pH-Dependent Complexation of Methacryloyloxydecyl Dihydrogen Phosphate (MDP) with Dipalmitoylphosphatidylcholine (DPPC) Liposomes: DSC and NMR Measurements

Seiichiro Fujisawa[1]*, Mariko Ishihara[1] and Yoshinori Kadoma[2]

[1]Meikai University School of Dentistry, 1-1 Keyakidai, Sakado, Saitama 350-0283, [2]Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-Ku, Tokyo 101-0062.

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Abstract

Motivation. Chelatable methacrylates with phosphate groups, such as MDP, have been used as tooth-bonding agents in dentistry [1, 2]. MDP has shown acceptable pulp response and clinical performance. However, the effect of MDP on biological membranes remains unknown. Liposomes have been employed in model systems at the membrane level to study the interaction of lipid-soluble drugs with biological membranes, and DSC and NMR spectroscopy have been used as powerful, yet relatively rapid and inexpensive, techniques for these studies. In the present study, we investigated the DSC phase-transition properties of DPPC liposomes induced by MDP and the changes in NMR chemical shifts of MDP associated with DPPC liposomes at various pH values.

Method. DPPC/MDP (2:1 molar ratio) liposomes were prepared. Phase transition temperature (Tm) and enthalpy of DPPC/MDP liposomes were determined by DSC. ^{1}H-NMR chemical shifts and coupling constants of DPPC/MDP liposomes were investigated in D_2O at pH 2.4 and pH 7.0 at 25, 35 and 50 °C. HOMO, LUMO, and partial charges for COSMO (water) and non-COSMO (vacuum) were calculated by the PM3 semiempirical methods..

Results. The DSC main peak for DPPC/MDP at pH 2.4 was markedly broadened with a considerably decreased enthalpy, whereas a peak with a shoulder at 35 °C appeared at pH 7.0. At pH 2.4, ^{1}H-NMR signals of MDP associated with DPPC liposomes were not found because of shielding, but those of the phosphodiester groups of DPPC were clearly apparent above or below the Tm. In contrast, at pH 7.0, signals of MDP together with those of DPPC appeared at 35 °C, above the Tm. The appearance of signals of MDP associated with DPPC liposomes was dependent on pH and Tm

Conclusions. The DSC and NMR measurements suggest that DPPC-MDP complexation under acidic conditions probably occurs by the formation of hydrogen bonds between un-ionized dihydroxy phosphate groups of MDP and the phosphodiester functions of the DPPC surface. The PM 3 calculations contributed to interpretation of the NMR aspects of the interaction between MDP andliposomes.

Keywords. DPPC liposomes; tooth adhesive material; interaction; NMR chemical shifts; DSC; PM3.

Abbreviations and notations

DPPC: L-α-dipalmitoylphosphatidylcholine; D_2O: deuterium oxide; DSC: differential scanning calorimetry

NMR: nuclear magnetic resonance; HOMO: the highest occupied molecular orbital; LUMO: the lowest unoccupied molecular orbital.

1 INTRODUCTION

MDP has been widely used as a tooth bonding agent in dental adhesive resin restorative systems [1, 2].

The functional properties of this compound include its ability to interact with calcium in the hydroxyapatite around tooth collagen, together with a methacryloyl function for intermolecular polymerization. However, the biological activity of this compound is incompletely understood. In studies of the mechanisms of biological activity, liposomes formed from natural or synthetic phospholipids are widely used as models for biological membranes [3, 4]. We have previously used DSC and NMR spectroscopy to phase-transition properties and NMR chemical shifts are valid indicators of interactions between biological membranes and dental materials such as methacrylates. Specifically, we have previously investigated the interaction of MDP with DPPC liposomes as assessed by DSC and NMR spectroscopy, but the mechanism of the interaction remained unknown [6]. The application of computational chemistry to dental materials is an exciting and rapidly expanding area [7]. We recently used the PM 3 semiempirical calculation method to interpret the mechanism of interaction between methacrylates and liposomes, suggesting that descriptors such as LUMO and HOMO energies and partial charges are useful tools for the interpretation of experimental results [8]. In the light of these recent developments, we have further investigated by ^{1}H-NMR spectroscopy the interaction between MDP and DPPC liposomes, relating the descriptors calculated by PM 3 methods to the ^{1}H NMR chemical shifts of MDP associated with DPPC liposomes at various pH values.

2 MATERIALS AND METHODS

2.1.1 Chemicals

The following chemicals and reagents were obtained from the indicated companies: DPPC, Sigma Chemical Co., St. Louis, Mo., USA; D_2O, NaOD, KD_2PO_4, $CDCl_3$, and 3-(trimethylsilyl)propionic acid sodium salt-d_4 (TMSPA), Merck Chemical Co., Darmstadt, Germany.

2.1.2 Synthesis of MDP

10-Hydroxydecyl methacrylate was prepared from 1,10-decanediol and methacryloyl chloride. The product was isolated by column chromatography on silica gel. The purified alcohol and triethylamine were slowly added to phosphorous oxychloride. After the termination of the reaction, the mixture was extracted with ether and the solvent was evaporated. The resulting viscous MDP was washed several times with *n*-hexane and dried (yield about 74%). The purity of the MDP was verified by NMR and infrared spectroscopy [9]. The chemical structure of MDP, with significant atoms or groups identified by letters, is shown in Fig. 1A.

^{1}H-NMR in D_2O buffer at pH 7.0 (δ, ppm): 1.31 (f; 12H, br s), 1.64 (e and g; 4H, m), 1.92 (c; 3H, s), 3.84 (h; 2H, double t, J = 6.60, 6.60 Hz), 4.17 (d; 2H, t, J = 6.6 Hz), 5.67 (a; 1H, br s), 6.10 (b; 1H, br s).

^{1}H-NMR in $CDCl_3$ (δ, ppm): 1.29 (12H, br s), 1.67 (4H, m), 1.94 (3H, m), 4.03 (2H, double t, J = 6.59, 6.60 Hz), 4.14 (2H, t, J = 6.60 Hz), 5.55 (1H, m), 6.10 (1H, m), 8.61 (OH).

2.1.3 Preparation of multilamellar liposomes

An appropriate amount of DPPC was dissolved in chloroform and dried under vacuum. MDP was added to the dried lipid film, and the mixture was dispersed in D_2O by vortex-mixing on a Vortex shaker at 45 °C for 2-3 min followed by sonication under a nitrogen atmosphere for 10 min at 45 °C. The molar ratio of DPPC to MDP was 0.5. Suspensions containing approximately 10% DPPC liposomes were prepared.

2.1.4 NMR spectroscopy

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^{1}H-NMR spectra were measured at 25, 30, 35 and 50°C with JEOL (Tokyo, Japan) JNM-GX270 or ALPHA 500 instruments at resolutions of 0.01 ppm and 0.0013 ppm, respectively.

2.1.5 pH measurement

pH was measured with a COM-8 pH meter (Denki Kagaku Keiki Co., Ltd, Tokyo, Japan). A solution of 0.1M KD_2PO_4/D_2O was adjusted to pH 7.0.

2.1.6 DSC studies

Samples for NMR measurements were used. Each 10-microL sample was sealed in a DSC specimen container. The samples were scanned in a sealed container on a DSC-Rigaku calorimeter (Rigaku Denki Co., Ltd, Tokyo, Japan) operating at a heating rate of 5 °C /min with a range setting of 0.5 mcal/s. Measurement of enthalpy was performed as described previously [5].

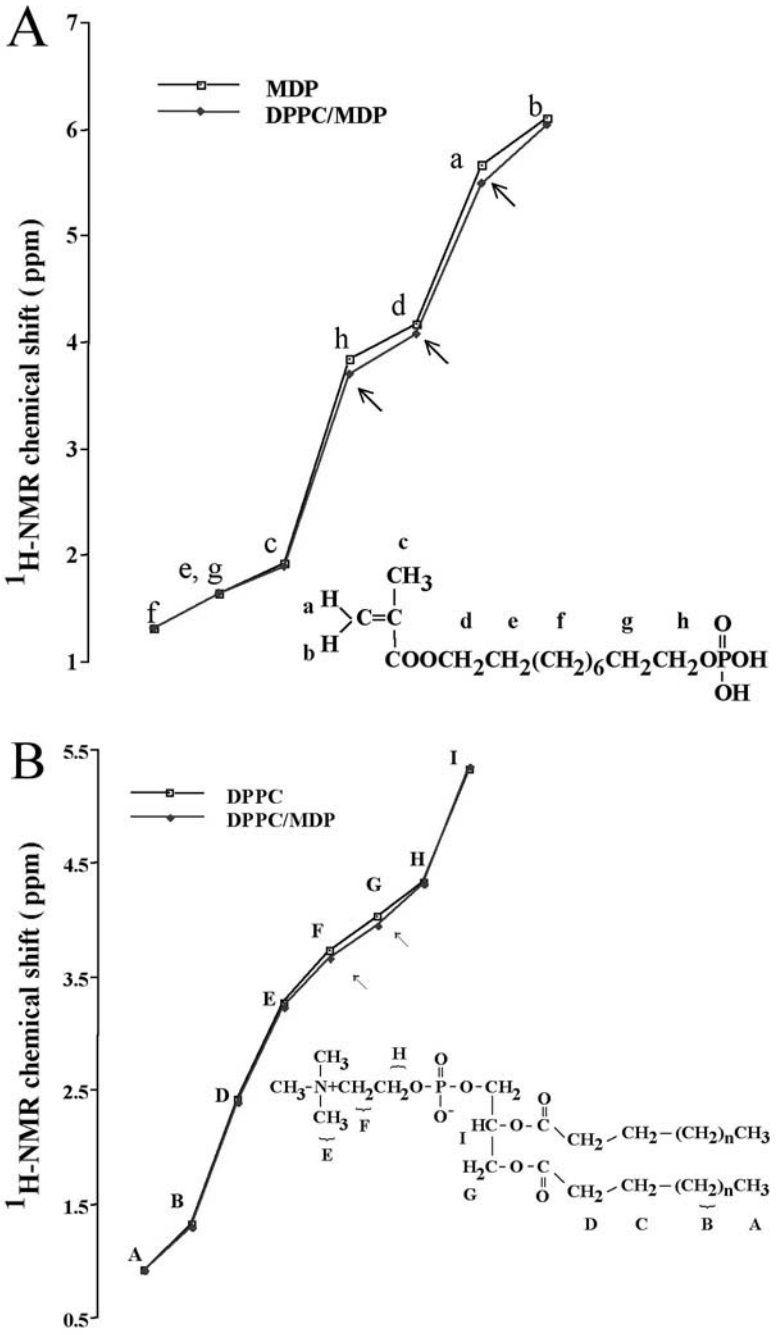
2.1.7 PM3 calculation

Theoretical calculations were carried out by the restricted Hartree-Fock level (RHF) PM3 semiempirical method, as implemented in the MOPAC program on a Tektronix Cache Workstation (version 3.8). Solvent effects for MDP were calculated using the COSMO (Conductor-like Screening Model) methodology. For the dielectric constant of the medium, we assumed water (e = 78.4) as solvent. HOMO, LUMO, and partial charges for COSMO and non-COSMO (vacuum) were calculated by the PM 3 semiempirical method.

2.2 Chemical Data

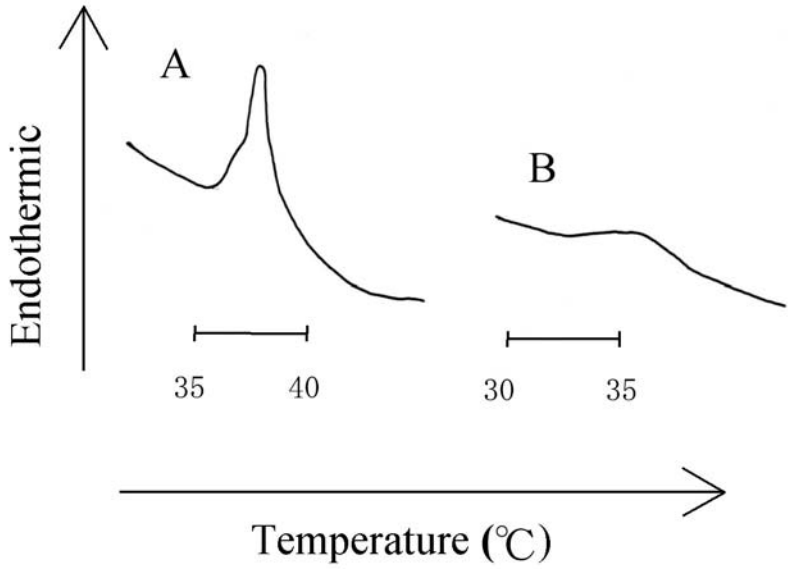
2.2 .1Chemical shift difference

^{1}H-NMR chemical shifts of MDP alone versus DPPC/MDP (2:1) liposomes (A), and of DPPC liposomes versus DPPC/MDP (2:1) liposomes (B) (Fig. 1). Letters correspond to significant atom or groups, as shown on the chemical structurea in the insets.→indicates a significant difference.



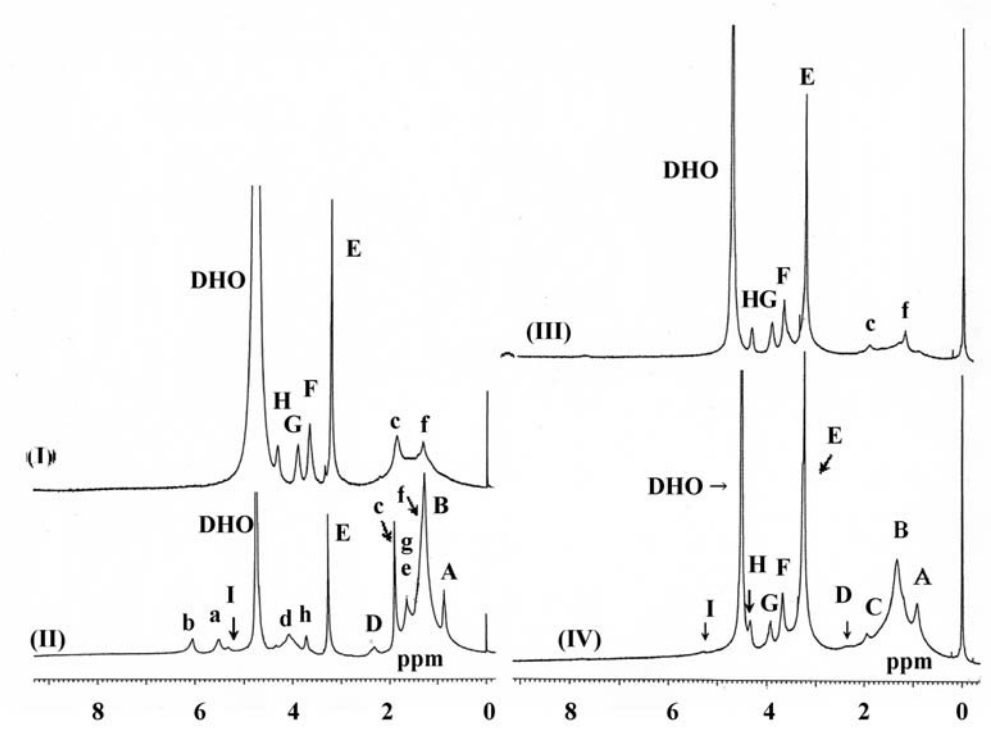
2.2.2 DSC curves

DSC curves for DPPC/MDP (2:1) liposomes at pH 7.0 (A) and pH 2.4 (B) (Fig.2).



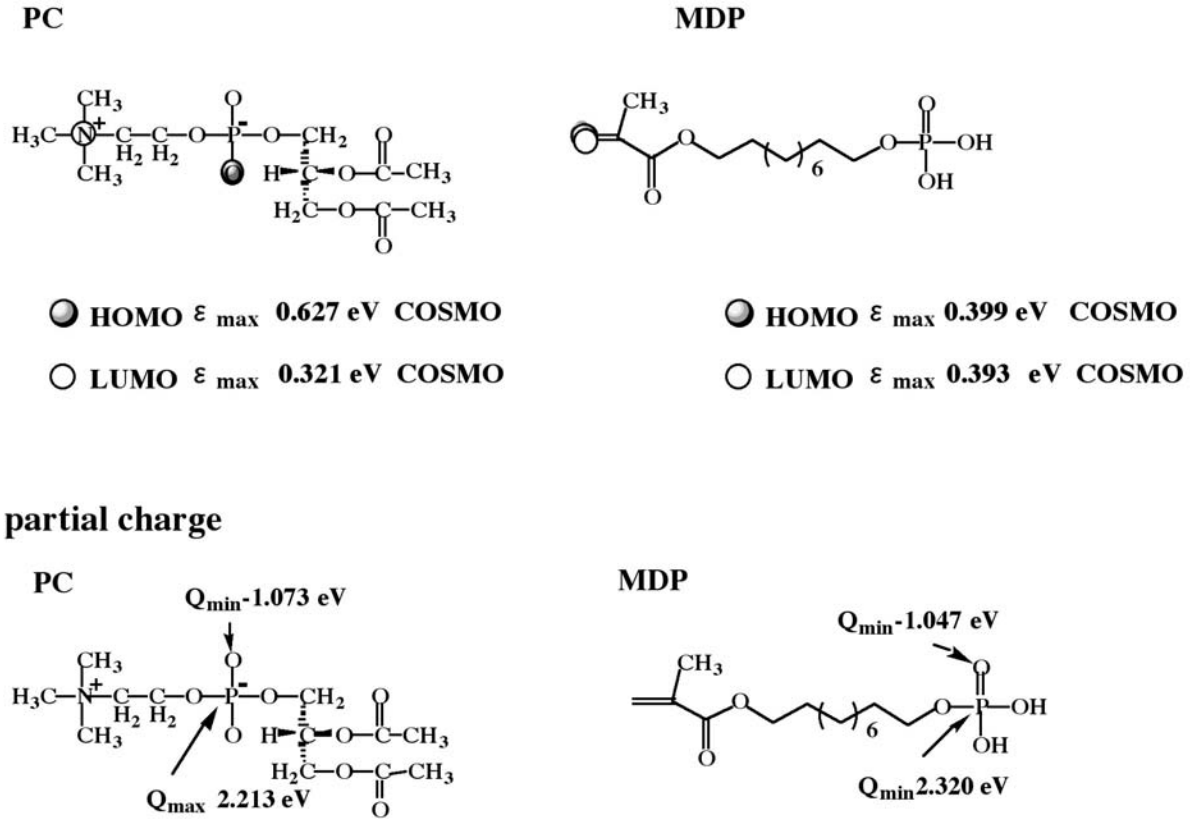
2.2.3 NMR spectra

^{1}H-NMR spectra of DPPC/MDP (2:1) liposomes at pH 7.0 at 25 °C (I) and at 35°C (II) , and at pH 2.4 (30 °C (III) and at 50 °C (IV) (Fig. 3). For identication of signals, see Fig.1.



2.3 4 Computational data

HOMO, LUMO, and partial charge for PC and MDP (Fig. 4).



3 RESULTS AND DISCUSSION

The samples of DPPC/MDP (2:1, molar ratio) liposomes in D_2O or D_2O buffer (pH 7.0) that were assayed by NMR spectroscopy were also scanned by DSC. The DSC curves are shown in Fig. 2. The pH (pD) of DPPC/MDP liposomes in D_2O was approximately 2.4, and the DSC curve of this sample was markedly broad, suggesting that disruption of the bilayers occurs at low pH values with consequent reduction of enthalpy to zero. In contrast, in D_2O buffer at pH 7.0, the DPPC/MDP liposome sample showed an endothermic peak with a shoulder. The main Tm for DPPC/MDP at pH 7.0 was 35.0 °C and its enthalpy was approximately 8.20 kcal/mol, which was about 10% less than that of the control (Tm 41.0 °C and enthalpy approximately 8.9 kcal/mol) [5]. It is clear from this finding of a markedly broadened DSC peak that DPPC-MDP complexation occurs at low pH values.

^{1}H-NMR spectra of MDP associated with DPPC liposomes in D_2O or D_2O buffer (pH 7.0) at 25, 30, 35 and 50 °C are shown in Fig 3. Chemical shift differences between MDP or DPPC liposomes and DPPC/MDP liposomes are summarized in Figs 1A and B.

In the NMR spectrum of DPPC/MDP liposomes at pH 7.0 at 25 °C (I), only broad signals for c and e of MDP molecules were apparent, and other proton signals for MDP were not found. In contrast, the signals for E, F, G and H of the phosphodiester groups of DPPC molecules appeared clearly at 25 °C, but signals H, G and F were abolished at 35 °C (II). At this temperature, the signals of acyl chains (B) and terminal methyls (A) appeared together with signals for MDP (II). As shown in Fig.

2, DPPC/MDP liposomes exist in the liquid phase above 35 °C. At this temperature, MDP incorporated into lipid bilayers could appear at the surface of liposome membranes as a result of their enhanced fluidity and mobility. Fig. 1A shows considerably greater up-field shifting in DPPC/MDP liposomes of the proton signals (a, b, d) of the methacryloyloxy group of MDP, H_2C=C-$COOCH_2$-, compared with other MDP signals (c, e, g and f), which suggests an extensive interaction between the methacryloyloxy groups of MDP and the phosphodiester groups of DPPC. The signal of h was also significantly shifted up-field, possibly due to the effect of the adjacent dihydrogen phosphate group. On the other hand, in D_2O at 30 °C at pH 2.4 (III), only high-intensity signals for c and f of MDP were found whereas, conversely, signals for the phosphodiester group of DPPC (F, G, H and E) were clearly apparent. At 50 °C (IV), no signals for MDP were found, whereas signals for DPPC (A, B, C, D, E, F, G, H and I) were observed together with a clear increase in the fluidity of acyl chains (signal B). Fig 1B shows the chemical shift differences for DPPC molecules between DPPC liposomes and DPPC/MDP liposomes in D_2O at pH 2.4, with a particular increase in up-field shifting of the F and G signals in DPPC/MDP liposomes compared with the corresponding signals in DPPC liposomes.

It is clear from the present findings that MDP associated with DPPC liposomes is extensively incorporated into DPPC lipid bilayers under acidic conditions. The broad DSC peak of DPPC/MDP liposomes at pH 2.4 suggests the formation of mixed DPPC-MDP micelles or other aggregates. In general, it has been suggested that the attractive force for DPPC-MDP interaction may arise from (1) hydrogen bonding involving un-ionized phosphate groups of MDP and from (2) consequently increased hydrophobic interactions between the hydrocarbon portion (decyl methylenes) of MDP and the acyl chains of DPPC. Charge-dipole interactions are probably of restricted importance for a minor driving force for MDP-DPPC interactions. Note: As is known, the C = O group in DPPC or MDP molecules has a permanent electric dipole. The dipole moment for DPPC and MDP was computed by the CAChe Ext Heuckel application, indicating that the moment for corresponding compounds is 18.5 deby and 6.41 deby, respectively. Hence, the C = O groups in DPPC may create some additional dipole-dipole interaction between adjacent phospholipids or MDP.

The appearance of a shoulder on the DSC curve for DPPC/MDP at pH 7.0 shifted the Tm to a lower temperature. The formation of such a shoulder for 2,4-dinitrophenols was previously reported to be due to an interaction occurring at the surface between hydrophobic and hydrophilic domains in the glycerol backbone of lipids [4].

We could not calculate the HOMO and LUMO energies for DPPC with large acyl chains by the PM 3 method, because the DPPC molecule is too large, and therefore we performed the calculation for phosphatidylcholine (PC) with α-methylene groups instead of DPPC, as shown in Fig. 4. The PM 3 calculation in COSMO suggested that the HOMO for DPPC probably interacts preferentially with the LUMO for MDP because of the lower energy between PC_{HOMO} and MDP_{LUMO}; the difference of energy for [$PC\varepsilon_{HOMO}$ (-10.760 eV) - $MDP\varepsilon_{LUMO}$ (-0.274 ev)] is 10.486 ev, whereas that for [$PC\varepsilon_{LUMO}$ (0.123 eV) - $MDP\varepsilon_{HOMO}$ (-10.506 ev)] is 10.629 ev. The relationships between HOMO and LUMO for the DPPC/MDP interaction were similar in both COSMO and non-COSMO. Additionally, partial charges for the MDP molecule were calculated in COSMO. The charges for the most negative atom and for the most positive atom (Q_{min} and Q_{max}, respectively) in the MDP and PC molecules in COSMO are associated with the P atoms, and their values in both compounds are similar. On the basis of the relationships between HOMO and LUMO for each molecule, the beta-carbon in the methacryloyloxy group in MDP preferentially interacts with the phosphodiester function on the DPPC bilayer surface, as shown in Fig. 3. Therefore, signals, a, b and c were strongly shifted up-field or were shielded in DPPC/MDP liposomes. Also, the chemical shift differences for DPPC molecules between DPPC and DPPC/MDP liposomes in D_2O at pH 2.4 showed strong up-field shifting of F (N-CH_2) and G (glycerol CH_2), which are located in the

phosphodiester head function, compared with the corresponding signals in DPPC liposomes. This agrees with the findings of the HOMO-LUMO calculations.

4 CONCLUSIONS

The complexation of DPPC-MDP is strongly dependent on the pH of medium. The strong complexation occurred at pH 2.4, resulting from the broadening of DSC peak of DPPC/MDP liposomes and the shielding of proton signals for MDP. A driving force for complexation is suggested to be the formation of hydrogen bonds between un-ionized MDP and the phosphodiester functions of DPPC surface at a low pH. The NMR chemical shifts of MDP associated with DPPC liposomes could be interpreted in terms of molecular descriptors obtained from PM 3 calculations.

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Biographies

Dr. S. Fujisawa is Professor of Dentistry and Chairman of the Department of Oral Diagnosis at Meikai University. His major research projects are the free radical mechanisms of toxicity, catalysis and protection. Dr. M. Ishihara is a chemist and lecturer at Meikai University School of Dentistry. Dr. Y. Kadoma is a chemist and Associate Professor at Tokyo Medical and Dental University Institute of Biomaterials and Bioengineering. His major research projects are preparation and evaluation of synthetic medical polymers**.**