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Hyaluronan-binding properties of human serum hemopexin

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Abstract Hemopexin, the heme-binding serum glycoprotein, exhibits a complex electrophoretic pattern on two-dimensional immunoelectrophoresis on agarose gels into which hyaluronic acid is incorporated in the first and monospecific anti-hemopexin in the second dimension. This heterogeneity reflects a range of interactions of hemopexin isoforms with hyaluronic acid. Electrophoretic patterns of individual human sera greatly differ in their contents of hyaluronan-interacting hemopexin species. Hemopexin itself has no hyaluronidase activity.

Key words: Hemopexin; Hyaluronic acid; Affinity electrophoresis

1. Introduction

Hemopexin is the heme-binding serum glycoprotein which is responsible for the transport of heme released into the blood stream from hemolysed erythrocytes to the liver for further metabolism [1]. The physiological sense of this metabolic pathway is to save iron which would otherwise escape from the body [2] and to prevent toxic oxidative effects of free heme on the tissues [3]. The heme scavenging property of hemopexin is well known [4]. Additional physiological functions of hemopexin have recently been suggested, i.e., involvement in neuron repair [5,6] and the hyaluronidase activity (the ability to hydrolyze polymeric hyaluronic acid). The latter function was derived from the observed identity of hemopexin and serum hyaluronidase [7].

The present communication presents evidence that purified hemopexin as well as the variant fraction of hemopexin contained in human serum have an affinity to hyaluronan. This property may be responsible for its accumulation in healing neuronal tissues. No hyaluronidase activity was, however, observed in purified hemopexin. This activity occurred in some hemopexin preparations due to contaminating hyaluronidases.

2. Materials and methods

2.1. Materials

Hyaluronic acid from human umbilical cord and chondroitin sulfate A from bovine trachea were purchased, as the sodium salts, from Sigma-Aldrich (Czech Republic).

Human hemopexin was isolated from the serum of healthy individuals by sequential chromatography on S-Sepharose FF and Blue Sepharose CL-6B [8].

2.2. Electrophoretic methods

Crossed affinity immunoelectrophoresis in agarose gel was performed according to Weeke [9] using a Multiphore apparatus (Pharmacia, Sweden). Hyaluronic acid (0.5 mg/ml) was incorporated into the agarose gel in the first and 150 μl of polyclonal anti-human hemopexin antiserum (SEVAC, Prague, Czech Republic) into the second dimension.

Substrate gel electrophoresis (hyaluronan-PAGE) was performed in 7.5% native polyacrylamide gels containing 0.17 mg/ml hyaluronic acid [10] using Mini-Protean II apparatus (BioRad, USA). 15 μl of 2.5 mg/ml hemopexin solution or of 1/20 diluted human serum was applied. The hyaluronidase activity was assessed by Alcian blue staining of the gels incubated for 12 h at 37°C in 0.1 M Na formate [11]. In parallel, the gels were stained with Coomassie blue R-250.

3. Results

The pattern of hemopexin, of human sera (Fig. 1A,B) and purified protein (Fig. 1C), in crossed affinity immunoelectrophoresis with hyaluronan, shows bi- to multiphasicity. Omission of hyaluronan from the first dimension gel resulted in a single symmetrical immunoprecipitation arc, observed for the human sera (Fig. 1a,b) and purified hemopexin (Fig. 1c). Components immunologically identical or largely homologous with hemopexin appear to be present in the sera which exhibit hyaladherent properties demonstrated by the inhomogeneous retardation of hemopexin on hyaluronan-containing gels. Both patterns and contents of anodically slower components differ markedly among individual sera as well as among individual hemopexin isolations, comprising 10–60% of total hemopexin. Such a complex immunoprecipitation pattern as that of hemopexin was not observed for any other serum protein. All serum proteins, however, are to some extent retarded on gels containing hyaluronan as compared to gels without hyaluronan (not shown), undoubtedly due to a high electroosmotic flow caused by immobilized negatively charged hyaluronic acid.

To investigate whether the microheterogeneity of hemopexin is specific for its interaction with hyaluronan we also incorporated into the first dimension chondroitin sulfate, another negatively charged glycosaminoglycan. The presence of this compound causes neither a shift in net mobility nor hemopexin heterogeneity. We conclude that the observed multiphasic electrophoretic patterns reflect specific hyaluronan–hemopexin interactions.

Several preparations of purified hemopexin were subjected to hyaluronan-PAGE and developed with Alcian blue for hyaluronidase activity (Fig. 2A) and with Coomassie blue staining for protein (Fig. 2B). Hyaluronidase (clear zones in Fig. 2A) is present in three out of seven hemopexin preparations. The electrophoretic mobility of the enzyme by itself, however, is distinctly slower than that of hemopexin and equal to that of hyaluronidase contained in the human serum (well 8, Fig. 2A). Since hemopexin has some affinity to Alcian blue (dark zones in Fig. 2A), a comparison can also be made on the Alcian blue stained gels. Obviously, hemopexin by itself has no hyaluronidase activity. Rather, serum hyaluroni-

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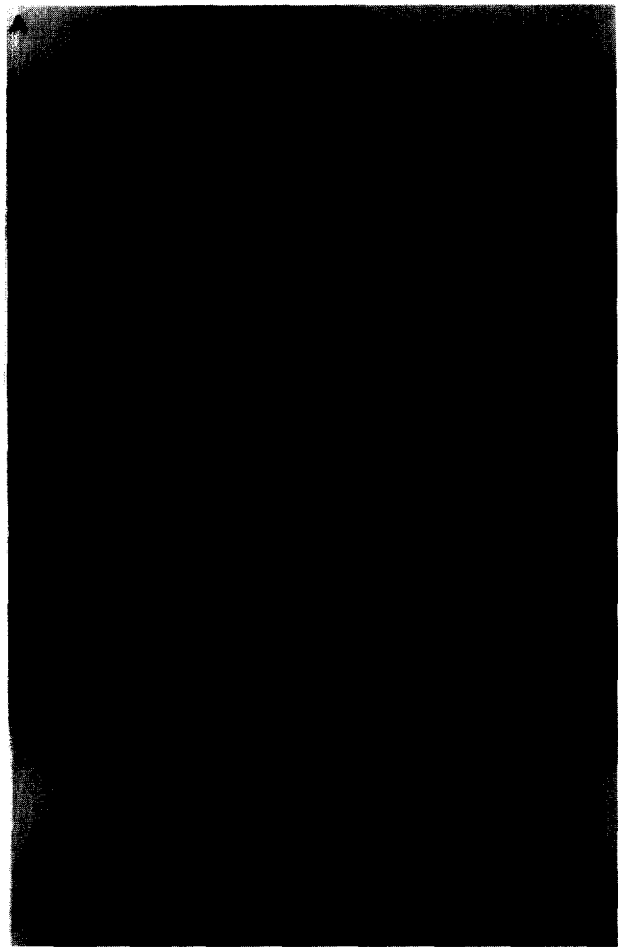

Fig. 1. Electrophoretic patterns of hemopexin, in serum or purified. Crossed immunoelectrophoresis of human sera (A, B) and of purified human hemopexin (C) performed with gels that contained hyaluronic acid (0.5 mg/ml) in the first dimension and polyclonal anti-human hemopexin antiserum in the second dimension. Panels a–c show control experiments performed with gels not containing hyaluronic acid. 3 μl of serum or 1 mg/ml hemopexin was applied.

case as a contaminant or possibly a complex may escort hemopexin during isolation procedures.

4. Discussion

Hemopexin is a heme-scavenging serum glycoprotein. Its carbohydrate moiety consists of five oligosaccharide chains [12] and comprises about 20% of the protein molecular mass [13]. Hemopexin single polypeptide chain is composed of two homologous domains joined by a hinge region [14]. A 'hemopexin domain' is also contained in a number of proteins functionally unrelated to hemopexin, such as matrix degrading metalloproteinases (collagenases, gelatinases, stromelysins) [15] and the cell adhesion plasma protein vitronectin [16]. Recently, a common hyaluronan binding motif $B(X_7)B$ (B is Arg or Lys and X any non-acidic amino acid residue) was found in the hyaluronan-binding proteins RHAMM, CD44 and the link protein [17] as well as in human, rat, rabbit and pig hemopexins [7]. The $B(X_7)B$ motif may be responsible for the hyaluronan-binding property of hemopexin. The proportion of this binding property in individual human sera as well as in isolated hemopexin preparations ranges from 10 to 60% of the total hemopexin. As only a fraction of hemopexin molecules is retarded on hyaluronan-containing gels the hyaluronan-binding motif cannot be solely responsible for the multiphasicity of the hemopexin patterns. The well-known hemopexin microheterogeneity in composition of five oligosaccharide chains [18] may result in subtle conformational differences of hemopexin polypeptide which are reflected in heterogeneous ligand binding. Consequently, the individual hemopexin populations are to various extents retarded on hyaluronan-containing gels. The great variety in the contents of HA-interacting hemopexin species in the individual sera will further be explored.

The observed interactions of hemopexin(-like?) species with hyaluronan may explain the long-term accumulation of hemopexin in hyaluronic acid rich tissues, i.e. healing neurons [5,6] and brain [19].

Hyaluronidase activity, if present in hemopexin preparations, was due to contaminanting hyaluronidases. It was a minor contaminant as 40 μg of protein had to be applied per well of hyaluronan-PAGE the hyaluronidase activity to be observed.

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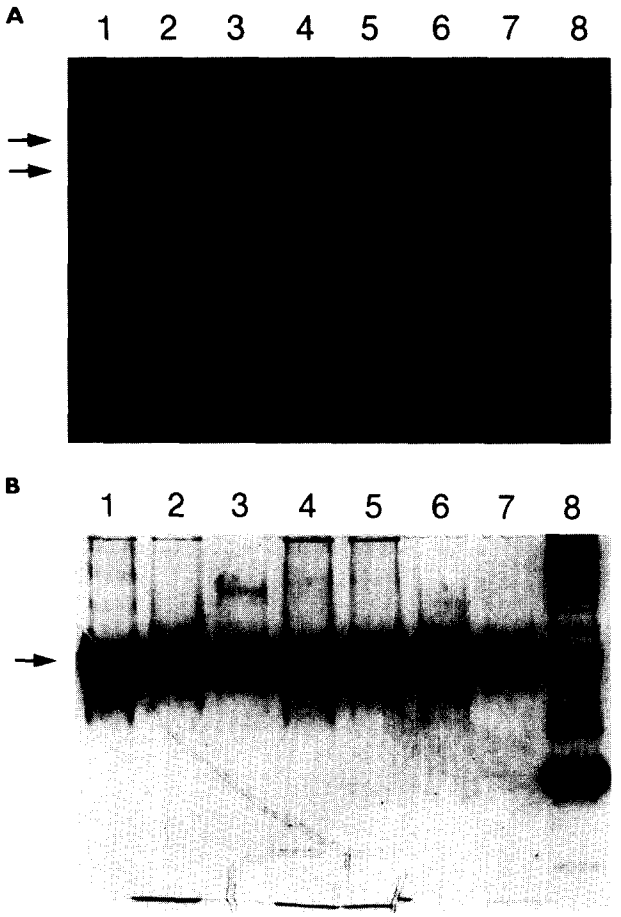

Fig. 2. Hyaluronidase activity testing of hemopexin preparations and a representative serum. A: Individual hemopexin preparations (lanes 1–7) and a representative human serum (lane 8) on substrate-PAGE. The 7.5% 'native' polyacrylamide gel contained 0.17 mg/ml hyaluronic acid and was stained for hyaluronidase activity with Alcian blue. The positions of hyaluronidase activity (clear bands) and of hemopexin (dark bands) are depicted. B: Control PAGE gel stained with Coomassie blue R-250.

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