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Partitioning of propranolol in the phospholipid bilayer coat of anionic magnetoliposomes

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Abstract

This work deals with the partitioning of the cationic amphiphilic drug, propranolol, in the coating of so-called magnetoliposomes (MLs), which consist of nanometre-sized, magnetizable iron oxide cores covered with a phospholipid bilayer. MLs of two types were used: either the ML coat consisted entirely of anionic dimyristoylphosphatidylglycerol, or it was mixed with zwitterionic dimyristoylphosphatidylcholine in a 5/95 molar ratio. To separate sorbed from non-sorbed propranolol, high-gradient magnetophoresis was used. The sorption profiles clearly show that electrostatic interactions play a key role in the sorption process as drug incorporation in the ML coat was favoured by increasing the anionic character of the ML envelope and by reducing the salt concentration of the medium. Also, upon drug binding some phospholipid molecules were expelled from the ML coat. The observations may be of relevance in the biomedical field, i.e. in the development of ML-based, intracellular theranostics.

1. Introduction

During the last few decades, magnetic fluids have been successfully introduced in selected biomedical applications. Within this context, magnetoliposomes (MLs), i.e. magnetic cores covered with a bilayer of phospholipids, may enjoy a privileged position since the coating material is strongly chemisorbed and, moreover, demonstrates excellent biocompatibility features (De Cuyper and Joniau 1988, Garcia *et al* 2002). In the past, we have investigated in depth the mechanism of their formation and thoroughly characterized these nanocolloids from a physico-chemical point of view, including their magnetic properties (De Cuyper and Joniau 1991, De Cuyper *et al* 2003). In addition, we exploited these nanocolloids to visualize anatomical structures by magnetic resonance imaging (Bulte *et al* 1999). Besides their diagnostic value, MLs also have great potential as therapeutic tools, for instance, as a stable delivery system for poorly water soluble drugs which can be hosted in the ML coat (Koneracká *et al* 2005). Knowledge of the partitioning of the latter molecules in the immobilized phospholipid bilayer, however, is of prime importance for optimizing MLs as efficient and reliable drug carriers. As biological membranes are negatively charged, we deliberately worked in the present study with an anionic ML coat built up of dimyristoylphosphatidylglycerol (DMPG), either pure or mixed with the zwitterionic dimyristoylphosphatidylcholine (DMPC) in a 5/95 molar ratio. Cationic (*R*, *S*)-propranolol (Ppn), used in medicine as a non-selective β-blocker for treating systemic hypertension, was chosen as a representative drug molecule (figure 1). It is assumed that the hydrophobic naphthalene portion of the substance is buried in the acyl-chain interior of the bilayer while the polar headgroup stays near the surface of the membrane (Avdeef *et al* 1998). To get some insight into the binding features, the adsorption profiles of the drug were constructed as a function of the charge density of the ML coat and of the salt concentration.

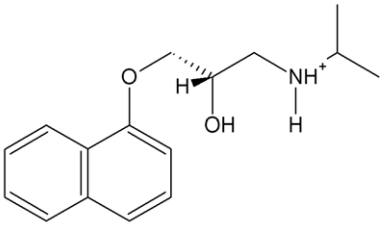

Figure 1. Chemical formula of the *R*-configurational isomer of propranolol. In this work the racemic *R*, *S* mixture was used.

2. Materials and methods

2.1. Materials

(*R*, *S*)-propranolol [1-(isopropylamino)-3-(naphthalene-1-yloxy)propane-2-ol] hydrochloride was obtained from Acros Organics (Geel, Belgium). DMPC and the sodium salt of DMPG were used as received from Avanti Polar Lipids (Alabaster, USA). *N*-Tris(hydroxymethyl)-methyl-2-amino-ethane sulfonic acid (TES) (Merck, Darmstadt, Germany) was used to prepare the buffer at pH 7.0. All chemicals used were of *pro analysis* grade.

2.2. Magnetoliposome preparation

Fe_3O_4 particles (diameter about 13 nm (De Cuyper *et al* 2003)) were prepared by wet precipitation of Fe^{2+} and Fe^{3+} chlorides with ammonia, and then coated with lauric acid molecules (Khalafalla and Reimers 1980, De Cuyper and Joniau 1988, 1991). The stock solution of this magnetic fluid contained 43 mg Fe_3O_4/ml. The magnetic fluid particles were then transformed into MLs by replacing the lauric acid envelope with a phospholipid bilayer following a procedure earlier developed in our laboratory (De Cuyper and Joniau 1988). Briefly, sonicated phospholipid vesicles made of DMPG, either pure or mixed with DMPC, were incubated in excess with the lauric acid stabilized magnetite particles (phospholipid/magnetite weight ratio = 5), and dialysed against buffer solution (5 mM TES, pH 7.0) for 4 days at 37 °C. Excess, non-adsorbed phospholipids were then removed by high-gradient magnetophoresis (figure 2). To this end, the mixture was pumped through glass tubes of about 2 mm inner diameter which were plugged with magnetizable steel wool and placed in the 5 mm gap between the two conical poles of an electromagnet (Bruker BE15, Bruker, Karlsruhe, Germany) operating at 30 A and 80 V. Hereby, the MLs were captured on the magnetic filter, while the conventional liposomes were not retained and simply float through (figure 2, step A). Then, the magnetic field was switched off and the MLs were washed out in a buffer stream at high speed (500 ml h^{-1}) (figure 2, Step B).

2.3. Experimental set-up for studying propranolol partitioning in the ML coat

The practical set-up used to study partitioning of the drug into the ML coat went as follows. Ppn was incubated at different concentrations with the ML dispersion at 37 °C for 12 h. The incubation medium consisted of 5 mM TES buffer, pH 7.0, containing 0, 5, 10 or 75 mM KCl. The sample size was about 1.5 ml. Then, the magnetoliposomes were captured from the mixture by high-gradient magnetophoresis (see above). Since it was found in a separate experiment that Ppn strongly binds to plastic material, care was taken that vials, conduit tubings, etc were made of glass. The eluate contained non-sorbed Ppn as well as Ppn embedded in a small amount of iron oxide-free, classical liposomes which obviously were not retained by the magnetic field.

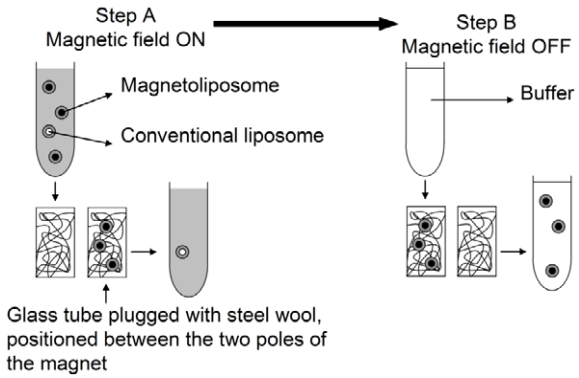

Figure 2. Schematic overview of the sequence in separating magnetoliposomes from iron oxide-free vesicles by high-gradient magnetophoresis.

2.4. Calculation of the amount of Ppn sorbed

The Ppn concentration in the eluate $[\text{Ppn}]_{\text{eluate}}$ was determined by recording the UV absorption between 250 and 340 nm. This spectrum was fitted to the spectrum of a standard Ppn solution in the same buffer. Hereby, a background curve which exponentially decreased upon increasing the wavelength was introduced as a correction for the light scattered by the liposomes present in the eluate. Thus, the following expression was fitted to the spectrum obtained:

$$P1 \times (\text{absorbance standard Ppn solution}) + P2 \times \exp(-P3 \times \text{wavelength}) \quad (1)$$

where $P1$, $P2$ and $P3$ are adjustable parameters. The squared difference between this expression and the experimentally obtained absorbance of the eluate was calculated for all wavelengths. Then the sum of these squared differences was minimized by adjusting $P1$, $P2$ and $P3$. The product of $P1$ and the concentration of the standard solution represents the concentration in the eluate. A typical spectrum of an eluate is shown in figure 3, curve A. The two components obtained by the fit are shown as well: the Ppn component, $P1 \times$ (absorbance standard Ppn solution) (figure 3, curve B) and the light scattering component, $P2 \times \exp(-P3 \times \text{wavelength})$ (figure 3, curve C). The phospholipid concentration of the eluate $[\text{PL}]_{\text{eluate}}$ was derived from a phosphate analysis. The amount of phospholipids in the eluate never exceeded 15% of the amount added (see further). The concentration of bound

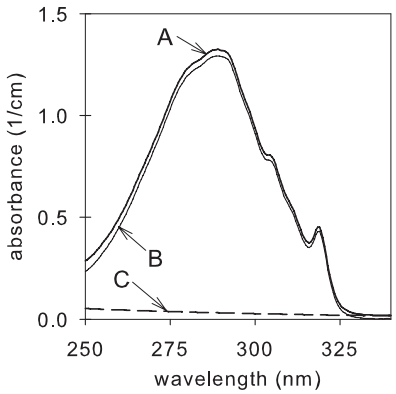

Figure 3. The fitting of a typical spectrum. A: spectrum of an eluate containing 0.224 mM Ppn, B: Ppn component, C: light scattering component.

Ppn $[\text{Ppn}]_{\text{bound}}$ can be expressed using a mass balance both in the retentate:

$$\frac{[\text{Ppn}]_{\text{bound}}}{[\text{PL}]_{\text{tot}}} = \frac{[\text{Ppn}]_{\text{tot}} - [\text{Ppn}]_{\text{eluate}}}{[\text{PL}]_{\text{tot}} - [\text{PL}]_{\text{eluate}}} \tag{2}$$

and in the eluate:

$$\frac{[\text{Ppn}]_{\text{bound}}}{[\text{PL}]_{\text{tot}}} = \frac{[\text{Ppn}]_{\text{eluate}} - [\text{Ppn}]_{\text{free}}}{[\text{PL}]_{\text{eluate}}} \tag{3}$$

where $[\text{Ppn}]_{\text{free}}$ and $[\text{Ppn}]_{\text{tot}}$ are the total non-bound and total added Ppn concentrations, respectively, and $[\text{PL}]_{\text{tot}}$ is the total phospholipid concentration. Combining the two equations allows us to calculate $[\text{Ppn}]_{\text{free}}$:

$$[\text{Ppn}]_{\text{free}} = \frac{[\text{Ppn}]_{\text{eluate}}[\text{PL}]_{\text{tot}} - [\text{PL}]_{\text{eluate}}[\text{Ppn}]_{\text{tot}}}{[\text{PL}]_{\text{tot}} - [\text{PL}]_{\text{eluate}}}. \tag{4}$$

The concentration Ppn bound, expressed in mol m^{-2} phospholipid, is then calculated from $[\text{Ppn}]_{\text{free}}$, $[\text{Ppn}]_{\text{tot}}$ and $[\text{PL}]_{\text{tot}}$. The phospholipid surface area was calculated on the basis of the total phospholipid concentration and on a projected surface area of 60 Å^2 for DMPC (Egorova 1994) and 62 Å^2 for DMPG (Toko and Yamafuji 1980) (see section 3).

2.5. Miscellaneous methods

The phospholipid content of MLs was derived from phosphate analysis, which was done spectrophotometrically according to the method of Vaskovsky *et al* (1975). Iron content was assessed using Tiron (Acros Organics, Geel, Belgium) as a complexing dye according to a protocol optimized earlier in our laboratory (De Cuyper and Joniau 1991).

3. Results and discussion

3.1. Characterization of anionic magnetoliposomes

Partitioning of Ppn was studied in two different ML types. A first one was produced starting from mixed DMPC/DMPG (95/5; molar ratio) vesicles; the coating of a second one consisted solely of DMPG. In both cases, the phospholipid/magnetite mass ratio equalled 0.79. Since both DMPG and DMPC, either pure or in a mixture, adopt a bilayer configuration upon covering a magnetite core (De Cuyper and Joniau 1988, 1991, De Cuyper *et al* 2006), and since the cross sectional areas of the two lipid types do not differ significantly, it can safely be assumed that the two particle types expose an identical surface area to the aqueous medium. Furthermore, assuming a phospholipid bilayer thickness of 3.5 nm when using myristoyl chains for the fatty acyl moieties in the phospholipids (as we did) (Marra and Israelachvili 1985) ultimately led to a phospholipid bilayer density of 0.934 g cm^{-3}. On the basis of this value and taking into account a density of 5.18 g cm^{-3} for magnetite (Lide 1992), the phospholipid/magnetite volume ratio of the MLs is calculated to be 4.3. This results in a magnetite core diameter of 9.4 nm which is in between 8.4 and 13.1 nm, the sizes determined using transmission electron microscopy by Peng *et al* (2003) and De Cuyper *et al* (2003), respectively, after applying the same preparation method.

3.2. Sorption of propranolol in magnetoliposomes

First, the time course of Ppn adsorption in the lipidic coat of MLs was investigated at pH 7.0 and 37 °C with the mixed DMPC/DMPG (95/5) MLs. Incubation of 0.08 mM Ppn with the MLs for various time periods (up to 48 h), however, showed that equilibrium was already established within the first 20 min, which in our experimental set-up is the shortest possible measurement time. The amount of Ppn sorbed was 0.246, 0.233, 0.240, 0.238 and 0.254 μmol m^{-2} phospholipid after 0.37, 2.73, 6.03, 24.00 and 48.00 h, respectively.

Next, the amount of Ppn sorbed in both DMPG/DMPC (5/95; molar ratio) and pure DMPG MLs was determined in 5 mM TES buffer, pH 7.0 as a function of Ppn and salt concentration (figure 4). Irrespective of the ML type used, it is found that Ppn binding decreased upon increasing the salt concentration. Furthermore, significantly higher Ppn amounts were captured by the 100% DMPG MLs. Apart from the occurrence of a significant hydrophobic interaction component (Avdeef *et al* 1998), both observations point to the importance of electrostatics. In interpreting these results, a crucial point deals with the degree of ionization of propranolol. The pKa of Ppn in water at 37 °C equals 9.14 (Balon *et al* 1999), but in the stationary buffer layer near the surface of negatively charged MLs, the pH may be drastically lowered below the bulk pH (7.0) because of electrostatic attraction of protons (Kramer *et al* 1998, Cocquyt *et al* 2007). This event may further favour protonation of Ppn molecules residing at the ML surface (Kramer *et al* 1998), and thus will additionally improve Ppn uptake by negatively charged MLs. On the other hand, the impact of the interface features on the pKa of sorbed Ppn is difficult to assess in a clear-cut way. The bilayer–water interface is a highly anisotropic region of roughly 10–15 Å thickness (Bernik and Negri 1998) and the accompanying difference in dielectric constant between the bulk water and the sorption site in the bilayer (74 versus ≈30 for neutral DMPC membranes (Cocquyt *et al* 2007)) undoubtedly will favour deprotonation of bound Ppn (corresponding to a pKa decrease),

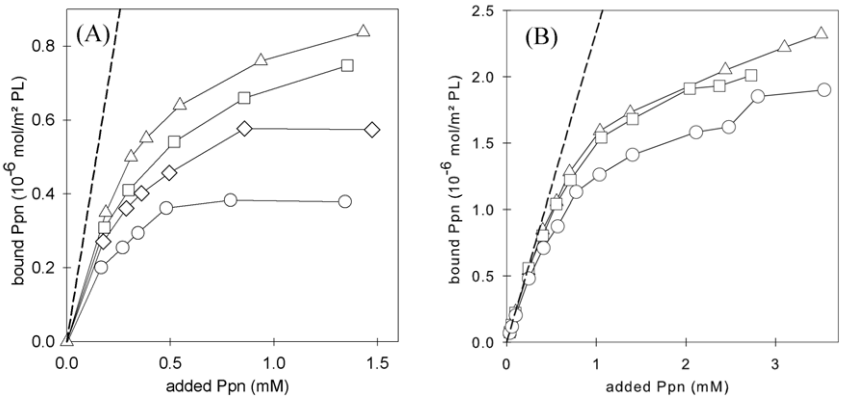

Figure 4. Binding profiles of Ppn in the bilayer coat of MLs, measured at 37 °C in 5 mM TES buffer, pH 7.0. The solid lines represent the amounts of Ppn sorbed in magnetoliposomes whose coating consisted of either DMPC/DMPG (95/5 mass ratio) (a) or DMPG (b). Besides buffer components, the medium also contained no added KCl (△), 5 mM KCl (□), 10 mM KCl (◇) and 75 mM KCl (○). The dashed lines represent the case where all Ppn should be sorbed. Note the difference in scales on the *x*- and *y*-axes between (a) and (b).

whereas the presence of the negative charge on the phosphate moiety of neighbouring DMPG molecules may increase the Ppn's pKa. Even though the overall results point to strong electrostatic attractions between the positively charged Ppn and the negatively charged DMPG, the amounts sorbed still deviate strongly from the hypothetical amounts corresponding to 100% sorption (dashed line in figures 4(a) and (b)).

3.3. Preservation of ML coat integrity during Ppn adsorption

A most interesting observation with respect to Ppn incorporation into the ML coat is illustrated in figure 5. It depicts, at various ionic strength conditions, the fraction of DMPG released from MLs into the eluate solution (recovered by high-gradient magnetophoresis), after sorption of different amounts of Ppn into pure DMPG MLs (1.14 mM) has reached equilibrium. The results show that the highest amount of DMPG in the eluate is found in conditions where Ppn adsorption reaches the highest level (see figure 4 (b), Δ). Thus, in concert with these data, it is very likely that the ML coat does not allow an expansion upon Ppn adsorption. Rather, to minimize structural membrane perturbations, it seems that uptake of Ppn has to be counterbalanced by a proportional expulsion of DMPG molecules. This behaviour of the ML coating contrasts with that of the bilayer of classical vesicles. The latter, indeed, is much less susceptible to geometric constraints and can easily take up Ppn molecules, but when a certain threshold uptake is surpassed, the vesicle's membrane, ultimately, is much more vulnerable (Božič *et al* 2006, De Carlo *et al* 2004, Rogers *et al* 1986) as compared to a ML's phospholipid bilayer which is strongly anchored on the surface of Fe_3O_4 grains.

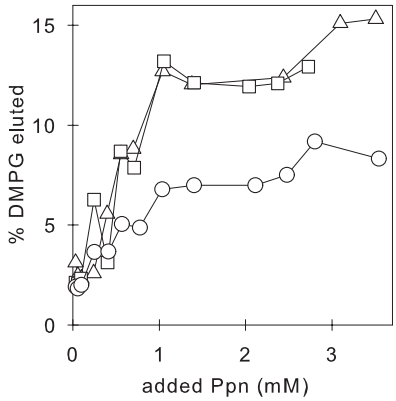

Figure 5. Fraction DMPG eluted during the magnetophoresis step after incubating 1.14 mM DMPG magnetoliposomes with different amounts of Ppn in 5 mM TES buffer at pH 7 and at 37 °C without any added salt (△), 5 mM KCl (□) and 75 mM KCl (○).

4. Conclusion and perspectives

In conclusion, the present results show that MLs are unique nanocolloids to be used as a vehicle with a cargo of amphiphilic drugs. In particular, we showed here that the amount of sorbed molecules can easily be fine-tuned by changing the phospholipid composition of the ML coat. In fact, the ML coat can be constructed using a wide variety of (phospho)lipid molecules making these structures extremely versatile. Since experiments, not discussed here, delivered the 'proof of principle' that anionic MLs are easily taken up by biological cells (e.g. 3T3 fibroblasts), interesting perspectives are opened to generating powerful intracellular theranostics which combine diagnostic MRI features with a therapeutic entity (De Cuyper *et al* 2002, Al-Jamal and Kostarelos 2007, McCarthy *et al* 2007).

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References

Al-Jamal W T and Kostarelos K 2007 *Int. J. Pharmacol.* **331** 182–5

Avdeef A, Box K J, Comer J E A, Hibbert C and Tam K Y 1998 *Pharmacol. Res.* **15** 209–15

Balon K, Riebesehl B U and Muller B W 1999 *J. Pharm. Sci.* **88** 802–6

Bernik D L and Negri R M 1998 *J. Colloid Interface Sci.* **203** 97–105

Božič B, Kralj-Iglič V and Svetina S 2006 *Phys. Rev.* E **73** 041915

Bulte J W M, De Cuyper M, Despres D and Frank J A 1999 *J. Magn. Reson. Imaging* **9** 329–35

Cocquyt J, Saveyn P, Van der Meeren P and De Cuyper M 2007 *Langmuir* **23** 1959–64

De Carlo S, Fiaux H and Marca-Martinet C A 2004 *J. Liposome Res.* **14** 61–76

De Cuyper M, Caluwier D, Baert J, Cocquyt J and Van der Meeren P 2006 *Z. Phys. Chem.* **220** 133–41

De Cuyper M, Hodenius M, Lacava Z G M, Azevedo R B, da Silva M D, Morais P C and Santana M H A 2002 *J. Colloid Interface Sci.* **245** 274–80

De Cuyper M and Joniau M 1988 *Eur. Biophys. J.* **15** 311–9

De Cuyper M and Joniau M 1991 *Langmuir* **7** 647–52

De Cuyper M, Muller P, Lueken H and Hodenius M 2003 *J. Phys.: Condens. Matter* **15** S1425–36

Egorova E M 1994 *Electrophoresis* **15** 1125–31

Garcia V, Lacava L M, Kückelhaus S, Azevedo R B, Da Silva M D, Morais P C, De Cuyper M and Lacava Z G M 2002 *Eur. Cells Mater.* **3** (Suppl. 2) 154–5

Khalafalla S E and Reimers G W 1980 *IEEE Trans. Magn.* **16** 178–83

Koneracká M, Kopčanský P, Sosa P, Bagel'ová J and Timko M 2005 *J. Magn. Magn. Mater.* **293** 271–6

Kramer S D, Braun A, Jakits-Deiser C and Wunderli-Allenspach H 1998 *Pharmacol. Res.* **15** 739–44

Lide D 1992 *Handbook of Chemistry and Physics* vol 73 (Boca Raton, FL: CRC press)

Marra J and Israelachvili J 1985 *Biochemistry* **24** 4608–18

McCarthy J, Kelly K, Sun E and Weissleder R 2007 *NanoMedicine* **2** 153–67

Peng Z, Hidajat K and Uddin M S 2003 *Korean J. Chem. Eng.* **20** 896–901

Rogers J A, Cheng S and Betageri G V 1986 *Biochem. Pharmacol.* **35** 2259–61

Toko K and Yamafuji K 1980 *Chem. Phys. Lipids* **26** 79–99

Vaskovsky V E, Kostetsky E Y and Vasendin I M 1975 *J. Chromatogr.* **114** 129–41