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Diphosphoryl Lipid A from *Rhodobacter sphaeroides* Inhibits Complexes That Form In Vitro between Lipopolysaccharide (LPS)-Binding Protein, Soluble CD14, and Spectrally Pure LPS

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An early event in septic shock is the activation of macrophages by a complex consisting of lipopolysaccharide (LPS), LPS-binding protein (LBP), and the cell surface antigen CD14. The complexes that form between [^{3}H]ReLPS (ReLPS is deep-rough-chemotype hexacyl LPS from *E. coli* D31m4), soluble CD14 (sCD14), and LBP were analyzed by two independent methods, native (nondenaturing) gel electrophoresis and size-exclusion high-performance liquid chromatography (HPLC). This is the first reported use of HPLC to purify and study LPS-protein complexes. The binding of [^{3}H]ReLPS to LBP and sCD14 was inhibited by preincubation with diphosphoryl lipid A from *Rhodobacter sphaeroides* (RsDPLA), a potent LPS antagonist. In addition, [^{3}H]ReLPS bound to LBP and to a truncated form of sCD14 [sCD14$_{(1–152)}$] that contained the LPS binding domain. Binding to both proteins was blocked by RsDPLA. Thus, RsDPLA competes in a 1:1 ratio for the same or nearby binding sites on ReLPS complexes. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of aggregated ReLPS eluting from the HPLC indicated that only LBP, not sCD14, was bound to the aggregated ReLPS. This finding supports the binary model of LPS complex formation with LBP and sCD14.

Septic shock (endotoxic shock) is responsible for significant morbidity and mortality in the United States (3). Lipopolysaccharide (LPS) from gram-negative bacteria, such as *Escherichia coli* and *Salmonella*, activates macrophages, leading to the release of proinflammatory cytokines that may trigger septic shock. An international effort is under way to find an effective treatment, although to date, clinical trials have not yet supported the efficacy of any one therapeutic agent (17). A naturally occurring, potent LPS antagonist is diphosphoryl lipid A from *Rhodobacter sphaeroides* (RsDPLA) (18). RsDPLA blocked the release of the LPS-induced proinflammatory cytokines, tumor necrosis factor alpha and interleukin-1β, in both murine and human cells (18). These continued research efforts are essential to reveal the underlying mechanisms of pathogenesis in septic shock.

During the early events of sepsis, it is thought that LPS interacts first with LPS-binding protein (LBP), a 60-kDa serum glycoprotein, and then complexes with the 55-kDa leukocyte differentiation antigen CD14 (10, 11, 24) on cell membranes (mCD14). Amino acids 57 to 64 of recombinant, soluble CD14 (sCD14) have been shown to be necessary for LPS binding, as demonstrated by protease protection studies (16) and by analysis of mutant sCD14 lacking that region (12). The binding of LPS to mCD14 for internalization by immune cells and the transduction of the LPS signal into those cells for subsequent cell activation are separate events blockable by two different monoclonal antibodies (7). LPS antagonists, such as deacylated LPS (15) and *R. sphaeroides* LPS (1), have been shown, respectively, to compete with LPS for binding to LBP (15) and to inhibit neutrophil response to LPS by decreasing serum LBP levels (1).

Previous studies of proteins that bind to LPS have used a variety of methods to demonstrate and quantify binding. For example, fluorescently labeled LPS has been used to study LPS binding to LBP and sCD14 (23, 24). Other workers have biosynthetically labeled LPS with an isotope and used a nondestructive method of analysis, such as native gel electrophoresis (12). Size-exclusion high-performance liquid chromatography (HPLC) is a second nondestructive method for studying protein binding. The advantage of the size-exclusion HPLC technique is that it neither structurally alters the LPS by adding a fluorescent adduct nor disrupts the native state of the proteins LBP and sCD14. In addition, the size-exclusion HPLC method can measure the size of LPS aggregates, determine an exclusion limit, and resolve CD14 from LBP, none of which is possible by the sucrose density gradient method (23).

To further elucidate the mechanism by which LPS interacts with LBP and sCD14, we developed a novel method to resolve and purify LPS complexes by size-exclusion HPLC. It had previously been shown (14) that in the presence of serum, RsDPLA blocked the binding of ^{125}I-labeled LPS to intact macrophage-like cells. Therefore, we decided to assess with HPLC the ability of RsDPLA to compete with LPS for binding to purified LBP, sCD14, and truncated sCD14 [sCD14$_{(1–152)}$]. The results of these studies support the binary model (23) of LPS-LBP binding rather than the ternary complex model (7, 24) of LPS-LBP-CD14 interaction.

(A portion of these data was presented as a poster at the Fourth Conference of the International Endotoxin Society in Nagoya, Japan, in October 1996.)

MATERIALS AND METHODS

Reagents. sCD14, sCD14$_{(1–152)}$, and LBP were obtained from Amgen. Deep-rough-chemotype hexaacyl LPS from *E. coli* D31m4 (ReLPS) and RsDPLA were purified in our lab as described by Qureshi et al. (19–21) and are designated spectrally pure to denote that mass spectral analyses are routinely performed on

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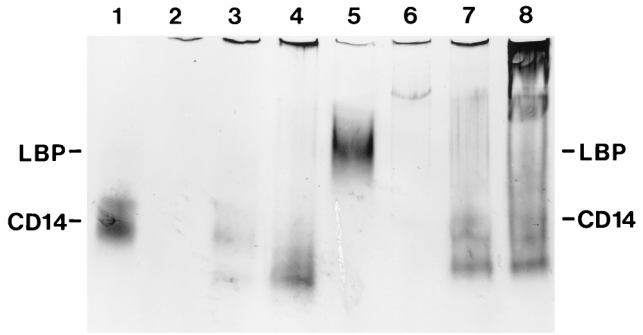

FIG. 1. A 5 to 20% gradient native polyacrylamide gel was stained with silver and shows complexes formed between LBP, sCD14, and ReLPS or RsDPLA. Lanes: 1, 5 μg of sCD14; 2, 4 μg of ReLPS; 3, 4 μg of ReLPS preincubated with 5 μg of sCD14; 4, 4 μg of ReLPS, 5 μg of sCD14, and 2 μg of LBP; 5, 2 μg of LBP; 6, 4 μg of RsDPLA; 7, 4 μg of RsDPLA and 5 μg of sCD14; 8, 4 μg of RsDPLA, 5 μg of sCD14, and 2 μg of LBP.

the preparations as a check for purity. Unless LPS is spectrally pure, phospholipids and proteins in LPS preparations confound the interpretation of one's subsequent results. All glass and plastic ware were rendered pyrogen-free by heating at 120°C overnight.

Preparation of [^{3}H]ReLPS. An overnight culture of *E. coli* D31m4 was diluted 40-fold into 200 ml of fresh LB medium. Fifty millicuries of [^{3}H]acetate (ICN) was added, and the culture was grown to an optical density at 650 nm of 0.75. The cells were harvested and extracted by the method of Galanos et al. (6) as modified by Qureshi et al. (20). The [^{3}H]ReLPS (1.9 mg, 7.26×10^8 dpm/mg) was treated with 0.1 M EDTA (pH 7.0) and stirred for 1.5 h at room temperature. The LPS was recovered as described previously (21).

The [^{3}H]ReLPS was then dissolved in chloroform-methanol (4:1, vol/vol) and applied to a 2- by 15-cm DEAE-cellulose column (acetate form). The column was washed with 30 ml of chloroform-methanol-water (2:3:1, vol/vol/vol). [^{3}H]ReLPS was eluted from the column with a linear gradient of 200 ml each of 0.04 M to 0.12 M ammonium acetate in chloroform-methanol-water (2:3:1, vol/vol/vol). Five-milliliter fractions were collected. Fractions numbered 23 through 29 were pooled and desalted. The predominantly hexaacyl [^{3}H]ReLPS was converted to the free-acid form by passage through a Chelex 100 (Na^+) and Dowex 50 (H^+) two-layered column in chloroform-methanol (4:1, vol/vol), as described previously (21). The specific activity of the final [^{3}H]ReLPS product, after an assay for phosphate esters (2), was 6.2×10^5 dpm/μg. [^{3}H]ReLPS and RsDPLA were both either suspended in pyrogen-free saline to yield the sodium salt or dissolved in 0.5% triethylamine in pyrogen-free water to yield the triethylamine salt. These solutions were then sonicated for 1 min.

Native polyacrylamide gel electrophoresis (PAGE) assay. To visualize the binding to sCD14, 4 μg of ReLPS or 4 μg of RsDPLA was incubated in a glass tube for 2 h at 37°C with 10 μl of water, sCD14 (5 μg in 5 μl), and/or LBP (2 μg in 5 μl). LBP and sCD14 were stored in phosphate-buffered saline (Dulbecco's PBS without Ca or Mg [GIBCO BRL 14190-144]) plus 20% glycerol (9). sCD14 and LBP in separate lanes served as markers for mobility shifts among the complexes. The resulting complexes were visualized on a native (nondenaturing) polyacrylamide gel, as described previously (12), and stained with silver (Bio-Rad). The 5 to 20% gradient gel had an internal pH of 8.6, while the electrode buffer pH was 8.3.

HPLC assay. Complexes with sCD14 (10 μg; 182 pmol in 2 μl) and LBP (10 μg; 170 pmol in 10 μl) were formed by incubating each protein or RsDPLA (10 μg; 6.6 nmol in 2 μl) with [^{3}H]ReLPS (1.3 μg; 565 pmol in 7 μl), both suspended in pyrogen-free saline or in 0.5% triethylamine in pyrogen-free water. Complexes were incubated at 37°C for 1 h in a total volume of 21 μl. Thus, a threefold excess of [^{3}H]ReLPS relative to protein was used for the binding experiments. Since LBP and sCD14 were stored in PBS plus 20% glycerol (9), this was the only buffering agent present. Incubation time and buffer were the same as that previously optimized by Hailman et al. (9). When [^{3}H]ReLPS and RsDPLA were incubated with LBP and sCD14, RsDPLA was added 30 min before ReLPS. These complexes were analyzed by HPLC on a Waters Protein Pak SW 300 gel filtration column in an isocratic 0.1 M KPO_4 buffer at pH 7.0. The HPLC hardware consisted of a Waters 6000A solvent delivery system, a Waters U6K universal liquid chromatography injector, and a Perkin-Elmer model LC-85B variable-wavelength detector set at 280 nm. Fractions of 0.5 ml were collected at a flow rate of 1 ml/min and analyzed by liquid scintillation counting. After binding of the [^{3}H]ReLPS to the proteins, aliquots of these samples were counted in liquid scintillation fluid to determine total radioactivity prior to HPLC. Based on these counts, the recovery from the HPLC column was 80 to 100%.

Complexes separated by HPLC and visualized by sodium dodecyl sulfate (SDS)-PAGE were formed by incubating 20 μg of nonradioactive ReLPS with 20 μg of LBP and 20 μg of sCD14 by the procedures described previously. However, the HPLC buffer was changed to 0.02 M $NaPO_4$ (pH 7.0). The fluid eluting at the midpoint of each peak was collected in a glass tube, freeze-dried, and resuspended in electrophoresis sample buffer. One-half of each sample was loaded on the gel. Reducing SDS-PAGE was done with procedures, a minigel apparatus, and molecular weight standards from Bio-Rad.

Competitive binding. For experiments in which RsDPLA competed with [^{3}H]ReLPS for binding to sCD14 and LBP, a native polyacrylamide gel was used to assay complex formation. A given amount of RsDPLA in saline was preincubated with 2 μg of LBP (2 μl of PBS) and 3 μg of sCD14 (6 μl of PBS) at 37°C for 30 min in glass tubes. Then, 1.3 μg of [^{3}H]ReLPS in 7 μl of saline was added and the mixture was incubated for 1.5 h at 37°C. Glycerol, water, and tracking dye were added to bring the volume to 20 μl, and the sample was loaded onto the gel. After electrophoresis, the gel was stained with Coomassie blue, soaked in Amplify (Amersham), and dried. The distribution of counts per minute was visualized by fluorography. The [^{3}H]ReLPS-CD14 bands were then excised from the gel and counted in a liquid scintillation counter. Recovery of sCD14-bound [^{3}H]ReLPS from the gel was 10 to 15% of the total [^{3}H]ReLPS loaded on the gel. The remaining [^{3}H]ReLPS in the gel was not counted.

RESULTS

The experiments described here were undertaken to determine the relative roles of LBP and sCD14 in forming complexes with ReLPS and RsDPLA. The ReLPS used for this study was the free acid form and was highly aggregated. Using a previously described assay (12) for native PAGE, we confirmed that ReLPS binds to sCD14 and shifts sCD14 lower in the gel (Fig. 1, lane 3). (Unlike SDS gels in which all proteins have a net negative charge from the SDS, in native gels, molecules migrate according to both mass and charge. At the pH of 8.3 used here, most, but not all, proteins migrate into the gel.) RsDPLA, when incubated with sCD14 (Fig. 1, lane 7), shifted sCD14 to a lower position in the gel. Thus, we conclude that RsDPLA also formed a complex with sCD14 and thereby changed the migration of sCD14. Alone, neither ReLPS (Fig. 1, lane 2) nor RsDPLA (Fig. 1, lane 6) is visible in the gel, in part because the silver stain used (Bio-Rad) primarily stains proteins and in part because not all of these complexes entered the gel very far, as observed by fluorography (data not shown). For points of reference, one lane was loaded with sCD14 alone (Fig. 1, lane 1) and one lane contained only LBP. When all three components, i.e., ReLPS, sCD14, and LBP, were added together (Fig. 1, lane 4), the complex migrated even further into the gel. The complex of RsDPLA, sCD14, and LBP seemed to have a decreased migration in this gel (Fig. 1, lane 8).

The native PAGE assay can also be used to measure [^{3}H]

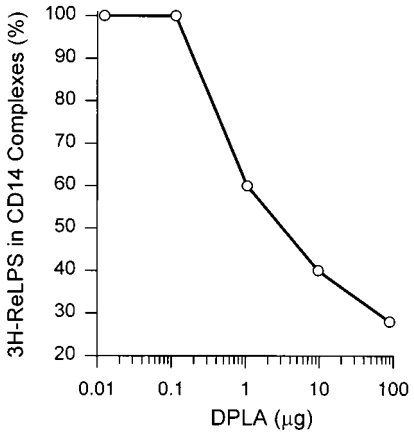

FIG. 2. Graph showing inhibition of [^{3}H]ReLPS binding to LBP and sCD14 after preincubation of sCD14 and LBP with various amounts of RsDPLA at 37°C. Data are from a typical experiment.

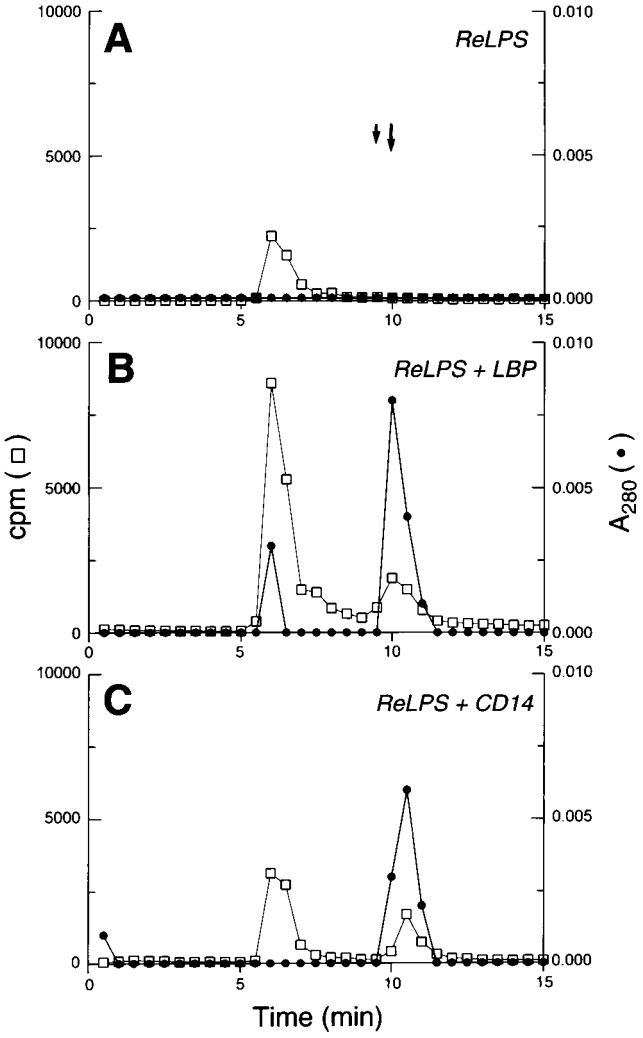

FIG. 3. HPLC elution profiles with counts per minute for [^{3}H]ReLPS and the absorbance at 280 nm (A_{280}) for both LBP and sCD14 proteins after complex formation for 1 h at 37°C. (A) [^{3}H]ReLPS at 1.3 μg (8×10^5 dpm); (B) 1.3 μg of [^{3}H]ReLPS plus 10 μg of LBP; (C) 1.3 μg of [^{3}H]ReLPS plus 10 μg of sCD14. The short arrow indicates the position of LBP elution, and the long arrow indicates that for sCD14.

ReLPS binding to sCD14 (12). Therefore, we tested whether RsDPLA could compete with [^{3}H]ReLPS for binding to sCD14. (The gel is not shown, but the data are presented in Fig. 2.) Less than 1 μg of RsDPLA did not compete for binding to sCD14. One to 100 μg of RsDPLA competed with 1.3 μg of [^{3}H]ReLPS for binding to the LBP-CD14 complex and decreased the binding of [^{3}H]ReLPS from 60 to 30% of the maximum, respectively (Fig. 2). Therefore, RsDPLA apparently displaced LPS from sCD14 in a 1:1 ratio, since 1 μg of RsDPLA yielded half-maximal competition with 1.3 μg of ReLPS.

The native PAGE assay was useful in visualizing the complexes that formed between sCD14 and disaggregated LPS, but to study and purify complexes between aggregated or disaggregated LPS and proteins, we developed an HPLC assay. Data from the HPLC assay were much easier to interpret. Using this assay, we determined the relative binding of [^{3}H]ReLPS to sCD14 and LBP. Aggregated [^{3}H]ReLPS eluted at 6 min (Fig. 3). When each protein was run separately, LBP eluted at 9.5 min and sCD14 eluted at 10 min (Fig. 3). The incubation of [^{3}H]ReLPS with LBP shifted some of the protein to the aggregated [^{3}H]ReLPS peak. In contrast, the incubation of [^{3}H]ReLPS with sCD14 did not. Both LBP and sCD14 increased the number of counts per minute of [^{3}H]ReLPS that eluted from the column. Individually, only LBP bound to the aggregated [^{3}H]ReLPS. However, both LBP and sCD14 bound to the disaggregated [^{3}H]ReLPS in the peaks eluting at 10 to 11 min. Significantly, sCD14 did not need LBP to bind to [^{3}H]ReLPS.

When [^{3}H]ReLPS was incubated with sCD14 and LBP together (Fig. 4), maximal binding of [^{3}H]ReLPS to sCD14 and/or to LBP was observed. Preincubation with RsDPLA blocked [^{3}H]ReLPS binding to LBP and sCD14 while shifting most of the radioactivity back to the position of the aggregated [^{3}H]ReLPS, i.e., away from the sCD14 protein peak. We also observed that some protein coeluted with the peak of aggregated [^{3}H]ReLPS. When [^{3}H]ReLPS was solubilized in 0.5% triethylamine (instead of saline) and then incubated with LBP and sCD14, the results were qualitatively similar (Fig. 5). However, as shown in Fig. 5A, there was a dramatic threefold increase in counts with disaggregated [^{3}H]ReLPS bound to sCD14 and LBP (peak appearing at 9 to 10 min). Presumably, the greater solubility of the triethylamine salt of [^{3}H]ReLPS (in contrast to the sodium salt) resulted in more efficient binding to the proteins. Preincubation with RsDPLA once again blocked the binding of [^{3}H]ReLPS to LBP and sCD14, as shown in Fig. 5B.

Unlike sCD14, which eluted 0.5 min later than LBP (Fig. 3A), $sCD14_{(1-152)}$ eluted 1 min later than LBP (Fig. 6A). This greater separation allowed us to determine more accurately

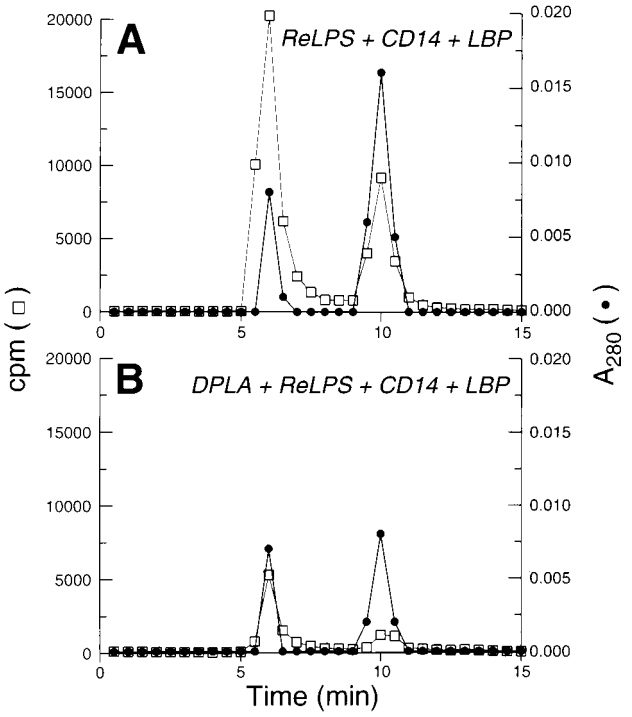

FIG. 4. HPLC elution profiles with counts per minute for [^{3}H]ReLPS and the absorbance at 280 nm (A_{280}) for both LBP and sCD14 proteins after complex formation for 1 h at 37°C. [^{3}H]ReLPS and RsDPLA were suspended in pyrogen-free saline. (A) [^{3}H]ReLPS at 1.3 μg, 10 μg of sCD14, and 10 μg of LBP; (B) 10 μg of RsDPLA, 10 μg of sCD14, and 10 μg of LBP preincubated for 30 min before addition of 1.3 μg of [^{3}H]ReLPS for a further 1-h incubation.

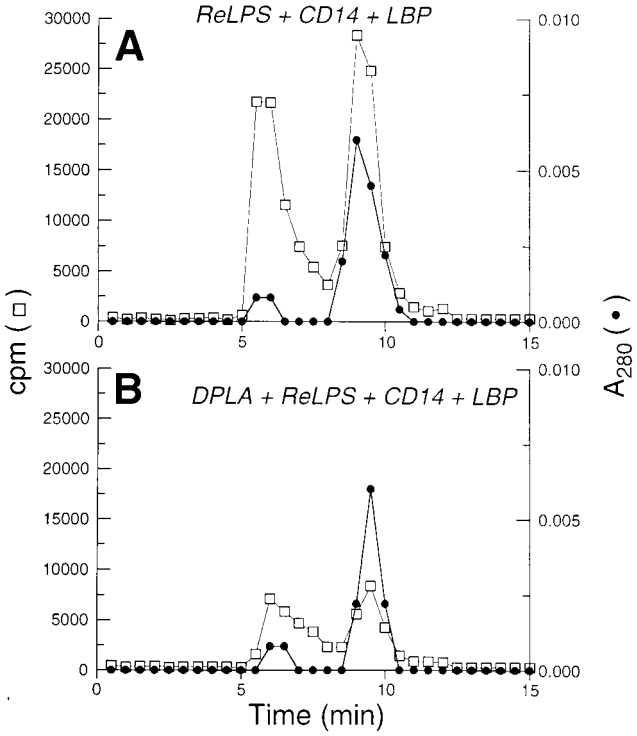

FIG. 5. HPLC elution profiles with counts per minute for [^{3}H]ReLPS and the absorbance at 280 nm (A_{280}) for both LBP and sCD14 proteins after complex formation for 1 h at 37°C. Both [^{3}H]ReLPS and RsDPLA were dissolved in 0.5% triethylamine in pyrogen-free water. (A) [^{3}H]ReLPS at 1.3 μg, 10 μg of sCD14, and 10 μg of LBP; (B) 10 μg of RsDPLA, 10 μg of sCD14, and 10 μg of LBP preincubated for 30 min before addition of [^{3}H]ReLPS for a further 1-h incubation.

that aggregated [^{3}H]ReLPS bound to LBP and thereby reduced the LBP absorbance 10-fold (Fig. 6B), while the peak height of sCD14$_{(1–152)}$ remained unchanged. However, disaggregated [^{3}H]ReLPS did bind to sCD14$_{(1–152)}$. By preincubating with RsDPLA (Fig. 6C), once again most of the radioactivity was shifted back to the position of the aggregated [^{3}H]ReLPS, i.e., away from the sCD14$_{(1–152)}$ protein peak, and some protein coeluted with the peak of aggregated [^{3}H]ReLPS. To identify the proteins in the early- and late-eluting peaks, the proteins from the individual peaks were collected and subjected to SDS-PAGE. LBP was the only detectable protein that eluted early, in peak 1, after forming a complex with aggregated [^{3}H]ReLPS (Fig. 7, lane 4). The remainder of the LBP and all of the detectable sCD14 eluted later, with the disaggregated material in peak 2 (Fig. 7, lane 5).

DISCUSSION

We utilized a native PAGE assay to show that both [^{3}H]ReLPS and its antagonist RsDPLA bound to sCD14, even in the absence of LBP (Fig. 1). Furthermore, RsDPLA competed with [^{3}H]ReLPS for binding to LBP-CD14 complexes (Fig. 2), suggesting that both ReLPS and RsDPLA were binding to the same or similar sites on sCD14 and LBP. Unlike Kawata et al. (13), who used ^{125}I-LPS and a synthetic RsDPLA designated E5531, our native RsDPLA and [^{3}H]ReLPS readily inhibited LBP-mediated LPS binding. The ratio of 1 μg of RsDPLA to 1.3 μg of [^{3}H]ReLPS illustrated in Fig. 2 is similar to that used (8) to block tumor necrosis factor alpha production in human monocyte-like THP-1 cells (1 μg of RsDPLA to 1 μg of ReLPS). Thus, these competitive binding studies were done with RsDPLA concentrations shown to have been effective in cellular cytokine inhibition. Similarly, Kirikae et al. (14) showed that a 1:1 ratio of RsDPLA/^{125}I-LPS was sufficient to block ^{125}I-LPS binding to macrophages. In contrast, it took Rose et al. (22) 25-fold more of the synthetic analog of RsDPLA (RSLA or 2′-cis-LA) to achieve half-maximal blocking of ^{125}I-LPS binding to macrophages (2.5 μg of RSLA per ml to 100 ng of ^{125}I-LPS per ml). Evidently, synthetic RSLA was not as effective as native RsDPLA.

A novel HPLC assay was devised to demonstrate that [^{3}H]ReLPS bound sCD14 without LBP present. This is the first reported use of HPLC to study LPS-protein complexes. However, the HPLC assay also revealed that more total [^{3}H]ReLPS was solubilized with LBP present and was thus able to pass through the molecular-sieve HPLC column. Both LBP and sCD14, however, were necessary for maximum solubility. For example, when LBP and sCD14 were present, 6.0×10^4 cpm eluted with the sodium salt of [^{3}H]ReLPS and 1.7×10^5 cpm eluted with the triethylamine salt. These HPLC data are 10- to 100-fold-higher counts per minute values than those reported as recovered by the sucrose density gradient method (23). LBP

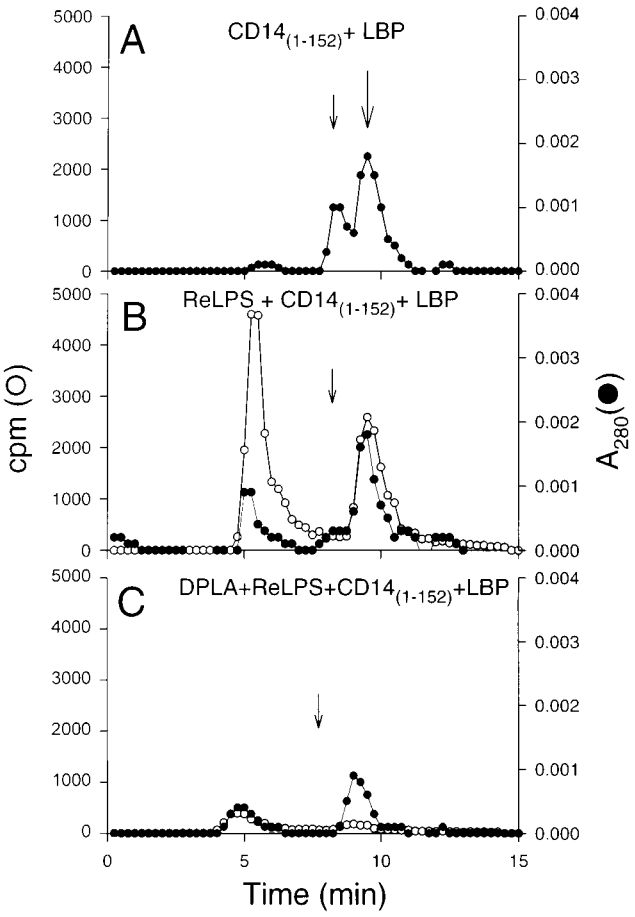

FIG. 6. HPLC elution profiles with counts per minute for [^{3}H]ReLPS and the absorbance at 280 nm (A_{280}) for both LBP and sCD14$_{(1–152)}$ proteins after complex formation for 1 h at 37°C. (A) LBP plus sCD14$_{(1–152)}$ (2.5 μg each) without [^{3}H]ReLPS; (B) 1.3 μg (8×10^5 dpm) of [^{3}H]ReLPS plus 2.5 μg each of LBP and sCD14$_{1–152}$); (C) 2.5 μg of LBP, 2.5 μg of sCD14$_{(1–152)}$, and 10 μg of RsDPLA preincubated for 30 min before addition of [^{3}H]ReLPS for a further 1-h incubation. The short arrow indicates the position of LBP elution, and the long arrow is that for sCD14$_{(1–152)}$.

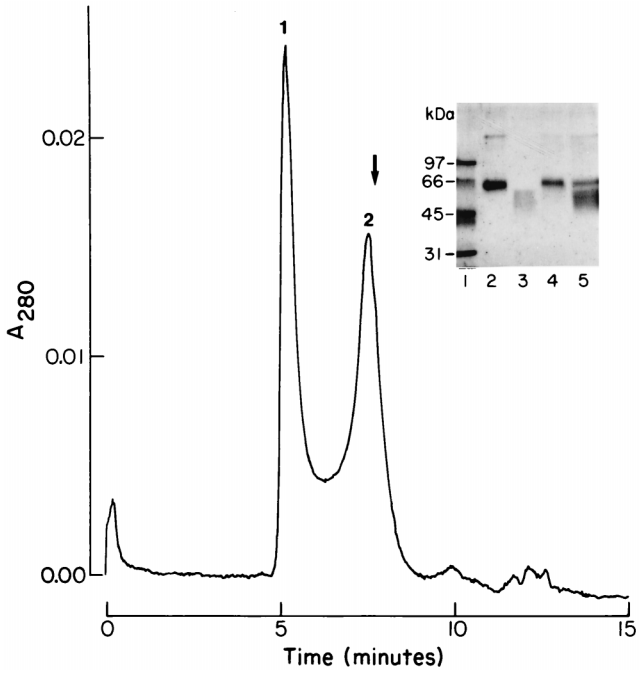

FIG. 7. Elution profile of an HPLC separation of ReLPS incubated with LBP and sCD14 for 1 h at 37°C. Inset, a 9% SDS reducing electrophoretic gel stained with Coomassie blue and containing the protein(s) that eluted from the HPLC at the midpoints of peak 1 (aggregated ReLPS position) and peak 2 (soluble protein position). The arrow indicates the position of sCD14 elution. Lanes for the gel: 1, molecular size standards; 2, 1 μg of LBP; 3, 0.5 μg of sCD14; 4, protein in HPLC peak 1; 5, proteins in HPLC peak 2.

was able to bind both to the aggregated [^{3}H]ReLPS (early-eluting fractions) and to the disaggregated [^{3}H]ReLPS (later-eluting fractions). In contrast, sCD14 bound only to the disaggregated [^{3}H]ReLPS. This observation supports the idea that LBP may disaggregate [^{3}H]ReLPS for subsequent binding by sCD14 (24). The HPLC data in Fig. 3 to 5 complement those from the native gel in Fig. 1. Thus, two independent methods are in agreement that sCD14 and LBP bind either to RsDPLA or to [^{3}H]ReLPS, thereby accounting in part for the antagonistic properties of RsDPLA. (In fact, [^{3}H]ReLPS did bind to both LBP and $sCD14_{(1–152)}$ [Fig. 6]). While aggregated [^{3}H] ReLPS bound to LBP, disaggregated [^{3}H]ReLPS bound to $sCD14_{(1–152)}$. RsDPLA blocked the binding to both proteins. Since $sCD14_{(1–152)}$ contains the LPS binding domain (12, 16), it is possible that amino acids 57 to 64 of CD14 (12, 16) may bind to [^{3}H]ReLPS and RsDPLA.

HPLC followed by SDS-PAGE was then used to identify the proteins that bind to aggregated ReLPS eluting in the HPLC peak 1 of Fig. 6. From SDS-PAGE, it is evident that only LBP was detected as being bound to aggregated ReLPS. However, this method of analysis does not detect transient or unstable binding components. All of the visible sCD14 and the remainder of the LBP eluted in peak 2, as expected for the behavior of soluble proteins in this system. Thus, sCD14 apparently did not bind to the ReLPS aggregates or did not participate in the formation of a stable ternary complex (7, 24) of ReLPS-LBP-sCD14, because this complex would have migrated on HPLC either with peak 1 or before peak 2. However, our data support the binary model (23) for LPS-LBP binding. The intriguing implications of the binary mechanism will affect future studies of the roles of LPS and mCD14 in macrophage activation.

In the in vitro experiments presented here, it can be observed that RsDPLA bound to sCD14 and inhibited binding by ReLPS. There is, however, a body of evidence (4, 5) that indicates that mCD14 may be incapable of transducing an LPS-binding signal into the cell. Those with this viewpoint posit a signal transducer molecule that associates with mCD14. Until such a molecule is identified, we believe that CD14 is the most likely LPS cell surface binding site. Indeed, our size-exclusion HPLC technique may help answer this larger question of signal transduction.

ACKNOWLEDGMENTS

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