Plasmid DNA Binds to the Core Oligosaccharide Domain of LPS Molecules of E. coli Cell Surface in the CaCl₂-Mediated Transformation Process

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In the standard procedure for artificial transformation of E. coli by plasmid DNA, cellular competence for DNA uptake is developed by suspending the cells in ice-cold CaCl₂ (50–100 mM). It is believed that CaCl₂ helps DNA adsorption to the lipopolysaccharide (LPS) molecules on E. coli cell surface; however, the binding mechanism is mostly obscure. In this report, we present our findings of an in-depth study on in vitro interaction between plasmid DNA and E. coli LPS, using different techniques like absorption and circular dichroism spectroscopy, isothermal titration calorimetry, electron and atomic force microscopy, and so on. The results suggest that the Ca(II) ions, forming coordination complexes with the phosphates of DNA and LPS, facilitate the binding between them. The binding interaction appears to be cooperative, reversible, exothermic, and enthalpy-driven in nature. Binding of LPS causes a partial transition of DNA from B- to A-form. Finer study with the hydrolyzed products of LPS shows that only the core oligosaccharide domain of LPS is responsible for the interaction with DNA. Moreover, the biological significance of this interaction becomes evident from the observation that E. coli cells, from which the LPS have been leached out considerably, show higher efficiency of transformation, when transformed with plasmid-LPS complex rather than plasmid DNA alone.

Introduction

The outer leaflet of the outer membrane of E. coli predominantly consists of certain proteins and a class of macroamphiphile, the LPS, whereas the inner leaflet is composed of phospholipids and proteins. One bacterial cell contains approximately 3.5 × 10⁶ LPS molecules occupying an area of 4.9 μm². As the surface of an E. coli cell amounts to 6.7 μm², it appears that three-quarters of bacterial surface consist of LPS, the remaining area being filled by proteins.¹ LPS has three covalently linked domains: (1) the lipid A moiety, a glucosamine-based phospholipid (instead of the classical glycerol-phospholipids of membrane), serves as the hydrophobic membrane anchor of LPS, (2) the core region, a nonrepeating oligosaccharide decorated with several phosphate-containing substituents, and (3) the O-antigen, a distal repeating oligosaccharide.²³

This communication mainly deals with the in vitro interaction between E. coli LPS and plasmid DNA. The biological importance of this study lies at the step of DNA adsorption on cell surface in the artificial transformation process of Gram-negative bacteria like E. coli. In gene technology, transformation is an important basic technique, which involves binding of DNA to the cell surface followed by its uptake across the wall-membrane complex into the cytoplasm. E. coli cells can be made competent for DNA uptake by suspending cells in ice-cold CaCl₂ (50–100 mM) and then subjecting them to a brief heat shock at 42 °C for 90 s.⁴⁵ However, the exact mechanism of this widely used CaCl₂-mediated artificial transformation procedure is still unknown. It is just believed that the CaCl₂ helps DNA adsorption to the competent cell surface and the heat-shock step facilitates penetration of the adsorbed DNA into the cell cytosol. In the process of transformation of the hidden mechanism of transformation, our previous findings suggest (1) the initial step of DNA adsorption on the cell surface, perhaps, involves the binding of DNA to the LPS molecules via the divalent cation Ca(II)⁶–⁸ and (2) the brief heat shock step facilitates entry of adsorbed DNA into the cell cytoplasm (a) by releasing lipids and thereby forming pores on the outer membrane⁹ and (b) by depolarizing the inner membrane and thereby decreasing the potential barrier for movement of negatively charged DNA molecules into cell interior.¹⁰ The results of the present in-depth study with plasmid pUC19 DNA and E. coli LPS reveal that the calcium ions, forming coordination complexes with the phosphates of DNA and LPS, facilitate DNA-LPS binding and the interaction is cooperative, reversible, exothermic and enthalpy-driven in nature; moreover, the “core oligosaccharide” (COS) part of LPS is the site, which binds with DNA. The biological significance of this in vitro interaction becomes evident from the fact that the E. coli cells, from which the LPS molecules are leached out, can be transformed more by DNA-LPS complex than by DNA alone.

Materials and Methods

Bacterial Strain and Plasmid. The E. coli XLI-Blue,¹¹ a genetically engineered highly transformable strain and the plasmid pUC19,¹² a cloning vector were used for this study.

Fine Chemicals and Biochemicals. Different fine- and biochemicals, used in this study, were of molecular biology grade and were procured from different companies like Sigma-Aldrich (U.S.A.), Pharmacia-Amersham (Sweden), and Sisco Research Laboratories (India).

Isolation and Purification of Plasmid DNA. Plasmid DNA was isolated from the transformed strain of E. coli (XLI-Blue + pUC19) by the method of alkaline lysis with SDS and purified by the method of polyethylene glycol.¹³¹⁴ The purity of plasmid DNA was checked by measuring its absorbance at 260nm/(absorbance at 280nm); the ratio attained the value of about 1.90 in our case. Moreover, the absence of any LPS in the purified DNA sample was checked by the KDO test [the colorimetric estimation of unique sugar 2-keto-3-deoxyoctulosonate.
present in LPS).\textsuperscript{15} When the purified plasmid DNA was visualized after electrophoresis in 1% agarose gel,\textsuperscript{16} more than 90% of the plasmid population was in the circularly closed covalent (CCC) form.

**Isolation and Purification of LPS.** LPS was isolated from *E. coli* XL1-Blue cells by the phenol-water extraction method\textsuperscript{17} and purified by incubating with DNase, RNase, and protease.\textsuperscript{18,19} The purified LPS was finally collected as lyophilized powder form and was assayed by KDO estimation.\textsuperscript{15} The average molecular weight of purified LPS was determined to be about 10 kDa using Q-TOF micro (Waters) mass spectrometer.\textsuperscript{20} According to requirement, the powdered LPS was homogeneously suspended by sonication (using Cole Parmer ultrasonic homogenizer 4710 and keeping its power set at 20 watts; sonication was allowed for 10 min placing the sample on ice) in aqueous solutions of different salts like CaCl\(_2\), MgCl\(_2\), NaCl, KCl, and so on of different molarities to prepare maximum working stock of 3.0 mg/mL, that is, 0.3 mM. Spectral scan of 0.1 mM suspension of LPS in different salts showed no characteristic absorption peak at 260 and 280 nm, indicating absence of DNA/RNA and protein, respectively.

**Fragmentation of LPS.** The three components of LPS, the lipid A moiety, the COS part, and the O-antigen, were fragmented, isolated, and purified as described in ref 21. LPS was hydrolyzed in a 2% (v/v) freshly prepared acetic acid solution in water for 7 h at 100 °C on an oil bath with mild stirring under nitrogen atmosphere. The precipitated lipid A was removed by centrifugation at 10000 rpm for 20 min at 10 °C. The aqueous part containing O-antigen, COS, and monosaccharides were fractionated by gel permeation chromatography in Bio-Gel P-10 column using water as eluent, at a flow rate of 8 mL/h. The eluents were monitored using Shimadzu differential refractometer (RID-10A). Fractions were collected using Pharmacia fraction collector (FRAC-100) and were finally lyophilized.

**UV Spectrophotometric Study of DNA—LPS Interaction.** Spectrophotometric studies were carried out at 25 °C in Shimadzu (UV-160-A) spectrophotometer using 1 cm path length rectangular quartz cuvette. To study the changes in absorption spectrum of DNA due to interaction with LPS, LPS was added stepwise in each of the sample (containing DNA) and reference cuvettes. Suspensions of LPS in different salts had a negligibly small optical density in wavelength region 200–800 nm and this was due to scattering. However, as LPS was added in each of the sample and reference cuvettes, contribution due to the scattering of LPS in DNA spectra had been automatically eliminated.

The binding isotherm for DNA—LPS interaction was obtained by plotting the fraction of DNA bound to LPS (θ) versus the total LPS concentration; θ was calculated as

\[
\theta = (A_0 - A) / (A_0 - A_a)
\]

where \(A_0\), \(A\), and \(A_a\) were (absorbance)\(_{260nm}\) of DNA in the absence of LPS, at any LPS concentration, and at LPS concentration for which maximum binding took place, respectively.\textsuperscript{22}

A better understanding of the strength of LPS binding to DNA came out from the analysis of Hill plot\textsuperscript{22,23} of absorbance data. For any macromolecule–ligand interaction, Hill equation was represented as

\[
\log \theta / (1 - \theta) = \gamma \log K_\theta + \gamma \log C_L
\]

where \(K_\theta\) = binding constant, \(\gamma\) = Hill coefficient, \(\theta\) = fraction of macromolecule bound to ligand, and \(C_L\) = concentration of free ligand. The plot of log \(\theta / (1 - \theta)\) versus log \(C_L\) represented Hill plot. The values of \(K_\theta\) and \(\gamma\) were determined from the intercept in x-axis (where log \(C_L\) = −log \(K_\theta\)) and the slope in region of the intercept respectively of Hill plot. The physical significance of \(K_\theta\) implied that its value was reciprocal to the ligand concentration corresponding to the half-saturation value of the binding isotherm, and the greater the value of \(K_\theta\), the greater was the affinity of the macromolecule (DNA) toward the ligand (LPS). The importance of \(\gamma\) was that it characterized the nature of cooperativity, that is, \(\gamma < 1\), \(\gamma = 1\), \(\gamma > 1\) signified negative cooperative, noncooperative, and positive cooperative nature of binding, respectively.

Circular Dichroism (CD) Spectrometric Study of DNA—LPS Interaction. The study was carried out at 25 °C in Jasco (J-720) spectropolarimeter using 0.1 cm path length cylindrical quartz cuvette. CD of DNA, LPS, and DNA—LPS complex, each in 1 mM CaCl\(_2\), were measured separately in wavelength region of 200–230 nm. Although the LPS itself had no CD in the said wavelength region, during processing of data, the negligible contribution due to LPS was subtracted from the CD data of DNA—LPS complex.

**Study of DNA—LPS Interaction by Isothermal Titration Calorimetry (ITC).** The study was performed using a VP-ITC calorimeter (Microcal Inc.). Both DNA and LPS were separately taken in 1 mM CaCl\(_2\), and calorimetric titration was carried out at 25 °C as described in refs 24 and 25. The titration involved 30 injections of LPS (5 μL for each first two injections and 10 μL each for the rest of the injections) at 4 min intervals into the sample cell (volume 1.4359 mL) containing pUC19 plasmid DNA. The titration cell was stirred at 394 rpm. The heat of LPS dilution in buffer alone was subtracted from the titration data for LPS with DNA. The data was analyzed to determine binding stoichiometry (\(N\)), affinity constant (\(K_a\)), and other thermodynamic parameters of the DNA—LPS interaction using Origin software.

**Agarose Gel Electrophoresis Study.** To compare the mobility of DNA—LPS complex with that of DNA only, they were loaded on two separate grooves of a 1% agarose gel. Electrophoresis was then allowed using 1× tris-acetate buffer supplemented with 1 mM CaCl\(_2\) and applying a voltage of 5 V/cm between the electrodes. The gel was stained in a solution of ethidium bromide (10 μg/mL) for 35 min, destained with deionized water for 2 h,\textsuperscript{26} and was then scanned by Typhoon 9210 (GE Healthcare).

DNA supercoiling was studied using the technique of two-dimensional agarose gel (17 × 17 cm) electrophoresis.\textsuperscript{29} In the first dimension, DNA was subjected to electrophoresis through 1.2% agarose gel in 1× tris-acetate (TA) buffer under an electric field of 40 V for 7 h at 4 °C. The gel was then soaked in TA buffer supplemented with chloroquine (4.5 μg/mL) for 2 h. The gel was turned clockwise by 90° and run in second dimension at 20 V for 12 h in TA buffer containing 4.5 μg/mL of chloroquine. The gel was subsequently stained and destained as described above.

**Immuno-Electron Microscopy of DNA—LPS Complex.** For electron microscopy, DNA—LPS bound complex was prepared by Bac method of Vollenweider et al.\textsuperscript{17} Grids were rotary shadowed with platinum in a Jeol JEE 400 high vacuum evaporator. Grids were then treated with 1% bovine serum albumin (BSA) for 15 min at room temperature, washed with phosphate buffer saline (PBS), and treated with anti-LPS antibody diluted in 1% BSA for 1 h at room temperature. Grids were again washed with PBS and treated with 5 nm gold-conjugated protein A (Sigma) for 1 h. Grids were washed again with PBS, dried in air, and examined in a FEI Tecnai 12 Bio TWIN electron microscope fitted with a mega-view III CCD camera.

**Production of Anti-LPS Antibody.** The anti-LPS antibody was raised according to the method of Oliver and Beckwith\textsuperscript{30} by injecting a rabbit interdermally at four sites with purified *E. coli* LPS (100 μg per rabbit) emulsified with complete and incomplete Freunds adjuvant for primary injection. Two subsequent injections of 50 μg LPS and one booster dose of 100 μg LPS, each emulsified with incomplete Freunds adjuvant, were pushed at intervals of 10 days. After three days of injecting the booster dose, blood was drawn by punching the heart without killing the rabbit. The blood serum was subsequently isolated and decomplexed by keeping at 55 °C for 30 min. This antiserum was used without further purification.

**Preparation of Competent Cells and their Transformation with Plasmid DNA.** Cells, competent for DNA uptake, were prepared by the standard method of CaCl\(_2\) treatment.\textsuperscript{27,29} Cells were initially grown to log phase [up to (OD)\(_{600nm}\) = 0.10 i.e. ~5 × 10\(^7\) cells/mL] in Luria Broth (LB),\textsuperscript{29} washed with and ultimately concentrated 25 times in ice-cold 100 mM CaCl\(_2\) to obtain competent cells.

For transformation, plasmid DNA (no more than 50 ng in a volume of 10 μL) was added to 200 μL of competent cell suspension and was
allowed to incubate at 0 °C for 30 min. Cell–DNA complex was then transferred to 42 °C for exactly 90 s and was rapidly chilled in ice. LB (800 μL) was next added and the cells were incubated at 37 °C for 45 min for the expression of antibiotic resistance marker (β-lactamase) encoded by the plasmid. These transformed cells were serially diluted in chilled tryptone broth (TB). A total of 100 μL of cells from properly diluted samples were spread on agar-LB medium with ampicillin (50 μg/mL) to obtain the number of transformants. The transformation efficiency (TR) was calculated as the number of transformants per μg of DNA added.

Atomic Force Microscopy of DNA Bound with LPS on Competent E. coli Cell Surface. Surface morphology of E. coli XL1 Blue cell was studied, as described in ref 30 using a scanning probe microscope (Vecco, AP 0100) equipped with a scanner having a maximum XY range of 10 × 10 μm. Cell aliquot of 100 μL was taken and cell surface morphology was observed by fixing the cells at 4 °C with 2.5% gluteraldehyde overnight. At the time of microscopy, fixed cells were washed twice and finally suspended in water; the drop and dry method was used to transfer the bacteria from suspension to a solid support for microscopy. To study structural features of individual cell surface, we used submonolayer coverage. For each specimen, 20 μL of bacterial suspension (total number of cells ∼9 × 10^8) was placed on a 1 cm² glass slide. To enhance cell adhesion, the glass slide was pretreated with Piranha solution (1:3 ratio of 30% H₂O₂/concentrated H₂SO₄) and washed copiously with deionized water before placing the cells. The surface was then washed three times with distilled water (40 μL) and dried in air. Height and phase data were captured using tapping mode AFM with standard Vecco phosphorus-doped silicon cantilevers having resonance frequencies of 245–284 kHz. Root mean square (rms) values of roughness were calculated for plane-fitted, flattened images and were used to measure the standard deviation of the height of a given line. All the measurements were done using Vecco ProScan 1.8 software.

Results and Discussion

The (absorbance)_{260nm} of plasmid pUC19 DNA in presence of 1 mM CaCl₂ decreased gradually with the increasing addition of LPS and two clear isosbestic points appeared in the spectra at 237 and 275 nm (Figure 1A), indicating some interaction between DNA and LPS. The binding isotherm of DNA–LPS interaction, represented by Figure 1B, was sigmoidal in shape, signifying the cooperative mode of interaction.²² The isotherm showed that with an increase in the concentration of LPS up to 15 μM, θ did not change much, implying an initial weak binding state of DNA. In between 15 and 50 μM of LPS concentration, θ had changed rapidly with the maximum slope, and this signified that the DNA had switched over from its initial weak binding state to a strong binding one. With a further increase in concentration of LPS beyond 50 μM, the change in θ was so small that it indicated the saturation of binding sites of DNA with LPS. Therefore, binding of LPS to the first or first few sites on a DNA molecule changed the earlier weak binding conformation of DNA to the strong binding one, which had increased the binding affinity of LPS for the remaining sites of DNA molecule and thus the interaction obeyed the cooperative mode of binding. Figure 1C represents the Hill plot, drawn from the absorbance data of DNA–LPS complex formed in presence of 1 mM CaCl₂. The values of K₆ (binding constant) and γ (Hill coefficient), as determined from the plot, were 2.95 × 10^6 M⁻¹ and 2.72, respectively. As the value of γ was greater than 1.0, it confirmed that the binding was positive cooperative in nature.

Figure 2 shows that for any particular concentrations of DNA (143 μM) and LPS (55 μM), the binding interaction had increased with gradual increase of the concentration of CaCl₂ (0–10 μM). Beyond 1 mM, a small decrease in interaction was observed up to 10 mM. The binding reached a saturation state above 10 mM CaCl₂. This result suggested that the maximum binding between DNA and LPS had taken place in presence of 1 mM CaCl₂.

To find out the reason behind the decrease in DNA–LPS interaction with increase of CaCl₂ concentration above 1 mM, supercoiled topoisomers of pUC19 DNA, dissolved in different molarities of CaCl₂, were analyzed using the technique of two-dimensional agarose gel electrophoresis. In this method, the direction of electrophoresis in the second dimension was perpendicular to that in the first dimension and the conditions used for both the dimensions were identical except for the concentration of a DNA intercalating agent, chloroquine, which
had altered the topology of circularly closed covalent (CCC) plasmid populations. The fact that the divalent metal cations altered the stability of DNA duplex and induced DNA aggregation through cross-linking of DNA strands, led us to carry out the 2D-gel experiment to investigate whether there was any change in the DNA compactness by the presence of different molarities of CaCl₂. Figure 3A shows that when pUC19 DNA was run in ordinary tris-acetate buffer, without any presence of Ca ion, more than 20 DNA spots had been resolved on the gel. Besides CCC topoisomers of different supercoil numbers and open circular (OC) monomeric forms of zero supercoiling, the spots might contain multimers, catenanes as well as θ-like replication intermediates, as reported in ref 32. Supercoiled pUC19 topoisomers were separated in first dimension; the most supercoiled molecules, due to most compactness, had the fastest mobility, while fully relaxed molecules (OCs) had run slowest through the gel matrix. In the second dimension, chloroquine relaxed natural occurring negative supercoils in CCC DNA and introduced positive supercoiling depending on its concentration. Intensity of the spots in Figure 3A indicated that the more compact forms were abundantly present in DNA population. Figure 3B shows that when pUC19 DNA was run in tris-acetate buffer containing 1 mM CaCl₂, there had been a lesser number of DNA spots than in Figure 3A, and DNA populations had shifted toward higher compactness. A further increase of CaCl₂ concentration to 10 mM had caused more compaction of DNA populations resulting in almost a single stretched spot (Figure 3C), which implied a distribution of DNA populations with very close supercoil numbers. Therefore, this experimental result clearly suggested that with the increase of molarity of CaCl₂ in the DNA environment, the supercoiling or compactness of CCC plasmid DNA had been increased. Because no change in the linking number of DNA occurred here, the increase of DNA compactness had perhaps resulted from the alteration in DNA twist by Ca²⁺-mediated cross-linking of DNA strands. Therefore, the reason behind the occurrence of the maximum extent of interaction between DNA and LPS in the presence of 1 mM CaCl₂ can be explained as follows: at low molarity, up to the concentration of 1 mM of CaCl₂, the binding interaction increased with the increase of molarity of CaCl₂ (Figure 2), that is, with the availability of more Ca ions for the formation of DNA-LPS complex. On the other hand, with increase of the molarity of CaCl₂, the compactness of DNA increased gradually, which eventually made more phosphate ions of DNA inaccessible for interaction with LPS via Ca ions. Thus, the result, represented by Figure 2, can be interpreted as, up to 1 mM CaCl₂, the availability of Ca ions had the dominating role over the DNA compactness for the formation of DNA-LPS complex and so the interaction had increased with an increase
in the molarity of CaCl$_2$. On the contrary, between 1−10 mM CaCl$_2$, DNA compactness, rather than the availability of the Ca ions, had the deciding role on DNA−LPS interaction and so the interaction decreased with the increase of compactness. Above 10 mM of CaCl$_2$, the compactness perhaps attained a saturation value (Figure 3C) and consequently the interaction did not alter with a further increase of the molarity of CaCl$_2$ (Figure 2).

The role of Ca-ion on DNA−LPS interaction was further substantiated from the reversal of the interaction by addition of EDTA to the DNA−LPS complex. When EDTA was stepwise added to a complex of DNA (143 μM) and LPS (55 μM) formed in 1 mM CaCl$_2$, (absorbance)$_{260}$, of DNA (bound with LPS) increased gradually with increasing concentrations of EDTA up to 0.9 mM, beyond which no considerable increase had taken place (Figure 4). EDTA, being a chelating agent for divalent cations, indeed chelated Ca ions and so DNA and LPS fell apart causing the rise of (absorbance)$_{260}$. This result, therefore, showed strong evidence that the binding between DNA and LPS had been mediated through divalent cation Ca(II); moreover, the removal of the interaction by addition of EDTA also indicated that it was reversible in nature.

The DNA−LPS interaction was found to be more pronounced ($K_b$ ≈ 3.1 × 10$^4$ M$^{-1}$) in the presence of Mg(II) ions, whereas there was hardly any interaction in the presence of monovalent cations Na(I), K(I), Li(I), and so on (data not shown). The group II elements (calcium, magnesium) were better at forming complexes than group I elements (sodium, potassium, lithium) due to higher charge, smaller size, and availability of suitable empty orbitals of lower energy; therefore, Ca$^{2+}$ and Mg$^{2+}$ showed the tendency to form coordination complexes in solution especially with the oxygen-donor ligands, namely, PO$_4^{3-}$-oxygen. The COS domain of LPS was known to be composed of heptose and 2-keto-3-deoxyoctonic acid, which were, in general, substituted by the charged group, such as, phosphate, pyrophosphate, 2-aminoethyl phosphate, and 2-aminoethyl pyrophosphate. Thus, the divalent cation calcium, forming stable coordination complex with phosphates, facilitated the association of two phosphate-rich structures, DNA and LPS. Moreover, DNA−LPS interaction was found to be much higher in presence of lanthanide group element like cerium (Ce$^{3+}$) than in Ca/Mg ions ($K_{b_{Ca(II)}}$ = 5.3 × 10$^4$ M$^{-1}$, ($K_{b_{Ca(II)}}$ = 2.95 × 10$^4$ M$^{-1}$, ($K_{b_{Mg(II)}}$ = 3.1 × 10$^4$ M$^{-1}$), since the lanthanide group elements were known to form mostly coordination complexes, this result further confirmed the formation of DNA−LPS complex through coordination with calcium ions.

The calorimetric titration study of DNA−LPS interaction exhibited (i) a monotonic decrease in exothermic heat of binding with successive injections until the saturation had been achieved (Figure 5A) and (ii) the incremental heats per mol of LPS added (Figure 5B). The different parameters of DNA−LPS interaction, determined from ITC measurements (using single site fit model), were as follows: change in enthalpy ($\Delta H$) = (−)14.77 kcal M$^{-1}$, change in entropy ($\Delta S$) = (−)4.75 × 10$^{-3}$ kcal M$^{-1}$ K$^{-1}$, binding constant ($K_b$) = 2.76 × 10$^4$ M$^{-1}$ and binding stoichiometry (N) = 0.0193. The results show that the interaction was an exothermic process and was more enthalpy-driven than entropy-driven. Moreover, the value of the binding constant (2.76 × 10$^4$ M$^{-1}$) obtained from this experiment was close to that (2.95 × 10$^4$ M$^{-1}$) obtained from the spectrophotometric titration.

The CD spectral study was performed to investigate if LPS binding had altered DNA conformation. The CD spectrum of pUC19 DNA in 1 mM CaCl$_2$ showed the characteristics of B-DNA conformation (figure 6). LPS, suspended in 1 mM CaCl$_2$, showed no chirality in the wavelength region of the CD spectrum of DNA. However, when LPS was added to DNA, CD spectra of DNA had changed considerably. With increase of LPS concentration, the spectra exhibited an attenuated positive lobe at 280 nm and an increased negative lobe at 245 nm with a red shift of the crossover point. Such spectral changes together with the occurrence of deep CD minima at short wavelength (∼212 nm) indicated a perturbation of secondary conformation, as observed in case of less hydrated DNA molecules, for which a transition from B to A form of DNA was known to occur. Moreover, the decrease of CD band of DNA around 280 nm implied occurrence of short single-stranded segments containing unpaired bases. Therefore, the LPS binding caused (i) DNA conformational change from its natural

![Figure 4](image)

**Figure 4.** Reversal of the DNA−LPS interaction with the gradual addition of EDTA. The initial complex was formed mixing 143 μM DNA and 55 μM LPS, both in 1 mM CaCl$_2$.

![Figure 5](image)

**Figure 5.** Calorimetric titration of pUC19 DNA with LPS in the presence of 1 mM CaCl$_2$ at 25 °C. The upper panel shows raw data obtained from 30 injections (5 μL for first two and 10 μL each for the rest) of LPS. The LPS concentration in the syringe was 300 μM, where as the DNA concentration in the cell was 134 μM. The lower panel shows the plot of total energy exchange (as kcal M$^{-1}$ of injectant) as a function of the molar ratio of LPS to DNA.
occurring B to A form and (ii) DNA strand separation forming small denatured regions.

The formation of DNA-LPS complex in presence of Ca ions was evident from the gel retardation study also. Because DNA and LPS was bridged up through Ca ions and EDTA was a chelator of Ca ions, instead of standard tris-acetate-EDTA, tris-acetate was used as the gel-running buffer. Moreover, to stabilize the DNA-LPS complex in gel, tris-acetate buffer was supplemented with 1 mM CaCl$_2$. The result of this study, represented by Figure 7, showed that the DNA-LPS complex had a retarded mobility than DNA alone, when run through 1% agarose gel. This was because the molecules of the DNA-LPS complex had a larger size than that of the naked DNA molecules.

To visualize the DNA-LPS bound complex, an immuno-electron microscopic study was carried out. Figure 8 represents the electron microscopic picture, where in addition to the supercoiled impression of DNA, the LPS-bound regions of DNA (as dark spots) were clearly visible. The picture also showed that the LPS did not bind homogeneously all over the DNA, rather bound to a continuous stretch of DNA as well as to some randomly scattered short regions (marked by arrows in Figure 8). However, electron microscopic picture could not give any clear idea about the regions of DNA, which were more accessible for LPS binding and it requires further investigation.

Structurally, LPS consisted of three important domains, namely, lipid A moiety, COS (core oligosaccharide) and OPS (O-specific polysaccharide). The above findings on DNA-LPS interaction led us to ask whether LPS in total or any of its domains was responsible for the binding with DNA. For this, LPS were fractionated to its three components. Solutions of COS (3 mg/mL) and OPS (3 mg/mL) domains were made separately in 1 mM CaCl$_2$, and the suspension of the lipid A moiety (3 mg/mL) was prepared in 1 mM CaCl$_2$ by sonication. Figure 9 shows that the stepwise addition of the lipid A suspension or the OPS solution to the plasmid DNA (143 µM DNA dissolved in 1 mM CaCl$_2$) had no considerable effect on the DNA absorbance at 260 nm, whereas the addition of the COS solution decreased the absorbance at 260 nm. Figure 2 also shows that, for interaction, the minimum required concentration of COS component was about 150 µg/mL and the concentration, above which the interaction reached the saturation state, was about 615 µg/mL. Because the COS component of LPS contained negatively-charged phosphate groups, it can justifiably be stated that LPS by its COS domain had interacted with the phosphate backbone of DNA via divalent cation Ca(II).

To investigate whether the above results on in vitro interaction between DNA and LPS had any in vivo importance behind the DNA adsorption step of the standard transformation procedure, experiment was carried out to scan the outer surface of the CaCl$_2$-treated competent E. coli cell by atomic force microscope.
(AFM), before and after adsorption of pUC19 DNA. In the standard protocol for artificial transformation of *E. coli* with plasmid DNA, 50 ng of DNA is generally added to 200 µL of competent *E. coli* cells in 100 mM CaCl$_2$ and the cell–DNA complex is incubated in ice for 30 min to allow the adsorption of DNA to the competent cell surface. In our AFM study, to visualize adsorbed DNA on competent cell surface, 10 times higher amount of DNA, that is, 500 ng of DNA was added to 200 µL of competent *E. coli* cells. After 30 min of DNA adsorption, cells were washed with 100 mM CaCl$_2$ to remove the unadsorbed DNA. Cells were then fixed with gluteraldehyde and finally suspended in water for AFM scanning. Figure 10 shows that the AFM images of competent cells with DNA (Figure 10C,D) and without DNA (Figure 10A,B). A scan of 2 × 2 µm$^2$ showed individual bacterium (images A and C) with rod-shaped character, with a typical length of 2–3 µm; corresponding width and height were 1 and 0.8 µm, respectively. At nanometer resolution (0.35 × 0.35 µm$^2$), images B and D exhibited bumps with lateral dimensions of 25–60 nm and an average surface roughness of 5–10 nm on cell surface. The bumps were reported to be LPS assembly on cellular outer membrane and the difference in heights of the bumps was suggested to be due to different O-antigen units at LPS termini.$^{30}$ Surface morphology of image D differed from that of image B by the presence of few thread-like structures in D. In some places the threads were in singular form and in some places they were in aggregated form. Whatever the form is, the heights of these threads were ranged from 5 to 8 nm. Because the diameter of a DNA double helix was about 2 nm, therefore, in the highly coiled-overcoil (supercoil) structure (in 100 mM CaCl$_2$), height of a DNA molecule lying on a surface, as scanned by an AFM, was expected to be of the order of the sizes of thread-like structures in image D. Therefore, the thread-like structures were nothing but the plasmid DNA molecules, and Figure 10D clearly shows that plasmid DNA molecules had been adsorbed to the LPS assembly on the cell surface. Moreover, most of the DNA molecules were found to adsorb in deep interspace between two LPS bumps. The reason was that the COS components, the DNA-binding sites of LPS molecules, were accessible at interspace of LPS bundles.

The biological importance of DNA–LPS complex formation in artificial transformation procedure was revealed from the result of the following experiment, where the transformation efficiency (TR)$_E$ of competent *E. coli* cells (from which LPS molecules were leached out) had been investigated by adding preformed complex of DNA and isolated LPS. LPS-leached *E. coli* XL1 Blue cells were prepared by treating them with ethanol. It was reported earlier$^7$ that the incubation of CaCl$_2$-treated competent *E. coli* cells with 5% v/v ethanol for a period of 30 min had caused leaching of about 40% LPS molecules from the cell surface to the extracellular medium. In this case, competent cells were first incubated with 5% v/v ethanol for
30 min and were then centrifuged at 8000 rpm for 10 min; the cell pellet was redissolved in 100 mM CaCl$_2$ containing 5% v/v ethanol to incubate further for 30 min. The cycle of ethanol treatment and centrifugation was repeated thrice and, after each cycle, the supernatant was assayed for the presence of LPS. Finally, LPS-leached cells were washed and resuspended in 100 mM CaCl$_2$. Two aliquots, each of 200 µL of the final cell suspension, were taken. In one, 50 ng pUC19 DNA was added and in the other, preformed DNA-suspension, were taken. In one, 50 ng pUC19 DNA was added and in the other, preformed DNA–LPS complex (by mixing 143 µM DNA and 55 µM LPS, both dissolved in 100 mM CaCl$_2$) was added in such a quantity that the DNA amount in complex became 50 ng. Subsequent steps of transformation were performed according to the standard protocol, described in Materials and Methods. Figure 11A indicates that, as the cycle of ethanol treatment was increased, release of LPS molecules from cell surface had increased proportionately. After the third cycle of ethanol treatment, no release of assayable LPS was observed. Figure 11B shows that after the third cycle of ethanol treatment, when cells were allowed to transform by pUC19 DNA and pUC19–LPS complex separately, (TR)$_h$ in the later case was about six times higher than that in the former case. Therefore, the addition of DNA alone to the cells (containing very low amount of LPS) resulted in low efficiency of transformation, whereas the addition of LPS-bound DNA to the same competent cells resulted in better efficiency of transformation, overcoming the problem of LPS shortage on the cell surface. This result clearly implied that the binding of DNA on 50 ng CaCl$_2$-treated competent cells resulted in better efficiency of transformation.

We also observed that the membrane had been rigidified due to release of its lipid molecules to extracellular medium, which had ultimately produced pores on the cell surface. These pores perhaps facilitated the passage of adsorbed DNA into cell cytosol during transformation. Therefore, the (TR)$_h$ increased with the increase of membrane rigidity, that is, with the increase of CaCl$_2$ concentration. Thus, at 1 mM CaCl$_2$, where the binding interaction between DNA and LPS was maximum, the outer membrane rigidification by the heat-pulse step was not significant enough for resulting considerable (TR)$_h$; whereas at 50–100 mM CaCl$_2$, although the DNA–LPS interaction was slightly lower than its maximum extent at 1 mM CaCl$_2$, the outer membrane rigidification by the heat-pulse step was maximum to make the (TR)$_h$ optimally high.

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References and Notes


