

Fattori della coagulazione: Biochimica e fisiologia

DETERGENT AND GANGLIOSIDE GM1-BINDING PROPERTIES OF PLATELET FACTOR 4 AS ASSESSED BY CHARGE-SHIFT ELECTROPHORESIS

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Background and Aims. platelet factor 4 (PF4) is a 7.8 kDa chemokine comprising 70 amino acids. Three anti-parallel β-sheets defining a hydrophobic region characterise the N-terminus; two pairs of contiguous oxidizable lysine residues defining a β-helix with amphiphilic properties characterised the carboxyl-terminus. During coagulation, the positively charged moieties of PF4 bind with high affinity to the negatively charged heparan sulphate (HS) and glycosaminoglycans (GAGs) neutralising their anticoagulant effect. Herein we detailed the amphiphilic and HS and ganglioside GM1-binding properties of PF4 and their possible modification after oxidation and nitration.

Methods. charge-shift electrophoresis (CSE) (1% agarose gels (0.05 M glycine/NaOH, 0.1 M NaCl, pH 9.0) in the presence of: 1) a detergent mix of composed of neutral TritonX-100 (TX100) and anionic deoxycholate (DOC) and in the presence of 2) the cationic detergent cetyltrimethylammonium bromide (CTAB); in vitro oxidation with 1mM 4-hydroxy-2-nonenal and in vitro nitration with 60 μM peroxynitrite (ONOO-).

Results. 1) PF4 shifted markedly in the presence of TX-100 plus DOC, indicating its ability to participate in the formation of mixed micelles with TX-100 and DOC compared to TX-100 alone or no detergent at all. 2) In the presence of TX-100 plus CTAB, PF4 exhibited no cathodic shift. The incorporation of Sudan Black B proved that mixed micelles of TX-100/DOC and TX-100-CTAB were formed under the conditions employed. However, the anodic shift of PF4 in the presence of DOC, with unaltered migration in the presence of CTAB are testimony to the electrostatic nature of the interaction of PF4 with DOC, with no participation of PF4 in the formation of mixed detergent micelles. 3) The migration of PF4 was shifted to the anod also in the presence of ganglioside GM1 at concentrations from 0.33 to 1.33 mg/mL, exceeding the GM1 critical micellar concentration. The formation of GM1 micelles was also documented by the SBB incorporation. GM1 binding to PF4 was dose-dependent, reaching saturation at the GM1:PF4 ratio of 1.2:1 by mass, or 23:1 on a molar basis. The formation of GM1 micelles with multiple negative charges was indispensable for shifting PF4 toward the anod, since the interaction of PF4 with the sole GM1 pentasaccharide mojety was unable to cause the same effect. 4) By the same CSE methodology in the absence of detergents, but with the addition of either HS or chondroitin sulfate (CS), PF4 shifted to the anode more markedly than in the presence of DOC and GM1; in addition, the PF4-CS complexes were highly poly-dispersed compared to the PF4-HS complexes. 5) In vitro oxidation with 1 mM 4-hydroxy-2-nonenal or nitration with 60 μM peroxynitrite (ONOO-) made PF4 undetectable in the agarose gels, likely due to aggregation/precipitation in the reaction vials.

Conclusions. we document the ability of PF4 to establish electrostatic interactions with negatively charged amphiphilic molecules, such as the negatively charged surfactant DOC and ganglioside GM1, as well as polyanionic polysaccharides. This is relevant in physiological PF4 interactions with the annular lipid shells that are in contact with the hydrophobic surfaces and support the structural stability and functions of integral membrane proteins, such as HS proteoglycans expressed on endothelial cells and activated platelets, perlecan and chemokine receptor CXCR3B at the surface of endothelial cells. The destabilization of PF4 upon reaction with 4-hydroxy-2-nonenal and peroxynitrite indicates that in the course of oxidative and nitrative stress occurring in inflammation the physiologic functions of PF4 could be lost.