Functional Immunoliposomes Harboring a Biosynthetically Lipid-Tagged Single-Chain Antibody†

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ABSTRACT: An anti-2-phenyloxazolone single-chain antibody was expressed in *Escherichia coli* as a lipoprotein fusion in order to generate a biosynthetically lipid-tagged molecule [Laukkanen et al. (1993) *Protein Eng. 6,* 449–454]. For purification, a hexahistidinyl tag was introduced to the C-terminus of the protein. The resulting antibody, termed Ox lpp-scFv-H6, was membrane-bound, displayed hapten-binding activity, and contained the lipoprotein-specific lipid modification as indicated by metabolic [^{3}H]palmitic acid labeling. The Ox lpp-scFv-H6 was purified by immobilized metal affinity chromatography followed by hapten-based affinity chromatography to essential homogeneity with a yield of 0.4–1.6 mg/L of culture. In detergent dialysis, the purified antibody partitioned quantitatively into phospholipid liposomes. The immunoliposome preparation consisting of a homogeneous population of unilamellar 100–200 nm vesicles displayed specific hapten-binding activity as measured by using ELISA and surface plasmon resonance (SPR)-based real-time biospecific interaction analysis. In SPR experiments, the immunoliposomes exhibited virtually irreversible binding to immobilized hapten compared to soluble antibody fragments, consistent with the predicted multivalent binding. Biosynthetic lipid-tagging of antibodies may prove useful for immunoliposome-based diagnostic and therapeutic applications.

Liposomes bearing antibody molecules on their surface have been shown to specifically bind to their appropriate molecular targets both *in vitro* (Gregoriadis & Neerunjun, 1975; Huang et al., 1980; Harsch et al., 1981; Martin et al., 1981) and *in vivo* (Hughes et al., 1989; Ahmad et al., 1993). This has aroused considerable interest into the use of immunoliposomes as vehicles for targeted drug delivery and for gene therapy (Heath et al., 1983; Wang & Huang, 1987; Maruyama et al., 1990). Moreover, immunoliposomes have been used in the design of novel formats for immunoassays (Kung et al., 1985; Plant et al., Pashkov et al., 1992) and as signal amplifiers in more conventional immunoassays (Ishimori & Rokugawa, 1993). Apart from these biotechnological applications, liposomes endowed with specific binding functions have been used as simplified model systems to study ligand–membrane receptor interactions (Egger et al., 1990; Lee et al., 1993).

Immobilization of antibody molecules to the surface of liposomes and planar lipid bilayers has been obtained through *in vitro* chemical conjugation to reactive lipid derivatives (Huang et al., 1980; Martin et al., 1981; Pinnaduwage & Huang, 1992; Lee et al., 1993). Because of their relative hydrophobicity, the fatty acylated antibody molecules associate stably with lipid membranes. Recent progress in the bacterial expression of functional antibodies as Fab fragments and as single-chain molecules (Hoogenboom et al., 1992; Plückthun, 1992; Skerra, 1993) prompted us to use genetic engineering to convert antibodies into membrane-bound molecules for immunoliposome applications. We expected that this approach would yield a defined product which can be effectively produced in bacteria and, at the same time, would obviate the tedious and potentially hazardous chemical treatments. We exploited the well-characterized machinery for the biosynthesis of *Escherichia coli* lipoproteins (Ichihara et al., 1981; Ghrayeb & Inouye, 1984; Choi et al., 1986; Gennity & Inouye, 1991) to design and produce a lipoprotein–single-chain antibody fusion protein (Laukkanen et al., 1993). We showed that this fusion antibody was fully active, was membrane-associated, and behaved like a membrane protein in detergent phase separation and in liposomal reconstitution experiments. In the present study, we describe the purification and reconstitution into liposomes of a biosynthetically lipid-tagged and polyhistidinylated anti-2-phenyloxazolone single-chain antibody and characterization of the immunoliposome–hapten interactions.

MATERIALS AND METHODS

Materials. Escherichia coli strain RV308 (su$^-$,Δ*lac*X74,*gal*-ISII::OP308,*str*A) was used as host for expression. Plasmids pKKtac and pML5 have been described earlier (Takkinen et al., 1991).

Egg yolk phosphatidylcholine, phosphatidylethanolamine, cholesterol, and *n*-octyl β-D-glucopyranoside were from Sigma. Triton X-100 was from Boehringer Mannheim. [9,10(N)-^{3}H]Palmitic acid (1.91 TBq/mmol) was purhased from Amersham. CNBr-activated Sepharose 4B and Chelating Sepharose Fast Flow were Pharmacia products. Lysozyme and DNA-modifying enzymes were products of Boehringer Mannheim and New England Biolabs. Sensor chips (CM5) and other reagents for BIAcore analysis were purhased from Pharmacia Biosensor AB.

Cloning of the Lipid-Tagged Antibody with a Hexahistidinyl Tail. Standard recombinant DNA techniques were used (Sambrook et al., 1989). The expression plasmid for the lipid-tagged antibody, pML3.7, contains the coding sequences for the signal peptide and nine N-terminal amino acid residues

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of lpp fused to the anti-2-phenyloxazolone single-chain Fv[1] fragment (Laukkanen et al., 1993). A hexahistidinyl tail was introduced into this construct by using polymerase chain reaction (Saiki et al., 1988). Primers were 5′-GCGCCGACATCATAACGGTTC-3′(5′-primer, sense), which is complementary to the sequence in the promoter region of pKKtac, and 5′-AAGATAAGCTTCTAATGATGGTGATGATGATGTTTCAGCTCCAGCTTGGTCCCAGCACC-3′(3′-primer, antisense, nucleotides coding for histidine residues are underlined). The oligonucleotides were synthesized in Applied Biosystems 391 DNA synthesizer. The correctness of the 3′-modification was confirmed by DNA sequencing (Sanger et al., 1977), and the expression construct was designated as pML3.7H.

Purification of Lipid-Tagged Antibody. Bacterial expression of Ox lpp-scFv-H6 was carried out as described earlier (Laukkanen et al., 1993). Briefly, *E. coli* RV308 cells harboring plasmid pML3.7H were grown in TB medium (Sambrook et al., 1989) with 100 μg/mL ampicillin. An overnight culture was diluted 1:50 and grown to an OD_{600} of 1.5, and then isopropyl β-D-thiogalactoside (IPTG) was added to a final concentration of 1 mM. The induction was continued overnight at 30 °C.

Cells harvested from 1-L overnight culture were suspended in 50 mL of lysis buffer (10 mM HEPES, pH 7.4, 1 mM EDTA, 0.5 M NaCl, 0.1 mM PMSF, and 0.1 mg/mL lysozyme). After a 15-min incubation at room temperature, the lysis of the cells was completed by a brief sonication using a probe-type sonicator. The cell envelopes were collected by ultracentrifucation (150000*g*, 1 h, 4 °C). The pellet was suspended in buffer A [10 mM HEPES, pH 7.4, 1 M NaCl, 10% (v/v) glycerol, and 0.1 mM PMSF] and recentrifuged as above. The pellet was resuspended in 15 mL of buffer A containing 1% (w/v) Triton X-100. The solubilization was carried out overnight at 4 °C with continuous rotation, and cleared by ultracentrifugation (150000*g*, 1 h, 4 °C).

For immobilized metal affinity chromatography (IMAC; Porath & Olin, 1983; Hochuli et al., 1988; Smith et al., 1988), the solubilized preparation was diluted 1:5 in buffer A. The final concentration of Triton X-100 was 0.2% (w/v) at this stage. The sample was incubated overnight at 4 °C in 50-mL total volume and constant mixing with 0.5–1 mL of Chelating Sepharose Fast Flow charged with Ni^{2+} as described previously (Porath & Olin, 1983). To decrease the binding of endogenous *E. coli* proteins, 1 mM imidazole was included in the buffer. The resin was loaded into a column and washed in a stepwise manner with 10 bed volumes each of buffer A containing 0.2% (w/v) Triton X-100 and 1, 50, 75, 100, and 250 mM imidazole.

For hapten-based affinity chromatography, BSA containing approximately 21 molecules of oxazolone, Ox_{21}BSA (Mäkelä et al., 1978), was coupled to CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's protocol. The pooled 75 and 100 mM imidazole fractions containing most of the Ox lpp-scFv-H6 were diluted 1:5 in buffer A containing 0.2% (w/v) Triton X-100 and incubated with Ox_{21}BSA resin in suspension as above. Washing and elution steps in hapten-based affinity chromatography were performed in a column as described previously (Takkinen et al., 1991; Harlow & Lane, 1988) except that 0.2% (w/v) Triton X-100 or 1% (w/v) *n*-octyl β-D-glucoside (OG) was present in the buffers.

Preparation of Liposomes. Pure egg yolk phospholipid/cholesterol mixture (10 mg, PC/PE/Cho, 10:1:5 molar ratio) was dissolved in 5 mL of 1% (w/v) OG with or without 40 μg of the purified Ox lpp-scFv-H6. The detergent was removed by dialyzing against 10 mM HEPES (pH 7.4) either overnight in cellulose dialysis bags (cutoff 12–14 kDa) with two buffer changes or in a LIPOSOMAT dialyzer (Dianorm, Munich, Germany) with cellulose membranes (cutoff 10 kDa). After detergent removal, the liposomes were collected by ultracentrifugation (150000*g*, 1 h, 4 °C) and suspended in 5 mL of 10 mM HEPES (pH 7.4).

Surface Plasmon Resonance Analysis of Liposomes. Binding properties of liposomes to the hapten were also characterized by using the BIAcore instrument (Pharmacia Biosensor AB, Uppsala, Sweden) employing the surface plasmon resonance (SPR) phenomenon (Löfås et al., 1991; Sjölander & Urbaniczky, 1991). The result of the measurement is converted into a sensorgram which shows the time development of the arbitrary resonance unit (RU) value which relates to the change in the incident angle of total internal reflection giving rise to SPR and is proportional to the mass interacting with the evanescent wave field in the dextran layer. A sensor chip (CM5) with a carboxymethylated dextran layer was used to immobilize Ox_{16}BSA and BSA. The immobilization was carried out according to the manufacturer's instructions using the Amine Coupling kit (Pharmacia Biosensor AB). Briefly, 35 μL of 75 μg/mL Ox_{16}BSA or 5 μg/mL BSA in 10 mM sodium acetate buffer (pH 4.0) was injected into carboxymethylated dextran activated with 35 μL of a 1:1 mixture of *N*-hydroxysuccinimide (0.1 M) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.4 M). Finally, the remaining activated carboxyl groups were blocked with injection of 35 μL of ethanolamine hydrochloride (1 M, pH 8.5). Noncovalently bound protein was removed by injecting 20 μL of 100 mM HCl. Immobilization levels of Ox_{16}BSA and BSA were approximately 2000 and 5000 resonance units (RU), respectively. The immobilization and the subsequent binding analyses were performed under a constant flow rate of 5 μL/min of 10 mM HEPES (pH 7.4), 3.4 mM EDTA, and 0.15 M NaCl. Liposome samples (30 μL of a 1:3 dilution unless otherwise stated) were injected onto the sensing surface followed by a buffer flow. Regeneration of sensor chips was performed with 50 μL of 0.5% (w/v) Triton X-100.

Electron Microscopy. The liposome samples were concentrated 10-fold by ultracentrifugation (150000*g*, 1 h, 4 °C) and resuspension in 10 mM HEPES, pH 7.4. A drop of liposomes was dried on a carbon-coated copper grid (150–200 mesh) and stained with 1% potassium phosphotungstate, pH 7.4. Negative-stained liposomes were observed with a Jeol JEM-100CX transmission electron microscope at 60 V.

Other Methods. Protein samples were analyzed by SDS–PAGE according to Laemmli (1970). Metabolic [^{3}H]palmitic acid labeling and immunoblotting using a polyclonal antiserum raised against the parental Ox scFv were otherwise carried out as described earlier (Laukkanen et al., 1993; Towbin et al., 1979). Hapten-binding activity measurements using ELISA were performed as described previously (Laukkanen et al., 1993). Protein content was determined by using a modified Amido Black method (Kaplan & Petersen, 1989).

RESULTS

Bacterial Expression of Lipid-Tagged Antibody with a Hexahistidinyl Tail. Our initial attempts in using hapten-

[1] Abbreviations: BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; Fab, antigen-binding fragment (Fd and L chains); Fv, variable region fragment; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethanesulfonyl fluoride; PC, phosphatidylcholine; PE, phosphatidylethanolamine; scFv, single-chain Fv; SDS, sodium dodecyl sulfate; TB, terrific broth.

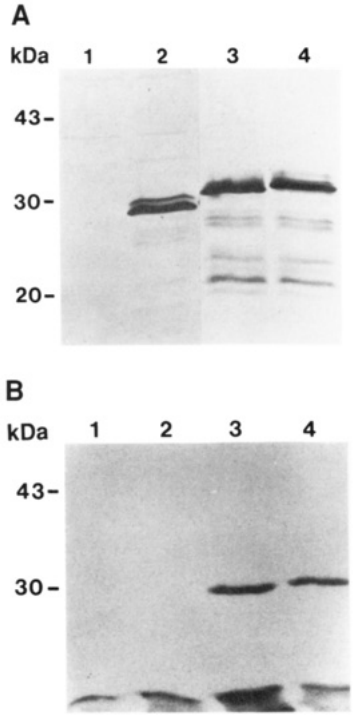

FIGURE 1: Bacterial expression of Ox lpp-scFv-H6. Whole-cell samples were analyzed by SDS–PAGE (15%) followed by immunoblotting using anti-Ox scFv antiserum (A) or by fluorography (B). *E. coli* RV308 cells harboring the plasmid pKKtac (vector control, *lanes 1*), pML5 (expression of the soluble Ox scFv, *lanes 2*), pML3.7 (expression of Ox lpp-scFv, *lanes 3*), pML3.7H (expression of Ox lpp-scFv-H6, *lanes 4*) were induced with IPTG for protein expression in the absence (A) or presence (B) of [^{3}H]palmitate.

based affinity chromatography to purify the lipid-tagged antibody, Ox lpp-scFv, from the crude detergent extract of bacterial membranes were unsuccessful. Therefore, we introduced a stretch of six histidinyl residues into the C-terminus of the antibody to serve as an affinity tag in immobilized metal affinity chromatography (IMAC). The resulting construct, pML3.7H, was expressed in *E. coli* RV308 as a 30-kDa species recognized by the antiserum raised against the purified soluble Ox scFv (Figure 1A). The slightly larger size of Ox lpp-scFv-H6 as compared to Ox lpp-scFv (28 kDa) which lacks the polyhistidinyl tag is consistent with the expected contribution of six weakly basic histidinyl residues to the electrophoretic mobility. The Ox lpp-scFv-H6 antibody was cell- and membrane-bound since very little if any immunoreactive Ox lpp-scFv-H6 was detected in the culture supernatant (data not shown). This is in good agreement with our earlier results showing the tight membrane association of Ox lpp-scFv mediated by the N-terminal lipid tag (Laukkanen et al., 1993). In metabolic labeling experiments, [^{3}H]-palmitate was incorporated into a 30-kDa species present in Ox lpp-scFv-H6-expressing cells but not in control cells expressing the soluble antibody (Figure 1B), indicating the biosynthetic incorporation of fatty acid into the lpp-fusion antibody.

Purification of Lipid-Tagged Antibody. The fusion protein was solubilized from the bacterial cell envelopes in 1% (w/v) Triton X-100. More than 95% of the solubilized Ox lpp-scFv-H6 was bound to Ni^{2+}-charged Chelating Sepharose as determined by ELISA (data not shown). The elution was performed in a stepwise manner with increasing imidazole concentrations. Most of the antibody eluted with buffers containing 75 and 100 mM imidazole. Silver-stained SDS–PAGE of the eluted material showed a major 30-kDa band and several minor bands of smaller size (Figure 2, *lane 4*).

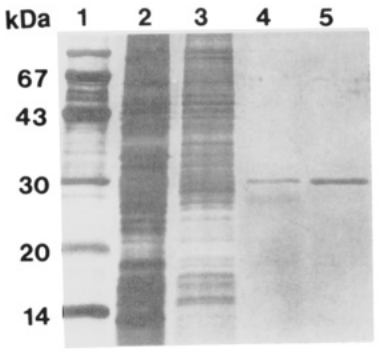

FIGURE 2: Purification of Ox lpp-scFv-H6. The silver-stained SDS–polyacrylamide gel (15%) shows the protein pattern at different stages of purification. Lanes: *1*, molecular mass markers; *2*, whole cell lysate; *3*, Triton X-100 extract; *4*, pooled 75–100 mM imidazole eluates from the Ni^{2+} chelating Sepharose column; *5*, low-pH eluate of the Ox_{21}BSA–Sepharose affinity column.

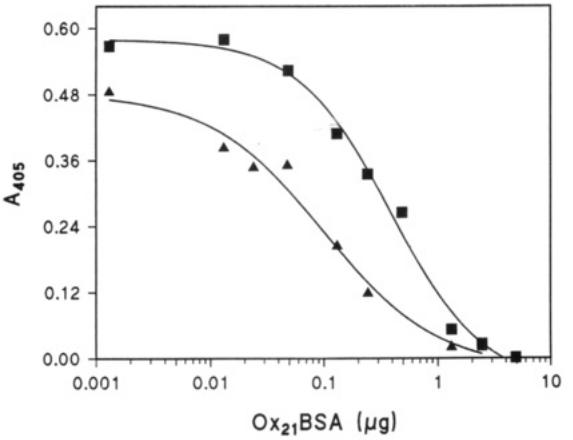

FIGURE 3: Hapten-binding activity of purified Ox lpp-scFv-H6. Purified Ox scFv (0.6 μM, ▲) and Ox lpp-scFv-H6 (1.1 μM, ●) and different amounts of soluble hapten (Ox_{21}BSA) were analyzed by ELISA as described earlier.

The lipid-tagged antibody was further purified by using hapten-based affinity chromatography in which Triton X-100 was exchanged to octyl glucoside for later reconstitution experiments. From 80% to 95% of the hapten-binding activity in the pooled 75–100 mM imidazole fractions was retained by an Ox_{21}BSA–Sepharose affinity column and subsequently eluted in low-pH buffer (100 mM glycine hydrochloride, pH 2) in the presence of OG. The purified antibody migrated as a single 30-kDa species in SDS–PAGE (Figure 2, *lane 5*). The recovery of the purified protein varied from 0.4 to 1.6 mg from a 1-L culture. The hapten-binding activity of the purified antibody was measured in ELISA, where the binding of Ox lpp-scFv-H6 and the parental Ox scFv to immobilized Ox_{21}-BSA was inhibited by soluble hapten at the same concentration range, indicating the absence of any major differences between these two antibody forms (Figure 3). The purified lipid-tagged antibody retained 66–75% of the hapten-binding activity after a 1-week incubation at room temperature or at 4 °C (Table 1).

Preparation of Immunoliposomes. The purified Ox lpp-scFv-H6 was reconstituted into phospholipid/cholesterol (PC/PE/Cho, 10:1:5 molar ratio) liposomes by removal of detergent in dialysis. The reconstitution mixture was then subjected to ultracentrifugation, and the resulting liposome pellet and supernatant were analyzed by immunoblotting (Figure 4A). No immunoreactivity (or hapten-binding activity) was detected in the supernatant (*lane 1*), whereas the 30-kDa Ox lpp-scFv-H6 band was quantitatively recovered in the liposome pellet

Table 1: Stability of Purified Lipid-Tagged Antibody and Immunoliposomes[a]

	A_{405}			
sample	control	4 °C	RT	37 °C
purified Ox lpp-scFv-H6	0.550	0.416	0.366	0.010
immunoliposomes	0.341	0.374	0.166	0.074

[a] Values are means of duplicate measurements. The hapten-binding activities were measured by using ELISA. Comparison of A_{405} values between a freshly made sample (control) and samples after a 1-week incubation in different temperatures is presented. No significant binding to BSA was observed.

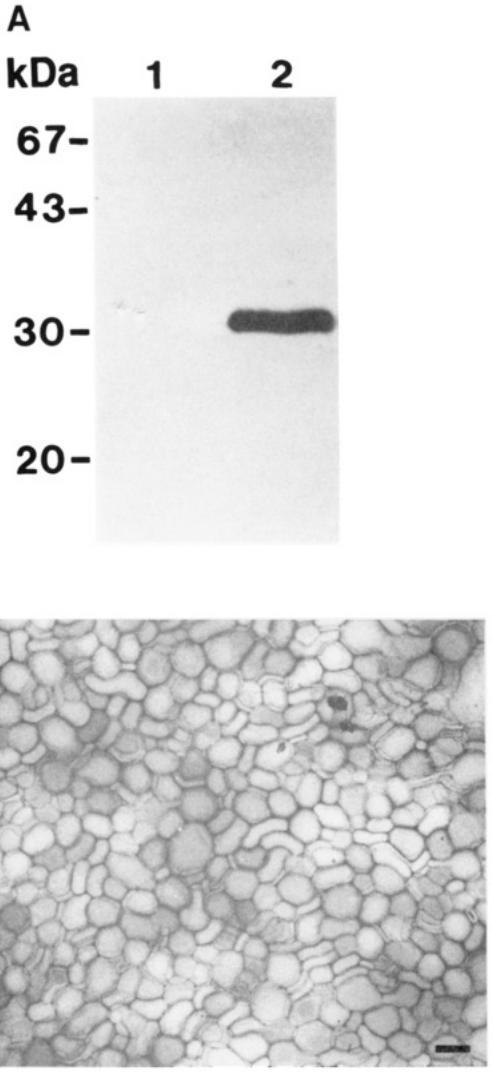

FIGURE 4: Reconstitution of Ox lpp-scFv-H6 into liposomes. (A) Immunoblot of the supernatant (lane 1) and liposome pellet (lane 2) of the ultracentrifuged reconstitution mixture. (B) Electron micrograph of the negative-stained liposome pellet (bar, 200 nm).

(*lane 2*), indicating efficient incorporation of the antibody into liposomes. In a negative-stained electron micrograph, the liposome preparation appeared as a relatively homogeneous population of vesicular structures with 100–200 nm diameter (Figure 4B). No signs of multilamellar structures were evident in these or in freeze–fracture (data not shown) EM pictures. The appearance of the immunoliposomes analyzed by EM is in keeping with the known unilamellarity and size range of liposomes prepared from octyl glucoside solutions (New, 1990). We calculated the average number of antibody molecules per liposome to be about 2000 by using the size and contents of protein and lipid in the immunoliposomes and by assuming full recovery. Half of the antibody molecules would be on the outer surface as the detergent dialysis method is expected to lead to a 50/50 distribution in terms of inward/outward orientation of the reconstituted protein. In recent experiments, we have reconstituted the lipid-tagged antibody to premade liposomes by diluting the antibody preparation in octyl glucoside directly into a large excess (10–20× volume) of liposomal suspension followed by overnight incubation and ultracentrifugation. Also, this approach leads to efficient incorporation of the antibody into the liposomes (data not shown).

Functional Characterization of Immunoliposomes. The hapten-binding activity of the immunoliposomes was analyzed by using BIAcore which monitors in real time small changes in the refractive index of the sensing layer caused by association/dissociation of macromolecules to a derivatized dextran matrix encased in a sensor chip (Löfås et al., 1991; Sjölander & Urbaniczky, 1991). Figure 5A shows a sensorgram of the binding of Ox lpp-scFv-H6-containing liposomes to Ox_{16}BSA during the 6-min injection of the liposome preparation (steps *1–3*). After the association phase (step *2*), the sample is replaced by buffer flow (steps *3–5*), and finally the sensor surface is regenerated with Triton X-100 (step *5*). In contrast to the binding of the immunoliposomes, the liposomes lacking the antibody did not bind to Ox_{16}BSA (Figure 5B). However, the immunoliposomes displayed low but significant binding to BSA (Figure 5B) which was not affected by the presence of soluble hapten (data not shown). The absence of any apparent dissociation of the immunoliposomes from Ox_{16}BSA contrasts the relatively rapid dissociation of soluble Ox scFv and is consistent with the multivalent nature of the hapten–antibody interaction in the immunoliposomes (Figure 5C). The binding of the immunoliposomes is mediated by the specific hapten–antibody interaction as it is displaced in a concentration-dependent manner by soluble hapten (Figure 6). By using BIAcore, the binding studies of purified Ox lpp-scFv-H6 to immobilized hapten in the presence of detergent (Triton X-100 and OG) have been unsuccessful, most likely due to optical interference of detergent micelles with the measurement.

The stability of the immunoliposomes was studied by incubating the immunoliposomes in HEPES buffer at different temperatures followed by ultracentrifugation and hapten-binding activity measurements in ELISA. No loss of hapten-binding activity was observed after an 8-day incubation at 4 °C whereas in samples incubated at room temperature and at 37 °C activity losses of 50% and 80%, respectively, were observed (Table 1). The partial loss of activity at room temperature and at 37 °C was not associated with the release of the lipid-tagged antibody from the liposomes or with major proteolytic degradation as evidenced by immunoblots of the supernatant and liposome pellets (data not shown).

DISCUSSION

The recent advances in bacterial expression of antibodies and in phage display technology now allow rapid isolation of desired antibodies and their large-scale production in *E. coli* as functional Fab and Fv fragments and as single-chain Fv fragments (Barbas, 1993; Skerra, 1993). The successful use of phage antibodies and bacterially expressed antibody fragments as immunological reagents has been demonstrated and is expected to largely replace the hybridoma-based monoclonal antibody technology (Nissim et al., 1994). By further engineering of antibody fragments, it is possible to introduce tags for easy detection and purification or to drive dimerization for higher avidity (Pack & Plückthun, 1992;

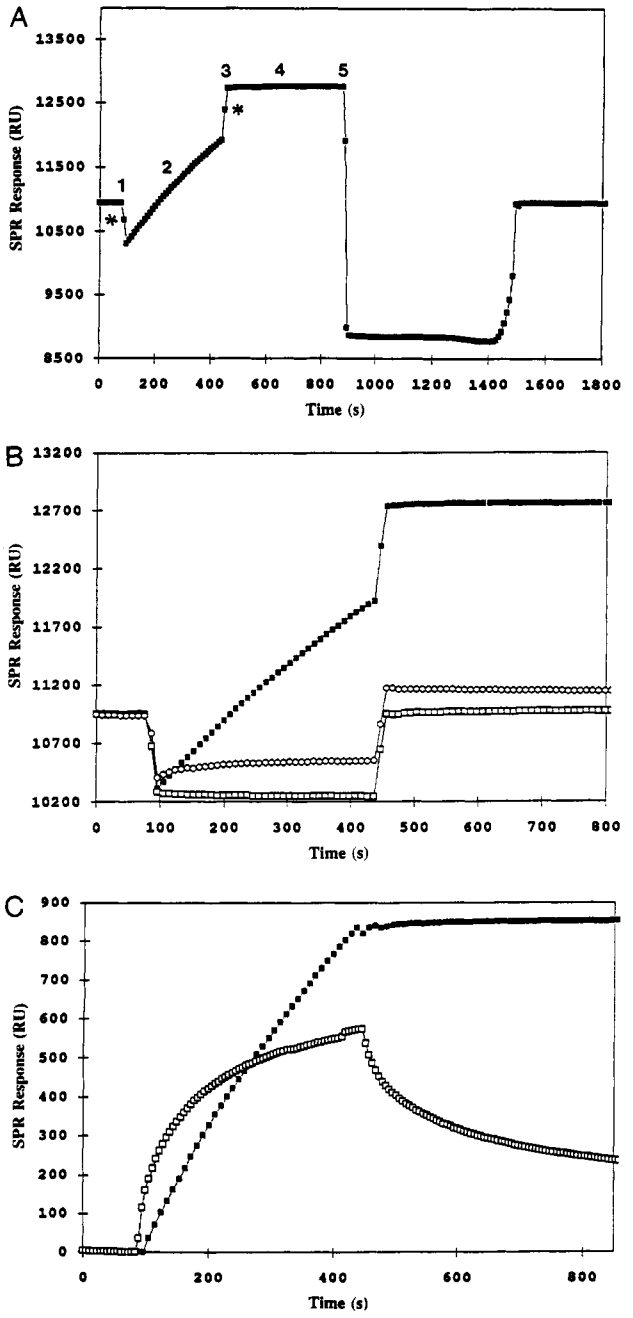

FIGURE 5: SPR analysis of immunoliposome binding. (A) A BIAcore sensorgram showing the injection of immunoliposomes (1) and the association phase (2), and after injection, the sample is replaced by buffer flow (3) and the apparent dissociation phase (4) followed by regeneration (5) of the sensor surface. The asterisk shows the bulk effect caused by the liposome suspension. (B) Binding of immunoliposomes (■) and liposomes without protein (□) to Ox_{16}BSA and binding of immunoliposomes (○) to BSA only. (C) A sensorgram of binding of 1:6 diluted immunoliposomes (■) and 600 nM Ox scFv (□) to Ox_{16}BSA. The data in panel C are corrected for the bulk effect.

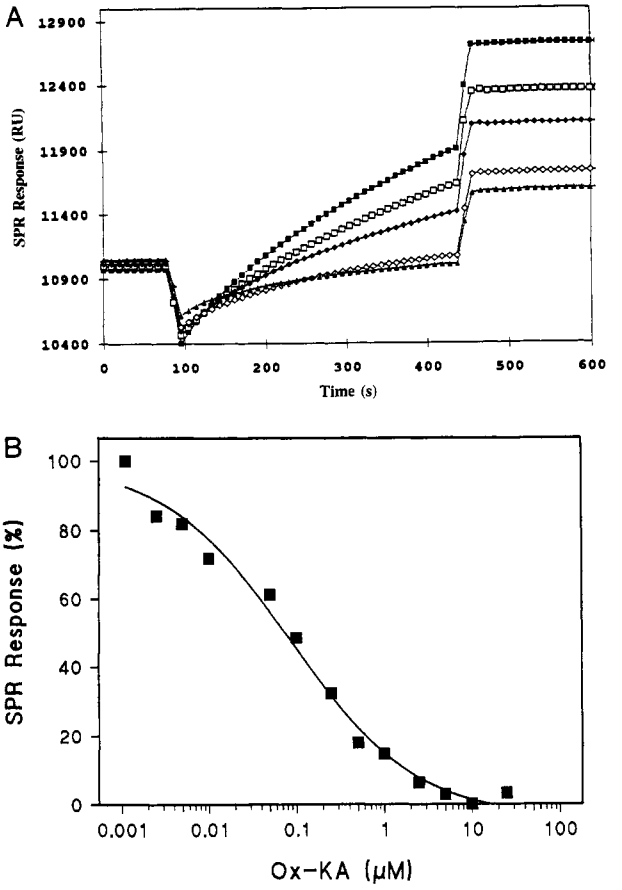

FIGURE 6: Specificity of immunoliposome binding in the SPR measurement. Immunoliposomes were injected in the presence of increasing concentrations of a soluble hapten, caproic acid conjugate of 2-phenyloxazolone (Ox-KA). (A) Overlay plot of sensorgrams obtained with immunoliposome samples containing 0.001 (■), 0.01 (□), 0.1 (♦), 1 (◇), and 10 μM (▲) Ox-KA. (B) Decrease of the specific SPR response as a function of Ox-KA concentration.

Skerra et al., 1991; Schmidt & Skerra, 1993). Furthermore, whole proteins or functional domains have been fused to bacterially expressed antibodies to generate hybrids with alkaline phosphatase (Wels et al., 1992; Ducancel et al., 1993), protein kinase (Ueda et al., 1992), protein A (Tai et al., 1990), or metallothionein (Das et al., 1992). These antibody fusion proteins may find use in immunoassays, immunotherapy, and medical imaging.

An obvious possibility to further increase the practical utility of bacterially expressed antibodies is to introduce tags to provide oriented immobilization to different matrices. We described earlier the bacterial expression of a single-chain antibody fused to the signal sequence and nine N-terminal amino acids of *E. coli* major lipoprotein (lpp) (Laukkanen et al., 1993). This design leads to the biosynthetic incorporation of a lipid moiety into the antibody and anchoring of the antibody stably to the bacterial membrane. The lipid-tagged antibody can be solubilized in detergents and transferred to proteoliposomes with retention of the hapten-binding activity.

The purification of the bacterially expressed and membrane-bound lpp–antibody fusion by hapten affinity chromatography proved difficult. This was probably due to the interference of the complex bacterial detergent extract with the purification scheme developed earlier for a soluble antibody fragment (Takkinen et al., 1991). Encouraged by earlier work (Skerra et al., 1991), we subsequently introduced a stretch of six histidines into the C-terminus of the lipid-tagged single-chain antibody to serve as an affinity tag in IMAC. The tag worked remarkably well, facilitating substantial purification of the Ox lpp-scFv-H6 from the detergent extract of bacterial membranes. The minor contaminants in the preparation were then removed by hapten-based affinity chromatography. Without any optimization, the recovery of purified antibody from 1 L of shake flask culture was about 1 mg. It is likely that substantially higher yields can be achieved by using high cell density fermentation (Pack et al., 1993).

The rationale of our work is to develop a tag for stable and oriented immobilization of purified antibodies to lipid mem-

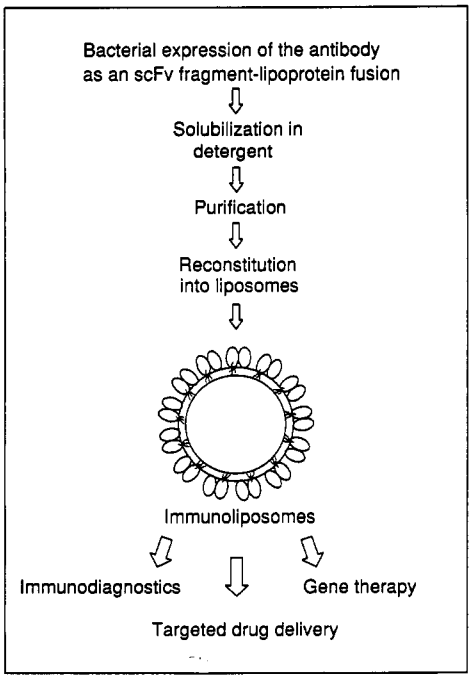

FIGURE 7: Biosynthetic lipid-tagging of antibodies. A schematic presentation showing the principal and major technical steps involved. See the text for more details.

branes. Here, the high potency of the biosynthetically generated lipid tag for immobilization was demonstrated by the very efficient incorporation of the purified lipid-tagged antibody into phospholipid liposomes by using detergent dialysis as judged by immunoblotting. Importantly, adsorption of the lipid-tagged antibody directly to premade liposomes was also possible. This method is more compatible with applications which involve encapsulation of water-soluble material with the liposomes, for which sonication and high-pressure extrusion methods are widely used (Olson et al., 1979; Hope et al., 1985). The immunoliposomes displayed specific hapten-binding activity in an SPR-based assay. The binding was inhibited by soluble hapten (caproic acid conjugate) in a concentration-dependent manner. This shows that the immunoliposomes harboring a biosynthetically lipid-tagged single-chain antibody are able to recognize and bind to the appropriate molecular target. Regarding the use of immunoliposomes as immunological reagents or as vehicles for drug delivery, the strength of the antibody–antigen interaction mediating the binding of liposomes to the antigen is important. Multivalent binding, obtained through the presence of many antibody molecules in the liposome, is expected to lead to higher binding avidity. The very slow dissociation that we observed in BIAcore experiments is in good agreement with the expected multivalent binding of immunoliposomes.

Presently, there is considerable interest in the use of liposomes as efficient and well-tolerated drug carriers for *in vivo* applications (Gregoriadis & Florence, 1993). Specific binding of immunoliposomes charged with drugs, DNA, or imaging agents may be exploited in the design of novel therapeutic and diagnostic approaches. Furthermore, use of immunoliposomes as specific reagents in various kinds of immunoassays has been described. The practical utility of bacterially produced lipid-tagged antibodies for any of the above-mentioned applications still remains to be demonstrated. However, we envision that the biosynthetic lipid-tagging approach as described herein [Figure 7; see also Keinänen and Laukkanen (1994)] may become an alternative to the presently used chemical conjugation methods for functional immobilization of antibodies to liposomes for targeted drug delivery and immunodiagnostic and other applications. In conclusion, the present study demonstrates the bacterial expression, purification, and functional reconstitution into liposomes of a lipoprotein–single-chain antibody fusion molecule, illustrating a novel method for stable and oriented immobilization of antibodies to lipid bilayers.

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