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Note

Development of a new method to characterize (SMBV™) antigen formulations using surface plasmon resonance technology

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Abstract

Supra Molecular Biovectors (SMBV™) are nanoparticles composed by a polysaccharidic core surrounded by a lipid bilayer. They are designed for drug delivery and vaccine and can be administrated by nasal route. The association rate and the stability of association between active principle (AP) or antigens (Ag) with SMBV™ can be evaluated using the plasmon resonance technology using a BIAcore X system and a HPA hydrophobic sensor chip. AP, Ag and/or adjuvant molecule solutions are injected over SMBV™ saturated HPA sensor chip surface. Using a very small quantity of material, this technique allows us to quickly have an overview of complex formulations using SMBV™. It is also the fastest screening technique to select the best SMBV™ for each Ag and the best formulation process.

Keywords: Supra Molecular Biovector™; Optical biosensor; Surface plasmon resonance

Supra Molecular Biovectors (SMBV™) are 60 nm cross-linked spherical cationic nanoparticles surrounded by a phospholipid/cholesterol bilayer developed to improve drug and antigen (Ag) delivery (De Miguel et al., 2001). The reticulated polysaccharide core (PSC) can be positively or negatively charged using quaternary ammonium functions and/or phosphate ones. This characteristic can be adapt according the active principle (AP) to associate or to optimize the bio-distribution. SMBV™ are designed to associate different types of active therapeutic products (proteins, peptides, nucleotides) and/or adjuvant molecules used in human and animal health care (Betbeder et al., 2000; Berton et al., 1997). These particles can be administered by inhalation (mucosal route administration) or injected (subcutaneous route) and designed for vaccine or drug delivery (El Mir et al., 2001; Von Hoegen, 2001). Formulations are realized by mixing of Ag with premade SMBV™ system. Kinetic of association between Ag and SMBV™ is fast and a complete equilibration is obtained after few minutes depending the Ag properties, associated Ag are inside, in the hy-

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drophilic core or stay in the lipid layer. Variable electrostatic charge (anionic or cationic) allows strong ionic interactions with Ag.

Because of the low quantity available and the cost of most of Ag, classical methods of separation (microfiltration, sucrose cushion) to evaluate the association of several compounds with these SMBV™ were not appropriate. An original method, using surface plasmon resonance (SPR) technology was chosen. BIAcore X is a system for real-time biomolecular interaction analysis (BIA) using SPR technology. Real-time BIA monitors the formation and dissociation of biomolecular complexes on a sensor surface as the interaction occurs. This is a non-invasive optical measuring technique which measures the mass concentration of biomolecules on the sensor surface (Rich and Myszka, 2000; Evans and MacKenzie, 1999; Malmqvist, 1999). All studies were conducted with a biosensor BIAcore X (BIAcore, Uppsala, Sweden) on a long-chain alkanethiol molecules that form a flat, quasi-cristalline hydrophobic layer HPA sensor chip (BR-1000-30, BIAcore). Daily running buffer was 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3.4 mM EDTA, 0.005% Surfactant P20 (HBS EP, BIAcore). Data were computer treated with the BIA evaluation 3.1 software (BIAcore).

To characterize interactions between Ag and SMBV™, after a first treatment with a saline-detergent solution (named 'cocktail' see next), we modify the HPA surface to create a monolayer of SMBV™ surface. 20 μl of SMBV™ (50 μg/ml) in 0.3 X saline phosphate buffer (PBS) are injected on the HPA surface under a 5 μl/min flow. After 4 min all the suspension is injected. 60 s later a wash procedure is done. The resonance unit (RU) signal is registered. Then, a second injection is conducted on a same way to insure the surface saturation and to avoid no specific interactions between Ag and the HPA alkanethiol surface. When SMBV™ are injected on the HPA sensor chip surface, the signal shown a progressive increase of RU intensity and a stabilization at about 1000 RU demonstrating the immobilization of SMBV™. A second similar injection of SMBV™ shown a limited increase of the signal to some RU. There is no more association observed with this second injection. The sensor chip surface is then considered as a saturated SMBV™ surface.

The SMBV™ association capability was realized observing the direct association of the free Ag in solution with SMBV™ immobilized on the sensor chip (Fig. 1). Ag in solution are carried over the immobilized SMBV™. If interaction occurs Ag are retained. The signal measured is proportional to the quantity of material retained on the sensor chip. Using increasing concentration of an Ag, it is possible to verify the capability of SMBV™ to associate this kind of Ag and to determine the most convenient Ag concentration to use for SPR analysis. As shown Fig. 2, the RU response is proportional to the Ag concentration injected on the sensor chip SMBV™ surface. The response is linear from 5 to 40 μg/ml concentration. Above it, the signal does not increase indicating the saturation concentration. For further studies a concentration equal or lower than 40 μg/ml have to be used. Because of the high level of response a lower concentration (20 μg/ml) was chosen.

To evaluate the association rate, formulations are injected over the SMBV™ immobilized on the sensor chip surface using the same conditions: 20 μl, 5 μl/min flow and a wash delay of 60 s (Fig. 3). Because of electrostatic repulsion, only the part of free Ag inside the formulation is able to associate the immobilized SMBV™. This result was demonstrate using a centrifugation on a sucrose cushion with a formulation. The sample fraction of free Ag is retained on the SMBV™ sensor chip surface, but the formulation one not (data non shown). The association rate is determined by comparison between the RU response obtained for free Ag and a formulation. A simple mathematics equation allows us to evaluate the associa-

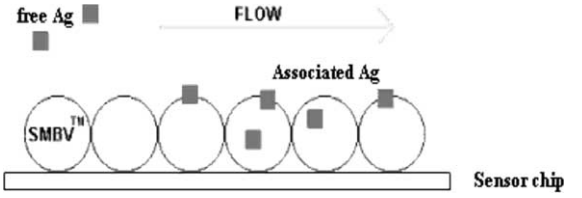

Fig. 1. Principle of Ag association with immobilized SMBV™.

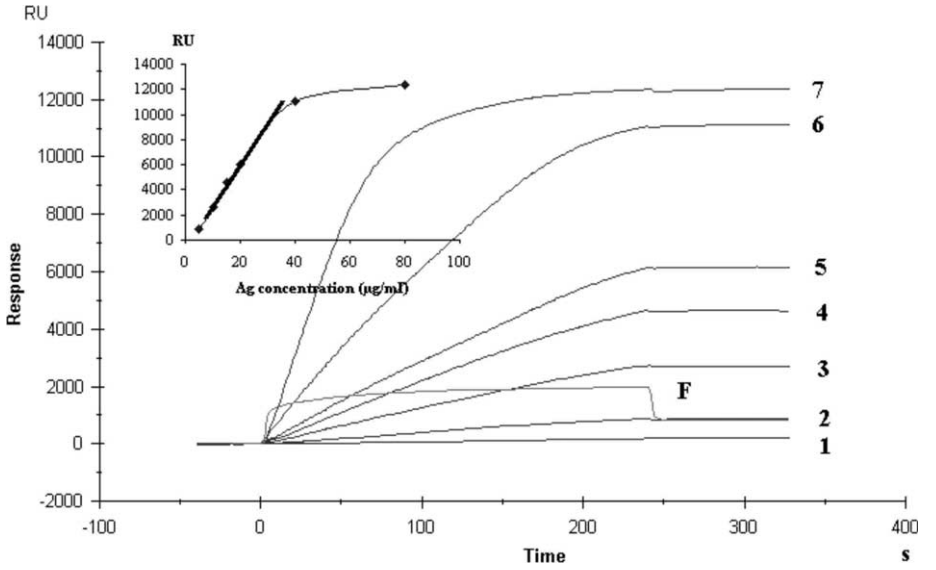

Fig. 2. Free Ag association with SMBV™ and formulation. Lines 1–7: increasing concentration of free Ag (HA) injected on immobilized SMBV™. 1 µg/ml; 5 µg/ml; 10 µg/ml; 15 µg/ml; 20 µg/ml; 40 µg/ml and 80 µg/ml. Between 5 and 40 µg/ml curve is linear (little graph over the sensorgramme). Line F: formulation Ag/BVSM™ with 20 µg Ag/ml.

tion rate: Association rate = 100 − [(formulation RU response/free Ag response) × 100]. The signal obtained for free Ag (control) and formulation at the same Ag concentration are directly compared. As shown Fig. 2, the control response at 20 µg/ml (lane 5) is higher than the formulation one (lane F): 6013 RU for free Ag and 798 RU for the formulation. The association rate level is 100 − (798/6013 × 100) = 86.7%.

When the Ag are associate it is strongly difficult to dissociate them from SMBV™ without alterate the SMBV™. It was necessary to completely regenerate the sensor chip surface to avoid any artefact signal. It means eliminate immobilized SMBV™ but respect the HPA surface to preserve its properties. The regeneration is realized using a preparation composed by 10 mM *n*-octyl-b-glucopyranoside (OGP, Sigma), 0.05% Empigen BB detergent 30% solution (Calbiochem) and 1 M NaCl (Fluka). This preparation named cocktail was injected on the sensor chip surface like samples at the end of each Ag/SMBV™ interaction (conditions of use: volume 10 µl; flow: 10 µl/min). The regeneration cocktail allows us to repeat the SMBV™ immobilization without any modification of the base signal and with a constant SMBV™ immobilization signal level. At least five different injections of increasing Ag concentration can be done successively and responses are directly proportional to the Ag concentration (Fig. 4). This result was confirmed by the possibility to do more than 40 complete analyses, immobilization, sample injection and regeneration procedure without any signal degradation (data no shown).

The originality of this method is to realize a preservative immobilization of the hydrophobic

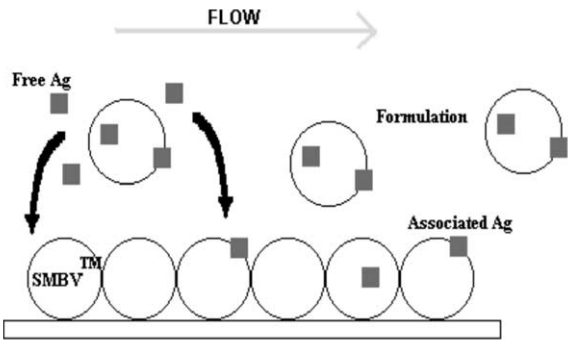

Fig. 3. Schematic view of the interactions between Ag/SMBV™ formulation and immobilized SMBV™.

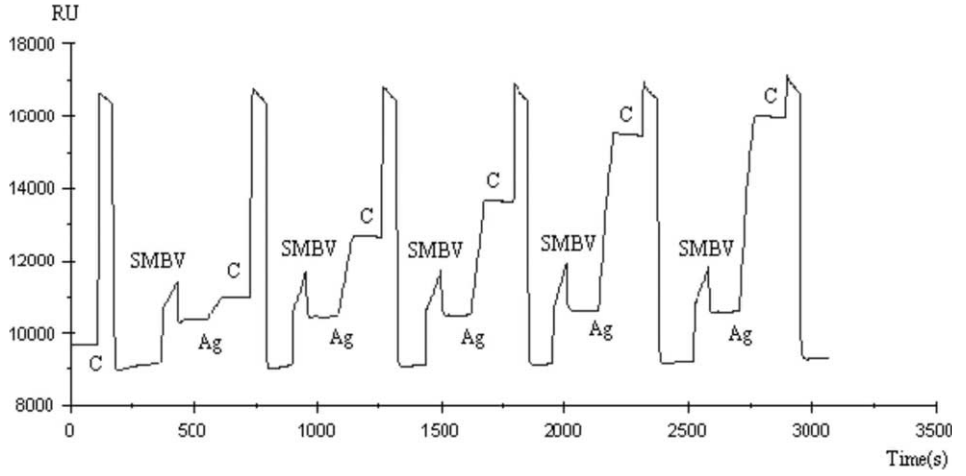

Fig. 4. Regeneration of the HPA sensor chip surface. C, cocktail; Ag, increasing concentration of Ag (from left to right); SMBV™, SMBV™ immobilization. After each regeneration, the basic signal is recovered.

particles on the HPA sensor chip surface to measure very sensitively the interactions of free injected Ag with the original structure of SMBV™.

We demonstrate that SMBV™ is able to associate a large different kinds of molecules and several molecules at the same time like AP and adjuvant. The screening of both Ag and SMBV™ to associate, the process of formulation to use, and the Ag/SMBV™ characterization are fast realized using this new method. SPR allows us to screen with very few row materials, a large variety of SMBV™ (with different electrostatic charge level, lipid bilayer compounds or core type). This method represents a highly interesting pre-screening step in the SMBV™ formulation and was used in several studies.

Closely, for a selected Ag/SMBV™ formulation, experimental process of formulation can be selected with this method as Ag/SMBV™ optimal ratio and ionic strength. When different molecules are used in the same formulation like Ag and adjuvant or two Ag, SPR allows us to determine the best process to associate them according the immune response. Particularly, it is possible to verify if there is any competition between the two Ag for SMBV™, if the second Ag shift the first associated and if there are interactions between both Ag limiting their association with SMBV™.

High yields of loading are generally obtained (over 85%) with these compounds. This pluripotent property is very competitive compared to others carriers like PACA nanoparticles (Balland et al., 1996) for which this method could be applied.

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