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Molecular Architecture of Nanocapsules, Bilayer-Enclosed Solid Particles of Cisplatin

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Abstract: Cisplatin nanocapsules represent a lipid formulation of the anticancer drug *cis*-diamminedichloroplatinum(II) (cisplatin) characterized by an unprecedented cisplatin-to-lipid ratio and exhibiting strongly improved cytotoxicity against tumor cells in vitro as compared to the free drug (Burger, K. N. J., et al. *Nat. Med.* **2002**, *8*, 81−84). Cisplatin nanocapsules are prepared by the repeated freezing and thawing of an equimolar dispersion of phosphatidylserine (PS) and phosphatidylcholine (PC) in a concentrated aqueous solution of cisplatin. Here, the molecular architecture of these novel nanostructures was elucidated by solid-state NMR techniques. ^{15}N NMR and ^{2}H NMR spectra of nanocapsules containing ^{15}N- and ^{2}H-labeled cisplatin, respectively, demonstrated that the core of the nanocapsules consists of solid cisplatin devoid of free water. Magic-angle spinning ^{15}N NMR showed that ∼90% of the cisplatin in the core is present as the dichloro species. The remaining 10% was accounted for by a newly discovered dinuclear Pt compound that was identified as the positively charged chloride-bridged dimer of cisplatin. NMR techniques sensitive to lipid organization, ^{31}P NMR and ^{2}H NMR, revealed that the cisplatin core is coated by phospholipids in a bilayer configuration and that the interaction between solid core and bilayer coat exerts a strong ordering effect on the phospholipid molecules. Compared to phospholipids in liposomal membranes, the motion of the phospholipid headgroups is restricted and the ordering of the acyl chains is increased, particularly in PS. The implications of these findings for the structural organization, the mechanism of formation, and the mode of action of cisplatin nanocapsules are discussed.

Introduction

Cisplatin is one of the most widely used anticancer drugs, which kills cells by interacting with DNA.[1−4] The clinical use of cisplatin is restricted by serious dose-limiting toxicities and rapid inactivation of the drug due to its high reactivity. In principle, these problems can be reduced by shielding the drug from the extracellular environment by a lipid coating. However, due to the low water solubility and low lipophilicity of cisplatin, liposomal formulations of cisplatin suffer from relatively low drug-to-lipid ratios and limited antitumor efficacy in clinical trials.[5,6] In our laboratory, a new and simple method was developed to efficiently encapsulate cisplatin in a lipid formulation.[7] The method involves repeated freezing and thawing of a concentrated aqueous solution of cisplatin in the presence of both phosphatidylcholine and phosphatidylserine. Examination by electron microscopy revealed the presence of nanocapsules of cisplatin, small electron-dense particles containing cisplatin and lipids. The nanocapsules have an unprecedented drug-to-lipid ratio and an in vitro cytotoxicity toward tumor cells up to 1000-fold higher than that of the free drug.[7] It was shown that both negatively charged phospholipids and positively charged aqua species of cisplatin are necessary for the formation of nanocapsules.[7]

The molecular organization of the cisplatin nanocapsules is not known. Yet this information will give insight into the mechanism of formation and mode of action of the cisplatin nanocapsules as well as into the general principles of enclosing precipitates in lipid layers. We analyzed the molecular organization of the nanocapsules by various solid-state NMR techniques sensitive to lipid[8−11] and cisplatin[12−14] structural organization and composition. The results demonstrate that water-free solid

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cisplatin forms the core of the nanocapsules that is surrounded by and interacts with a bilayer of lipids that are immobilized by interaction with the core.

Experimental Section

Materials. 1,2-Dioleoyl-*sn*-glycerophosphocholine (DOPC) and -phosphoserine (DOPS) were purchased from Avanti Polar Lipids. 1,2-[11,11′-$^2H_4$]-Dioleoyl-*sn*-glycerophosphocholine (DOPC-d_4) and -phosphoserine (DOPS-d_4) were synthesized as described previously.[15,16] Cisplatin [cis-diamminedichloroplatinum (II), *cis*-$PtCl_2(NH_3)_2$] was obtained from Sigma−Aldrich. ^{15}N-Labeled cisplatin was a kind gift of Professor J. Reedijk (Leiden University, Leiden, The Netherlands). Deuterated *cis*-$[PtCl_2(ND_3)_2]$ was prepared by dissolving cisplatin in D_2O (5 mM) and incubation in the dark overnight at 37 °C to ensure full proton−deuterium exchange. After addition of sodium chloride, deuterated cisplatin was crystallized at 4 °C. Crystals were collected by centrifugation and washed three times with D_2O at 4 °C, and residual D_2O was removed in a vacuum.

Preparation of Nanocapsules. Cisplatin was dissolved in MilliQ water and incubated overnight in the dark at 37 °C. Nanocapsules were prepared by hydrating dry lipid films (1.2 μmol) consisting of DOPC/DOPS 1/1 (mol/mol), with 1.2 mL of the 5 mM cisplatin solution at 37 °C for 30 min, followed by 10 freeze−thaw cycles in ethanol/dry ice at −70 °C and a water bath at 37 °C.[17] Free cisplatin and multilamellar liposomes were removed by low-speed centrifugation (4 min at 470*g*). Subsequently, nanocapsules were washed by resuspension in 1 mL of water and centrifugation as above. Nanocapsules containing deuterated cisplatin were prepared in D_2O as above and subsequently washed with MilliQ water to remove excess D_2O.

NMR Measurements. NMR spectra were recorded on a Bruker Avance 500 WB spectrometer (Karlsruhe, Germany). ^2H NMR spectra were obtained by a quadrupole echo technique. Static ^{31}P and ^{15}N NMR spectra were recorded by use of cross polarization (CP) and spin−echo techniques with proton decoupling in a 7 mm CP probe. Magic-angle spinning (MAS)^{15}N NMR spectra were recorded with CP in a 4 mm CP MAS probe. For static experiments, the samples of nanocapsules contained 2−4 μmol of phospholipid (and 20−40 μmol of cisplatin) in 300 μL of water. For ^{15}N NMR MAS experiments, pellets of nanocapsules corresponding to at least 10 μmol of ^{15}N-labeled cisplatin were transferred in a 4 mm rotor. All NMR spectra of nanocapsules, crystalline cisplatin, and aqueous lipid dispersions were recorded at 4 °C. ^{15}N NMR spectra of 5 mM cisplatin in water were recorded at 37 °C by use of the DEPT pulse sequence with a 1/73 s ($1/J_{^1H-^{15}N}$) refocusing delay.

Mass Spectrometry. The measurements were performed on an electrospray ionization time-of-flight (ESI-ToF) instrument (LC-T; Micromass Ltd., Manchester, U.K.), operating in the positive ion mode and equipped with a Z-spray nano-flow ESI source. The dispersion of nanocapsules in water was sprayed by use of a capillary voltage of 1000 V and a cone and extraction voltage of 50 V.

Results

Cisplatin Organization in Nanocapsules: Physical State and Composition. To characterize the physical state and the chemical composition of cisplatin in the nanocapsules, ^{15}N NMR experiments were carried out on nanocapsules prepared from ^{15}N-labeled cisplatin. ^{15}N NMR was used rather than ^{195}Pt NMR because the chemical shift anisotropies (CSA) for ^{195}Pt in square planar coordination complexes are too large to enable solid-state NMR experiments.[18,19] The ^{15}N NMR spectrum of the nanocapsules (Figure 1a) shows a broad line shape with a CSA of about 135 ppm, which is nearly identical to the line shape of solid ^{15}N-labeled cisplatin (Figure 1b). ^{15}N-Labeled cisplatin dissolved in water gives high-resolution signals (Figure 1c) originating from different cisplatin species due to averaging of the CSA by the rotational motion of the molecules. Such signals cannot be detected in the spectrum of the nanocapsules despite the fact that the nanocapsules are prepared from and are present in an aqueous environment. These results directly demonstrate that all cisplatin in the nanocapsules is solid and must be shielded from the excess aqueous phase.

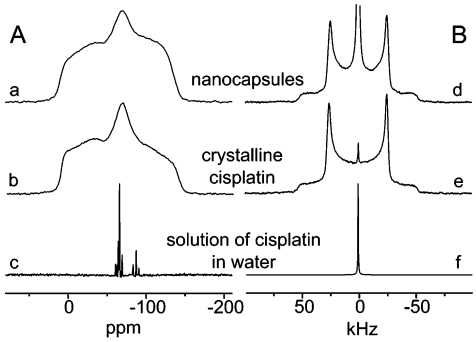

Figure 1. Organization of the core of cisplatin nanocapsules: (A) ^{15}N NMR spectra and (B) ^2H NMR spectra of ^2H-labeled cisplatin, in nanocapsules (a, d), in the crystalline state (b, e), and in aqueous solution (c, f).

To compare the ordering of cisplatin in nanocapsules to that in the crystalline state we used ^2H NMR, which is known as a sensitive technique for studying molecular order.[8] In the solid state, deuterated *cis*-$[PtCl_2(ND_3)_2]$ shows the characteristic doublet line shape with a residual quadrupole splitting ($\Delta\nu_Q$) of 49.5 kHz (Figure 1e), which can be calculated to result solely from fast (on the NMR time scale) rotation around the Pt−ND_3 bond in the solid state. The nanocapsules give rise to an identical doublet line shape (Figure 1d), demonstrating that the cisplatin in the nanocapsules is a solid, in agreement with the ^{15}N NMR results. The isotropic signal superimposed on the doublet (Figure 1d) originates from residual D_2O remaining in the preparation (see Experimental Section). In aqueous solution, deuterated cisplatin exhibits an isotropic signal due to isotropic movement of the cisplatin species and the water molecules (Figure 1f).

The intensity of the anisotropic doublet in the spectrum of the nanocapsules did not decrease during 3 weeks of storage at 4 °C despite the presence of excess water (data available as Supporting Information), demonstrating the stability of the nanocapsules at 4 °C. The virtual absence of D−H exchange in the cisplatin core of the nanocapsules is due to its solid nature, as deuterated cisplatin above its solubility limit in aqueous suspension is not subject to D−H exchange either (data available as Supporting Information). This indicates that water molecules do not penetrate into solid cisplatin. Although the lipid coat of the nanocapsules is most likely permeable for water,[20] it poses a barrier against leakage of cisplatin species, thus protecting the solid core from dissolving.

Hydrolysis of cisplatin is essential for its antitumor activity, because the aqua species rather than the dichloride form of

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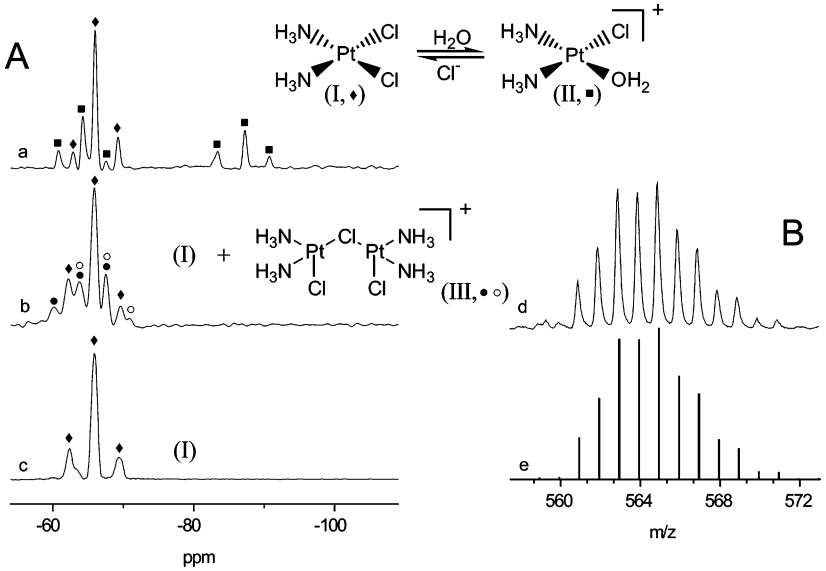

Figure 2. Chemical composition of the core of cisplatin nanocapsules. (A) ^{15}N NMR spectra of ^{15}N-labeled cisplatin in 5 mM aqueous solution (a), enclosed in nanocapsules (b), and as crystalline powder (c). The spectra were recorded by DEPT (a) and CP MAS (b, c). Hydrolysis of cisplatin and the structure of the chloride-bridged dimer are shown as insets in spectra a and b, respectively. The positions of the central peak and satellites of the different species of cisplatin are marked by ◆ (dichloride), ● (monoaqua), and ●, ○ (dimer). (B) Mass spectrum of the cluster ion at m/z 564$^+$ (d) and calculated spectrum (e) of the M$^+$ ion of the chloride-bridged dimer (III).

cisplatin react with DNA.[1,2] The most abundant hydrolysis product is the monoaquated $[PtCl(H_2O)(NH_3)_2]^+$ cation (Figure 2a), which is known to be much more reactive than cisplatin. Therefore, knowledge of the chemical composition of the nanocapsules is essential for understanding their mode of action.

^{15}N NMR spectroscopy has proven extremely powerful in elucidating the chemistry of ^{15}N-labeled platinum ammine complexes.[12−14] Therefore, we used this technique to analyze the composition of the nanocapsules. Since about 34% of platinum is ^{195}Pt (spin quantum number $I = {}^1/_2$) Pt−^{15}N peaks are tripletlike with one central peak arising from ^{194,196}Pt−$^{15}NH_3$ (spin quantum number of ^{194}Pt and ^{196}Pt $I = 0$) and two satellites from ^{195}Pt−$^{15}NH_3$ due to J coupling of about 350 Hz. In water, the ^{15}N NMR spectrum of cisplatin shows both the dichloride (I) and the monoaqua (II) forms (Figure 2a). The dichloride form (I) exhibits one signal from two equivalent ^{15}N nuclei at −65.9 ppm with two ^{195}Pt satellites. The monoaqua form (II) exhibits two signals from two nonequivalent ^{15}N nuclei at −64.2 ppm (^{15}N trans to chloride) and −87.2 ppm (^{15}N trans to oxygen), both with ^{195}Pt satellites. The ratio of the neutral dichloride to monoaqua species is 3:2. Analysis of the chemical composition of the nanocapsules in terms of cisplatin species is complicated because cisplatin and its hydrated species possess very high reactivity toward nucleophilic compounds, including polar solvents,[21] leading to a loss of the initial composition upon dissolution of the nanocapsules. To avoid this problem, we analyzed the composition of intact nanocapsules by MAS NMR, which enhances the spectral resolution in solids.[22]

The MAS ^{15}N NMR spectrum of cisplatin nanocapsules (Figure 2b) reveals that the main cisplatin component corresponds to the dichloride species as evidenced by comparison with the spectrum of the crystalline powder of cisplatin (Figure 2c). The spectrum of the nanocapsules (Figure 2b) also shows the absence of any aqua species of cisplatin (compare with Figure 2a). However, a minor (8−12%) additional component is discerned in the MAS ^{15}N NMR spectrum of the nanocapsules. This component shows two signals from two nonequivalent ^{15}N nuclei at −63.7 and −67.5 ppm with ^{195}Pt satellites (Figure 2b), indicating that the ^{15}N nuclei are in chemically nonequivalent environments in a platinum coordination complex. A chemical shift value of ^{15}N around −65 ppm is characteristic for platinum coordination complexes with NH_3 and Cl ligands.[12,13] To elucidate the structure of this component, we analyzed the nanocapsules by ESI-ToF mass spectrometry.[23] The mass spectrum revealed the presence of the M$^+$ cluster of the chloride-bridged dimer (III) around m/z 564 (Figure 2d) with a characteristic cluster pattern resulting from the isotopes of platinum (^{194}Pt, ^{195}Pt, and ^{196}Pt) and chlorine (^{35}Cl and ^{37}Cl) as predicted from calculation (Figure 2e). The structure of the chloride-bridged dimer (III) is in agreement with the ^{15}N NMR data. The chloride-bridged dimer of cisplatin is not stable. After disruption of the lipid coat by sonication, the core transforms in a mixture of the dichloride and aqua species (data available as Supporting Information). The MAS ^{15}N NMR spectrum of a lyophilized sample of an equilibrated aqueous solution of cisplatin was similar to that of the nanocapsules (data available as Supporting Information), indicating that the composition of the cisplatin precipitates formed upon freezing does not depend on the presence of lipids.

Lipid Organization in Nanocapsules. To elucidate the lipid organization in the coat of nanocapsules, we compared the ^{31}P NMR spectrum of nanocapsules to that of liposomes. The ^{31}P NMR spectrum of the DOPC/DOPS liposomes exhibits a line shape characteristic of a liquid crystalline bilayer[11] and consists of two overlapping signals with resolved high-field peaks and low-field shoulders, one from DOPC with a CSA of 41.7 ppm and the other from DOPS with a CSA of 54.6 ppm (Figure 3a).

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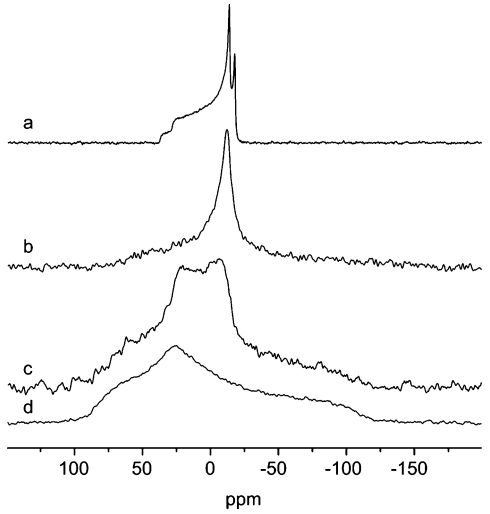

Figure 3. Ordering of the lipid headgroup in the coat of nanocapsules. ^{31}P NMR spectra (a−d) of an equimolar mixture of DOPC/DOPS dispersion, in water (a) and in cisplatin nanocapsules (b, c), and of the crystalline powder of DOPC/DOPS (d). The spectra were recorded by use of spin−echo (a, b) and CP with a contact time of 0.8 ms (c, d).

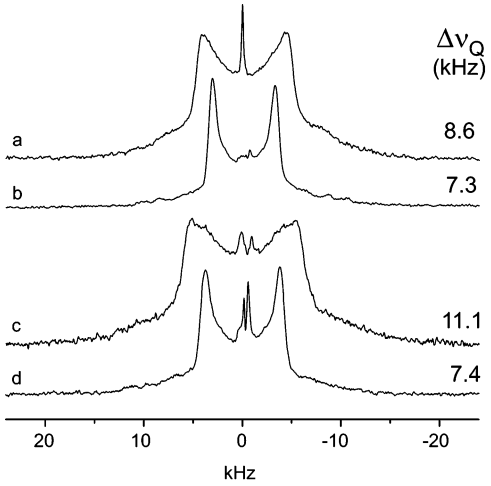

Figure 4. Ordering of the acyl chains in the coat of nanocapsules. ^{2}H NMR spectra of nanocapsules prepared from DOPC-d_4/DOPS (a) and DOPC/DOPS-d_4 (c) mixtures are compared to DOPC-d_4/DOPS (b) and DOPC/DOPS-d_4 (d) aqueous dispersions. Corresponding values of $\Delta\nu_Q$ are indicated.

In contrast, the ^{31}P NMR spectrum of nanocapsules does not show resolved peaks (Figure 3b). Analysis of the spectrum reveals the presence of two overlapping signals. One signal shows a line shape similar to that of the liquid crystalline bilayer with a CSA corresponding to that of DOPC, indicating the presence of mobile phospholipids in the coat of the nanocapsules. The other signal exhibits a broad line shape, which is characteristic of an immobile phospholipid. The line shape of the immobile component is better visualized in the CP-edited ^{31}P NMR spectrum of nanocapsules (Figure 3c), in which the intensity of the immobile component is increased with respect to the mobile component by using a short contact time for cross polarization.[10] The line shape of the immobile component is nearly identical to the spectrum of the crystalline powder of DOPC/DOPS (Figure 3d). The relative content of the immobile component was typically 22−24% as determined by integration of the spin−echo spectrum (Figure 3b). CP ^{31}P NMR spectra recorded with different contact times[9] revealed that the motion of the mobile component in nanocapsules is also restricted compared with the DOPC/DOPS aqueous dispersion (data available as Supporting Information).

To avoid the overlap of lipid signals and to get more insight in the molecular order of DOPC and DOPS in nanocapsules, we applied ^{2}H NMR to deuterium-labeled DOPC-d_4 and DOPS-d_4 with both acyl chains labeled at the 11-position.[15,16] This approach allows the separate detection of the order of these lipids in mixtures with unlabeled lipids. ^{2}H NMR spectra of nanocapsules and aqueous dispersions prepared from DOPC-d_4/DOPS and DOPC/ DOPS-d_4, and the values of $\Delta\nu_Q$, which are directly related to the order parameter,[8] are shown in Figure 4. In mixed DOPC/DOPS bilayers, the values of $\Delta\nu_Q$ of the deuterium labels in DOPC-d_4 and DOPS-d_4 coincide (Figure 4b,d), reflecting similar order and homogeneous mixing of the lipids. In nanocapsules, the values of $\Delta\nu_Q$ are increased for both DOPC-d_4 and DOPS-d_4 compared to those of the aqueous dispersions (Figure 4), reflecting that the order of both lipids is increased in nanocapsules. However, the increase in $\Delta\nu_Q$ is larger for DOPS-d_4 as compared to that of DOPC, indicating a stronger ordering of DOPS due to interaction with the core of the nanocapsules.

DOPS can interact with the positively charged core of the nanocapsules via electrostatic attraction. In addition, up to 50% of DOPS reacts with cisplatin during the preparation of nanocapsules, yielding a stable coordination complex.[17,24] The DOPS−cisplatin complex itself does not account for the observed immobilization of the phospholipids, as the ^{31}P NMR spectrum of bilayers prepared from a 1:1 mixture of DOPC and DOPS−cisplatin complex did not show an immobile component (data available as Supporting Information). The ^{31}P NMR spectrum of DOPC/DOPS liposomes in the presence of an aqueous solution of cisplatin does not contain an immobilized component either (data available as Supporting Information). These data indicate that the interaction of DOPS and/or the DOPS−cisplatin complex with the solid core of the cisplatin nanocapsules causes the immobilization.

Discussion

Molecular Organization of Cisplatin Nanocapsules. Cisplatin nanocapsules were found to consist of a core of solid cisplatin virtually devoid of free water, as evidenced by the ^{15}N and ^{2}H NMR results, coated by a phospholipid bilayer. This conclusion is in agreement with the correspondence of the experimentally determined cisplatin-to-lipid molar ratio of the nanocapsules of 10−12 (ref 7) to that calculated on the basis of the geometry of the nanocapsules and the solid, water-free nature of the core. When the nanocapsules were modeled as cylinders bounded by two hemispheres, with an average width and length of 46 and 86 nm, respectively,[7] and values for the coat thickness of 5 nm,[25] for the coat density of 1 (ref 25), and for the density of solid cisplatin of 3.7 (Merck Catalog, 2002) were used, the ratio between the numbers of molecules occupying the volumes of the core and of the bilayer coat, respectively, was calculated, yielding a cisplatin-to-lipid molar ratio of 11.

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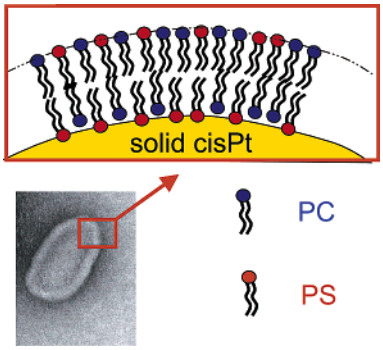

Figure 5. Model for the molecular organization of cisplatin nanocapsules (see Discussion). The negative stain electron micrograph shows a typical nanocapsule.

Analysis of the chemical composition of the core of the nanocapsules by MAS ^{15}N NMR and mass spectrometry revealed that the core consists mainly of the dichloride species of cisplatin with a minor contribution of the chloride-bridged dimer. A similar species distribution was found upon lyophilizing an equilibrated aqueous solution of cisplatin, indicating that there is no selective incorporation of cisplatin species by interaction with the lipids during the preparation of the nanocapsules. Although the aqua species of cisplatin are known to form hydroxo-bridged dimers or trimers,[2,26] the chloride-bridged dimer identified here has not been reported before. We speculate that the chloride-bridged dimer is formed as a result of the increasing concentrations of the dichloride and monoaqua species in the remaining fluid during the freezing of water, which facilitates the reaction between these species.

^{31}P NMR has provided information about the organization of the interface between the cisplatin core and the bilayer coat. The ^{31}P NMR spectra of the nanocapsules revealed the presence of an immobile component corresponding to 22−24% of the phospholipids present. This spectral component most likely originates from the DOPS molecules in the inner leaflet of the bilayer coat, as the ^{2}H NMR data indicate that DOPS is more strongly affected by the interaction with cisplatin than DOPC. The immobilization suggests that the phosphate moiety of these PS molecules is in an anhydrous environment, pointing to a direct interaction between the PS headgroup and the solid core as depicted in the model presented in Figure 5. The immobilization of the phosphate moieties was shown to depend on the interaction of the bilayer with the solid core of the nanocapsules, as it could not be accounted for by the interaction of PC/PS bilayers with cisplatin species in solution nor by the presence of the DOPS−cisplatin coordination complex. We therefore conclude that the arrest of the molecular rotational and lateral diffusion of the PS molecules in the inner leaflet results from electrostatic interaction between the positively charged chloride-bridged dimer at the surface of the core and the negatively charged DOPS and/or from the interaction of the DOPS with the solid core via complex formation.

Apart from the immobilized component, the ^{31}P NMR data revealed a general restriction of motion of the phospholipids in the coat of the nanocapsules as compared to their counterparts in liposomal membranes. The ^{2}H NMR data showed the accompanying increase in ordering of the acyl chains in the bilayer coat of the nanocapsules. This effect was significantly larger for DOPS than for DOPC, due to the stronger interaction of DOPS with the core of nanocapsules discussed above. Contrary to the headgroups, the acyl chains of DOPS in the inner leaflet are not immobilized and still undergo fast motion on the NMR time scale, resulting from acyl chain rotation. Similar spectral changes were also observed in PS/PC bilayers interacting with a positively charged peptide[27] and in dehydrated PC bilayers upon interaction with the polysaccharide fructan.[28] The strong binding of DOPS molecules in the inner leaflet to the solid surface of the core (Figure 5) interferes with the motion of DOPC, resulting in increased lipid ordering. Interestingly, the restriction of motion and increased acyl chain ordering is not confined to the inner leaflet of the bilayer coat but extends to the phospholipids present in the outer leaflet of the bilayer coat. This interlayer coupling was indicated by the NMR line shapes being broadened rather than resolved in separate signals from inner and outer leaflet. The occurrence of increased lipid ordering and interleaflet coupling in bilayers on solid supports has also been observed in bilayers tethered to a solid support.[29,30] In contrast, bilayers deposited on glass beads do not exhibit interlayer coupling,[31] because there is no strong interaction between the lipid molecules and the solid support.

Mechanism of Nanocapsule Formation. The present results have implications for the mechanistic model for the formation of cisplatin nanocapsules, proposed previously.[7] According to that model, cisplatin is concentrated in the residual fluid during freezing, giving rise to nanoprecipitates of dichloro-cisplatin covered by positively charged aqua species. Subsequently, the negatively charged DOPC/DOPS vesicles interact with the positively charged nanoprecipitates and reorganize to wrap them. The resulting nanocapsules do not redissolve upon thawing. The present data infer that the chloride-bridged dimers rather than the aqua species confer positive charge to the nanoprecipitates. Furthermore, the absence of free water molecules in the nanocapsules implies that the bilayer coat forms a very tight cover around the solid cisplatin core. This poses constraints on the flexibility of the bilayer coat. Indeed, replacing the unsaturated DOPC and DOPS by their more rigid saturated counterparts impaired the formation of nanocapsules.[17] At the temperature of nanocapsule formation, a bilayer of DOPC/DOPS is still in the liquid crystalline state whereas the saturated species form a rigid gel-state bilayer,[32] which is not flexible enough to cover the cisplatin nanoprecipitates.

Nanocapsules have a characteristic bean shape with a variable length and a typical diameter of about 40−50 nm.[7] This indicates that the diameter size of the cisplatin nanoprecipitate poses another constraint on nanocapsule formation. In general, small precipitates are expected to be more easily completely covered by a lipid bilayer than large precipitates. However, the intrinsic packing properties of the phospholipid molecules[33] and the tightness of the coat put an upper limit on the surface curvature

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of the cisplatin nanoprecipitates to be wrapped in a phospholipid bilayer. As a result, the nanoprecipitates of cisplatin are not covered by the DOPC/ DOPS bilayer during the formation of nanocapsules, unless their diameter is at least 30−40 nm, which is only slightly larger than the diameter of sonicated vesicles, the most strongly curved bilayers formed by phospholipids.[34] The virtual absence of nanocapsules with larger diameters suggests that the diameter size of the nanoprecipitate is rate-limiting for nanocapsule formation. We speculate that the elongated rather than spherical geometry of the nanocapsules originates from a preferential direction in cisplatin crystal growth under the conditions of preparation.[35] For nanocapsules to form, the interaction between the cisplatin nanoprecipitate and the bilayer coat is crucial. This method of encapsulation may be extended to other compounds if conditions can be created where bilayers can interact with and subsequently enclose growing nanoprecipitates.

Efficacy against Tumor Cells. Analysis of the chemical composition of the cisplatin nanocapsules provides further insight into the reported increased cytotoxicity of the cisplatin nanocapsules toward tumor cells in vitro as compared to that of the free drug.[7] The absence of the reactive and toxic aqua species in the nanocapsules excludes these as primary cause for the improved efficacy. The high antitumor efficacy of a number of dinuclear Pt compounds is well established.[4] However, the newly identified chloride-bridged dimer present in the nanocapsules is unlikely to be responsible for the increased cell killing as it was found to be unstable in water. This leaves the different mechanism of uptake of the nanocapsules compared to that of the free drug as the most likely explanation for the increased in vitro cytotoxicity. Nanocapsules could enter the cell via endocytosis, delivering a high concentration of cisplatin inside the cell (see ref 7).

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Summary and Conclusions

In this paper, we have elucidated the molecular architecture of lipid-coated cisplatin nanocapsules, a novel type of nanostructures. ^{15}N NMR and ^{2}H NMR spectra of nanocapsules containing ^{15}N- and ^{2}H-labeled cisplatin, respectively, demonstrated that the core of the nanocapsules consists of solid cisplatin devoid of free water. Magic-angle spinning ^{15}N NMR showed that ∼90% of the cisplatin in the core is present as the dichloro species. The remaining 10% was accounted for by a newly discovered dinuclear Pt compound that was identified as the positively charged chloride-bridged dimer of cisplatin. The interaction between solid core and bilayer coat exerts a strong ordering effect on the phospholipid molecules. Compared to phospholipids in liposomal membranes, the motion of the phospholipid headgroups is restricted and the ordering of the acyl chains is increased, particularly in PS, which can interact with the positively charged core of the nanocapsules via electrostatic attraction and via formation of a coordination complex with cisplatin. For nanocapsules to form, the interaction between the cisplatin nanoprecipitate and the bilayer coat is crucial. We believe that this method of encapsulation may be extended to other compounds with limited solubility in water, provided that conditions can be created where bilayers can interact with and subsequently enclose a nanoprecipitate.

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Supporting Information Available: Five figures with ^{2}H, ^{15}N, and ^{31}P NMR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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