1H NMR Determination of Liposomal Encapsulation Efficiency

X. M. Zhang[1], A. B. Patel[1], R. A. de Graaf[1], K. L. Behar[1]

[1]Yale University School of Medicine, New Haven, CT, United States

Synopsis

The liposome is a useful model system to study effects of cellular and subcellular compartmentation on NMR parameters of metabolites measured in vivo. A simple ^1H NMR method was developed to determine the encapsulated and unencapsulated metabolite markers in liposomes under chemically defined conditions without the need to physically separate the internal and external components. The method relies on the use of the pH-sensitive chemical shift of a marker metabolite while maintaining a stable pH difference between internal and external environments. Comparison of the encapsulation efficiencies determined for various sized liposomes with a chemical shift reagent gave remarkable similar results.

Introduction

Encapsulation efficiency is a key parameter for the characterization and optimization of liposomes as a drug and gene delivery vehicle. The majority of reported experimental methods to determine liposome encapsulation efficiency involves quantitative measurements of the encapsulated marker inside the vesicles after removal of the free (unencapsulated) marker using column chromatography, centrifugation, or dialysis (1).

Method

Multilammellar vesicle liposomal dispersion was prepared according to the dry film hydration procedure with DPPC phospholipid. The aqueous medium is phosphate buffer solution with pH of 7.5. The unilammellar vesicle liposomal dispersion was prepared from the multilammellar liposomal dispersion through the repetitive freeze-thawed cycles and extrusion through polycarbonate membranes with the desired pore size (2). ^1H NMR was performed at 11.7 Tesla (AM-500 NMR Bruker Spectrometer).

Results and Discussion

The proton resonances for the internal and external homocarnosine marker in different pH media are shown in Figure 1. The chemical shift for the external peak was found to shift downfield from 6.98 to 7.13 ppm when the pH value of the external medium was changed from 8.0 to 6.5. Thulium (III)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra-(methylene phosphonic acid) ($TmDOTP^{5-}$) was also found to shift the proton resonances of the external homocarnosine from that of the internal homocarnosine as shown in Figure 2. The chemical shift change caused by addition of $TmDOTP^{5-}$ was linearly correlated with the shift reagent concentration with a slope of 0.133 ppm/mM (R^2=0.998), indicating that 0.3 mM of $TmDOTP^{5-}$ was sufficient to resolve the two resonances without significant line broadening.

The encapsulation efficiencies determined directly from the separated internal and external proton resonance integrations are summarized in Table 1 for the DPPC liposomes prepared by extrusion through 50, 200 and 1000 nm polycarbonate membranes. Examination of Table 1 showed that the encapsulation efficiency increases as the liposome size increases, and the results from the two different experimental approaches were remarkably consistent with each other.

Table 1. Encapsulation Efficiencies[a]

Liposomal Dispersion[b]	pH Gradient		0.3 mM ($TmDOTP^{5-}$)	
	Entrapped. %[c]	µl/µmol[d]	Entrapped, %[c]	µl/µmol[d]
50 nm	5.8±0.3	0.83±0.04	5.8±0.3	0.832±0.04
200 nm	12.6±0.6	1.80±0.10	12.3±0.6	1.76±0.10
1000 nm	20.1±1.0	2.87±0.15	20.6±1.0	2.94±0.15

[a]The total lipid concentration was 51 mg/ml. [b]The pore size of the polycarbonate membranes. [c]Encapsulation efficiency in percentage [100 x $I_{int}/(I_{int}+I_{ext})$). [d]Encapsulation efficiency in µl/µmol.

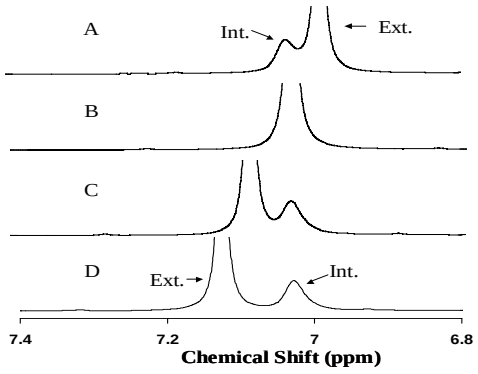

Figure 1. Ring proton resonances for the internal and external homocarnosine in different pH media. A: pH=8.00; B: pH=7.5; C: pH=7.15; D: pH=6.8.

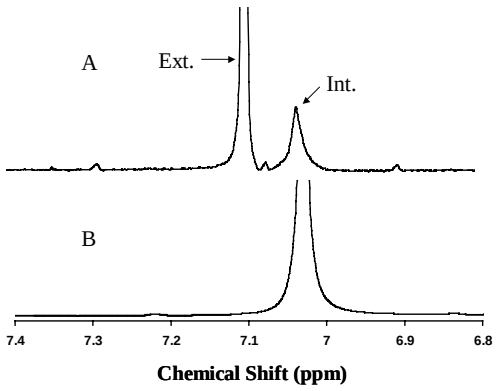

Figure 2. Ring proton resonance for the internal and external marker (A) with 0.3 mM shift reagent ($TmDOTP^{5-}$); (B) without the shift reagent.

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