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Appropriate Coating Methods and Other Conditions for Enzyme-Linked Immunosorbent Assay of Smooth, Rough, and Neutral Lipopolysaccharides of *Pseudomonas aeruginosa*

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Smooth, rough, and neutral forms of lipopolysaccharide (LPS) from *Pseudomonas aeruginosa* were used to assess the appropriate conditions for effective enzyme-linked immunosorbent assay (ELISA) of LPS. Each of these forms of well-defined LPS was tested for the efficiency of antigen coating by various methods as well as to identify an appropriate type of microtiter plate to use. For smooth LPS, the standard carbonate-bicarbonate buffer method was as efficient as the other sensitivity-enhancing plate-coating methods compared. The rough LPS, which has an overall hydrophobic characteristic, was shown to adhere effectively, regardless of the coating method used, to only one type of microtiter plate, CovaLink. This type of plate has secondary amine groups attached on its polystyrene surface by carbon chain spacers, which likely favors hydrophobic interactions between the rough LPS and the well surfaces. Dehydration methods were effective for coating microtiter plates with the neutral LPS examined, which is composed predominantly of a D-rhamnan. For the two dehydration procedures, LPS suspended in water or the organic solvent chloroform-ethanol was added directly to the wells, and the solvent was allowed to dehydrate or evaporate overnight. Precoating of plates with either polymyxin or poly-L-lysine did not give any major improvement in coating with the various forms of LPS. The possibility of using proteinase K- and sodium dodecyl sulfate-treated LPS preparations for ELISAs was also investigated. Smooth LPS prepared by this method was as effective in ELISA as LPS prepared by the hot water-phenol method, while the rough and neutral LPSs prepared this way were not satisfactory for ELISA.

Enzyme-linked immunosorbent assay (ELISA) has been well exploited for the detection of pathogens and contaminants in the medicine and food industries. ELISA techniques allow many samples to be tested at low cost. A useful antigen for detecting the presence of gram-negative bacterial pathogens is endotoxin or lipopolysaccharide (LPS) (17). However, the structural and physical properties of LPS are highly heterogeneous. As a result, ELISAs involving LPS can be problematic because of poor reproducibility and a need for large amounts of antigen to sensitize microtiter plates for the assay (33).

The basis for the heterogeneity could be the capping efficiency of O antigen repeats, which result in variable chain lengths (or sizes) of LPS molecules (3). Heterogeneity in LPS sizes has been observed in *Pseudomonas aeruginosa* (8) and in members of the family *Enterobacteriaceae* (21, 31). Another feature of the heterogeneity is the unique chemical nature of the sugars present in the O antigenic repeats, which are used to separate particular strains of species into serotypes (22). Among the organisms that are known to have short-chain LPSs, namely, lipooligosaccharides (20), for example, *Bordetella pertussis* and *Neisseria meningitidis* (7, 15, 16), chemical differences among the sugars in the distal portions of the polysaccharide also contribute to heterogeneity and serospecificity. These forms of heterogeneity in turn influence the net charge, the distribution of charged domains, and the dominance of hydrophilic or hydrophobic regions in the LPS molecules (35).

In this study, we addressed the question of whether the chain length and physical properties of LPS and the method used to coat microtiter wells with antigen have any influence on the efficiency of ELISA of LPS. The LPS antigens chosen for the study were prepared from wild-type and mutant strains of *P. aeruginosa* to yield well-defined antigens that ranged from smooth LPS, composed of long, amino sugar-rich O antigenic polymers (9), to rough LPS, which is devoid of the O antigen (24), as well as the neutral A band common-antigen LPS, which is composed predominantly of α-linked D-rhamnan (2). In various ELISA studies, the focus has been placed on examining a particular coating method, and the influence of the heterogeneous chemical nature of LPS was not addressed (30, 37). Thus, it was necessary to determine the suitability of various antigen-coating methods for each of the LPS antigens. The coating methods that we assessed include the use of the routine carbonate-bicarbonate buffer (10), the use of polymyxin B as a specific absorbent for LPS (17, 30), direct dehydration of LPS onto microtiter wells, and evaporation of the solvent from LPS that was suspended in a chloroform-ethanol solution (12). The proteinase K-sodium dodecyl sulfate (SDS) treatment described by Hitchcock and Brown (19) has been well exploited as a fast method for preparing LPS from a large number of gram-negative isolates. We will also assess the effectiveness of LPS prepared by this method in ELISAs.

MATERIALS AND METHODS

Chemicals. All chemicals, unless otherwise stated, were purchased from Fisher Scientific (Pittsburgh, Pa.).

Bacterial strains and isolation of LPSs. *P. aeruginosa* PAO1 (serotype O5) was used for the isolation of smooth LPS (B-band LPS) (18) by the standard hot phenol-water extraction

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method (38). *P. aeruginosa* AK1401 was the source for neutral A-band LPS (4), and the A-band LPS was also prepared by the phenol-water extraction method (23). Rough LPS was isolated from strain 21-1 (9), a core-deficient derivative of strain PAO1, which was isolated by infecting the parent strain with LPS-specific phage E79 (29). The method used for the preparation of rough LPS was the petroleum ether-chloroform-phenol extraction procedure described by Galanos et al. (13). For each form of the coating antigen, 10-mg/ml (wt/vol) stock solutions were made with pyrogen-free water obtained from a MilliQ Millipak water filter (Millipore, Mississauga, Canada). These stock solutions were stored at −20°C in small aliquots until required.

All three forms of LPS antigens were also prepared by proteinase K-SDS treatment described by Hitchcock and Brown (19) with some modifications. For this procedure, the amount of cells was standardized as follows. An overnight culture of the bacteria was grown in tryptic soy broth (TSB) and diluted with sterile TSB to give an optical density at 600 nm (OD_{600}) of 1.00. An aliquot of 1.5 ml of this cell suspension was then centrifuged at room temperature for 2 min at 7,000 × *g*. The pellet was resuspended in 80 μl of lysis buffer (2% SDS, 4% 2-mercaptoethanol, 10% glycerol, 1 M Tris-HCl [pH 6.8], 0.05% [wt/vol] bromphenol blue) and steamed for 30 min. The mixture was allowed to cool to room temperature before 10 μl of a 10-mg/ml solution of proteinase K was added. The preparation was completed by incubating the mixture at 60°C for 3 h. After insoluble cell debris was sedimented (5 min, 14,000 × *g*), the supernatant was stored at −20°C until use.

ELISA plates. The ELISA plates used were Immulon 2 U-bottomed plates (Dynatech), Nunc Polysorp U plates, Nunc Polysorp C plates, and Nunc CovaLink plates. The properties of these plates, according to technical literature from the manufacturers, are as follows. Among the microtiter plates that we examined, Immulon 2 was shown to have a high affinity for proteins and may also bind peptides and DNA. The Nunc Polysorp U plate was designed to bind antigens of a more polar nature (11). The Nunc Polysorp C plate has the same formulation as the Nunc Polysorp U plate except for the shape of the well. The C plate is designed to give the washing advantages of a U-bottomed plate along with the better reading properties of a flat-bottomed plate. The CovaLink plate contains secondary amine groups attached to its surface via carbon chain spacers. The plates can be coated with antigens by interaction with the secondary amines, or the antigens can be covalently linked to them via various chemical reactions (11).

Antigen-coating procedures. (i) Carbonate method. Appropriate amounts of purified and lyophilized LPS were diluted in carbonate coating buffer (0.15% [wt/vol] Na_2CO_3, 0.1% [wt/vol] $MgCl_2 \cdot 6H_2O$, 0.3% [wt/vol] $NaHCO_3$ [pH 9.8]) to a concentration of 5 μg/ml. Proteinase K-SDS-treated LPS preparations were diluted to 10 μl/ml (1:100 dilution) with carbonate buffer. An aliquot of 100 μl was added to each ELISA well. The plates were then incubated at 4°C overnight.

(ii) Polymyxin-carbonate enhancement. The procedure used for enhancement was that described by Hancock and Poxton (17). Briefly, a mixture containing 1 mg of polymyxin B sulfate (Sigma, St. Louis, Mo.) and 10 mg of LPS per ml was prepared in pyrogen-free water and stirred at room temperature for 30 min. The mixture was then dialyzed with a molecular weight cutoff of 3500 (SpectraPor tubing; Spectrum, Houston, Tex.) overnight against double-distilled water. A 1:16 dilution (625 ng of LPS per ml) was made in carbonate buffer. Of this antigen solution, 100 μl was added per well, and the ELISA plates were incubated at 4°C overnight.

(iii) Precoating with polymyxin. The precoating procedure was performed by the method of Mertsola et al. (30). LPS antigen was solubilized in carbonate buffer as described above to a concentration of 5 μg/ml. Aliquots (100 μl) of the antigens were added to the ELISA wells and allowed to incubate for 2 h at 37°C.

(iv) Direct dehydration method. An antigen concentration of 5 μg of the pure antigen or 10 μl of the proteinase K-SDS-treated LPS preparation per ml was made in double-distilled water. An aliquot of 100 μl was applied to each well, and the samples were allowed to dehydrate completely by incubation at room temperature for 2 days.

(v) Chloroform-ethanol evaporation method. The evaporation procedure was performed as described by Freudenberg et al. (12). LPS was diluted to 5 μg/ml in chloroform-ethanol (1:10, vol/vol). For the proteinase K-SDS-treated LPS preparations, 1:100 dilution in chloroform-ethanol was used. An aliquot of 100 μl of LPS in organic solvent was added per well and allowed to evaporate to dryness by incubation at room temperature for 24 h.

(vi) Precoating with poly-L-lysine. The procedure for the precoating was described by Takahashi et al. (37) and was followed by the addition of aliquots of the LPS antigens suspended in phosphate-buffered saline (PBS; 0.8% NaCl, 0.02% KH_2PO_4, 0.29% Na_2HPO_4, 0.05% KCl [pH 7.4]) at 5 μg/ml. Aliquots (100 μl per well) were incubated on the plate for 2 h at 37°C.

ELISA procedure. For performing the ELISAs, we followed the protocol described by Mutharia and Hancock (33) with modifications. Murine monoclonal antibodies (MAbs) specific for the O antigens of O5, A-band, and rough LPS have been described previously (9, 26, 27); these antibodies are MAb MF15-4 (immunoglobulin M [IgM]) (26), MAb N1F10 (IgM) (27), and MAb 177 (IgM) (9), respectively. All MAbs were produced as culture supernatants. Standard curves of the antibody dilutions were plotted against constant amounts of antigen in order to determine the antibody ranges required for the comparative study. Antibody dilutions were made in PBS with 1% skim milk.

Smooth LPS was tested with MAb MF15-4 (the dilutions used were 1:200, 1:400, 1:800, 1:1,600, 1:2,000, and 1:4,000). A-band LPS from strain AK1401 was tested with MAb N1F10 tissue culture supernatant (the dilutions used were 1:1, 1:5, 1:10, 1:20, 1:40, and 1:80), and rough LPS from *P. aeruginosa* 21-1 was tested with MAb 177 (anti-lipid A) tissue culture supernatant (the dilutions used were 1:5, 1:25, 1:50, 1:100, 1:200, and 1:400). For the proteinase K-SDS-treated LPS preparations, antigen solution was flicked out of the wells after the incubation time and replaced for 30 min by blocking solution (250 μl of PBS-Tween with 3% skim milk powder [Difco, Detroit, Mich.] per well) in order to dilute the effect of the SDS in the LPS sample. Then plates were washed seven times with PBS–0.1% Tween 20 (Sigma) (PBS-Tween) with a plate washer (EL403; BIO-TEK Instruments, Mandel Scientific Supplies, Guelph, Canada). Free binding sites were blocked with 250 μl of PBS-Tween with 3% skim milk powder per well for 60 min at 37°C. After blocking, the plate was washed again as described before. Then, 100 μl of primary antibody was added per well, and the plates were incubated at 37°C for 2 h. After being washed seven times with PBS-Tween, the plates were incubated with 100 μl of secondary antibody [1:5,000 dilution of goat anti-mouse $F(ab')_2$ conjugated to alkaline phosphatase (Jackson Immunoscientific, Richmond, Va.)] per well at 37°C for 1 h and washed as described before. This enzyme-conjugated second antibody reacts equally well with light chains and Fab fragments of both IgG and IgM, as

TABLE 1. Effectiveness of coating method in ELISAs with purified smooth, neutral A-band, and rough LPS antigens of *P. aeruginosa*

Coating method	ELISA reaction[a] with LPS:		
	Smooth	Neutral	Rough
Carbonate-bicarbonate	++	++	++
Polymyxin enhancement	−	−	++
Direct dehydration	++[b]	−	++
Chloroform-ethanol	+	++	++
Polymyxin precoating	+	++	−
Poly-L-lysine precoating	++	++	−

[a] ++, acceptable at an OD_{405} of > 0.3; +, weak at an OD_{405} of 0.3 to 0.1; −, not acceptable at an OD_{405} of < 0.1. The signals are taken from the given range of antibody dilutions used in this study. The ratings apply to all types of microtiter plates unless stated otherwise.

[b] Acceptable signal observed only when the CovaLink plate was used.

described previously (25, 26). The substrate added to the wells at 100 μl per well was *p*-nitrophenyl phosphate (1 mg/ml) (Sigma) in diethanolamine buffer (9.6% [vol/vol] diethanolamine, 0.01% [wt/vol] $MgCl_2 \cdot 6H_2O$ [pH 9.8]). After incubation for 1 h at 37°C, the OD_{405} was determined with a Titertek Multiskan (Flow Laboratories, Mississauga, Canada). Wells without antigen and wells without specific antibody were used as negative controls. All experiments were performed in triplicate. The averages for the triplicates were plotted as bar graphs. Standard deviation values were calculated for each triplicate.

RESULTS

In all the ELISAs performed in this study, each form of LPS was used to coat microtiter plates by different methods. The coated plates were then tested for dose-dependent reactions against a dilution series of the suitable MAb. The use of carbonate buffer or poly-L-lysine appeared to be equally effective for smooth LPS from strain PAO1, and strong signals were observed with MAb MF15-4 (Table 1). The efficiency of antigen coating by the chloroform-ethanol evaporation method was lower. Neither of the coating methods utilizing polymyxin enhancement (those of Hancock and Poxton [17] and Mertsola et al. [30]) nor the direct dehydration method gave a sufficient signal on all plates (Fig. 1a). ELISA reaction signals with the polymyxin enhancement method were comparable to those with the carbonate coating method when the LPS antigen concentration was raised from 0.625 to 5 μg/ml (data not shown). For smooth LPS, the direct dehydration method was effective only when CovaLink plates were used. The highest levels of sensitivity were observed on Immulon 2 plates coated by either the carbonate buffer or chloroform-ethanol method and on CovaLink plates coated by the direct dehydration method (Fig. 1a).

When the neutral A-band LPS was tested in ELISAs, effective antigen coating was obtained with carbonate buffer, chloroform-ethanol evaporation, and precoating with polymyxin or poly-L-lysine (Table 1). The polymyxin enhancement and the direct dehydration methods were inefficient for the A-band LPS. When different microtiter plates were tested for ELISA of A-band LPS, strong signals were observed on Polysorp C and U plates when the carbonate buffer coating method was used. Coating by the chloroform-ethanol method appeared to work equally well for Immulon 2 plates, Polysorp C plates, and CovaLink plates and somewhat less effectively by the carbonate method on Polysorp C and U plates. CovaLink was only effective for the neutral A-band LPS when the chloroform-ethanol evaporation method was used (Fig. 1b). No discernible differences were observed between Polysorp C and Polysorp U plates, which have flat- and U-bottomed well shapes, respectively.

For effective coating of microtiter plates with rough LPS, it was immediately apparent that CovaLink plates gave the strongest signals regardless of the coating method used (Fig. 1c).

LPS prepared by the proteinase K-SDS method of Hitchcock and Brown (19) has become increasingly popular because of the speed with which LPS coating antigens can be prepared. Thus, it was of interest to determine whether such LPS preparations could be used in ELISAs. Strong reaction signals were observed when proteinase K-SDS preparations of smooth LPS were reacted with a 1:800 dilution of MAb MF15-4 in ELISAs. An OD_{405} of greater than 0.3 was still recorded at an antibody dilution of 1:2,000 (Fig. 2a). All microtiter plates used appeared to be effective. Direct dehydration and evaporation with chloroform-ethanol were superior to the carbonate coating method with these LPS preparations (Fig. 2a), with positive OD signals even at the lowest antibody dilution (1:4,000) used. The effects of proteinase K-SDS treatment on the preparation of the three forms of LPS antigens for ELISA are summarized in Table 2.

No discernible binding of the proteinase K-SDS-treated LPS preparations was observed when the polymyxin coating method was used. There was no binding on Immulon 2, Polysorp U, or Polysorp C plates coated with carbonate buffer or with polymyxin enhancement (data not shown). When the neutral A-band LPS of strain AK1401 was prepared by proteinase K-SDS treatment, the only coating procedure that worked was the direct dehydration method (Fig. 2b). Attempts to coat the plates with rough LPS from strain 21-1 prepared by the proteinase K-SDS method were unsuccessful by any method tested in this study.

DISCUSSION

Effective ways to coat microtiter plates with antigens are essential for ELISAs, and various ways of attaching the antigen to the solid support have been reported. The majority of the studies dealt with protein antigens (5, 28, 32). For a number of studies that dealt with LPS, very little effort was made to distinguish between charged and neutral LPS forms. In this study, an attempt was made to compare different coating methods for LPSs of different chemical natures.

In order to elucidate the influence of different coating methods and plate types on the ability to detect LPSs in ELISAs, microtiter plates used for comparisons were coated with constant amounts of LPS antigens. A series of dilutions of each of the MAbs were used to determine the titer. These values allowed evaluation of the efficiency of plate coating for a given smooth, rough, or neutral form of LPS antigen.

We compared six methods for coating microtiter plates with different LPSs, namely, smooth LPS, rough LPS, and neutral A-band LPS. The first method, the use of carbonate buffer for antigen coating, is known to be very effective in coating polystyrene or polyvinylchloride plates with proteins. The high pH, 9.8, of the carbonate buffer might favor the ionic interaction of the anionic moieties of the LPS, including uronic acid residues in the O antigen of smooth LPS of serotype O5 and the 2-keto-3-deoxyoctonic acid residues as well as phosphate groups in the core. Indeed, this method was shown to be effective for the smooth form of LPS (Fig. 1a). This result is consistent with previously published data (33).

The second coating method, polymyxin enhancement, made

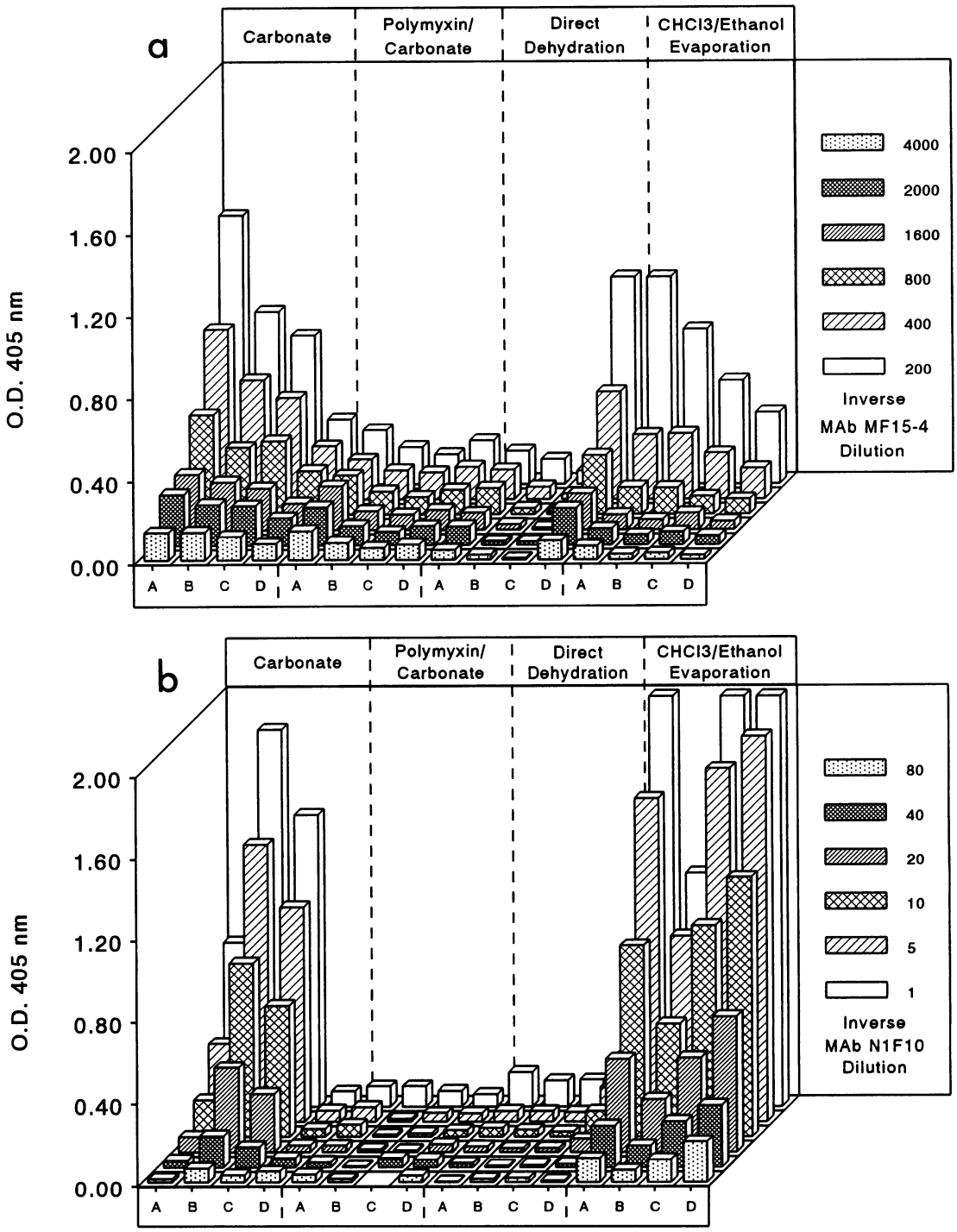

FIG. 1. Effect of coating methods on ELISAs of smooth (a), neutral A-band (b), and rough (c) LPS of *P. aeruginosa*. ELISA plate types: A, Immulon 2; B, Polysorp U; C, Polysorp C; D, CovaLink. The interaction of LPS antigen with specific MAbs is shown for four different coating methods. The MAbs used were MF15-4 (a), N1F10 (b), and 177 (c).

use of the natural affinity of the cationic polymyxin for LPS, which has been shown to be highly anionic. Polymyxin binds tightly to LPS, resembling the binding of polyamines and Ca^{2+} or Mg^{2+} to the LPS core (34, 35). Complex formation between LPS and polymyxin was shown by Hancock and Poxton to enhance the sensitivity of ELISA of LPS (17). We did not find any enhancement of the signal in this assay compared with that obtained by other methods; indeed, the signals were lower than with all other coating methods used. As described in Results, the polymyxin enhancement method gave reasonable ELISA reaction signals only when higher LPS antigen concentrations were used. It was obvious that the advantage of using polymyxin suggested by others diminished for the smooth LPS examined. Another principle used in this study was the dehyra-

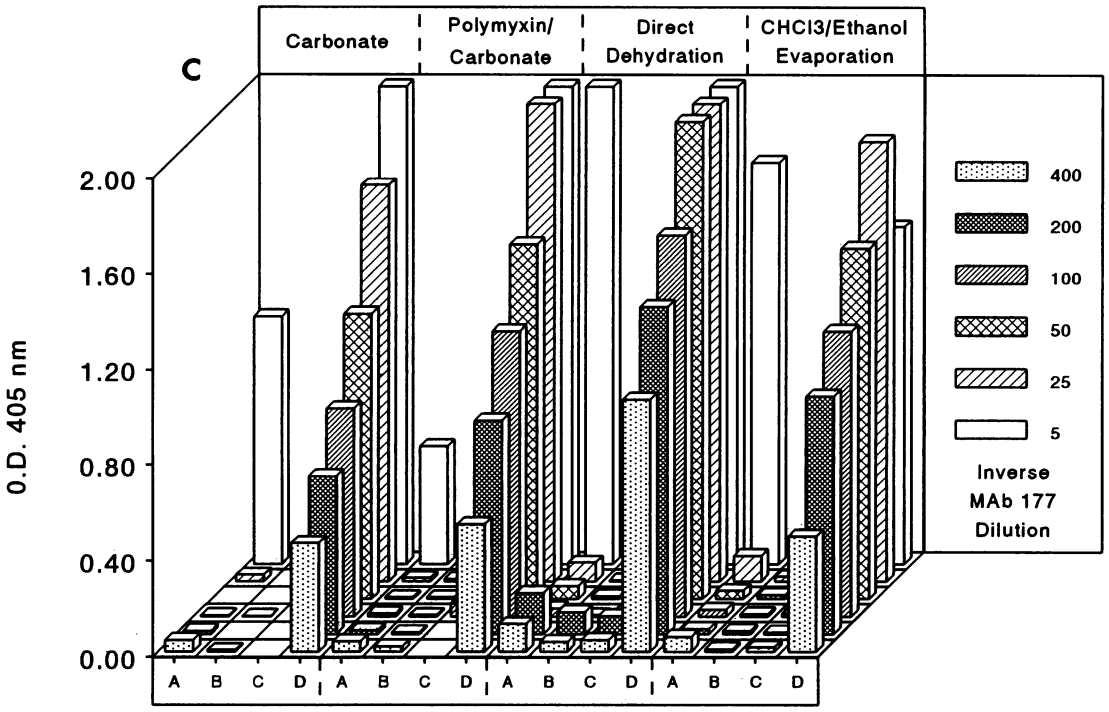

FIG. 1—*Continued.*

tion of LPS antigens onto the plates. This method may favor unspecified binding by hydrophilic moieties to the microtiter plate. The removal of water from the hydration shell surrounding the antigens and the concentration effect conferred upon the antigen by the drying process should lead to tight contact of an antigen with the plate surface (1, 6).

The dehydration effect was the basis of the fourth method described, drying of the antigen from a mixture of the organic solvents chloroform and ethanol onto a plate (12). This should favor the hydrophobic properties of the antigen. The chloroform would have a solvent effect on the polystyrene surface of the microtiter plates. Exposure of the plates to the organic solvent likely makes the surface of the wells rougher, creating a larger surface to facilitate the binding of hydrophobic regions of the LPS antigen substances in question.

Two other methods that exploited the anionic properties of the core of LPSs were described by Mertsola et al. (30) and by Takahashi et al. (37). In their studies, microtiter plates were precoated with either polymyxin or poly-L-lysine to create a positively charged surface for subsequent anchoring of the negatively charged portions of the LPS molecules. With these two methods, strong ELISA reactions signals were observed for the neutral A-band LPS but not for the rough LPS. For the smooth LPS, poly-L-lysine precoating gave results comparable to those with the carbonate-bicarbonate coating method (Table 1). The enhancement advantages of these precoating procedures were not apparent when the results observed were not better than those obtained by the use of the standard carbonate-bicarbonate antigen coating method.

The coating efficiency was influenced by the method and the plate material used. From our results, the type of microtiter plate used appeared to influence the efficiency of LPS coating. In this study, four different types of polystyrene plates with a mostly hydrophobic surface were used. Flexible polyvinyl chloride assay plates (Falcon 3911 MicroTest III), known to bind peptides very efficiently, were also examined (data not shown). Their use was discontinued because of the high frequency of strong background signals with LPSs as antigens.

Since smooth LPS is well endowed with both hydrophilic and hydrophobic domains, the coating of ELISA plates with purified smooth LPS from *P. aeruginosa* PAO1 can be achieved by using carbonate buffer, the chloroform-ethanol precipitation method, and poly-L-lysine- or polymyxin-coated plates. Interestingly, polymyxin enhancement and the direct dehydration method were found to be ineffective for the antigen concentrations used. Because of the reliability and the convenience of the standard carbonate buffer coating method, the use of this method with Immulon 2 plates is recommended for smooth LPS. We found that the other coating methods tested were also effective but did not demonstrate significant improvements over the carbonate coating method. However, for smooth and neutral LPS prepared by the proteinase K-SDS method, the direct dehydration method was shown to be superior to the carbonate method (Fig. 2a).

The A-band LPS of *P. aeruginosa* contains predominantly a polymer of D-rhamnose plus minor proportions of 3-*O*-methyl rhamnose, ribose, glucose, and mannose (2). No phosphate was found in this structure, but stoichiometric amounts of sulfate were found (36). The molecules are also different in size from the usual O side chain-containing (B-band) LPS. Estimates by SDS-polyacrylamide gel electrophoresis (PAGE) have shown that the average A-band LPS is shorter than the average smooth LPS (36). The A-band LPS is therefore a neutral polysaccharide. In this study, the most effective methods for coating plates with these neutral LPS molecules for ELISA were the direct dehydration and chloroform-ethanol evaporation methods. The Polysorp U microtiter plate appeared to be the plate of choice for this type of antigen.

Rough LPS contains lipid A with the complete core oligosaccharide residues. Coating of lipid A core antigens from strain 21-1 onto the Polysorp plates gave unacceptable results. Acceptable but weak OD signals were observed on Immulon 2

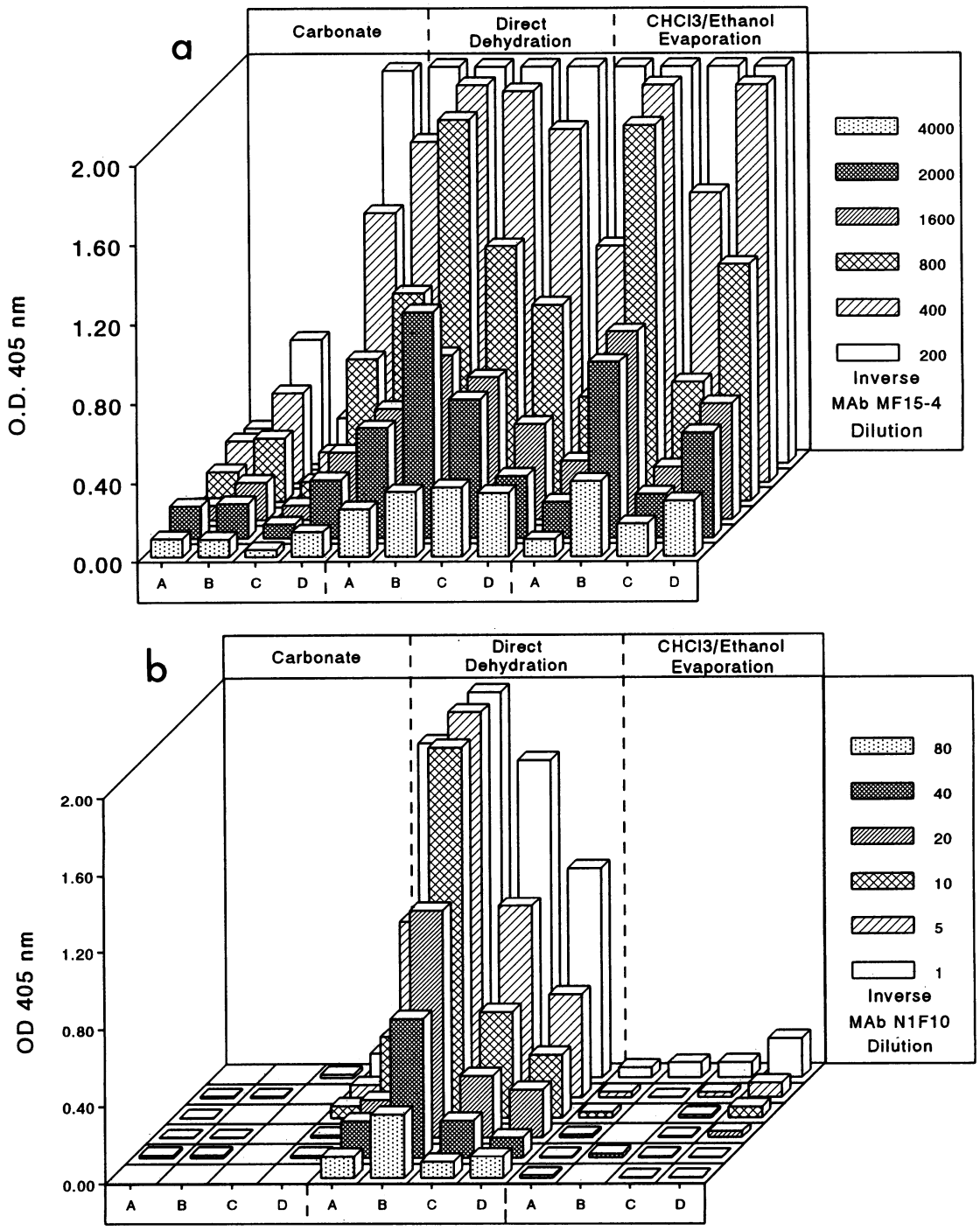

FIG. 2. Effect of coating method on ELISAs of smooth (a) and neutral A-band (b) LPS of *P. aeruginosa* prepared by proteinase K-SDS treatment. ELISA plate types: A, Immulon 2; B, Polysorp U; C, Polysorp C; D, CovaLink. The interaction of LPS antigen with MAb MF15-4 (a) or N1F10 (b) is shown for three different coating methods.

coated with rough LPS only when a high concentration of MAb 177 (1:5 dilution) was used (Fig. 1c). In contrast, the CovaLink plates with the grafted spacers appeared to be ideal for the rough LPS antigen. Strong ELISA signals were observed on CovaLink plates independent of the coating method used. Rough LPS contains proportionally higher amounts of lipid A, which could play a role in contributing to the hydrophobicity of the rough LPS molecules. From our results, hydrophobic interactions apparently accounted for the effectiveness of CovaLink plates for ELISA of rough LPS.

With proteinase K-SDS-treated preparations of rough LPS, no coating occurred on any of the plates with any of the methods at the antigen concentration employed (data not shown). This might be an effect of SDS in the preparations, to which hydrophobic substances were susceptible (14). Large and complex molecules with an abundant number of repeating

TABLE 2. Effectiveness of coating methods in ELISAs with proteinase K-SDS-treated smooth, neutral A-band, and rough LPS antigens of *P. aeruginosa*

Coating method	ELISA reaction[a] with LPS:		
	Smooth	Neutral	Rough
Carbonate-bicarbonate	+	−	−
Direct dehydration	++	++	−
Chloroform-ethanol	++	−	−

[a] See Table 1, footnote *a*.

epitopes (30 to 50 repeats), such as smooth LPS and A-band LPS, appeared to be less susceptible to the coating method than smaller molecules such as rough LPS.

In conclusion, because of the heterogeneous nature of LPS molecules, no one coating method for ELISA of LPS can be recommended. For successful coating of these antigens on polystyrene and modified derivative microtiter plates, one has to take into consideration the size, charge, and hydrophilic or hydrophobic properties of the LPS antigen before choosing the coating method and the type of ELISA plate. It was also shown that proteinase K-SDS-treated smooth LPS preparations commonly used for SDS-PAGE analysis can be used successfully in ELISAs. For smooth LPS, the coating of ELISA plates with carbonate buffer is reliable for all plate types used in this study. For neutral LPS, ELISA plates are best coated with the chloroform-ethanol evaporation method followed by the carbonate coating method. For rough LPS, it is obvious that only CovaLink microtiter plates can be recommended for use with any coating method.

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