Solubilization of Therapeutic Agents in Micellar Nanomedicines

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Electronic Supporting Information (ESI)

Materials

1,2-Distearoyl-sn-glycero-3-phosphatidylethanolamine-N- [methoxy(polyethyleneglycol) -2000] sodium salt (DSPE-PEG_{2000}) was purchased from Lipoid AG, and bexarotene was obtained from ChemieTek. All the other reagents were acquired from Fisher Scientific or Sigma-Aldrich. The HEPES buffered saline prepared in house contained 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and 135 mM NaCl. The PB buffer was prepared in house by mixing 0.003 g $NaHPO_3$ and 0.021 g NaH_2PO_4 per 100 mL of water. The PBS buffer was prepared by adding 0.9 g NaCl per 100 mL of PB buffer.

Preparation of SSM-BEX samples

Separate DSPE-PEG_{2000} and bexarotene stock solutions were prepared by direct dissolution of compounds in methanol, followed by 5 min of vortexing. The appropriate volumes of lipid and bexarotene stock solutions were combined in 100 mL round bottom flasks and vortexed for 2 min. The final lipid concentration in these flasks was set constant at 1 mM and the bexarotene concentration was varied, in the range $c_{bex} = 0 - 100$ μg/mL. The solvent from each solution was rotary-evaporated in vacuum under a stream of argon (650 mmHg, 150 rpm, 50 °C, 20 min) to form a thin film. To remove all the trace amounts of the solvent, the films were left to dry overnight under vacuum in the dark. We prepared the samples by rehydrating the films with HEPES buffered saline, vortexing them for 3 min at the maximum speed until complete dissolution, and sonicating them for 5 min. The samples were then flushed with argon and sealed. Micellar dispersions were let to equilibrate in the dark at 25 °C for 2 hours. A sample with the blank SSM was prepared by an identical procedure as described above, with the exception that no drug solution was added. All the prepared solutions were analyzed by dynamic light scattering (DLS), and the drug content was examined by high performance liquid chromatography (HPLC).

Preparation of aqueous bexarotene solutions

Intrinsic solubilities of bexarotene in HEPES buffered saline and pure water were determined as follows: 100 μg of the drug was mixed with 1 mL of the either solvent, and samples were shaken at 250 rpm for 24 hours at ambient temperature (average 23°C) followed by centrifugation at 14,000 g for 15 minutes. Supernatant solutions were analyzed by HPLC to determine the concentrations of the solubilized bexarotene.

Preparation of SSM-VIP samples

Weighed amounts of DSPE-PEG_{2000} and VIP were directly dissolved in either standard phosphate buffered saline (PBS, pH 7.4) or phosphate buffer (PB, 1 mM, pH=7.4, prepared in house), to obtain the solutions with the lipid concentrations ranging incrementally from 0 to 0.4 mM, and with constant VIP concentration $c_{VIP} = 4$ μM. The average sizes of the micelles in the used solvents were determined by the dynamic light scattering.

High performance liquid chromatography

Bexarotene content in all the prepared solutions (aqueous bexarotene solutions, blank SSM, and SSM-BEX solutions) was quantified by the reverse phase HPLC (RP-HPLC) using Shimadzu Prominence instrument equipped with a Varian Pursuit CRs C18 column and a diode array detector. The isocratic flow rate at 1.2 ml/min of mobile phase that contained methanol/acetonitrile/water at 47/47/6 ratio acidified with 0.1 % trifluoroacetic acid (TFA) was used. The UV absorbance at 254 nm was used for the detection of bexarotene. Concentration of the dissolved bexarotene was determined by comparison to a calibration curve of standard solutions with known bexarotene concentrations.

Particle size analysis by dynamic light scattering

Aliquots of the SSM-BEX, SSM-VIP, and blank SSM samples were transferred to drop cell cuvettes. Particle analyses of the samples were performed by the dynamic light scattering at the ambient temperature using the Agilent 7030 NICOMP DLS/ZLS instrument, equipped with a 100 mW He-Ne laser at 632.8 nm, set up at the 90° angle. We accumulated the analysis of the autocorrelation function over at least 15 min, from which we obtained particle size distributions and modality. The measurements were carried out at 23°C with autoadjusted light scattering intensity of 300 KHz and at the fixed detector angle of 90°.

Determination of lipid:VIP saturation molar ratio of association

The association curves of VIP with SSM were obtained by measuring the fluorescence intensity enhancement ratio (I/I_0) of VIP in SSM solutions, relative to the free VIP in PB and PBS. The measurements were performed on the solutions with the increasing lipid concentration, $c_{lip} = 0 - 0.4$ mM, and the constant VIP concentration, $c_{VIP} = 4$ μM, where the lipid:VIP molar ratios ranged from 0 to 100. All the fluorescence measurements were normalized to the measurements of VIP peptide in either PB or PBS solutions. The fluorescence measurements were taken using the SLM Aminco 8000 spectrofluorometer set at the excitation wavelength of 277 nm and the emission wavelength of 304 nm, corresponding to the tyrosine amino acid residue of VIP.

The data points from the association curves were fitted to a typical carrying capacity equation:

$$y = y_0 + \frac{ax}{x+b}, \tag{1}$$

The $lim_{x\to\infty} y = y_0 + a$, but since it is unreasonable to assume that x ever reaches infinity, we used the steady state approximation, and estimated that the association curves reach plateaus (saturation) at $y_p \approx y_0 + 0.9a$. Substituting y_p for y in Eq. 1 yields the lipid:peptide saturation molar ratio (N_{sat}).

From our previous study [1], we correlated the micelle size (hydrodynamic diameter d_h) to the number of monomers per SSM (N_{agg}). The dimensional analysis yields an approximation for the number of VIP molecules per SSM (loading efficiency, N_{eff}) as follows:

$$\frac{c_{lip}}{c_{SSM}} : \frac{c_{lip}}{c_{VIP}} = N_{eff} = \frac{N_{agg}}{N_{sat}}, \tag{2}$$

Partial Atomic Charges of Bexarotene

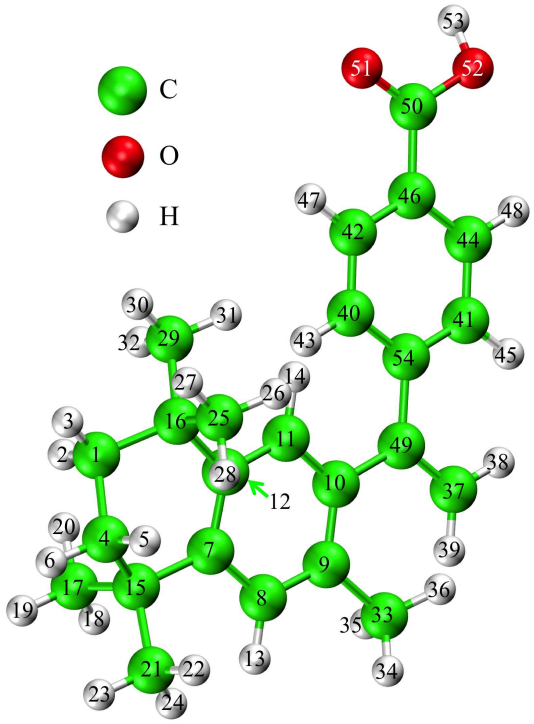

Figure 1: Bexarotene molecule with labeled atoms.

To prepare the parameters for bexarotene, we relied on the fact that all the bexarotene atoms have similar atom types already defined in the existing CHARMM27 force field. We split a bexarotene molecule into fragments that already exist in the CHARMM27 force field, and then, by analogy, assigned to all the bexarotene atoms the atom types, partial atomic charges and nonbonding and bonding parameters. Finally, we ensured that bexarotene molecule had no net charge. Below, we list the partial atomic charges of bexarotene atoms, labeled as marked in Fig. 1:

Atom label	*Atom type*	*Partial atomic charge*
C1	CT2	-0.180000
H2	HA	0.090000
H3	HA	0.090000
C4	CT2	-0.180000
H5	HA	0.090000
H6	HA	0.090000
C7	CA	0.000000
C8	CA	-0.115000
C9	CA	0.000000
C10	CA	0.000000
C11	CA	-0.115000
C12	CA	0.000000
H13	HP	0.115000
H14	HP	0.115000
C15	CT1	0.000000

C16	CT1	0.000000
C17	CT3	-0.270000
H18	HA	0.090000
H19	HA	0.090000
H20	HA	0.090000
C21	CT3	-0.270000
H22	HA	0.090000
H23	HA	0.090000
H24	HA	0.090000
C25	CT3	-0.270000
H26	HA	0.090000
H27	HA	0.090000
H28	HA	0.090000
C29	CT3	-0.270000
H30	HA	0.090000
H31	HA	0.090000
H32	HA	0.090000
C33	CT3	-0.270000
H34	HA	0.090000
H35	HA	0.090000
H36	HA	0.090000
C37	CE2	-0.420000
H38	HE2	0.210000
H39	HE2	0.210000
C40	CA	-0.115000
C41	CA	-0.115000
C42	CA	-0.115000
H43	HP	0.115000
C44	CA	-0.115000
H45	HP	0.115000
C46	CA	-0.030000
H47	HP	0.115000
H48	HP	0.115000
C49	CE1	0.000000
C50	CD	0.750000
O51	OB	-0.550000
O52	OH1	-0.600000
H53	H	0.430000
C54	CA	0.000000

Molecular Dynamics Simulations

In simulations of the empty SSM-10, SSM-20, and SSM-90, the monomers were initially spherically distributed and solvated in cubic solvent boxes in VMD [2]. SSM-10 and SSM-20 were simulated in pure water, and SSM-90 was simulated in 0.16M NaCl. In all the cases, water was described with a TIP3P water model. The resulting unit cells, containing $185,000-639,000$ atoms, were first minimized and then equilibrated within NAMD [3] for $5-10$ ns at $T=300$ K and $p=1$ atm (NpT ensemble) with periodic boundary conditions applied. The cutoff distance was set to 11Å. The short-range interactions were calculated every time step (1 fs), and the long-range electrostatic forces were computed every two time steps by the particle-mesh Ewald method [4]. The Langevin damping constant of $\gamma_{Lang}=0.01$ ps^{-1} was used for faster dynamics during the equilibration period. During the free energy calculations, the Langevin damping constant was set to $\gamma_{Lang}=1.0$ ps^{-1}.

1, 3 and 5 bexarotene drug molecules were placed and equilibrated within the SSM-90 core in 0.16M NaCl for

3.5 ns. A single bexarotene was equilibrated within the SSM-10 core in water for 3 ns. These equilibrated SSM-BEX systems were used as the initial structures for calculation of free energy profiles of bexarotene in SSM. For comparison, we also evaluated free energy profiles of bexarotene in three interfacial systems, containing octane and either water, 0.16 M NaCl, or hydrated PEG (70 w/w % of $CH_3[OCH_2CH_2]_4OCH_3$ polymers). Octane, water, and 0.16 M NaCl solution were each contained in the boxes 48 Å $\times$ 48 Å $\times$ 48 Å in size, whereas aqueous PEG solution was placed in a box 48 Å $\times$ 48 Å $\times$ 90 Å in size.

In all the systems, free energy profiles were evaluated by the adaptive biasing force (ABF) method [5–7]. The free energy profiles were obtained by integrating the average forces acting on bexarotene along the reaction coordinate ζ. In SSM, ζ was chosen as the distance (r) of the drug center of mass (COM) and the alkane core COM. The calculated profile was modified by the entropy correction for spherical systems, given by $\Delta S(r) = k_B \ln(r/r_0)^2$, where k_B is the Boltzmann constant, and r_0 is the reference point (where $\Delta G(r) = 0$) [8, 9]. In the interfacial systems, ζ was chosen as the z component of the distance between the bexarotene and the octane box COMs (normal to interfacial planes). Individual ABF simulations were run for 6 – 20 ns. In SSM-10 and SSM-90 with single bexarotene molecules, bexarotene was constrained in 4 Å-wide windows along the reaction coordinate. In SSM-90 with multiple bexarotene molecules, one of the drug molecules was constrained in 2 Å-wide windows. In interfacial systems, bexarotene was constrained in 6 Å-wide windows.

Separately, we also equilibrated a cluster of 11 bexarotene molecules in the SSM-90 core in 0.16M NaCl for 17 ns, to examine the hydrogen bond network formed between the bexarotene molecules.

The equilibrated SSM-20 (simulated in water, which approximates the low ionic strength solvent) and SSM-90 (0.16 M NaCl) were used as the starting structures for SSM-VIP simulations. In SSM-20, 2 VIP monomers were placed on the opposite sides of the micelle within the PEG palisade region. Positions of the VIP monomers were adjusted by steered molecular dynamics, so that the clusters of the positively charged VIP residues were brought to within 7 Å from the SSM interface. In SSM-90, we modified one of the DSPE-PEG_{2000} monomers into a DSPE-PEG_{3400} monomer with a VIP peptide grafted onto its distal end. We examined two cases for binding of VIP to SSM-90, where DSPE-PEG_{3400} monomer was positioned so that VIP can bind either on the side or on the top of the SSM core (Fig. 7 (a) in the main document). After minimization of the modified SSM-90, we used steered molecular dynamics to bring the positively charged VIP residues within 7 Å from the SSM interface. Following the steered MD, all the SSM-VIP systems were equilibrated for $\approx$ 30 ns.

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