Published in final edited form as:
Anal Biochem. 2007 April 15; 363(2): 312–314.

Characterization of heparin-living bacteria interactions by chemiluminescence electrophoretic mobility shift assay

Jonghoon Kang[&], **Myung Soog Lee**, and **David G. Gorenstein**[*]
Sealy Center for Structural Biology and Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, Texas 77555-1157, USA

Keywords

chemiluminescence; nonradioisotopic detection; EMSA; heparin-bacteria interactions; heparin; bacteria

The electrophoretic mobility shift assay [EMSA]1 is one of the most sensitive methods for studying DNA-protein interactions. Chemiluminescence [CL]1 has been used as an alternative to radioisotopic detection of samples in the EMSA [1,2], as it has advantages such as safety and stability (no isotopic decay) of the sample. In this study, we examined the feasibility of the application of CL EMSA to studying heparin-living bacteria interactions. As an example, binding of biotinylated heparin to *Escherichia coli* was examined.

The pathogenesis of most infections is initiated by microbial adhesion to host tissue. This adhesion is, therefore, a promising target for the development of new antimicrobial therapeutics [3]. In the adhesion, heparin or heparin-related oligosaccharides are one of the extracellular matrix molecules of the host recognized by cell surface proteins of bacteria [4–8].

Due to the lack of appropriate techniques for the study of the interactions of heparin-living bacteria, most heparin-bacteria binding studies have been conducted with isolated bacterial proteins [9,10]. Although useful information can be obtained from such studies, one potential drawback is the exclusion of membrane phenomena such as ligand-induced receptor oligomerization that can affect the overall binding affinity [11].

Therefore, it is desirable to examine the adhesion process using bacteria in the intact state to include the membrane phenomena. With this purpose, scintillation counting of radioisotope radiation [12] and sodium dodecyl sulfate-polyacrylamide gel electrophoresis [8] were used to assess the binding of heparin to living bacteria. More recently, atomic force microscopy was successfully used to investigate heparin-living bacteria interactions and producing quantitative data [13]. However, these methods are qualitative [8], require radioisotope labeling [12], an instrument, which may not be readily available in common laboratories [13].

EMSA has been widely employed to detect DNA-protein interactions since its first application [14]. In this study, CL EMSA is introduced to complement the existing methods to study heparin-bacteria interactions. CL has advantages such as safety and sample stability (no

*Corresponding author: David G. Gorenstein, Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, Texas USA 77555-1157, Telephone: 409-747-6800, Fax: 409-747-6850, E-mail: dggorens@utmb.edu
&Current address: Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA. 24061-0477 USA

isotopic decay), while still exhibiting linearity between signal and sample quantity [15]. It has been applied to a quantitative binding study of RNA-protein [16] and heparin-protein interactions [17]. The underlying mechanism of CL generation is that biotin attached to heparin is recognized by horseradish peroxidase-conjugated streptavidin. This enzyme catalyzes a chemical reaction to generate luminescence [15]. In this study, we examined the feasibility of CL EMSA to quantitatively study of heparin-living bacteria interactions using *E. coli* as a model system.

An overnight culture of a commercial *E. coli* strain, BL21 (DE3) carrying pLysS and pTYB1 (New England Biolab, Beverly, MA) was centrifuged and washed two times with PBS1 and resuspended in the buffer. Aliquots of the *E. coli* containing buffer were used for the measurement of OD600 (optical density at 600 nm) of the cells and in the heparin binding assay. Luria-Bertani broth and plates contained 50 μg/ml ampicillin and 34 μg/ml chloramphenicol. From the number of *E. coli* colonies on the plate after spreading the cells with a defined volume and the OD600, we obtained the relationship between OD600 and the *E. coli* concentration in PBS. Colony numbers (y) on the plates were fit to Eq. (1) by linear regression using *Mathematica* (Wolfram Research, Champaign, IL).

$$y = ax \tag{1}$$

where x is the volume (μl) multiplied by 10^{-6} × OD600 of the cell solution spread on a plate. The constant a was obtained as 0.46 ± 0.02 and R^2 for the fitting was 0.992. This suggests that one OD600 of the cells corresponds to 0.76 pM.

Binding of biotinylated heparin (Sigma, St Louis, MO) to *E. coli* was achieved by incubating 5 nM biotinylated heparin in PBS and a variable quantity of the bacteria in a 10 μl reaction volume for 1 h at room temperature. The total quantity of biotinylated heparin in the reaction mixture was 50 fmol, which is within the linear signal range [17]. Then, 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 trisborate/EDTA buffer at 100 V for 30 min. Heparin in the gel was transferred to Biodyne 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 LightShift Chemiluminescent EMSA kit (Pierce) following the manufacturer's protocol. Omission of the UV crosslinking step because of the absence of crosslinkable double bonds in heparin, unlike nucleic acids, did not cause a problem in the detection, indicating the electroblotting was sufficient for the attachment of heparin to a nylon membrane. The CL IDV1 of biotinylated heparin was quantitatively measured with a cooled CCD1 camera (Fluor Chem 8800 Imaging system, Alpha Innotech, San Leandro, CA) [18] with autobackground subtraction.

Obviously, the size of *E. coli* is huge so that any shifted bands were not detected (Fig. 1A). Degree of binding (B) of heparin to *E. coli* was assessed by Eq. (2):

$$B = \left(1 - \frac{IDV_C}{IDV_0}\right) \tag{2}$$

where IDV_C, and IDV_0 are the IDV of unbound heparin at the concentration C of the cell or without the cell, respectively.

Binding of heparin (H) to the protein (P) on the bacterial cell surface was modeled in the following way.

$$H + P \leftrightarrow HP. \tag{3}$$

The relationship between degree of heparin-protein binding (B_{HP}) and protein concentration [P] corresponding Eq. (3) is:

$$B_{HP} = \frac{[P]}{K + [P]} \tag{4}$$

where K is the dissociation constant. Here, the total protein concentration was used for [P] as the heparin concentration used in the titration was significantly less than the dissociation constants of most known heparin-protein bindings [9]. This is another advantage of CL EMSA, as it requires a very small quantity of biotinylated material for detection because of its high sensitivity [16,17]. It is desirable to express Eq. (4) using the experimental variable, total *E. coli* concentration [E] so that the experimental measurement (Eq. (2)) can be applied directly. If the number of the heparin-binding proteins on the cell surface of one bacterium is n, then Eq. (4) can be converted to Eq. (5) by substituting $n \times [E]$ for [P]:

$$B_{HP} = \frac{n[E]}{K + n[E]}. \tag{5}$$

Eq. (5) can be used to derive a degree of binding (B_{HE}) of a hypothetical one-to-one heparin-bacteria association (Eq. (6)) with apparent dissociation constant, K/n:

$$H + E \leftrightarrow HE. \tag{6}$$

The B_{HE} of Eq. (6) is then,

$$B_{HE} = \frac{[E]}{K / n + [E]}. \tag{7}$$

Of course, a single bacterium binds many heparin molecules. However, if there is no significant difference of affinity among the heparin-binding sites, then Eqs. (6) and (7) are appropriate for the description of binding performed in this study.

Eq. (7) was applied in the fitting of values obtained from Eq. (2) by nonlinear regression using *Mathematica*. K/n and R^2 values obtained from the fitting were 0.60 ± 0.06 pM and 0.927, respectively (Fig. 1B). Such high affinity is obviously attributed to the presence of multiple copies of the heparin-binding sites on the cell surface. The constant K/n obtained from the fitting is not a true thermodynamic equilibrium constant because of the multiplicity and potential heterogeneity of the heparin-binding sites on the cell surface. However, the value can be used for the comparison of relative heparin-binding affinity between different bacterial species if the titration is performed with the same concentration of heparin.

In summary, the applicability of CL EMSA for the quantitative assessment of heparin-living bacteria binding was examined with *E. coli* and biotinylated heparin. This method can be readily applied to heparin-bacteria binding study to assess relative heparin-binding affinity of bacteria as it has several advantages such as safety and stability of the sample, high sensitivity and the linearity of signal, and relative ease of performance. In addition, the binding conditions such as buffer composition and temperature can be varied. Most importantly, the binding can be examined with living bacteria. As in the study of DNA-protein interactions, CL EMSA is expected to be widely used for heparin-bacteria interaction studies complementing existing methods specially for the study of heparin binding of the commensal microorganisms as their huge diversity (500–1000 species in human gut) [19] requires a fast method.

Acknowledgements

This work was supported by grants from the U.S. Edgewood Chemical Biological Center (W911 SR-04-C-0065), NIH (AI27744 and U01 AI054827), NHLBI (N01HV28184), and the Welch Foundation (H1296). We thank David E. Volk for critical reading of the manuscript and valuable suggestions.

References

1. Berger R, Duncan MR, Berman B. Nonradioactive gel mobility shift assay using chemiluminescent detection. BioTechniques 1993;15:650–652. [PubMed: 8251167]
2. Ikeda S, Oda T. Nonisotopic gel-mobility shift assay using chemiluminescent detection system. BioTechniques 1993;14:878–880. [PubMed: 8333947]
3. Patti JM, Allen BL, McGavin MJ, Hook M. MSCRAMM-mediated adherence of microorganisms to host tissues. Annu Rev Microbiol 1994;48:585–617. [PubMed: 7826020]
4. Rostand KS, Esko JD. Microbial adherence to and invasion through proteoglycans. Infect Immun 1997;65:1–8. [PubMed: 8975885]
5. Aoki K, Matsumoto S, Hirayama Y, Wada T, Ozeki Y, Niki M, Domenech P, Umemori K, Yamamoto S, Mineda A, Matsumoto M, Kobayashi K. Extracellular mycobacterial DNA-binding protein 1 participates in mycobacterium-lung epithelial cell interaction through hyaluronic acid. J Biol Chem 2004;279:39798–39806. [PubMed: 15234978]
6. Ascencio F, Fransson LA, Wadstrom T. Affinity of the gastric pathogen Helicobacter pylori for the N-sulphated glycosaminoglycan heparan sulphate. J Med Microbiol 1993;38:240–244. [PubMed: 7682621]
7. Baron MJ, Bolduc GR, Goldberg MB, Auperin TC, Madoff LC. Alpha C protein of group B Streptococcus binds host cell surface glycosaminoglycan and enters cells by an actin-dependent mechanism. J Biol Chem 2004;279:24714–24723. [PubMed: 15044471]
8. Fleckenstein JM, Holland JT, Hasty DL. Interaction of an outer membrane protein of enterotoxigenic Escherichia coli with cell surface heparan sulfate proteoglycans. Infect Immun 2002;70:1530–1537. [PubMed: 11854241]
9. Capila I, Linhardt RJ. Heparin-protein interactions. Angew Chem Int Ed Engl 2002;41:391–412. [PubMed: 12491369]
10. Muñoz EM, Linhardt RJ. Heparin-binding domains in vascular biology. Arteriorscler Thromb Vasc Biol 2004;24:1549–1557.
11. Bormann BJ, Engelman DM. Intramembrane helix-helix association in oligomerization and transmembrane signaling. Annu Rev Biophys Biomol Struct 1992;21:223–242. [PubMed: 1326354]
12. Chen JC, Zhang JP, Stephens RS. Structural requirements of heparin binding to Chlamydia trachomatis. J Biol Chem 1996;271:11134–11140. [PubMed: 8626658]
13. Dupres V, Menozzi FD, Locht C, Clare BH, Abbott NL, Cuenot S, Bompard C, Raze D, Dufrene YF. Nanoscale mapping and functional analysis of individual adhesions on living bacteria. Nat Methods 2005;2:515–520. [PubMed: 15973422]
14. Garner MM, Revzin A. A gel electrophoresis method for quantifying the binding of proteins to specific DNA regions: application to components of the Escherichia coli lactose operon regulatory system. Nucleic Acids Res 1981;9:3047–3060. [PubMed: 6269071]
15. Kang J, Lee MS, Gorenstein DG. Quantitative analysis of chemiluminescence signals using a cooled charge-coupled device camera. Anal Biochem 2005;345:66–71. [PubMed: 16125123]
16. Kang J, Lee MS, Watowich SJ, Gorenstein DG. Chemiluminescence-based electrophoretic mobility shift assay of RNA-protein interactions: Application to binding of viral capsid proteins to RNA. J Virol Methods 2006;131:155–159. [PubMed: 16182384]
17. Kang J, Lee MS, Gorenstein DG. Chemiluminescence-based electrophoretic mobility shift assay of heparin-protein interactions. Anal Biochem 2006;349:156–158. [PubMed: 16352290]
18. Kang J. Application of a cooled charge-coupled device (CCD) camera for the detection of chemiluminescence signal. Biologicals. (In press)
19. Xu J, Gordon JI. Honor thy symbionts. Proc Natl Acad Sci USA 2003;100:10452–10459. [PubMed: 12923294]

Abbreviations used

EMSA
electrophoretic mobility shift assay

CL
chemiluminescence

PBS
phosphate-buffered saline

IDV
integrated density value

CCD
charge-coupled device

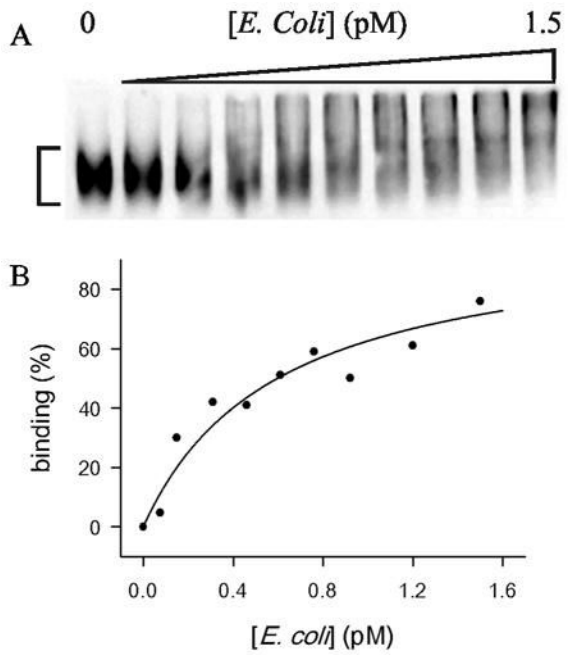

Fig 1.

CL EMSA of biotinylated heparin and *E. coli* interaction. (A) *E. coli* concentrations were increased up to 1.5 pM. The region for the measurement of the CL IDV of unbound biotinylated heparin is marked. The smearing between the well and unbound heparin in the gel indicates dissociation of bound heparin during electrophoresis. (B) Degree of binding (%) obtained from Eq. (2) was plotted against *E. coli* concentration in pM (closed circle). The data were fit to Eq. (7) (solid line).