Contributions of Polysaccharide and Lipid Regions of Lipopolysaccharide to the Recognition by Spike G Protein of Bacteriophage ϕX174

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A histidine-tagged G protein of bacteriophage ϕX174 (HisG) bound strongly with lipopolysaccharide (LPS) of Escherichia coli C, one of a ϕX174-sensitive Ra strain. The dissociation constant, \( K_d \), was measured to be 0.16 ± 0.04 μM by fluorometric titration. HisG showed slightly less affinity to LPSs of the insensitive Rc and Rd \( _2 \) strains having shorter R-core polysaccharide sequences than that of the sensitive Ra strains. The difference between the two types of LPS was demonstrated by CD spectra; LPSs of the sensitive strains increased the signal intensity for \( \beta \)-sheet, while the insensitive strains decreased it. The chemically degraded LPS derivatives lacking a hydrophobic lipid region showed much less affinity to HisG, indicating the importance of the lipid region of LPS for strong binding with HisG. On the other hand, since the chemically degraded derivatives increased the intensity of CD spectra, the polysaccharide region is thought to contribute to the conformation change of the protein.

Key words: lipopolysaccharide; spike G protein; ϕX174; recognition

Bacteriophage ϕX174 is a small icosahedral virus having a circular, single-stranded DNA and four capsid proteins, F, G, H, and J.\(^1\) In infection process, ϕX174 recognizes lipopolysaccharide (LPS) as a receptor.\(^2\) LPS, a major component of the outer membrane of Gram-negative bacteria, consists of three distinct regions: O-antigenic repeating unit, R-core polysaccharide, and lipid A. Host bacteria of ϕX174 are limited to some Ra strains of enterobacteria such as Escherichia coli C, Salmonella enterica serovar Typhimurium TV119, Shigella sonnei phase II, etc., those having complete sequences of R-core polysaccharides on their LPSs.\(^3–5\) Thus, the R-core region is thought to be the most important one for the recognition of LPS by ϕX174. An electron microscopic study revealed that an icosahedral-shaped ϕX174 attached onto the surface of host cell by one of the spikes consist of five G proteins and one H protein.\(^6\) From this observation, ϕX174 has been considered to recognize LPS by spike proteins G and/or H, however, the detail mechanism of recognition is still unknown.

In our previous study, we prepared the spike proteins H and G as hexa histidine-tagged fusions (HisH and HisG), and demonstrated their specific binding to LPS of a ϕX174-sensitive strains, E. coli C.\(^7,8\) H protein was shown to perform an entropy-driving binding with the LPS, and the conformation change caused by hydrophobic interaction with the LPS seemed to be a driving force of binding by HisH.\(^9\) On the other hand, HisG was also prepared and shown to bind with the LPS, however, the details of the recognition remained to be clarified. Here in this paper, we report characterization of the binding of HisG with LPSs of the ϕX174-sensitive and -insensitive strains and the chemically degraded LPS derivatives by fluorescence titration and circular dichroism (CD) spectra. The contributions of lipid and polysaccharide regions of LPS to the recognition by spike G protein was investigated by comparing the LPSs having various lengths of polysaccharide region and the LPS derivatives lacking some fatty acids of lipid A region of LPS.

Materials and Methods

Chemicals. LPS of E. coli C was extracted from cultured cells\(^{10}\) by the PCP-method.\(^{11}\) Other LPSs of S. enterica serovar Typhimurium TV119, SL684, and E. coli EH100 and F583 strains were purchased from Sigma (St. Louis, MO, USA). Unless otherwise speci-
hexa histidine tag (HisG) was expressed in *E. coli* according to the previously reported procedure,\(^\text{12}\) LPS of *E. coli* was suspended in 10 mL of 4 M KOH was heated in a sealed glass tube at 125°C for 18 h. Precipitated fatty acid was removed by centrifugation (3,500 × g, 30 min). The supernatant was neutralized with 6 M HCl, washed three times with CH₂Cl₂, and then lyophilized to afford crude *O*, *N*-deacylated LPS. The crude preparation was purified by gel chromatography using a Sephadex G-10 column (2.1 cm × 32 cm, Amersham Pharmacia Biotech, Tokyo, Japan). Saccharide-containing fractions were monitored by the phenol-H₂SO₄ method, and the combined fractions were lyophilized to give *O*, *N*-deacylated LPS (39.3 mg, 39%).

**CD spectra.** CD spectra were recorded from 200 to 250 nm using a Jasco J-720M spectropolarimeter with software provided by the manufacturer. Parameters were: sensitivity, 20 mdeg; scan speed, 10 nm/min; band width, 1.0 nm, respectively. Measurements were done at room temperature in a quartz cell (1 mm). HisG (5 μM) dissolved in PBS was titrated with 10 μM of LPSs or LPS derivatives in the same buffer.

Fluorometric titration. Fluorescence measurements were done on a Hitachi 650–560 spectrometer at 25°C using an excitation slit width of 1 nm and emission slit width of 18 nm. To the HisG solution (0.37 μM, 1.5 mL) in PBS, was added gradually the solutions of LPSs or LPS derivatives in the same buffer (final concentration was 12 μM) by a microsyringe (0–25 μL), and change of fluorescence intensity at 345 nm was monitored. The following simple bimolecular binding equilibrium is assumed between HisG and LPS;

\[
\text{HisG} + \text{LPS} \rightleftharpoons \text{HisG} \cdot \text{LPS} \quad \text{(eq.1)}
\]

Dissociation constant \(K_d\) of the HisG-LPS complex is;

\[
K_d = \frac{[\text{HisG}][\text{LPS}]}{[\text{HisG} \cdot \text{LPS}]} \quad \text{(eq.2)}
\]

Initial concentrations of the HisG [HisG]₀ and the LPS [LPS]₀ are;

\[
[\text{HisG}]_0 = [\text{HisG}]_0 + [\text{HisG} \cdot \text{LPS}]_0 \quad \text{(eq.3)}
\]

\[
[\text{LPS}]_0 = [\text{LPS}]_0 + [\text{HisG} \cdot \text{LPS}]_0 \quad \text{(eq.4)}
\]

\[
[\text{HisG} \cdot \text{LPS}]_0 = [\text{HisG}]_0 + [\text{LPS}]_0 + K_d - \sqrt{(\text{HisG}]_0 + [\text{LPS}]_0 + K_d) - 4[\text{HisG}]_0[\text{LPS}]_0} \quad \text{(eq.5)}
\]
From equations 2–4, [HisG - LPS] is given by;

The initial fluorescence intensity of the protein in the absence of the ligand is given by \( f_0[\text{HisG}]_0 \) where \( f_0 \) is the fluorescence intensity coefficient of the HisG proportional to the fluorescence quantum yield. When the HisG is titrated by the LPS, the observed fluorescence intensity \( F \) is expressed as;

\[
F = f_0[\text{HisG}] + f_{\text{LPS}}[\text{HisG} \cdot \text{LPS}] \quad (\text{eq.6})
\]

\[
\Delta F = \Delta F_{\text{max}}[\text{HisG}]_0 + [\text{LPS}]_0 + K_d - \frac{([\text{HisG}]_0 + [\text{LPS}]_0 + K_d)^2 - 4[\text{HisG}]_0[\text{LPS}]_0}{2[\text{HisG}]_0} \quad (\text{eq.8})
\]

From equations 5–7, \( \Delta F \) is given by;
Where \( \Delta F_{\text{max}} \) is defined as \( \{f_0 - f_{\text{LPS}}\}f_0 \times 100 \)

In equation 8, all the values except for \( K_d \) and \( \Delta F_{\text{max}} \) are experimentally known. We can obtain the values of \( K_d \) and \( \Delta F_{\text{max}} \) by the least squares method to minimize the deviation of the calculated values from the observed data.

**Results**

**Interaction of HisG with LPSs having various R-core polysaccharide chains**

The structures of LPSs used in this study are shown in Fig. 1.\(^{3,4,16-19} \) We evaluate the contribution of polysaccharide region to the recognition of LPS by HisG using these LPSs having various R-core polysaccharide chains. As seen in Fig. 2A, HisG in PBS (pH 7.4) showed a bell-shaped fluorescence emission spectrum with an emission maximum \( \lambda_{\text{max}} \) at 345 nm upon excitation at 280 nm. The addition of the LPS of *E. coli* C, one of a native host Ra strain of \( \phi X 174 \), decreased the intensity in fluorescence spectrum dose-dependently to LPS concentration. The intensity change at 345 nm, \( \Delta F \), was plotted against LPS concentration, where \( \Delta F \) is defined as a percentage of change in the fluorescence intensity relative to the intensity in the absence of LPS. A typical titration curve of HisG by the LPS of *E. coli* C is shown in Fig. 2B. Other LPSs also showed similar titration curves. The values of the dissociation constant, \( K_d \), and the maximum fluorescence intensity change, \( \Delta F_{\text{max}} \), were found by the least square fitting to eq. 8 (Table 1), assuming a bimolecular reaction between HisG and each LPS (see “Materials and Methods” for details). The parameters calculated for the complex of HisG with the LPS of *E. coli* C were \( K_d = 0.16 \pm 0.04 \mu M \) and \( \Delta F_{\text{max}} = -10.62 \pm 0.27\% \), respectively. Strong affinity was also observed for the LPSs of other \( \phi X 174 \)-sensitive Ra strains, *E. coli* EH100 and *S. enterica* serovar Typhimurium TV119. As the best receptor for \( \phi X 174 \), LPS is required to have a complete sequence of R-core polysaccharide.\(^{5} \) However, the LPS of *S. enterica* serovar Typhimurium SL684 is lacking four of five hexoses in the outer core,\(^{17} \) and the LPS of *E. coli* F583 is lacking all the hexoses in the outer core and two of three heptoses in the inner core.\(^{18,19} \) These LPSs from the \( \phi X 174 \)-insensitive strains were compared with those of the sensitive strains in the interaction with HisG. The values of \( K_d \) did not change as much as to become 0.26 \( \mu M \), but the \( \Delta F_{\text{max}} \) were reduced to about a half (\( -7.4\% \)) in comparison to those of the sensitive strains.

Changes of fluorescence intensity are generally attributable to conformation changes of proteins. HisG was supposed to perform a limited conformation change in the binding with the LPSs from \( \phi X 174 \)-insensitive strains, because the magnitude of fluorescence change was reduced in this case. Hence, we examined the circular dichroism (CD) spectra of HisG in the absence and the presence of the various LPSs. X-ray crystallographic studies showed that G protein in a phage capsid had an eight-stranded antiparallel \( \beta \)-barrel motif.\(^{20,21} \) HisG in PBS (pH 7.4) also showed a typical spectrum for \( \beta \)-sheet secondary structure; a positive absorbance around 200 nm and a negative peak at 218 nm were observed. We thereby believe that folding of HisG is comparable to that of the native G protein in a phage capsid. When 10 \( \mu M \) of the LPS of *E. coli* C was added to HisG, the negative peak at 218 nm was augmented significantly (Fig. 3). Such conformation change was also observed in the cases of the LPSs of other \( \phi X 174 \)-sensitive Ra strains, *E. coli* EH100 and *S. enterica* serovar Typhimurium TV119. In a sharp contrast, the negative peak at 218 nm decreased in the cases of the LPSs of insensitive Rc and Rd strains, *S. enterica* serovar Typhimurium SL684 and *E. coli* F583. Thus, the pattern of the conformation change of HisG caused by two types of LPSs was dramatically different.
**Interactions of HisG with deacylated LPS derivatives**

We prepared LPS derivatives which were modified in a lipid region and examined their interaction with HisG. The detailed structure of the lipid A region was analyzed for the LPS of *E. coli*. It comprises a β(1–6) linked disaccharide of GlcN, 3-hydroxytetradecanoic acids, 3-acyloxytetradecanoic acids, and phosphates (Fig. 4). This structure is common in enterobacteria including *E. coli* and *S. enterica* serovar Typhimurium. The LPS of *E. coli* C was chemically degraded by limited hydrolysis by the reported procedures. The cleavage at the ketoside linkages of KDO by 1 M aqueous acetic acid gave a polysaccharide part of LPS (PS). The treatments with anhydrous hydrazide and 4 M KOH removed ester-linked and amide-linked fatty acids to afford O-deacylated and O,N-deacylated derivatives, respectively. The structural integrity of the obtained LPS derivatives were cross-examined by chemical and biological approaches (data not shown).

The results of fluorometric titration of HisG with the deacylated derivatives are summarized in Table 2. In the case of O-deacylated LPS, less affinity of $K_d$ (0.42 μM), and apparently reduced magnitude of fluorescence change of $ΔF_{\text{max}}$ (~7.72%) were observed in comparison to that of the intact LPS. Moreover, in the cases of O,N-deacylated LPS and PS, affinities were decreased by one order and the magnitudes of $ΔF_{\text{max}}$ were further reduced to about ~5%. All the three derivatives showed the same tendency, that the fluorescence intensity of HisG decreased. This argues that a part of HisG around the tryptophan residue is exposed to a more hydrophilic environment in case of the binding with the deacylated derivatives as well as the intact LPS.
Recognition of Lipopolysaccharide by G Protein of ϕX174 873

CD spectra of HisG in the presence of the derivatives also revealed that all the derivatives caused conformation change of HisG in the direction of augmenting the negative peak at 218 nm. The intact LPS as well as the deacylated derivatives changed the conformation to increase the β-sheet content of HisG. These CD spectra confirmed a limited conformation change of HisG that was proposed from the observation of the decreased ΔF_{max} in the fluorometric titration. Consequently, whether hydrophobic fatty acids are present or not, if a complete R-core saccharide region is present, the deacylated LPS derivatives cause a similar conformation change of HisG.

Discussion

HisG bound strongly with the LPS of E. coli C, one of the ϕX174-sensitive strains, and the dissociation constant, K_d, of the complex was found to be 1.6 × 10^{-7} M. The observed affinity was about 44-fold stronger than that of the histidine-tagged H protein (HisH) toward the same LPS (7.0 × 10^{-6} M). In a ϕX174 particle, the spike G protein forms a star-shaped pentamer by interaction of the stretched N
and C terminals with an adjacent subunit, and the pentamers of G protein lie on a center of 5-fold vertices of an icosahedron which is made by twelve sets of pentamers of F protein. A hydrophilic channel is created inside of the pentamers of F and G proteins, and the phage DNA is thought to be ejected through the bacterial membrane along with the lipid region of LPS to the LPS recognition by G protein, and a pentamer simply provides a channel for penetration of the phage DNA.

A role of the polysaccharide region of LPS for the recognition by HisG was investigated using various LPSs of the \( \phi X174 \)-sensitive and -insensitive strains having different R-core polysaccharide sequences. HisG showed only a little difference in affinity \( K_d \) of approximately 0.2 \( \mu \)m to all the tested LPSs of Ra, Rc, and Rd strains. However, the values of \( \Delta F_{\text{max}} \) for the LPSs of insensitive Rc and Rd strains were about half of those of the sensitive Ra strains. The reduced magnitude of \( \Delta F_{\text{max}} \) values indicates a limited conformation change of HisG, hence, CD spectra of HisG were compared in the presence of various LPSs. The LPSs of sensitive strains caused conformational change of HisG to increase \( \beta \)-sheet content. In a sharp contrast, the LPSs of insensitive strains decreased \( \beta \)-sheet content of HisG (Fig. 3). A difference of conformation change was clearly demonstrated by CD spectra. In infection process, \( \phi X174 \) recognizes and attaches to a host cell, undergoes the alteration of three-dimensional organization of capsid proteins, and ejects the phage DNA with an H protein into the periplasmic space of the host cell through one of twelve spike channels (DNA penetration). Thus, G protein is needed to recognize LPS and then to induce conformation change in order to open the spike channel. If the conformation change caused by the LPSs of sensitive strains to increase \( \beta \)-sheet content is identified as a “favorable change”, the binding with the LPSs of insensitive strains to decrease \( \beta \)-sheet content is thought to be an “unfavorable change”, which prevents a desired conformation change for DNA penetration. Consequently, this is a part of answer that \( \phi X174 \) cannot infect the strains with shorter R-core polysaccharide sequences on their R-core.

When a lipid A part or fatty acids was removed from LPS, a large decrease in affinity for HisG was observed (Table 2). Thus, a significant contribution of the lipid region of LPS to the LPS recognition by HisG was confirmed. The intact LPS is an amphipathic molecule, and has ability to form aggregates such as micelles and bilayers. However, since \( O,N \)-deacylated LPS and PS are no longer amphipathic, the low affinity of these derivatives to HisG may be attributable to the loss of the hydrophobic lipid region and/or to the loss of ability for aggregate formation. Significant contribution of the lipid region had also been shown in the case of HisH where a great increase of fluorescence intensity \( \Delta F_{\text{max}} = \pm 79.8\% \) and shift of \( \lambda_{\text{max}} \) to the shorter wavelength were observed in the interaction with LPS. This shows hydrophobic interaction of HisH with LPS. In the DNA penetration process, one of twelve molecules of H protein was reported to penetrate through the bacterial membrane along with the phage DNA. On the other hand, spike G and capsid F proteins were found to remain outside of the cell as a vacant phage shell after DNA penetration. Our previous finding that the lipid region of LPS was significant in recognition by HisH is quite reasonable, because spike H protein has to intermingle with

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Table 2. Parameters for the Binding of HisG with the Deacylated LPS Derivatives

<table>
<thead>
<tr>
<th>Derivatives</th>
<th>( \Delta F_{\text{max}} ) (%)</th>
<th>( K_d ) (( \mu )m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( O )-deacylated LPS</td>
<td>(-7.72 \pm 0.20)</td>
<td>(0.42 \pm 0.09)</td>
</tr>
<tr>
<td>( O,N )-deacylated LPS</td>
<td>(-5.35 \pm 0.20)</td>
<td>(4.71 \pm 0.60)</td>
</tr>
<tr>
<td>PS</td>
<td>(-5.13 \pm 0.15)</td>
<td>(2.24 \pm 0.30)</td>
</tr>
</tbody>
</table>

Figure next to \( \pm \) are standard deviations. The molecular weights of the derivatives were calculated based on the structures in Fig. 4; for \( O \)-deacylated LPS (3478), \( O,N \)-deacylated LPS (3028), and PS (1904), respectively.
the hydrophobic membrane. Contrary to HisH, HisG showed reduced fluorescence intensity ($\Delta F_{\text{max}} = -10.62\%$) and no change in $\lambda_{\text{max}}$ by the interaction with the intact LPS. Moreover, the fluorescence intensity of HisG also decreased in the interaction with the deacylated derivatives. This indicates that a tryptophan residue in HisG was exposed to a more hydrophilic environment by the interaction with the intact LPS as well as the deacylated derivatives. Thus, we assume that the hydrophilic polysaccharide region of LPS would be more significant for HisG rather than HisH in order to change the protein conformation. The observation from CD spectra that the deacylated derivatives kept the “favorable change” of HisG provides a good support to the assumption. Thus, the conclusion is that 1) G protein recognizes both the polysaccharide and lipid regions of LPS, 2) the lipid region contributes to strengthen the recognition by G protein toward LPS, and 3) a complete R-core polysaccharide moiety drives a desired conformation change of G protein to switch on the transition into the DNA penetration process followed by adsorption. This study confirmed a different and significant contributions of polysaccharide and lipid regions of LPS for the host-recognition by a spike protein of bacteriophage $\phi X174$.

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