serum [40] and later found to be identical to alpha-l-cysteine proteinase inhibitor [41]. TK, unlike HK and LK, is not subject to kallikrein hydrolysis. Trypsin, however, cleaves TK and releases T-kinin (Ile-Ser-bradykinin) [42], while cathepsin D may release Met-T-kinin-Leu, but not T-kinin, from TK [43]. In addition cathepsin D may degrade and inactivate an isolated domain (D3) of kininogen as well human stefins A and B, and cystatin C [44,45]. Therefore TK has also been called thiostatin, a name thought to better fit its presumed biological function as a cysteine protease inhibitor [46]. TK exists in two closely related forms (TK-I and TK-II). The sequences of the mRNAs coding for the two TKs and rat LK are more than 90% homologous. However, TKs are generated by two distinct TK genes (T-I and T-II genes), which appears to have been created as a sidetrack evolutionary product through duplication of the K gene after the divergence of rat and mouse [30,47]. The rat Brown Norway–Katholiek strain carries a congenital kininogen deficiency (i.e. HK- and LK-deficient rats) while the production of TK remains unaffected. A puzzling observation is that the Katholiek strain does not show any physiological difference from parental, normal kininogen-producing (Brown Norway) strain as described in human kininogen-deficient counterparts [37].

The two major pathways for kinin generation, which fulfill different functions, are the plasma kallikrein–kinin system, which initiates the activation of the intrinsic coagulation cascade, and a simpler pathway involving tissue kallikrein [2,8,48,49]. Human tissue kallikrein (hK1 or KLK1) belongs to a family of 15 kallikrein-related peptidases (for a recently updated nomenclature see: [50]). Unlike other members of this family, hK1 releases Lys-BK from LK by cleaving the Met379–Lys380 and Arg389–Ser390 bonds, also in HK. The plasma kinin-forming system (contact system) typically involves three serine protease zymogens (factor XII or Hageman factor, factor XI, and plasma prekallikrein) and HK. Plasma kallikrein is synthesized in the liver and secreted by hepatocytes as an inactive zymogen predominantly associated with HK in the bloodstream. Contact of plasma with negatively charged surfaces (e.g. in sulfated proteoglycans or endotoxins) leads to a cascade of proteolytic episodes including the auto-activation of Hageman factor. Finally prekallikrein is converted to kallikrein by factor XIIa, and the newly formed plasma kallikrein cleaves HK to release BK [51]. Binding of HK to glycosaminoglycans blocks the release of BK into the plasma and on surfaces of endothelial cells [52]. Alternatively, activation may be initiated by binding of constituents of the contact activation cascade to the surface of leukocytes, platelets, or endothelial cells. This pathway involves a multi-protein receptor which consists of at least cytokeratin-1, urokinase plasminogen activator receptor, and gC1qR [48,53]. Proteases involved in the intrinsic clotting cascade and kinin generation may also trigger the activation of the complement system. The contact activation phase is regulated in plasma by inhibitors such as antithrombin III, C1-inhibitor, α2-macroglobulin and α1-proteinase inhibitor. The C1-inhibitor and α2-macroglobulin are the main plasma kallikrein inhibitors [54–56]. On the other hand hK1 may be regulated by kallistatin, a kallikrein-binding protein that is found in many tissues, cells, and fluids [57]. In research settings, both hK1 and plasma kallikrein are also inhibited by aprotinin from bovine organs [58].