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Plasma Hemopexin as a Potential Regulator of Vascular Responsiveness to Angiotensin II

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Abstract

This brief review focuses on the functional activities of plasma hemopexin recently recognized by several authors. In particular, the protease-like activity of hemopexin in vitro is linked with downregulation of the vascular angiotensin II receptor in vivo, leading to vascular expansion. Also a potential mechanism of inhibition of hemopexin activity by extracellular adenosine triphosphate is considered.

Keywords

hemopexin activity, angiotensin II receptor, vascular expansion, protease-like activity, ATP

Introduction

The plasma β1-glycoprotein hemopexin (Hx) is known as an acute phase protein that can bind free heme.[1-3] As free heme itself can cause oxidant stress due to its catalytic activity, removal of free heme from the circulation is essential.[4,5] Many plasma molecules can be considered as moonlighting proteins, showing various functions including enzymatic activity.[6] For instance, even a common plasma molecule like albumin shows phosphodiesterase activity.[7] Different functional roles have also been attributed to Hx, such as suppression of neutrophil necrosis,[8] inhibition of cellular adhesion,[9] or attenuation of inflammation.[10] Our group has observed serine protease–like activity in various purified Hx preparations from human plasma as well as in recombinant Hx samples.[11,12]

The isoform of Hx may not circulate in its active state, but interaction with the local microenvironment may promote Hx activity, as is true for many serine proteases in the circulation, for example coagulation factors.[13] Thus, the coagulation factors or certain enzymes belonging to the complement system, which are potentially harmful for the vessel wall in their activated form, may circulate in either zymogenic form or in a soluble complex with an inhibitor.[14] Normal blood plasma contains large amounts of serine protease inhibitors (SERPINS) that can silence potentially damaging proteases including proteases released by activated neutrophils.[13] In view of the potential effect of the active form of Hx upon the angiotensin II receptor availability,[15] it seems likely that the protease-like effect of this particular molecule is also regulated in vivo. Although the configuration of the possible inactivated Hx in the circulation of healthy individuals is unknown, in vitro data clearly point to an inhibiting capacity of nucleotides such as adenosine triphosphate (ATP).[12] Also recent observations in healthy pregnant women versus patients with preeclampsia (PE) support the notion that the balance between active Hx and its ''natural'' inhibitor, that is extracellular ATP, may be important.[15,16] The inhibitory effect of extracellular ATP upon the protease-like activity of plasma Hx is also supported by the observation in an experimental sepsis model.[17] The authors describe an Hx-mediated reduction in neutrophil migration in experimental sepsis in mice. They show reversal of the Hx effect upon neutrophil chemotaxis in vitro following ATP supplementation. Also decreased plasma ATP was observed in mice with severe sepsis.[17]

Active Hx and Vascular Expansion in Pregnancy

In pregnant women, active Hx rises from week 10 of gestational age until the end of pregnancy.[15] In addition, around the same gestational age, the vascular responsiveness on

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angiotensin II decreases, leading to an expanded vascular bed in healthy pregnant women.[18] In contrast, participants with PE do not show an increase in plasma Hx activity, whereas the vascular responsiveness upon angiotensin II is persisting in these patients, resulting in a contracted vascular bed and hypertension. Increased levels of plasma ATP occur in PE as compared to healthy pregnant individuals.[16] We observed that in PE the plasma Hx is inactivated by forming a complex with ATP, since treatment of PE plasma samples with soluble apyrase (ie, ATP/ADP phosphatase) leads to reactivation of Hx in these samples.[16] Conversely, ATP supplementation of plasma samples of healthy pregnant individuals inhibits its Hx activity as tested using our standard assay.

Mechanism of Vascular Expansion by Hx

Vascular tone is controlled by a myriad of systems. Both the autonomic neural and hormonal systems, such as the renin–angiotensin–aldosterone system (RAAS), are essential. Local vasodilatation is also mediated by the molecules derived from smooth muscle or endothelial cells, that is nitric oxide as well as products derived from the kinin system (bradykinin) or proinflammatory mediators.[19] Hemopexin may interact with the RAAS. Thus, incubation of endothelial cells carrying a receptor for angiotensin II (AT-1R) with Hx resulted in the shedding of AT-1R as demonstrated by flow cytometry and Western blotting.[16] Previously, we have also shown that Hx is able to affect extracellular matrix molecules (ECMs) such as glomerular anionic sites (sialoglycoproteins, heparin sulfate proteoglycans, and the ecto-enzyme CD39).[20] Interestingly, in participants with corticosteroid-responsive idiopathic nephrotic syndrome (INS) in relapse, loss of glomerular anionic sites occurs concomitantly with an increase in plasma Hx activity.[21] Proteinuria was also observed in rats following intrarenal infusion with active Hx; glomeruli of these animals showed a similar lesion as in human INS, that is loss of glomerular ECM including glomerular CD39, anionic sites, and effacement of glomerular podocytes as detected by electron microscopy.[22] Whether there is loss of AT-1R in patients with INS or in rats infused with active Hx remains to be established.

In view of the data regarding the in vitro activity of Hx, it is obvious that in healthy pregnant participants the loss of AT-1R may be (at least partly) due to their increased plasma Hx activity after week 10 of pregnancy. In other words, the active isoform of Hx may be considered as an endogenous factor controlling blood pressure by mediating the angiotensin II sensitivity of the vessel wall. This notion is supported by observations in healthy volunteers on low-salt versus high-salt diets. Their mean plasma Hx activity decreased after high sodium versus low sodium intake, suggesting that the availability of vascular angiotensin II receptors may be associated with their plasma Hx activity (Krikken JA, Lely AT, Bakker SJL, Borghuis T, Faas MM, van Goor H, Navis GJ, Bakker WW, unpublished data, 2011).

Purification and Measurement of Hx Activity

Advanced technical insight has led to various modifications regarding the purification of Hx from human plasma pool. Over the last decade, we used a relatively simple method.[11] In brief, plasma from EDTA blood was dialyzed and run over an ion exchange column using a saline gradient. The 0.08 mol/L fraction was concentrated and applied to a column of sepharose conjugated with polyclonal anti-Hx immunoglobulin G (IgG). This affinity column was subsequently eluted with an acid glycine buffer followed immediately by eluate neutralizing. Depending on the magnitude of Hx glycosylation, the molecular mass may vary between 75 and 85 kDa, using this method.[11]

Previously, we studied the enzymatic activities in plasma fractions with a molecular weight of approximately 100 kDa. This factor was termed 100KF.[11] The active moiety of 100KF appeared to be an isoform of plasma Hx. Using histochemistry or immunostaining, we showed that this factor was able to strip glomerular ECMs (ie, sialoglycoproteins) and the glomerular CD39 after incubation of cryostat sections of kidney tissue with this factor. This method, based on the loss of glomerular apyrase, was termed the ''apyrase stripping assay''. Next to this in vitro assay, it appeared that the protease activity of Hx could also be measured by standard amidolytic assays using chromogenic substrates.[12]

We routinely use the chromogenic substrate S2302 (ie, H-D-Pro-Phe-Arg-pNA.2HCl) for estimating the activity of purified human plasma Hx or recombinant Hx. Substrate S2251 (H-D-Val-Leu-Lys-pNa.2HCl) is used as a control substrate, with no (or minimal) digestion after incubation with active Hx. Also the ultra performance liquid chromatography (UPLC; Waters Co, Milford, Massachusetts) can be applied to show that Hx is able to clip substrate S2302, resulting in the formation of 2 fragments (see Figure 1).

Inhibition of Active Hx

The activity of Hx in vitro can be inhibited by regular serine protease inhibitors (antithrombin III, phenyl methyl sulfonyl fluoride [PMSF], soy bean trypsin inhibitor [SBTI]) or by α-2-macroglobuline and also, as stated before, by nucleotides like adenosine diphosphate (ADP) or ATP.[12,23] However, inhibition with ATP using the amidolytic assay with S2302 as a substrate showed inconsistent results. Apparently, to enable the inhibiting action of ATP, the microenvironment (ie, tissue factors present in the apyrase stripping assay) is essential. The question emerged as to which particular tissue factor is important. Based on the abundant presence of glomerular ectoapyrase in kidney tissue, our guess was that glomerular apyrase per se may facilitate the inhibiting effect of ATP. Indeed, supplementation of soluble apyrase resulted in the reproducible inhibition of Hx activity using the amidolytic assay with S2302. Apparently,

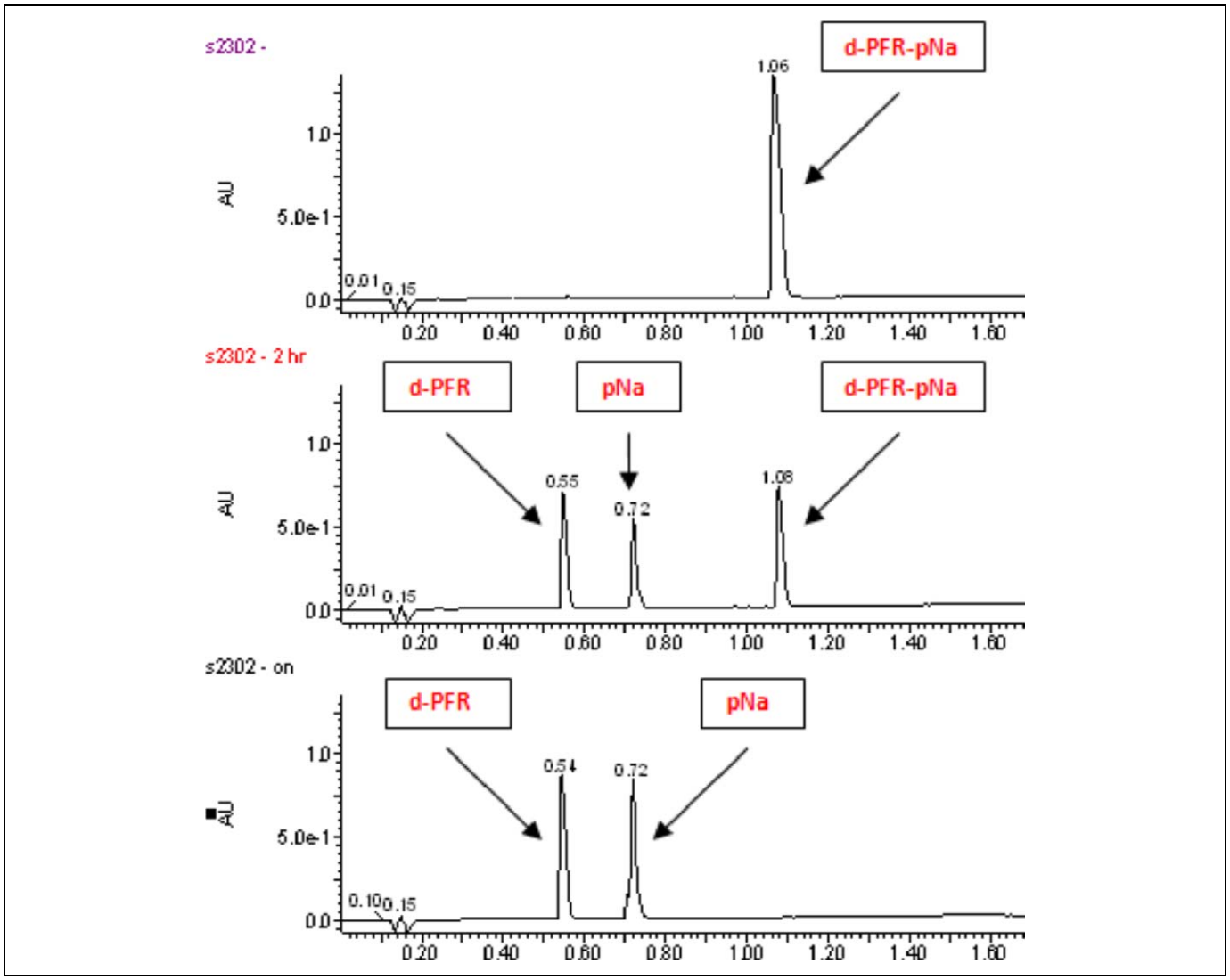

Figure 1. Digestion of synthetic substrate D-Pro-Phe-Arg-pNA.2HCl (S2302; 0.5 mg substrate/mL Tris-HCl; Chromogenix, Milan, Italy) by purified plasma hemopexin (Hx 300 μg/mL Tris) followed by Ultra Performance Liquid Chromatography (UPLC; Waters Co, Milford, Massachusetts). Peaks shown above are the diode array signal A running an ACN/H2O-gradient 5% to 55%/2 min. Upper panel shows the intact substrate identified by mass signal (Rt = 1.06) without Hx. The middle panel shows digestion of S2302, after 2 hours of incubation with Hx resulting in 2 products (Rt = 0.54; and Rt = 0.72) next to the original substrate. Identification of complying mass peaks shows exact cleavage at the C-terminus of the arginine of the starting material. The lower panel shows complete digestion of S2302 after overnight incubation with Hx. (X-axis: absorbance units [Aus] at 215 nm; Y-axis: time in minutes.) on indicates overnight; ACN, acetonitrile.

enzyme–substrate interaction is required for this particular ATP effect to occur in vitro.

The exact mechanism of Hx inactivation by ATP in the presence of apyrase remains to be studied. It is conceivable, however, that cleaving of the phosphate bondings of ATP by apyrase liberates (energy rich) phosphate in such a way that Hx is functionally inactivated by phosphorylation. This notion is supported by the observation that inhibition of Hx activity with ATP in the apyrase stripping assay cannot (or to significant lesser extent) be mimicked using ATPγS, a nondegradable ATP analogue. Also, using the amidolytic assay, replacement of ATP by ATPγS in the presence of apyrase leads to blunting of inhibition of Hx activity. This suggests that (depending on the ratios of substrate, enzyme, and digestion products in the microenvironment) hydrolysis of ATP molecules by apyrase may facilitate the potential of ATP to inhibit Hx activity.

Hx: Protease or Protease Like?

The question as to the exact functional properties of Hx remains to be resolved. The heme binding and transport function of Hx is widely recognized. Other putative functions such as Hx as a necrosis-suppressing factor or as an inhibitor of cellular adhesion or as an anti-inflammatory agent, have only recently been described.[8-10] We observed protease-like activity in both native and recombinant Hx, although alignment studies did not show primary sequence characteristics for proteases (unpublished data). As some authors claim that structure and function of proteins

should always be in line,[24] according to this concept, Hx cannot act like a protease. However, the structure–function dogma may be obsolete, since it has recently been shown that not the sequence alone, but rather the tertiary and quaternary structures of a protein molecule may determine its potential functional properties.[25-27] Whatever be the outcome of studies regarding the possible multiple functional characteristics of Hx, as Hx is able to act like a protease, we consider this plasma constituent as a protease-like endogenous factor, which is able to regulate the availability of vascular angiotensin II receptors.

Declaration of Conflicting Interests

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Authors' Note

CD39 or ectoapyrase is also termed ENTPD1 (ectonucleoside triphosphate diphosphohydrolase 1).

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