

Fig. 6. The Surface Hydration of Cationic Liposomes Containing Cationic Lipids with Carbamate or Amido Linker as Monitored by Laurdan Generalized Polarization (GP) in PBS

per Tween 80 would be much higher than those of MEL-A. The strong wetting ability of Tween 80 on MHAPC- and OH-Chol-liposomes might mask the positive charge of liposomes, thereby slightly decreasing the zeta potentials (Fig. 2).

Of great interest are the different behaviors of MEL-A on the hydration levels of MHAPC- and OH-Chol-liposomes. As shown in Fig. 5, MEL-A hydrated MHAPC-liposomes while it dehydrated OH-Chol-liposomes with an increased MEL-A amount. MEL-A always showed its wetting ability on MHAPC-liposomes since there was no hydrogen bond interaction with MHAPC. However, the interaction of sugars with lipid membranes may reflect on the dehydrating phenomenon of MEL-A on OH-Chol-liposomes.^{28,29} According to the water replacement hypothesis, sugars interact with lipid headgroups through hydrogen bonds and exclude water molecules from the lipid membrane, maintaining the "dry" membranes in a physical state similar to that seen in the presence of water.³⁰ In our case, the hydroxyl groups in MEL-A would form hydrogen bonds with the secondary amine of OH-Chol, thereby excluding water molecules from the liposomal surface and finally dehydrating OH-Chol-liposomes.

Cellular Association of Liposomes The cellular association of liposomes in A549 cells was studied to establish a relationship with zeta potentials and hydration levels of liposomes. As shown in Fig. 7, MHAPC-liposomes demonstrated much higher cellular association of liposomes than OH-Chol-liposomes. This phenomenon corresponded well with the higher zeta potentials (Fig. 2A) and "drier" surface (Fig. 5A) of MHAPC-liposomes as compared to OH-Chol-liposomes. Modification by MEL-A and Tween 80 could diminish the cellular association of both MHAPC- and OH-Chol-liposomes in PBS. We suppose that the relative hydrated surface of MEL-A-modified MHAPC-liposomes (Fig. 5A) may be responsible for the slightly decreased cellular association, since the zeta potentials of MEL-A-modified and -non-modified MHAPC-liposomes are similar (Fig. 2A). Modification of MHAPC-liposomes by Tween 80 resulted in a slightly wet surface (Fig. 5A) and lower positive charge (Fig. 2A), which may be reasons for the extremely low cellular association. In terms of the cellular association of OH-Chol formulations (Fig. 7), although MEL-A modification also decreased cellular association, it showed significant higher association than the Tween 80-modified formulation.

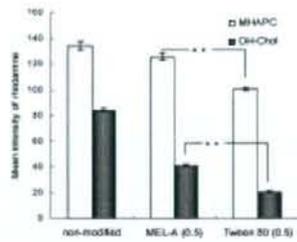


Fig. 7. Cellular Association of Rhodamine-Labeled Liposomes (+/- = 3/1) in A549 Cells Incubated for 2 h in PBS

Each result represents the mean \pm S.D. ($n=3$), ** $p < 0.01$ (Student's t test).

The decreased cellular association of MEL-A modified OH-Chol-liposomes was mainly due to the strongly negative zeta potential (Fig. 2B) as compared to non-modified formulations, however MEL-A modification significantly increased gene transfection efficiency of OH-Chol-liposomes. It might be due to membrane fusion between plasma membrane and cationic liposomes induced by MEL-A.^{12,14-17} It is hard to image how MEL-A-modified OH-Chol-liposomes exhibited higher cellular association than Tween 80-modified OH-Chol-liposomes, since the former had much lower zeta potential (Fig. 2B); however, the relatively dehydrated surface of MEL-A-modified OH-Chol-liposomes (Fig. 5B) seemed to compensate for the negative zeta potential of the interaction with the cell membrane and exhibited higher cellular association of liposomes than Tween 80-modified OH-Chol-liposomes.

Summary

The higher positive zeta potential and greater dehydrated surfaces may relate to the higher cellular association of MHAPC-liposomes than OH-Chol-liposomes. Modification by MEL-A and Tween 80 demonstrated that the tertiary amine in MHAPC was relatively "stable" for the zeta potential and surface pH, while the secondary amine in OH-Chol was greatly affected. Tween 80 had high wetting ability independent of cationic lipids, while MEL-A dehydrated the liposomal surface of OH-Chol-liposomes, possibly by hydrogen bond interaction with the secondary amine. The dehydration of MEL-A-modified OH-Chol-liposomes might compensate for the negative zeta potential for its cellular association of liposomes. We found that the hydrated surface of liposomes may be less effective than the dehydrated surface in the cellular association of liposomes.

Acknowledgments This work was supported in part by a grant for research on Regulatory Science of Pharmaceuticals and Medical Devices from the Ministry of Health, Labor and Welfare, and by the Open Research Center Project. The authors wish to thank Prof. Tsuneji Nagai for his valuable suggestions and The Nagai Foundation Tokyo for supporting Dr. Ding in a post-doctoral fellowship. We thank Prof. Gert Storm (UIPS, Utrecht University) for his advice on the experiments.

References

- 1) Miller A. D., *Curr. Med. Chem.*, **10**, 1195-1211 (2003).
- 2) Chesnoy S., Huang L., *Annu. Rev. Biophys. Biomol. Struct.*, **29**, 27-47 (2000).
- 3) Han S. E., Kang H., Shim G. Y., Suh M. S., Kim S. J., Kim J. S., Oh Y. K., *Int. J. Pharm.*, **353**, 260-269 (2008).

- 4) Takauchi K, Ishihara M, Kawazura C, Noji M, Furuno T, Nakanishi M. *FEBS Lett.*, **397**, 207–209 (1996).
- 5) Ding W, Hattori Y, Higashiyama K, Maitani Y. *Int. J. Pharm.*, **354**, 196–203 (2008).
- 6) Maitani Y, Yano S, Hattori Y, Furuhata M, Hayashi K. *J. Liposome Res.*, **16**, 359–372 (2006).
- 7) Felisek J, Gaedke L, DeRouchey J, Walker G. F., Nikol S, Wagner E. *J. Gene Med.*, **8**, 186–197 (2006).
- 8) Almoffi M. R., Harashima H., Shinohara Y., Almoffi A., Baba Y., Kiwada H. *Arch. Biochem. Biophys.*, **410**, 246–253 (2003).
- 9) Hirsch-Lerner D, Barenholz Y. *Biochim. Biophys. Acta*, **1461**, 47–57 (1999).
- 10) Luciani P, Bombelli C, Colone M, Giassanti L., Rytönen S. J., Saily V. M., Mancini G., Kinnunen P. K. *Biomacromolecules*, **8**, 1999–2003 (2007).
- 11) Meidan V M, Cohen J S, Amariglio N, Hirsch-Lerner D, Barenholz Y. *Biochim. Biophys. Acta*, **1464**, 251–261 (2000).
- 12) Ding W, Izumiya T, Hattori Y, Qi X, Kitamoto D, Maitani Y. *Int. J. Pharm.*, **32**, 311–315 (2009).
- 13) Kitamoto D, Yanagisawa H, Haraya K., Kitamoto H.K. *Biotechnol. Lett.*, **20**, 813–818 (1998).
- 14) Inoh Y, Kitamoto D, Hirashima N, Nakanishi M. *Biochim. Biophys. Res. Commun.*, **289**, 57–61 (2001).
- 15) Inoh Y, Kitamoto D, Hirashima N, Nakanishi M. *J. Controlled Release*, **94**, 423–431 (2004).
- 16) Ueno Y, Hirashima N, Inoh Y, Furuno T, Nakanishi M. *Int. J. Pharm.*, **30**, 169–172 (2007).
- 17) Ueno Y, Inoh Y, Furuno T, Hirashima N, Kitamoto D, Nakanishi M. *J. Controlled Release*, **123**, 247–253 (2007).
- 18) Hattori Y, Kubo H, Higashiyama K, Maitani Y. *J. Biomed. Nanotech.*, **1**, 176–184 (2005).
- 19) Igarashi S, Hattori Y, Maitani Y. *J. Controlled Release*, **113**, 361–368 (2006).
- 20) Zaidam N. J., Barenholz Y. *Biochim. Biophys. Acta*, **1329**, 211–222 (1997).
- 21) Parassini T, De Stasio G, Ravagnan G., Rusch R. M., Gratton E. *Biophys. J.*, **60**, 179–189 (1991).
- 22) Zaidam N. J., Barenholz Y., Minsky A. *FEBS Lett.*, **457**, 419–422 (1999).
- 23) Thompson B., Mignot N., Hoffend H., Lamoni D., Seguin J, Nicoluzzi C., de la Figuera N., Kazm R. L., Meng X. Y., Scherman D., Besacodes M. *Biocopy Chem.*, **16**, 608–614 (2005).
- 24) Tirush O, Barenholz Y., Katzhusler J, Prieu A. *Biophys. J.*, **74**, 1371–1379 (1998).
- 25) Song L. Y., Akhong Q. F., Rong Q., Wang Z., Ansell S., Hape M. J., Mei H. *Biochim. Biophys. Acta*, **1558**, 1–13 (2002).
- 26) Iaruelavilli J., Wimmerstrom H. *Nature (London)*, **378**, 219–225 (1996).
- 27) Zaidam N. J., Barenholz Y. *Int. J. Pharm.*, **183**, 43–46 (1999).
- 28) Crowe J. H., Crowe L. M., Carpenter J. F., Awell Wistrom C. *Biochim. J.*, **242**, 1–10 (1987).
- 29) Crowe J. H., Crowe L. M., Carpenter J. F., Rudolph A. S., Austell Wistrom C., Spargo R. J., Anchordoguy T. J. *Biochim. Biophys. Acta*, **947**, 367–384 (1988).
- 30) Caecla C., Hincha D. K. *Biophys. J.*, **90**, 2831–2842 (2006).