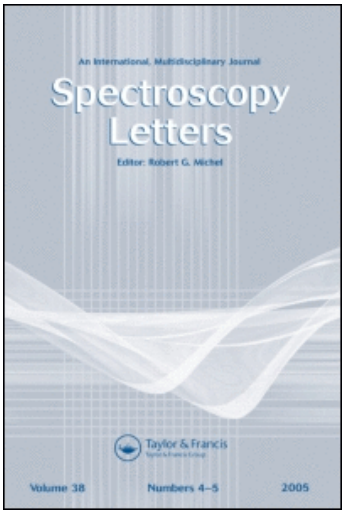

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Effects of N-Phosphoproline on the Phospholipid of Human Erythrocytes Membrane

Li Yan-Mei[a]; Zhao Yu-Fen[a]
[a] Bio-organic Phosphorus Chemistry Laboratory, Department of Chemistry, Tsinghua University, Beijing, P.R.China

To cite this Article Yan-Mei, Li and Yu-Fen, Zhao(2000) 'Effects of N-Phosphoproline on the Phospholipid of Human Erythrocytes Membrane', Spectroscopy Letters, 33: 5, 653 — 659
To link to this Article: DOI: 10.1080/00387010009350147
URL: http://dx.doi.org/10.1080/00387010009350147

SPECTROSCOPY LETTERS, 33(5), 653–659 (2000)

EFFECTS OF N-PHOSPHOPROLINE ON THE PHOSPHOLIPID OF HUMAN ERYTHROCYTES MEMBRANE

Key Words: Phosphoamino acid, Distribution and Conformation of Phospholipid, Human Erythrocyte Membrane

Yan-Mei Li, Yu-Fen Zhao*

Bio-organic Phosphorus Chemistry Laboratory, Department of Chemistry, Tsinghua University, Beijing 100084, P.R.China

ABSTRACT

The effects of phosphorylated proline on the packing and conformation of the membrane phospholipid have been studied. Generally it causes the polar parts of the phospholipid to become more mobile, the packing of the long chain of phospholipid to become more loose, the long chain to be in a lower trans/gauche ratio, and the end of the long chain to become more orderly. Any change in the level of phosphorylated proline affects the fluidity of the membrane phospholipid. Free proline and phosphorylated proline have different effects on the aliphatic chains of phospholipid.

INTRODUCTION

The Raman active C-C and C-H stretching modes of the lipids have been used to monitor the trans-gauche isomerism and lateral mobility in the systems of synthetic phospholipids, as well as for the mixed systems. The O-C-C-N^+ stretching modes have also been used to study the stability of the polar part of phospholipid in the membrane. They have been used for a long time to characterize the phospholipid interactions with polypeptides and proteins, e.g. with mellitin[1], myelin proteolipid apoprotein[2] and lysozyme[3]. The interactions between the

phospholipids and peptides or proteins are becoming clearer. It is also known that many kinds of phosphopeptides and phosphoproteins exist in or on the biomembrane. However, there have been very few investigations of the lipid distribution and membrane structure around the phosphopeptides or phosphoproteins. The specificity of lipid-phosphopeptide interactions, induced by phosphopeptides, may play an important role in cellular biology. An investigation of the interaction may give some clues to the specific requirement of phosphopeptides or phosphoproteins in or on biomembranes. Particularly, regarding some of the bioprocesses that are regulated by the phosphorylation and dephosphorylation of the membrane-associated peptides or proteins[4]. The goal of this work is to study the interactions between phosphopeptide and phospholipid. In order to simplify the research system, the interactions between the human erythrocytes as the membrane model and the single phosphoamino acids as the model for phosphopeptide were studied[5-6].

In this paper, the study has been focused on the effects of N-phosphoproline on membrane phospholipid systematically, especially the effects of N-phosphoproline's level, etc.

MATERIALS AND METHODS

Synthesis of phosphoproline as per literature[7-8]

Preparation of human erythrocyte membrane[5]

Fresh human blood samples collected in heparinized tubes were centrifuged immediately at 2000 rpm (4°C) for 5 min. and the plasma and buffy coat were removed by careful suction. The cells were resuspended in isotonic K_2HPO_4/KH_2PO_4 buffer (pH=7.35~7.45). After mixing well by inversion, the samples were centrifuged again at 2000 rpm for 5 min. at 4°C. The supernatants were removed by careful suction. This washing procedure was repeated three times more until the supernatants were no longer red.

The washed cells were suspended in the low osmolarity Tris buffer (10mM, pH=7.35~7.45, 4°C) and stirred thoroughly. The tubes were allowed to stand approximately 15 min. prior to centrifuging at 20,000 rpm for 25 min. at 4°C. The supernatants were decanted carefully. An additional osmolarity Tris buffer (10mM, pH=7.35~7.45, 4°C) was blown into the tube, and the suspensions were centrifuged for 25 min. at 20,000 rpm at 4°C. A total of three washes were necessary before the membranes were colourless.

Preparation of samples for Raman measurement

Phosphoproline solution was prepared at a concentration of 1.0×10^{-3}M. 5mL of the erythrocyte membrane, mentioned above, was mixed with 5 mL of phosphoproline solution

(1.0×10^{-3}M) and the mixture was centrifuged at 50,000 rpm for 1 hour at 4°C. After the supernatant solution was decanted, the samples were then incubated at 4°C for 10 hours.

Pure membrane in 10 mM Tris buffer (pH=7.35~7.45, 4°C) was used as a control.

Raman measurements

Raman spectra were recorded with a computerized Spex Model 1403 double monochromator with a spectral resolution of 2 cm^{-1}. Samples were excited with the 514.5 nm line of a Spectra-Physics argon ion laser; the laser power during sampling was 600 mW. Spectra were recorded digitally with an integrating period of 0.5 s. Capillaries containing samples were placed in a thermo-electrically regulated sample holder whose temperature was kept at 25±0.2°C.

Spectral data were collected at a 0.5 cm^{-1} interval over the spectral range and stored in the computer. Typically, 10 repeated scans were averaged to provide high-quality composite spectra. The composite spectra were subjected to a 13 point smoothing routine, which does not skew the spectral bands.

RESULTS AND DISCUSSION

N-phosphoamino acids are of importance not only in many biochemical reactions[9-10], but also in many biological processes. For example, in our experiment it was found that fresh pure DIPP-Pro (N-Diisopropylphosphoryl proline) might lead to a change in the conformation of the phospholipid constituted in the human erythrocytes membrane (i.e., the packing of the phospholipid and the mobility of the chain changed).

Effect of DIPP-Pro

First, the effects on the polar part of the phospholipid were studied. It was found that the intensity of the peak around 770 cm^{-1}, which is the contribution of the gauche conformation of O-C-C-N^+ [3], increased and that of the peak around 720 cm^{-1}, which is due to its all-trans conformation[3], decreased. So the intensity ratio of I_{720}/I_{770} dropped from 0.61 (pure membrane sample as reference) to 0.60 (Table I). The decrease of this ratio means that the lattice of the polar parts is more mobile and less ordered[3].

Second, the effects on the aliphatic chains on the nonpolar parts of the phospholipid were studied. In the CH_2-stretching region (2800-3000 cm^{-1}), the intensity ratio of $\gamma=I_{2890}/I_{2850}$ decreased and the lateral order parameter S_{lat} dropped from 0.40 (pure membrane sample as reference) to 0.32 (after adding DIPP-Pro), where $S_{lat}=(\gamma-0.17)/1.5$[11]. γ is affected by the packing of the chains. If the packing of the chains becomes orderly, the value of γ will

TABLE 1

Effects of DIPP-Pro on the Membrane Phospholipid Fluidity

Entity	I_{720}/I_{770}	γ	S_{lat}	I_{2940}/I_{2850}	I_{1130}/I_{1090}
Reference	0.61	1.30	0.40	1.72	1.77
DIPP-Pro	0.60	1.18	0.32	1.30	1.03

Notes:

1. The data in the table is the intensity ratio of the two peaks in the line of the table.
2. $\gamma=I_{2890}/I_{2850}$ and $S_{lat}=(\gamma-0.7)/1.5$
3. DIPP-Pro's concentration: 1.0×10^{-3}M

increase[11]. This indicates that DIPP-Pro caused an increase in the relative "looseness" of the lateral chain order and also in the chain mobility.

In the skeletal optical region (C-C stretching, 1050-1150 cm^{-1}), the intensity of the peak 1060 cm^{-1} and 1130 cm^{-1} (assigned to all-trans chain segments) increased, while the intensity of the peak 1090 cm^{-1} (assigned to the gauche configurations) decreased for the DIPP-Pro treated sample. Therefore, the intensity ratio of I_{1130}/I_{1090} decreased from 1.77 (pure membrane sample) to 1.03. This implies that DIPP-Pro causes a decrease in the long C-C chain of the phospholipid all-trans fraction (i.e. in the lower trans/gauche ratio[11]).

Finally, the effects on the $-CH_3$ ends of the long chain in the phospholipid were studied. In the CH-stretching region (2800-3000 cm^{-1}), the peak at 2940 cm^{-1} was attributed to the C-H stretch of the $-CH_3$ in the end of the long chain and the change of the intensity ratio of I_{2940}/I_{2850} means the change of the long chain end's mobility. In the study, it was found that after adding DIPP-Pro, the intensity ratio dropped from 1.72 (pure membrane sample) to 1.30. This means that the ends of the long chains in the phospholipid became less mobile and more ordered.

In conclusion, phosphorylated proline affected the packing and conformation of the membrane phospholipid. Generally, it causes the polar parts of the phospholipid to become more mobile, the packing of the long chain of phospholipid to become more loose, the long chain to be in a lower trans/gauche ratio, and the end of the long chain to become more orderly.

Effect of DIPP-Pro levels on the aliphatic chains

In the CH_2-stretching region, decrease in I_{2890}/I_{2850} peak-height intensity ratio was detected when DIPP-Pro levels were low (Table II, Figure I). This result indicates that little

TABLE 2

Effects of DIPP-Pro Levels on the Membrane Phospholipid Fluidity

DIPP-Pro Levels (M)	I_{1130}/I_{1090}	$\gamma=I_{2890}/I_{2850}$	I_{2940}/I_{2850}
Reference	1.77	1.29	1.25
1.0×10^{-5}	1.03	1.00	1.17
1.0×10^{-4}	1.05	1.07	1.04
1.0×10^{-3}	1.03	1.09	1.02
1.0×10^{-2}	1.03	1.06	0.96
1.0×10^{-1}	1.08	1.26	1.19

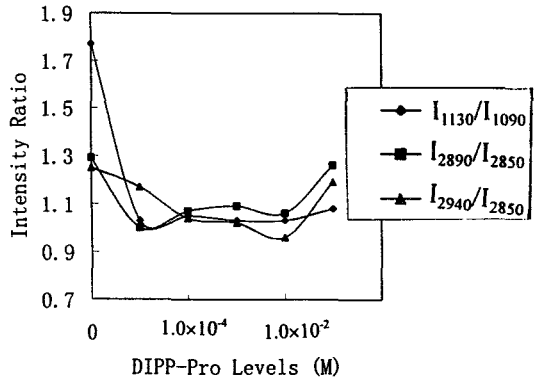

Figure I. Effects of DIPP-Pro Levels on the Membrane Phospholipid Fluidity

amounts of DIPP-Pro produces a decrease in the relative "crowding" of the packing of the chains. However, as the DIPP-Pro levels increased, the ratio of I_{2890}/I_{2850} increase (i.e., the packing of the chains became relative "crowding" comparing to the less amount of DIPP-Pro).

The mobility of $-CH_3$ ends of the long chain in the phospholipid is also affected by the DIPP-Pro level. From Table 2 and Figure 1, it can be seen that when the DIPP-Pro levels were low, the I_{2940}/I_{2850} ratio decreased compared to the pure membrane. When DIPP-Pro's level reached 1.0×10^{-2} M, the I_{2940}/I_{2850} ratio has the minimal value (0.96). But when DIPP-

TABLE 3

Effects of Pro and DIPP-Pro on the Membrane Phospholipid Fluidity

Entity	I_{1130}/I_{1090}	I_{2890}/I_{1850}	I_{2940}/I_{2850}
Reference	1.77	1.29	1.25
DIPP-Pro	1.03	1.09	1.02
Pro	1.26	1.09	1.04

Pro's levels were high (e.g. 1.0×10^{-1} M), the I_{2940}/I_{2850} ratio increased. The change of 2940 cm^{-1} in intensity relative to 2850 cm^{-1} indicated some changes in the long chain ends.

Table 2 and Figure 1 also demonstrates the changes in the all-trans fraction of the chains in different DIPP-Pro levels, by comparing the intensity of the peak 1130 cm^{-1} with that of the peak 1090 cm^{-1}. The result indicates that, in general, DIPP-Pro produces the long chain of membrane phospholipid containing less all-trans fractions. The higher the all-trans fraction, the more ordered are the long chains. When all the long chains are in all-trans configuration, it is the most ordered configuration. But generally, there exists a certain amount of gauche configuration, so that the long chains of membrane phospholipid have a certain fluidity.

Effects of free proline and phosphorylated proline on the aliphatic chains

From Table 3, it can be seen that the effects of free proline and phosphorylated proline are almost the same on the packing of the chains and the $-CH_3$ ends. However, DIPP-Pro has a greater effect on the all-trans fraction of the chains than free proline. This might be due to the fact that the existence of diisopropyl chains in DIPP-Pro caused the aliphatic chains of phospholipid to have less trans configuration (i.e., more gauche configuration).

CONCLUSION

Phosphorylated proline affects the packing and conformation of the membrane phospholipid. Generally, it causes the polar parts of the phospholipid to become more mobile, the packing of the long chain of phospholipid to become more loose, the long chain to be in a lower trans/gauche ratio, and the end of the long chain to become more orderly.

Any change in the level of phosphorylated proline has effects on the fluidity of membrane phospholipid. Free proline and phosphorylated proline have different effects on the aliphatic chains of phospholipid.

ACKNOWLEDGEMENT

The authors would like to thank the National Natural Science Foundation of China, the State Education Commission of China and the Cao Guangbiao High Technology Foundation for the financial support.

REFERENCES

1. M.Laufluer, J.F.Faucon, J.Dufourcq and M.Pezolet, *Biochim.Biophys.Acta* 1989; **908**:85
2. S.P.Verma, D.F.H.Wallach and J.D.Sakura, *Biochemistry* 1980; **19**:574
3. J.L.Lippert, R.M.Lindsay and R.Schultz, *Biochim.Biophys.Acta* 1980; **599**:32
4. O.M.Rosen and E.G.Krebs (eds), *Protein Phosphorylation*, Papers from a Conference, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1981
5. Y.M.Li, X.H.Wang, Y.F.Zhao, J.Y.Yu, M.Q.Wan and P.Zeng, *J.Bio.Phys.* 1993; **19**:85
6. Y.M.Li, Y.F.Zhao, J.Y.Yu, Q.Zhou, L.J.Yu and L.Xu, *Proceedings of International Symposium on Life Chemistry*, Beijing, China, Nov.19-23, 1996, pp348
7. Y.F.Zhao, G.J.Ji, J.N.Zeng and C.B.Xue, *Synthesis* 1988; **(6)**:444
8. Y.M.LI, Y.Jin, Y.C.Li and Y.F.Zhao, *Phosph.Sulf.Silic.* 1995; **101**:141
9. Y.M.Li, Y.W.Yin and Y.F.Zhao, *Int. J. Peptide & Protein Res.* 1992; **39(8)**:375
10. Y.M.Li, D.Q.Zhang, H.W.Zhang, G.J.Ji and Y.F.Zhao, *Bioorg.Chem.* 1992; **20(3)**:285
11. B.P.Gaber and W.L.Peticolas, *Biochim. Biophys. Acta* 1977; **465**:260

Date Received: January 7, 2000
Date Accepted: May 1, 2000