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Heparin–protein interactions: From affinity and kinetics to biological roles. Application to an interaction network regulating angiogenesis

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ABSTRACT

Numerous extracellular proteins, growth factors, chemokines, cytokines, enzymes, lipoproteins, involved in a variety of biological processes, interact with heparin and/or heparan sulfate at the cell surface and in the extracellular matrix (ECM). The goal of this study is to investigate the relationship(s) between affinity and kinetics of heparin–protein interactions and the localization of the proteins, their intrinsic disorder and their biological roles. Most proteins bind to heparin with a higher affinity than their fragments and form more stable complexes with heparin than with heparan sulfate. Lipoproteins and matrisome-associated proteins (e.g. growth factors and cytokines) bind to heparin with very high affinity. Matrisome-associated proteins form transient complexes with heparin. However they bind to this glycosaminoglycan with a higher affinity than the proteins of the core matrisome, which contribute to ECM assembly and organization, and than the secreted proteins which are not associated with the ECM. The association rate of proteins with heparin is related to the intrinsic disorder of heparin-binding sites. Enzyme inhibitor activity, protein dimerization, skeletal system development and pathways in cancer are functionally associated with proteins displaying a high or very high affinity for heparin (K_D < 100 nM). Besides their use in investigating molecular recognition and functions, kinetics and affinity are essential to prioritize interactions in networks and to build network models as discussed for the interaction network established at the surface of endothelial cells by endostatin, a heparin-binding protein regulating angiogenesis.

1. Introduction

Biomolecular interactions are connected to biological functions and the identification of interactions established by a biomolecule gives clues on its molecular functions and on the mechanisms of the biological processes it is involved in. More than 400 proteins (e.g. extracellular proteins, growth factors, chemokines, cytokines, enzymes, lipoproteins) involved in a variety of biological processes (extracellular matrix assembly, development, signaling, cancer, angiogenesis, amyloidogenesis, host-pathogen interactions) interact with heparin (HP) and/or heparan sulfate (HS), at the cell surface and in the extracellular matrix (ECM) (Capila and Linhardt, 2002; Esko and Linhardt, 2009; Lindahl and Li, 2009; Ori et al., 2011). In addition, heparin/heparan sulfate–protein interactions participate in the assembly of multicomponent complexes (Gallagher, 2006) and are potential targets for therapeutic interventions (Lindahl, 2007). It is thus important to characterize these interactions at the molecular level.

Numerous approaches have been used to study glycosaminoglycan–protein interactions such as solid-phase and filter-binding assays, affinity chromatography, electrophoretic techniques (Powell et al., 2004), surface plasmon resonance (SPR), X-ray crystallography, NMR spectroscopy (Laguri et al., 2011), molecular modeling (Ricard-Blum et al., 2004; Imberty et al., 2007; Ballut et al., 2013), dual polarization interferometry (Ricard-Blum et al., 2006a), affinity proteomics (Ori et al., 2011) and carbohydrate microarray (Marson et al., 2009; Rogers et al., 2011) including GAG arrays probed by SPR imaging (Faye et al., 2009a). Characteristic features of glycosaminoglycan–protein interactions include heparin-binding sequences, heparin/heparan sulfate sulfation and epimerization contributing to the binding (Kreuger et al., 2006), thermodynamic parameters (Gibbs free energy, enthalpy, entropy), kinetics (association and dissociation rates, ka and kd respectively) and affinity (K_D) reflecting the strength of the interactions.

The goal of this work is to investigate the relationship(s) between affinity and kinetics of heparin–protein interactions and the localization of heparin-binding proteins, their intrinsic disorder, their molecular functions and the biological processes or pathways they are involved in. We have also compared the affinity of proteins for heparin, low molecular weight (LMW) heparin and heparan sulfate. We built for this purpose a dataset of protein–heparin interactions characterized by SPR because this technique allows the calculation of kinetic parameters and affinity. Furthermore the selection of a single technical approach

Abbreviations: ECM, Extracellular matrix; FGF, Fibroblast growth factor; FGFR, Fibroblast growth factor receptor; GAG, Glycosaminoglycan; GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; HP, Heparin; HS, Heparan sulfate; LMW, Low molecular weight; MMP, Matrix metalloproteinase; SPR, Surface plasmon resonance; VEGFR, Vascular endothelial growth factor receptor.

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avoids the discrepancies that might have been introduced by including interactions characterized by different techniques in the dataset. Interactions were collected among the experimental data from our laboratory, queries of the extracellular matrix interaction database (MatrixDB, http://matrixdb.ibcp.fr/, Chautard et al., 2011, 2009) and manual curation of the literature.

2. Results

2.1. Heparin–protein interaction dataset

The dataset of protein–heparin interactions integrating affinity (K_D) and kinetics (ka, kd) was built using data from MatrixDB database (98 for HP, 40 for HS) (http://matrixdb.ibcp.fr/, Chautard et al., 2011, 2009; Orchard et al., 2012) and from manual curation of the literature (www.ncbi.nlm.nih.gov/pubmed/). We also included experimental data from the laboratory on protein–heparin/heparan sulfate interactions such as endostatin, a fragment of collagen XVIII (Ricard-Blum et al., 2004), collagens I and V (Ricard-Blum et al., 2006b), α5β1 and αvβ3 integrins (Faye et al., 2009b) and procollagen C-proteinase enhancer (PCPE-1, Weiss et al., 2010). We selected protein–heparin/heparan sulfate interactions characterized by SPR, both to build a dataset comprising kinetic data and to reduce as much as possible the heterogeneity of the dataset. We extracted the affinity (185 K_D values) and kinetic parameters (130 values of association and dissociation rates, ka and kd respectively) from 69 publications reporting interactions of proteins with heparin, low molecular weight heparin and heparan sulfate. We included interaction data for low molecular weight heparin (Mw < 9 kDa) and heparan sulfate only when interaction data for heparin were available for the same protein (Table 1). The dataset comprised 160 K_D values and 118 ka/kd values of protein–heparin interactions from 65 publications, 71 unique protein–heparin interactions, 49 unique fragment-heparin interactions and 5 lipoprotein particles-heparin interactions (Table 1). Heparin-binding sequences which have been experimentally determined were integrated in the dataset when available. The full dataset is available as supplementary material (Supplementary Table 1).

2.2. Do the affinities of proteins for heparin depend upon their categories?

We first determined if different categories of proteins exhibited differences in affinity for heparin and/or in their binding kinetics to heparin. Individual values and the results of statistical analyses with p values < 0.05 are provided in Supplementary Tables 2–6. The three nuclear proteins of the dataset bound to heparin with low affinity (mean K_D value = 1.15×10^{-5} M) but this group was too small to be included in the statistical analysis. Matrisome-associated proteins bound to heparin with higher affinity than the other protein categories, except for lipoproteins, which had a significantly higher affinity for heparin than all the other categories (Fig. 1A, Supplementary Table 3). Matrisome-associated proteins and lipoproteins bound faster to heparin than the other proteins of the dataset, the differences being significant between matrisome-associated proteins and fragments ($p < 0.01$, Fig. 1B, Supplementary Table 4) and between matrisome-associated proteins and non-ECM secreted proteins ($p < 0.05$, Fig. 1B, Supplementary Table 4). The dissociation rate of the complexes formed by matrisome-associated proteins with heparin was significantly higher than those formed by lipoproteins, non-ECM secreted proteins and fragments ($p < 0.05$, Fig. 1C, Supplementary Tables 5 and 6). The lowest dissociation rates, corresponding to the most stable complexes, were observed for lipoproteins and for non-ECM secreted proteins.

Table 1
Number of affinity and kinetics values per protein category in the heparin–protein interaction dataset. Number of K_D and kinetic parameter (association rate, ka, and dissociation rate, kd) values in our dataset of protein interactions with heparin, heparan sulfate and low molecular weight (LMW) heparin for each category of proteins as defined by Hynes group (Hynes and Naba, 2012; Naba et al., 2012).

	Protein categories	Heparin	LMW heparin	Heparan sulfate
Number of K_D values	Fragments	50	0	5
	Core matrisome	13	0	4
	Matrisome-associated	52	8	2
	Non-ECM secreted	32	0	2
	Membrane	5	0	1
	Nucleus	3	0	0
	Lipoproteins	5	0	3
	Total	160	8	17
Number of ka/kd values	Fragments	46	0	5
	Core matrisome	6	0	2
	Matrisome-associated	35	0	1
	Non-ECM secreted	24	0	1
	Membrane	1	0	0
	Nucleus	1	0	0
	Lipoproteins	5	0	3
	Total	118	0	12

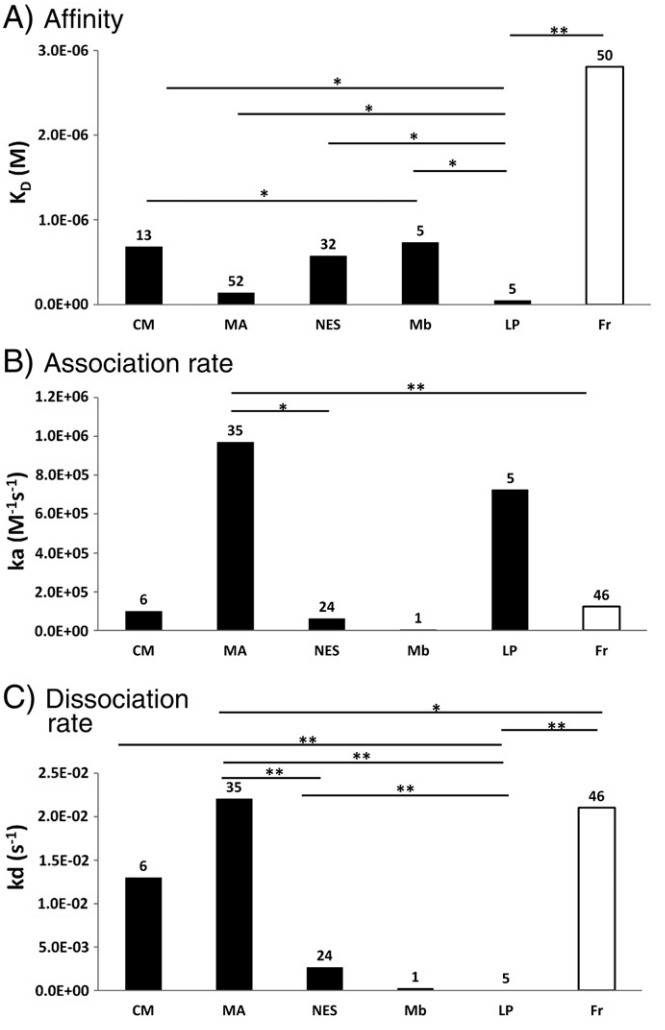

Fig. 1. Affinity and kinetics of heparin–protein interactions of protein categories. Mean affinity (A), association (B) and dissociation (C) rates of heparin–protein interactions calculated by SPR (Supplementary Table 1) as a function of protein categories defined as described by Hynes group (Hynes and Naba, 2012; Naba et al., 2012). CM: Core Matrisome, MA: Matrisome-associated, NES: Non-ECM secreted, Mb: Membrane, LP: Lipoproteins, Fr: Fragments. The number of values in each category and the statistical significance ($^*p < 0.05$, $^{**}p < 0.01$) are indicated (see Supplementary Tables 2–6 for the results of statistical analyses).

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