Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Inorganic Biochemistry 98 (2004) 925–930
JOURNAL OF Inorganic Biochemistry
www.elsevier.com/locate/jinorgbio

Mannose–*Escherichia coli* interaction in the presence of metal cations studied in vitro by colorimetric polydiacetylene/glycolipid liposomes

Chunyan Sun [a], Yuanjian Zhang [a], Yi Fan [b], Yajun Li [b], Jinghong Li [a,*]

[a] *State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China*

[b] *Laboratory of Excited State Process, Changchun Institute of Optic Fine Mechanics and Physics, Chinese Academy of Sciences, Changchun 130021, PR China*

Received 8 December 2003; received in revised form 11 March 2004; accepted 18 March 2004
Available online 22 April 2004

Abstract

Supramolecular assemblies of liposomes (vesicles) made of diacetylenic lipids and synthetic mannoside derivative glycolipid receptors were successfully used to mimic the molecular recognition occurring between mannose and *Escherichia coli*. This specific molecular recognition was translated into visible blue-to-red color transition (biochromism) of the polymerized liposomes, readily quantified by UV–visible spectroscopy. Some transition metal cations (Cd^{2+}, Ag^{+}, Cu^{2+}, Fe^{3+}, Zn^{2+} and Ni^{2+}) and alkali earth metal cations (Ca^{2+}, Mg^{2+} and Ba^{2+}) were introduced into the system to analyze their effects on specific biochromism. Results showed that the presence of Cd^{2+}, Ag^{+}, Ca^{2+}, Mg^{2+} and Ba^{2+} enhanced biochromism. A possible enhancement mechanism was proposed in the process of bacterial adhesion to host cells. However, Cu^{2+}, Fe^{3+}, Zn^{2+} and Ni^{2+} exhibited inhibitory effects that cooperated with diacetylene lipid with a carboxylic group and increased the rigidity of the liposomal outer leaflet, blocking changes in the side chain conformation and electrical structure of polydiacetylene polymer during biochromism.

Keywords: Polydiacetylene; Mannose; *Escherichia coli*; Metal cations; Liposomes; Molecular recognition

1. Introduction

Cell membranes, with their highly organized and self-assembled structures, perform fundamental cellular functions such as molecular recognition, pumping, gating, energy conversion and signal transduction. The design of ''smart'' materials based on cell membrane structures with specific functional properties is an emerging field of study [1]. Liposomes formed from amphiphilic lipids (either natural or completely synthetic) have spherically closed lamellar structures which enclose an aqueous compartment. Due to these structural features, they are considered the closest analogues of biological cell membranes.

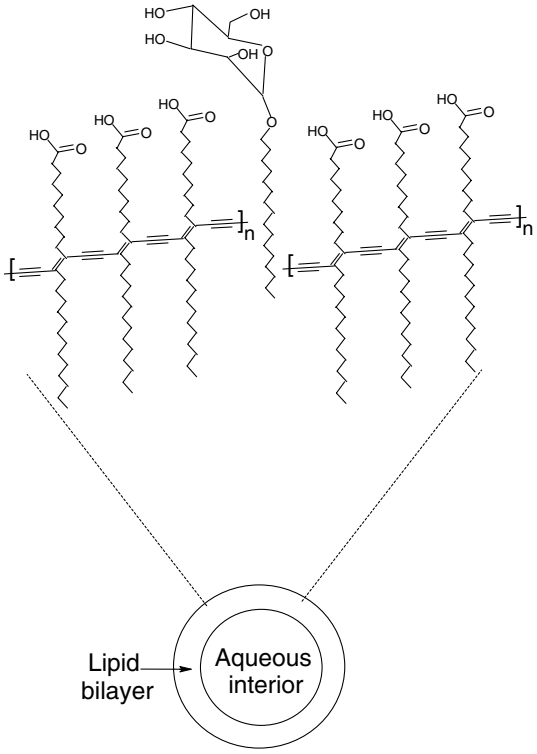
Lipids with a diacetylenic unit have many particular optical and electrical properties. Certain closely packed and properly designed assembly of diacetylene lipids, such as liposomes in solution or thin films on solid supports, can undergo polymerization upon UV irradiation at 254 nm via 1,4-addition reaction to form a blue colored polydiacetylene (PDA) polymer with a conjugated backbone of alternating triple and double bonds [2–4]. The most attractive advantage of using PDA as sensing matrices comes from the fact that a visible color change, from blue to red, occurs in response to changes in a variety of environmental factors, including temperature [5], mechanical stress [6], solvent [7], pH [8], and ligand–receptor interactions [9]. Moreover, PDA-based biosensors for the detection of biologically important species such as microbial toxins [10], oligopeptides [11], antibodies [12] and even bacteria [13] and

* Corresponding author. Tel./fax: +86-431-5262243.
E-mail address: lijingh@ciac.jl.cn (J. Li).

0162-0134/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2004.03.006

viruses [1], have been intensively studied in the last decade. It is well-known that metal cations play essential roles in modulating life actions within the organism. As far as we know, there have been no reports on the effect of metal cations on the bio-recognition between mannose and *Escherichia coli* (*E. coli*) in PDA liposomes.

In this study, we used the mixed liposomes, composed of conjugated polydiacetylene lipids and synthesized glycolipid receptors, as a simulative model to investigate the influence of metal cations on the specific interaction between mannose and *E. coli*. This specific molecular recognition translated into a blue-to-red transition that was quantified by UV–visible absorption spectroscopy. Metal cations included alkali earth metal ions Ca^{2+}, Mg^{2+} and Ba^{2+}, and transition metal cations Cd^{2+}, Ag^{+}, Cu^{2+}, Fe^{3+}, Ni^{2+} and Zn^{2+}. Results exhibited some regularity to a certain degree. The presence of Ca^{2+}, Mg^{2+}, Ba^{2+}, Cd^{2+} and Ag^{+} enhanced the response of PDA-glycolipid liposomes when incubated with *E. coli*. On the contrary, Cu^{2+} and Fe^{3+} inhibited molecular recognition, making the biochromism of the system similar to the background, whereas Ni^{2+} and Zn^{2+} slightly decreased biochromism. These phenomena had been repeated in multiple independent experiments. On the basis of these results, a preliminary mechanism was proposed.



Scheme 1. Schematic diagram of the mixed MC_{16}/PCDA vesicles used for biochromism studies, indicating part of the assembly of glycolipid and polydiacetylene. The conjugated polymer backbones of alternating double and triple bonds constitute the chromatic detection element. Mannose is the receptor for *E. coli*.

2. Materials and methods

2.1. Chemicals

Mannoside derivative (α-D-mannoside-hexadecyl, MC_{16}) (Scheme 1) was obtained from Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, China, and *E. coli K12* was a kind gift from Prof. Fangyu Hou (School of Basic Medicine, Jilin University, China). 10,12-Pentacosadiynoic acid (PCDA), purchased from Farchan Laboratories (USA), was recrystallized from petroleum ether (30–60 °C) twice before use. MC_{16} and PCDA were both dissolved in chloroform (1 mM). All other reagents were of analytical grade without further purification. Water used in the experiment was ultrapure water with a resistivity of 18.3 MΩ/cm.

2.2. Measurements

UV–visible spectra were recorded on a double-beam spectrophotometer UV-360 Spectrometer (Shimadzu, Japan). Resonance Raman (RR) Spectra were measured using a Raman Infinity Spectrophotometer (France) at a resolution of 4 cm^{-1}. A 488-nm line with 50-mW power from an argon ion laser was used as excitation source. A polarizing beam splitter composed of two half-wave plates and a polarizing cube was applied to control the power of the exciting radiation reaching the sample. The polarization of the 488-nm beam was perpendicularly oriented to the entrance slit of the spectrometer in order to generate RR scattering. Liposomes (typically 0.2 ml) were dropped onto a glass. The laser beam was focused to a 30-μm spot on the surface of the sample. To avoid the influence of the photoinduced thermochromism, the sample was set at lower temperature conditions.

2.3. Escherichia coli K12 preparation

Escherichia coli strain *K12* was cultured aerobically at 37 °C for 18 h on solid medium (pH 7.4) in 1 L broth containing 10% peptone, 5% NaCl and 20% agar. The solid medium was warmed up and solved. The autoclave was operated at approximately 15 lb/in^2 (at 121 °C) for 30 min. Bacterial solution was washed with normal saline thrice and centrifuged at 1700*g* for 10 min. Then, it was diluted with physiological saline to the required 9×10^8 cells/ml concentration, which was determined by turbidimetry.

2.4. Liposome formation

Liposomes of a mixture of polymerizable matrix lipid (PCDA) and receptor-containing lipid (MC_{16}) were

obtained by bath sonication. Both lipids (1 mM, in chloroform) were mixed in 0.05 molar ratio of MC_{16} to PCDA. The solvent was removed completely by nitrogen stream, and an equivalent amount of ultrapure water was added to the dried lipids. The suspension was sonicated for 30 min in the water bath at 72 °C and kept warm for 2 h. Then, after it cooled to room temperature, the solution was stored at 4 °C for 48 h to allow liposome formation. Prior to polymerization, the liposome suspension was purged with N_2 for 20 min. Polymerization was carried out at room temperature irradiating the solution with 254 nm UV light (1.8 mW/cm^2) for 285 s. The resulting blue liposome suspension was stored in the dark at 4 °C.

2.5. Colorimetric assay

For biochromism experiments, 0.2-ml *E. coli K12* suspension was added to 1-ml blue polymer vesicles (or vesicles containing metal ions of different concentrations pipetted with a microsyringe) and incubated at 37 °C for 2 min. Biochromism was evaluated by colorimetric response (CR) through UV–visible spectra, which is a quantitative value for the extent of blue-to-red transition [9]. The visible absorption spectrum of the liposome solution was analyzed as $B = I_{blue}/(I_{blue} + I_{red})$, where I is the absorption intensity of the blue and red phases. CR of a liposome solution is defined as the percentage change in B upon exposure to bacteria, $CR(\%) = [(B_0 - B_v)/B_0] \times 100\%$, where subscripts 0 and v represent prior and subsequent exposure to bacteria, respectively.

3. Results and discussion

Polymerized PCDA/MC_{16} liposomes appeared blue which arose from the ene–yne conjugated system that comprised the polymer backbone of polydiacetylene matrix, and exhibited an intense absorption maximum at 670 nm (blue-phase peak) and a weaker absorption at 574 nm (red phase peak) (Fig. 1, curve a). After incubation with *E. coli K12* (dispersed in 0.85% aqueous sodium chloride solution), a dramatic change in the visible absorption spectrum was observed; the blue-phase peak decreased with the concurrent increase of the red-phase peak, and the liposome suspension turned to purple or red depending on the concentration of bacteria (Fig. 1, curve b). No changes in color were observed when physiological saline alone was added to the same liposome suspension. Liposomes remained blue if the ligand lipid was removed from the molecular assembly. It has been demonstrated that this color change resulted from the specific bio-recognition between mannose inserted in bilayer vesicles and *E. coli*.

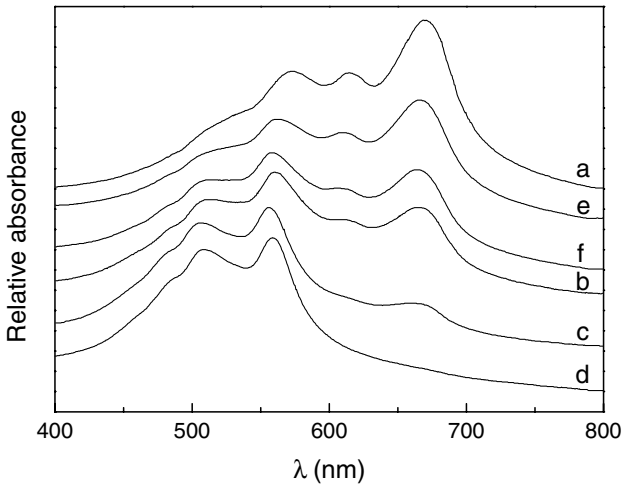


Fig. 1. Visible absorption spectra of PCDA/MC_{16} liposomes. (a) PCDA/MC_{16}; (b) PCDA/MC_{16}-*E. coli*; (c) PCDA/MC_{16}-*E. coli*-$CaCl_2$; (d) PCDA/MC_{16}-*E. coli*-$CdCl_2$; (e) PCDA/MC_{16}-*E. coli*-$CuCl_2$; (f) PCDA/MC_{16}-*E. coli*-$ZnCl_2$. Metal ion concentration was 1.0×10^{-4} M.

In order to examine the effect of metal cations on the biochromism described above, different concentrations of various metal cations were pipetted into the blue liposomes using a microsyringe (the volume of the injected solution could be ignored relative to the liposomes). Basically, the inherent absorption spectrum of original PCDA/MC_{16} liposomes was not affected by the addition of metal cations. However, when incubated with *E. coli*, liposomes containing various metal cations exhibited different signal responses. In the case of those containing 1.0×10^{-4} M Cd^{2+}, the biochromatic transition was highly improved and the system rapidly became carmine. As shown in Fig. 1(d), the blue-phase peak almost vanished and liposomes completely shifted to the red phase, which corresponded to a CR% of 100. CR evolutions in liposomes with and without Cd^{2+} incubated with *E. coli* at different time were compared in Fig. 2. Apparently, biochromism in liposomes containing Cd^{2+} became much more rapid in comparison with vesicles not carrying the metal. Therefore, it could be concluded that the presence of Cd^{2+} in liposomes greatly accelerated bio-recognition at their interfaces. Moreover, it was found that Ca^{2+}, Mg^{2+}, Ba^{2+} and Ag^{+} also enhanced the biochromatic degree of the system. An example can be observed in the UV–visible spectrum of the PCDA/MC_{16}-*E. coli*-Ca^{2+} system in Fig. 1, curve c. On the contrary, other transition metal cations such as Cu^{2+}, Fe^{3+}, Ni^{2+} and Zn^{2+} inhibited biochromism in PCDA/MC_{16} liposomes incubated with *E. coli*. For instance, curves e and f in Fig. 1 correspond to systems of PCDA/MC_{16}-*E. coli*-Cu^{2+} and PCDA/MC_{16}-*E. coli*-Zn^{2+}, respectively.

The influence of all these ions (1.0×10^{-4} M) on CRs of PCDA/MC_{16}-*E. coli* was compared in Fig. 3 (control means PCDA/MC_{16} without adding metal cations). Experimental results exhibited good repeatability and

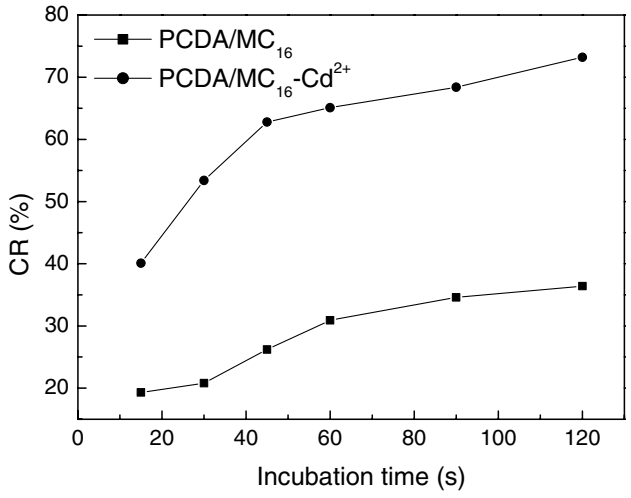


Fig. 2. CRs for $PCDA/MC_{16}$ vesicles and $PCDA/MC_{16}$ vesicles containing 1.0×10^{-5} M $CdCl_2$ vs. bacterial incubation time.

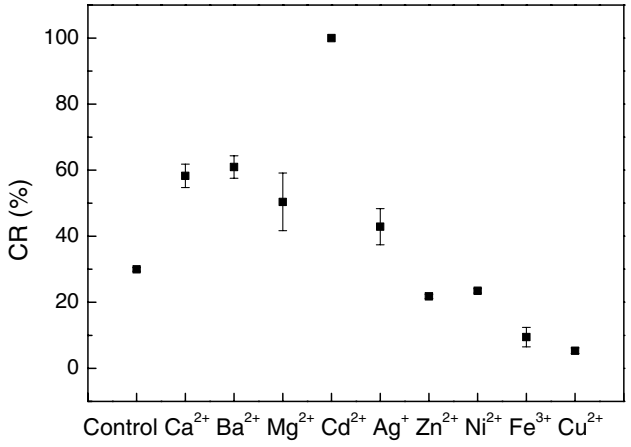


Fig. 3. Effect of the selected metal cations (1.0×10^{-4} M) on CRs of $PCDA/MC_{16}$-*E. coli*. Every CR value was the average of results obtained in three independent experiments.

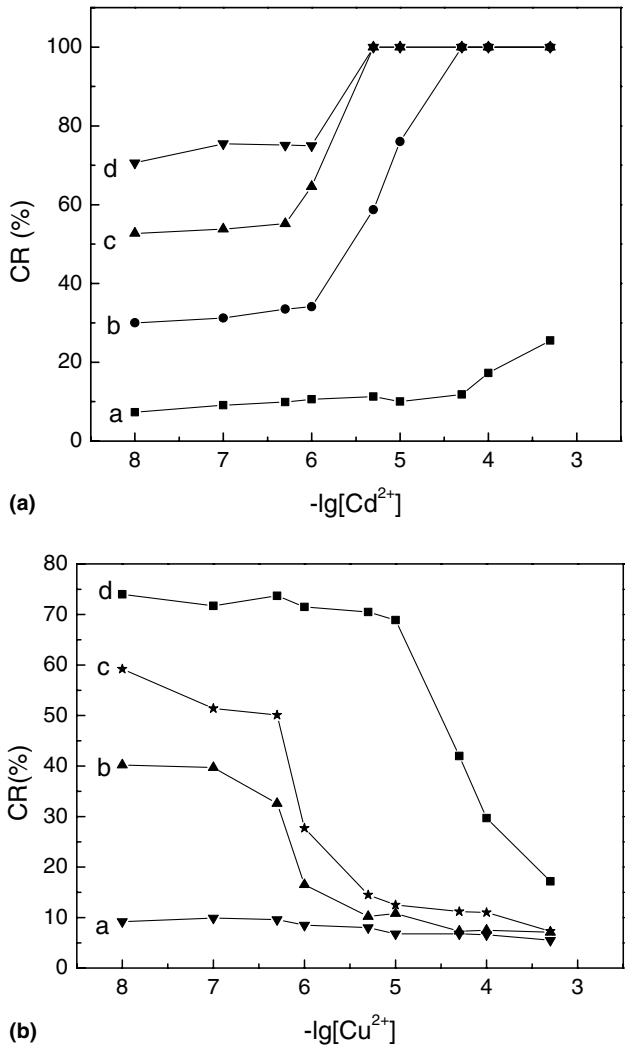


Fig. 4. Effect of Cd^{2+} and Cu^{2+} concentrations on CRs of $PCDA/MC_{16}$-*E. coli*. *E. coli* concentrations (cells/ml) were: (a) 0; (b) 9×10^5; (c) 1.8×10^6; (d) 9×10^8. Left dots in each line represent $PCDA/MC_{16}$ liposomes without adding Cd^{2+} or Cu^{2+} (control).

regularity. Briefly, the addition of Ca^{2+}, Mg^{2+}, Ba^{2+}, Cd^{2+} and Ag^{+} to liposomes obviously increased CR values, whereas Fe^{3+} and Cu^{2+} decreased the biochromatic degree almost to the system background (CR = 7%), and Zn^{2+} and Ni^{2+} slightly weakened biochromism. In the following experiments, Cd^{2+} and Cu^{2+} were, respectively, taken as examples for their enhancing and inhibitory roles on biochromism.

The effect of $CdCl_2$ and $CuCl_2$ from 1.0×10^{-7} to 5.0×10^{-4} M was tested for different *E. coli* concentrations (Figs. 4(a) and (b)). Both the enhancing effect of Cd^{2+} and the inhibitory effect of Cu^{2+} became more distinct at higher metal concentrations. There was a cooperative effect between metal ions and *E. coli* to modulate the blue-to-red transition in liposomes. Results suggested that detection speed and sensitivity in chromatic biosensors for *E. coli* might be greatly improved in the presence of metal ions such as Cd^{2+}. In addition, direct colorimetric sensors based on polydiacetylene self-assemblies could be applied to detect metal ions.

The mechanism involved in blue-to-red biochromatic transitions was also elucidated in the present study. RR spectrum changes of $PCDA/MC_{16}$ liposomes during biochromism were obtained by 488-nm laser light excitation (Fig. 5). The RR spectrum of the blue liposomes (Fig. 5, curve a) displays two groups of bands at 1449, 1515 and 2078, 2120 cm^{-1}, in which the first and the last values can be assigned to stretching vibrations for the double and triple bonds of the polymer backbone, respectively [14]. Blue liposomes presented a purple appearance after incubation with *E. coli*. The RR spectrum (Fig. 5, curve a′) showed the decrease of 1449 and 2078 cm^{-1} intensities, and the concurrent increase of 1515 and 2120 cm^{-1} values. In the presence of appropriate amounts of Cd^{2+}, the liposome suspension turned

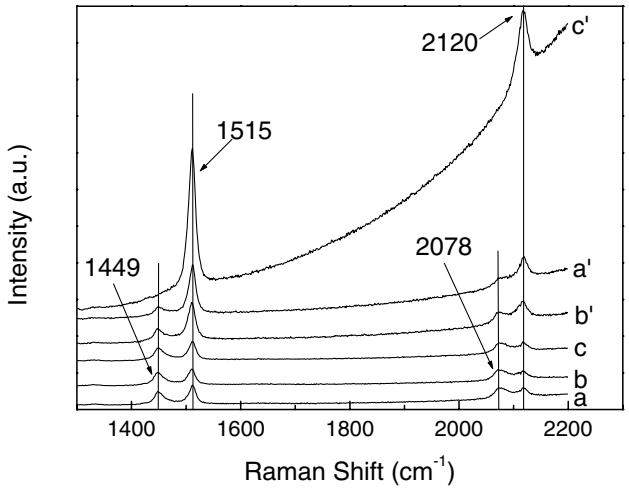
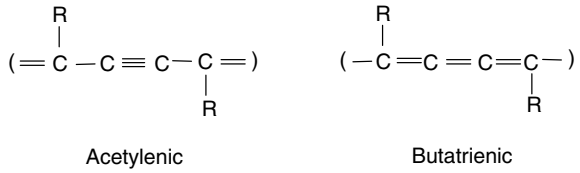


Fig. 5. Resonance Raman spectra of PCDA/MC_{16} liposomes. (a) PCDA/MC_{16}; (b) PCDA/MC_{16}-$CuCl_2$; (c) PCDA/MC_{16}-$CdCl_2$; a′, b′ and c′ are the results corresponding to a, b, and c incubated with *E. coli*. Metal ion concentration was 1.0×10^{-4} M.

completely to red after incubation. The corresponding spectrum (Fig. 5, curve c′) only contained the two major features at 1515 and 2120 cm^{-1}, whereas the bands at 1449 and 2078 cm^{-1} disappeared. However, when the liposome suspension containing Cu^{2+} was incubated with *E. coli*, it appeared blue. In the corresponding spectrum (Fig. 5, curve b′), 1449 and 2078 cm^{-1} band intensities slightly decreased whereas 1515 and 2120 cm^{-1} increased. By comparing all the RR spectra, it can be concluded that: (i) Double and triple bands simultaneously shifted to higher wave values during blue-to-red color transition; (ii) 1449 and 2078 cm^{-1} bands could be attributed to the blue phase and 1515 and 2120 cm^{-1} bands, to the red phase; (iii) the addition of Cd^{2+} or Cu^{2+} had little effect on the RR spectrum of original blue liposomes, thus it could be deduced that there was no direct interaction of metal cations with double or triple bonds in the polymer backbone in this condition and (iv) the intensity ratio of the double to the triple bonds in the red form increased as compared to the one in the original blue form, suggesting that the relative amount of the double bonds to the triple bonds increased during biochromism. Previous reports demonstrated that there were two resonance structures in the polydiacetylene backbone (Scheme 2), and that the acetylene structure was energetically more favorable than the butatriene structure in the ground state of long-chain polydiacetylene molecules [15–17]. It is, therefore, reasonable to conclude that the increase in the relative amount of the double bonds towards the triple bonds during biochromism could be attributed to a configuration transformation from acetylene to butatriene. Previous studies on PDA color transition generally accepted that the blue to red shift was associated with the reduction of the effective conjugated length of the ene–yne backbone and also with changes in the electronic properties of the polymer ene–yne backbone, which are strongly coupled to side chain conformation [18]. Both viruses and toxins are large macromolecules that bind to the cellular surface and begin their destructive journey attempting to merge with the cell membrane or to penetrate it. The mechanical stress provoked by toxins or viruses acts directly onto polymer vesicles causing polydiacetylene vesicles to turn from blue to red. Therefore, we can speculate that the specific *E. coli*–mannose interaction may alter side chain conformation, causing the electrical structure of the polymer backbone to change from acetylene to butatriene, and inducing blue-to-red transition in liposomes.



Scheme 2. Schematic diagrams of two resonance structures of PDA backbone.

Mannose binds specifically to FimH adhesin of bacterial type 1 pili in *E. coli* [19]. Type 1 pili are filamentous protein appendages that extend from the surface of many gram-negative organisms and are composed of FimA, FimF, FimG (structural pilus subunits), and FimH proteins (mannose-binding adhesin) [20]. FimA accounts for more than 98% of the pilus protein, and FimH is uniquely responsible for the binding to D-mannose [21,22]. FimH adhesin is a two-domain protein with a carboxy-terminal pilin domain, related to the incorporation into the pilus, and an amino-terminal mannose-binding lectin domain [23]. The overall process of bacteria adhesion to host cells is divided into two steps; aggregation and absorption, and specific adhesion. In the first step, which is reversible, bacteria approach the cellular surface and bind non-specifically to it. It is worth mentioning that, at this point, some kinds of bacteria show chemotaxis. In the second step (irreversible), bacteria bind specifically to cells, and receptors combine with ligands through chemical bonds. Some environmental factors, such as temperature or ion concentrations, and particularly divalent cation concentrations affect bacteria adhesion. Moreover, divalent cations show enhanced effects on bacteria adhesion depending on charge number and ion size [24]. It is generally accepted that charges may serve as ion bridges during the adhesin–acceptor interaction [25]. Amphiphilic diacetylene derivatives with a carboxylic group such as tricosa-10,12-diynoic acid, and PCDA were reported to form salts with transition metal ions Ag(I) and Cd(II) (which have the same electronic configuration), and alkali earth metal ions Ca(II), etc. [26]. Thus,

we speculated that in the liposome suspension, metal ions Cd(II), Ag(I), Ca(II), Mg(II), and Ba(II) existing in the form of dissociated ions, favored bacterial adhesion to the receptor on the liposome surface. Consequently, these ions could enhance the biochromatic degree of PCDA liposomes. Nevertheless, according to a previous report [27], amphiphilic diacetylenic acid could cooperate with transition metal ions such as Cu(II), Zn(II) and Ni(II), as can be observed in the phase transition regions of π-A isotherms of diacetylenic acid on the aqueous subphase containing these metal ions. In our view, this suggests that the rigidity of the liposomal outer leaflet was increased due to the formation of coordination compounds between PCDA and metal ions such as Cu(II), Fe(III), Zn(II) and Ni(II). The increased rigidity blocked the changes on the side chain conformation and electrical structure of PDA polymer during biochromism. At the same time, coordination compounds of these ions could not facilitate the bacterial adhesion as free ions. Therefore, the presence of these ions inhibited the blue-to-red transition in PCDA/MC_{16} liposomes. Furthermore, the addition of appropriate amounts of screening agent disodium ethylenediaminetetraacetate (pH 5.5) into PCDA/MC_{16} liposomes containing Cd(II) or Cu(II) eliminated both the enhancing effect of Cd(II) and the inhibitory effect of Cu(II) on biochromism. These results preliminarily supported our presumption.

4. Conclusion

In summary, metal cations were introduced into the supramolecular assembly (liposome) of glycolipid and polydiacetylene to study in vitro their effects on molecular recognition between mannose and *E. coli* through the unique blue-to-red biochromism of the PDA matrix. Cd(II), Ag(I) and alkali earth metal ions Ca(II), Mg(II), Ba(II) promoted color transition, whereas Cu(II), Fe(III), Zn(II) and Ni(II) exhibited inhibitory effects. Moreover, experimental results exhibited good repeatability and regularity, and thus the possible mechanism involved in biochromatic transitions was proposed. These discoveries would aid to broaden the practical applications of chromatic biosensors, improving detection speed and sensitivity for biological molecules, and developing a novel direct chromatic detection method for metal cations. Moreover, our results will contribute to better understand bio-molecular recognition functions of cell membranes.

Acknowledgements

This work was financially supported by National Natural Science Foundation of China (Nos. 60071013, 20125513 and 20345002), 100 People Plan from Chinese Academy of Sciences.

References

[1] A. Reichert, J.O. Nagy, W. Spevak, D.H. Charych, J. Am. Chem. Soc. 117 (1995) 829–830.
[2] B. Tieke, G. Lieser, J. Colloid Interf. Sci. 88 (1982) 471–486.
[3] D. Day, H. Ringsdorf, J. Polym. Sci., Polym. Lett. Ed. 16 (1978) 205–210.
[4] D. Bloor, R.R. Chance, Polydiacetylenes: Applied Science, NATO Advanced Study Institute Series E, Martin Nijhoff Publishers, Dordrecht, The Netherlands, 1985.
[5] H.W. Beckham, M.F. Rubner, Macromolecules 26 (1993) 5198–5201.
[6] R.A. Nallicheri, M.F. Rubner, Macromolecules 24 (1991) 517–525.
[7] R.R. Chance, Macromolecules 13 (1980) 396–398.
[8] Q. Cheng, R.C. Stevens, Langmuir 14 (1998) 1974–1976.
[9] D.H. Charych, J.O. Nagy, W. Spevak, M.D. Bednarski, Science 261 (1993) 585–588.
[10] J.J. Pan, D. Charych, Langmuir 13 (1997) 1365–1367.
[11] S. Kolusheva, T. Shahal, R. Jelinek, Biochemistry 39 (2000) 15851–15859.
[12] I. Gill, A. Ballesteros, Angew. Chem., Int. Ed. 42 (2003) 3264–3267.
[13] Z. Ma, J. Li, M. Liu, J. Cao, Z. Zou, J. Tu, L. Jiang, J. Am. Chem. Soc. 120 (1998) 12678–12679.
[14] M. Wenzel, G.H. Atkinson, J. Am. Chem. Soc. 111 (1989) 6123–6127.
[15] R.R. Chance, R.H. Baughman, H. Muller, C.J. Eckhardt, J. Chem. Phys. 67 (1977) 3616–3618.
[16] H. Eckhardt, C.J. Eckhardt, K.C. Yee, J. Chem. Phys. 70 (1979) 5498–5502.
[17] H.W. Beekham, M.F. Rubner, Macromolecules 26 (1993) 5192–5197.
[18] A. Lio, A. Reichert, D.J. Ahn, J.O. Nagy, M. Salmeron, D.H. Charych, Langmuir 13 (1997) 6524–6532.
[19] C.C. Linc, Y.C. Yeh, C.Y. Yang, C.L. Chen, G.F. Chen, C.C. Chen, Y.C. Wu, J. Am. Chem. Soc. 124 (2002) 3508–3509.
[20] G.E. Sato, S.J. Hultgren, J. Bacteriol. 181 (1999) 1059–1071.
[21] S.L. Harris, P.A. Spears, E.A. Havell, T.S. Hamrick, J.R. Horton, P.E. Orndorff, J. Bacteriol. 183 (2001) 4099–4102.
[22] K.A. Krogfelt, H. Bergmans, P. Klemm, Infect. Immun. 58 (1990) 1995–1998.
[23] S.D. Knight, J. Berglund, D. Choudhury, Curr. Opin. Chem. Biol. 4 (2000) 653–660.
[24] G. Xu, Molecular Medicine Bacteriology, Science Publishing Company, Beijing, 2000, p. 133.
[25] N. Bhosle, P.A. Suci, A.M. Baty, R.M. Weiner, G.G. Geesey, J. Colloid Interf. Sci. 205 (1998) 89–96.
[26] B. Tieke, G. Wegner, D. Naegele, H. Ringsdorf, Angew. Chem., Int. Ed. Engl. 15 (1976) 764–765.
[27] X. Huang, M.H. Liu, Chem. Commun. (2003) 66–67.