

LIPID ORGANIZATION IN CISPLATIN NANOCAPSULES: LIPID-COATED AGGREGATES OF CISPLATIN WITH HIGH CYTOTOXICITY

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Cisplatin (cis-diamminedichloroplatinum) is a commonly used anticancer drug. The clinical use of cisplatin faces three major problems: 1) serious dose-limiting toxicities; 2) rapid inactivation of the drug; 3) the frequent occurrence of cisplatin resistance. In general, these problems can be reduced by encapsulating the drug in a liposome. However, the low water solubility and low lipophilicity of cisplatin result in a liposomal formulation with a very low drug-to-lipid ratio. In our laboratory, a new method was developed to efficiently trap cisplatin in a lipid formulation [Chupin, V. *et al.* **J. Am. Chem. Soc.** 126 (2004) 13816]. The method involves hydration of a dry lipid film composed of equimolar amounts of zwitterionic 1,2-dioleoylphosphatidylcholine (DOPC) and negatively charged 1,2-dioleoylphosphatidylserine (DOPS), with a concentrated solution of cisplatin in water followed by 10 freeze-thaw cycles. Electron microscopy examination revealed the presence of nanocapsules of cisplatin – small electron-dense particles containing precipitates of cisplatin and coated by a lipid bilayer. The nanocapsules have an unprecedented drug-to-lipid ratio and an *in vitro* cytotoxicity up to 1000-fold higher than the free drug.

Nanocapsule formation requires the presence of both negatively charged lipids and positively charged species of cisplatin, indicating that electrostatic interactions play an important role. A saturated solution of cisplatin in H₂O contains a mixture of the neutral dichloride- and hydroxo-species of cisplatin and positively charged aquo-species of cisplatin. Based on these data the following mechanism for the formation of nanocapsules was proposed. During freezing, solutes are excluded from the expanding ice phase. The solubility limit of the neutral species of cisplatin is exceeded first and small aggregates form, which subsequently are covered by positively charged aquo-species of cisplatin. The positively charged cisplatin aggregates interact with the negatively charged lipid vesicles, and membranes reorganize to cover the surface of aggregates resulting in nanocapsules. Only those aggregates of cisplatin that are completely covered by lipid do not redissolve upon thawing.

To investigate the lipid organization in the coat of nanocapsules we used ³¹P NMR spectroscopy. The ³¹P NMR spectrum of nanocapsules significantly differs from the spectrum of a DOPC:DOPS dispersion. The ³¹P NMR spectrum of the DOPC:DOPS aqueous dispersion consists of two resolved signals, one of DOPC

with a chemical shift anisotropy (csa) of 38 ppm and another of DOPS with a csa of 51 ppm. Both signals have a line shape, which is characteristic of a liquid crystalline bilayer. In contrast, the ^{31}P NMR spectrum of nanocapsules consists of two signals with different line shapes, indicating the presence of two phospholipid phases in the bilayer coat of the nanocapsules. The analysis of the nanocapsules by ^{31}P NMR will be presented and the role of the interactions between the aquo-species of cisplatin and DOPS in the formation of nanocapsules will be discussed.