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Magnesium ion is an effective inhibitor of the binding of *Escherichia coli* to heparin

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Abstract

We investigated the effects of ions and temperature on the binding of *E. coli* to heparin using a chemiluminescence electrophoretic mobility shift assay. We found that magnesium ion is an effective inhibitor of the binding. The method can be readily applied to discover agents that can block the binding.

Keywords

Chemiluminescence; EMSA; Heparin; Heparin-bacteria interaction; Host-pathogen interaction; Magnesium

Microbial adhesion to host tissue is the first step in the pathogenesis of most infections. Therefore, this adhesion is a promising target for the development of new antimicrobial therapeutics (Patti et al., 1994; Thomas et al., 2007). Heparin and heparin-related oligosaccharides in the extracellular matrix of host cells are one of the molecules recognized by cell surface proteins of bacteria (Rostand and Esko, 1997; Aoki et al., 2004; Ascencio et al., 2003; Baron et al., 2004; Fleckenstein et al., 2002; Kim et al., 2004). Recently Hess et al. suggested that internalization of *Staphylococcus aureus* by intestinal epithelial cells is mediated by binding to the cell-surface heparan sulfate moiety, a structural homolog to heparin (Hess et al., 2006). With the discovery of increasing numbers of heparin or heparan sulfate-mediated bacterial infections, it is desirable to discover agents that can block this binding and lead to the development of therapeutics.

An electrophoretic mobility shift assay (EMSA) using chemiluminescence (CL) was recently introduced to complement the existing methods for the study of heparin-bacteria interactions (Kang et al., 2007). Because heparin has a high negative charge density with an average of 2.7 negative charges per disaccharide repeating unit (Capilar and Linhardt, 2002), it can be studied by EMSA. The underlying principle of CL EMSA for the quantitative measurement of heparin binding is that biotin attached to heparin is recognized by horseradish peroxidase-conjugated streptavidin. This enzyme catalyzes a chemical reaction to generate CL. The method has several advantages such as safety and stability of the sample, high sensitivity and the linearity of signal, and relative ease of performance (Kang et al., 2005). CL EMSA has been applied to quantitative

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binding studies such as RNA-protein (Kang et al., 2006a) and heparin-protein interactions (Kang et al., 2006b). Another advantage of using EMSA is the binding conditions such as buffer composition and temperature can be easily varied. This feature has been widely used to examine salt effects on DNA-protein interactions (for example, Jen-Jacobson et al., 1983). In this study, we examine binding of *E. coli* to heparin in several different conditions using CL EMSA to gauge the effects of three salts and temperature on the binding.

An *E. coli* strain, BL21 (DE3) carrying pLysS and pTYB1 (New England Biolab, Beverly, MA) was prepared and its concentration was obtained as described previously (Kang et al., 2007). Binding of *E. coli* to biotinylated heparin (B9806, Sigma, St Louis, MO) was achieved by incubating 5 nM biotinylated heparin and 4.58×10^6 bacteria in a 10 μ l PBS for 1 h at 25 °C (unless mentioned otherwise) with perturbation. The total quantity of biotinylated heparin in the reaction mixture was 50 fmol, which is within the linear signal range (Kang et al., 2006b). The binding mixtures were combined with 6x gel-loading buffer (0.25% bromophenol blue and 30% glycerol) and electrophoresed on an 8% polyacrylamide gel in 1x tris-borate/EDTA buffer at 100 V for 30 min. Heparin in the gel was transferred to nylon membranes (Pierce, Rockford, IL) by electroblotting at 100 V for 1 h using a Mini Trans-Blot Cell (Bio-Rad, Hercules, CA) and detected using a chemiluminescent EMSA kit (Pierce) following the manufacturer's protocol. The CL integral density value (IDV) of biotinylated heparin was quantitatively measured with a cooled charge-coupled device camera (Alpha Innotech, San Leandro, CA) with autobackground subtraction (Kang, 2007).

The perturbations on the intrinsic CL IDV of biotinylated heparin were found not to have a significant effect (Fig. 1A). The standard deviation of the variation was about 10%. Perturbation effects on the heparin-bacteria binding is shown in Fig. 1B. The binding was quantified using Eq. (1).

$$binding = \frac{IDV_0 - IDV}{IDV_0} \quad (1)$$

where *IDV* and *IDV*₀ represent IDV of unbound heparin with and without bacteria, respectively. Relative binding in each perturbation normalized to the control, which was set 100%, is shown in Fig. 1C.

MgCl₂ (100 mM) showed a specific inhibition by reducing the binding by 80%, which was not observed in the perturbation with added NaCl (200 mM). Therefore, one can conclude that the inhibition by MgCl₂ is caused by Mg²⁺ not Cl⁻. Mg²⁺ has the highest charge density of all the ions found in cells. Consequently, it readily binds to anionic sites, particularly to polyphosphates. Therefore, the inhibition by Mg²⁺ of the heparin-bacteria binding is likely due to electroneutralization by Mg²⁺ of heparin, which is a highly negatively charged polymer. The inhibition by Mg²⁺ *in vivo* is considered insignificant as its physiological concentration is low (~ 1mM) (Sperelakis, 1995). CaCl₂ (100 mM) was identified as an effective heparin-precipitating agent (lane 4 in Fig. 1A). While Mg²⁺ binding to heparin is purely electrostatic, Ca²⁺ binding is much stronger and more specific (Mattai and Kwak, 1988). The strong binding to heparin by Ca²⁺ may be responsible for the precipitation. The binding is not sensitive to the reaction temperature as seen in lanes 6 and 7 in Fig. 1B. Our results indicate that electrostatic interaction is the major driving force for the binding of bacteria to heparin, which can be effectively blocked by Mg²⁺. The method can be readily applied to identify agents that can block the binding of bacteria to heparin.

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Fig. 1.

Perturbation effects on the binding of *E. coli* to biotinylated heparin. (A) Perturbation effects on the intrinsic CL IDV of biotinylated heparin. (B) Perturbation effects on the binding. Binding of 5 nM biotinylated heparin with 0.76 pM *E. coli* in a 10 μ l reaction volume was achieved in several different conditions. Lane 1 is 5 nM biotinylated heparin only and lane 2 is the control binding without perturbations. Na, Mg, and Ca indicate perturbation with NaCl, MgCl₂, and CaCl₂, respectively. The lanes marked 37 and 4 represent the reaction temperature in Celcius, respectively. The region for the measurement of the CL IDV of unbound biotinylated heparin is marked. The smearing between the well and the unbound heparin in the gel indicates dissociation of bound heparin during electrophoresis. (C) Relative heparin-binding affinity of *E. coli* under variable conditions. Binding affinity obtained from Eq. (1) was normalized to the control binding.