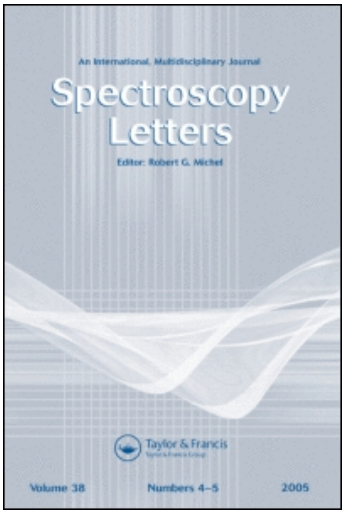

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Investigations on the Effects of Camp on Membrane Phospholipid of Human Erythrocytes by Laser Raman Spectroscopy

Yan-Mei Li[a]; Ming Wei[a]; Yu-Fen Zhao[a]; Jian-Yuan Yu[b]; Qun Zhou[b]
[a] Bioorganic Phosphorus Chemistry Laboratory, Department of Chemistry, Tsinghua University, Beijing, P.R., China [b] Analysis Center, Tsinghua University, Beijing, P.R., China

To cite this Article Li, Yan-Mei , Wei, Ming , Zhao, Yu-Fen , Yu, Jian-Yuan and Zhou, Qun(1999) 'Investigations on the Effects of Camp on Membrane Phospholipid of Human Erythrocytes by Laser Raman Spectroscopy', Spectroscopy Letters, 32: 2, 197 — 203
To link to this Article: DOI: 10.1080/00387019909349977
URL: http://dx.doi.org/10.1080/00387019909349977

SPECTROSCOPY LETTERS, 32(2), 197-203 (1999)

INVESTIGATIONS ON THE EFFECTS OF cAMP ON MEMBRANE PHOSPHOLIPID OF HUMAN ERYTHROCYTES BY LASER RAMAN SPECTROSCOPY

Key Words: Raman, Membrane Phospholipid, cAMP

Yan-Mei LI, Ming WEI and Yu-Fen ZHAO*
Bioorganic Phosphorus Chemistry Laboratory, Department of Chemistry, Tsinghua University, Beijing 100084, P.R.China

Jian-Yuan YU and Qun ZHOU
Analysis Center, Tsinghua University, Beijing 100084, P.R.China

Abstract: The effect of cAMP levels on the fluidity of human erythrocytes membrane phospholipid is studied. When the cAMP levels are low, cAMP produces phospholipid molecules less ordered. When the cAMP levels are high, it has the opposite function for the above mentioned part of phospholipid. Generally, cAMP causes the long nonpolar chains in higher gauche configuration fraction. The effects of cAMP levels on the packing of the long nonpolar chain are not very notable. cGMP has the negative function on the membrane phospholipid. ATP has no notable effect on the membrane phospholipid. But cGMP or ATP weakens the function of cAMP and even causes the mixture of cAMP-cGMP or cAMP-ATP having an opposite effect compared to that of pure cAMP.

1. Introduction

Biological membrane is a complex lipid mixture in which the proteins are embedded. Raman spectroscopy has been widely used to monitor the structure and packing of lipids in the systems of synthetic phospholipids, as well as for the mixed systems [1-3]. Changes in temperature [4], ions [5], pH [6], composition [7] and some exogenous compounds [1,2] have been shown to influence the peak positions and heights in the membrane Raman spectra, indicating changes in the structure or packing of the various membrane components.

Cyclic adenosine monophosphate (cAMP) plays an important role in regulating the cell cycles [8] and transmembrane channels [9,10]. One of the most important functions of cAMP is that it affects the properties of biomembrane, e.g., cAMP maintains the vertebrate oocyte in

prophase arrest by changing the fluidity of the membrane [11]. But there are few reports on the effect of cAMP on the fluidity of biomembrane, especially the effect of cAMP levels. The goal of this work is to study the effect of cAMP on the fluidity of biomembrane phospholipid, especially the effect of cAMP levels. Considering the fact that there exists the antagonistic effect among the levels of cAMP, cGMP and ATP [12], the effect of cAMP-cGMP or cAMP-ATP mixture were also reported.

2. Methods and Experimental

2.1 Membrane Model [1]

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 (10 mM, 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 low osmolarity Tris buffer (10 mM, 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.

2.2 Preparation of Samples for Raman Measurement

The solution of the tested compounds (in 10 mM Tris buffer, pH = 7.35 ~7.45, 4°C) was added into the membrane tube and stirred thoroughly. After standing for 30 min., the mixture was centrifuged at 50,000 rpm for 1 hr. at 4°C. After the supernatant solution was decanted, the sample was then stored at 4°C.

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

2.3 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 400 mW. Spectra were recorded digitally with an integrating period of 0.5 s. Capillaries containing the samples were placed in a thermoelectrically regulated sample holder whose temperature was kept at 25 ± 0.2 °C.

Spectral data were collected at 4 cm-1 internals 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 9 point-smoothing routine which does not skew the spectral bands.

3. Results And Discussion

The mammalian erythrocyte, or red cell, represents a readily available source of plasma membranes. In addition to the rather unlimited amounts of starting material, these cells contain no demonstrable subcellular organelles, thus providing a unique supply of pure plasma membranes. In this study, mammalian erythrocyte membranes were chosen as a membrane model to study the effect of cAMP on the biomembrane.

Phospholipids, the building-blocks of the biomembrane, are amphiphilic molecules which contain polar parts (choline, ethanolamine, etc ends) and nonpolar parts (aliphatic chains). The fluidity of the membrane lipids includes the fluidity of the polar parts and nonpolar parts. The mobility of the nonpolar parts also includes the packing of the chains, the all-trans fraction of the long chains and the mobility of the long chain ends'. The change in the mobility in each part of the lipid molecule can be detected by Raman spectra.

The Raman spectra for erythrocyte membranes which have undergone cAMP or cGMP-, ATP-contained mixture treatment were collected. Specific regions for analyses are the C-H stretch region (2800-3000 cm-1), C-C stretching region (1050-1150 cm-1) and O-C-C-N+ stretch region (700-850 cm-1).

In C-H stretch region (2800-3000 cm-1), Raman bands at 2850, 2890, 2940 and 2960 cm-1 have been assigned [13] to the symmetric CH2 stretch, asymmetric CH2 stretch, symmetric CH3 stretch and asymmetric CH3 stretch, respectively. A change in the $r = I_{2890}/I_{2850}$ peak-height intensity ratio and the lateral order parameter $S_{lat} = (r-0.17)/1.5$ are the measures of membrane lipid fluidity, especially of the packing of the chains. If the packing of the chains becomes ordered, the value of r and Slat will increase [1,3,14]. In this region, the peak at 2940 cm-1 is different from the peaks at 2850 and 2890 cm-1, because they are attributed to a different part of the long chains. According to Akutsu and his co-workers [15], 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} reflects the change in the mobility of the long ends. The increase in this ratio means that the ends of the long chain of the phospholipid become more mobile and less ordered [15]. It was also reported that the Raman band at 2940 cm-1 contains C-H stretch vibrations from a free amino acid side chain CH_2 group which can also undergo changes in intensity with temperature or external parameters [3,5,13].

In C-C stretch region (1050-1150 cm-1), Raman bands at 1060 and 1130 cm-1 have been attributed [1,14] to the contribution of all-trans chain segments and peak at 1090 cm-1 has been attributed [1,14] to that of gauche configuration. The corresponding cAMP or cGMP-, ATP-cAMP solutions were used as a reference. The contribution of cAMP, cGMP and ATP's symmetric OPO stretching mode on 1090 cm-1 band could be ignored. The higher the intensity ratio of I_{1130}/I_{1090} indicates the higher all-trans fraction, i.e. the less fluidity of the C-C chain [14].

In the 700-850 cm-1 range, the Raman band around 770 cm-1 is the contribution of the gauche conformation of O-C-C-N+ and band around 720 cm-1 due to the all-trans conformation [16]. Because phosphatidylcholine is the main part of membrane phospholipid, the changes in the intensity ratio I_{720}/I_{770} can be used as a probe to measure the mobility of the polar parts. The decrease in the intensity ratio I_{720}/I_{770} means that the lattice of the polar parts is more mobile and less ordered [16].

3.1 The effect of cAMP

First, the effect of cAMP on the polar part of the phospholipid was studied. It can be seen from the experimental results (table I, Figure I) that after adding a small amount of cAMP, the ratio of I_{720}/I_{770} dropped from 0.69 (pure membrane sample, reference) to 0.56 (10-6M cAMP), 0.45 (10-5M cAMP), 0.43 (10-4M cAMP) and 0.59 (10-3M cAMP) respectively. But when the cAMP level reached 10-2M, the ratio of I720/I770 increased to 0.77. This indicates that cAMP in lower levels causes the polar parts of membrane phospholipid to be more mobile, i.e. less ordered and cAMP in higher levels causes them to be less mobile, i.e. more ordered (Table II).

Second, the effects of cAMP on the aliphatic chains (the nonpolar parts) of the phospholipid were studied (Table I, Figure I). In this region, a slight increase in I2890/I2850 peak-height intensity ratio was detected when cAMP levels were low. This result indicates that a small amount of cAMP produces an increase in the relative "crowding" of the packing of the chains. But when the cAMP levels reached 10-2 M, the ratio of I2890/I2850 dropped to 1.02 (compared with the

TABLE I. Effect of cAMP levels on the Membrane Phospholipid Fluidity

cAMP levels	Pure Membrane	10-6M	10-5M	10-4M	10-3M	10-2M
I_{720}/I_{770}	0.69	0.56	0.45	0.43	0.59	0.77
I_{2890}/I_{2850}	1.14	1.14	1.22	1.48	1.19	1.02
I_{1130}/I_{1090}	1.94	2.04	1.29	1.24	1.30	1.61
I_{2940}/I_{2850}	1.17	1.21	1.47	1.33	1.46	1.06

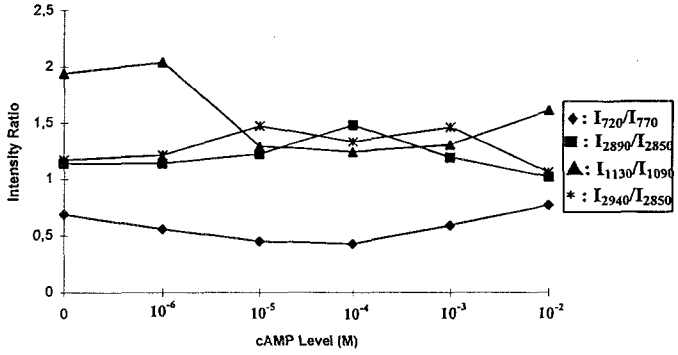

Figure I. Effect of cAMP levels on the Membrane Phospholipid Fluidity

TABLE II. Changes of Membrane Phospholipid Fluidity

cAMP levels	Polar Parts (I_{720}/I_{770})	Packing of the Chains (I_{2890}/I_{2850})	All-trans Fraction of the Chains (I_{1130}/I_{1090})	Long Chain Ends (I_{2940}/I_{2850})
low levels	less ordered	slightly more "crowding"	less	changed
high levels	more ordered	slightly less "crowding"	less	changed

pure membrane). This indicates that there is less "crowding" in the packing of the chains (Table II). But by and large, the changes in the packing of the chains is not very notable.

Table I and figure I also demonstrates the changes in all-trans fraction of the chains in different cAMP 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 cAMP produces the long chains of membrane phospholipid containing less all-trans fraction (Table II). The higher the all-trans fraction, the more ordered the long chains are. When all the long chains are in all-trans configuration, it is the most ordered configuration of the long chains. But generally, there exists a certain amount of gauche configuration, so that the long chains of membrane phospholipid have certain fluidity. The existence of gauche configuration in the long chains and the transportation of gauche configuration

along the long chains are one of the basic manners which control the transportation of some molecules through the biomembrane [17,18]. cAMP causes less all-trans fraction, i.e. higher gauche configuration fraction. It might lead to the easier transportation of some molecules from one side of the membrane to the other .

Finally, the effects of cAMP on the -CH3 ends of the long chain in the phospholipid were studied. From Table I and Figure I, it can be seen that when the cAMP levels were low, the I2940/I2850 ratio increased compared to the pure membrane. On the other hand, when the cAMP levels were high (e.g. 10-2M), the I2940/I2850 ratio decreased. The change of 2940 cm-1 in intensity relative to 2850 cm-1 indicated some changes in the long chain ends. It also indicated that perhaps there happened some conformation changes of the membrane proteins.

The fluidity of the membrane phospholipid includes the fluidity of the polar parts and nonpolar parts. And the mobility of the nonpolar parts includes the packing of the chains, the all-trans fraction of the long chains and the mobility of the long chain ends. From the experimental results, it can be seen that, when the cAMP levels are low, cAMP will produce polar parts that are more mobile and less ordered. The long chains tend to be in higher gauche configuration fraction. These changes might allow the membrane proteins, which embed in or on membrane phospholipid lattice, to change their shapes easily. Some of the molecules which take part in life-processes might be easy to transport through the biomembrane. When the concentration of cAMP is high, cAMP might have the opposite function on the mobility of the polar parts and long chain ends. But generally cAMP ------ no matter how concentrated they are ------ produces the long nonpolar chains in lower all-trans fraction. The effect of cAMP levels on the packing of the long nonpolar chain is not very notable either. But the effect on the long chain ends is not negligible.
It is noticeable in Figure I that the effects of cAMP levels have an extreme point. At this concentration (near 10-4 M), cAMP has the greatest effect on the fluidity of membrane phospholipid. It might be concluded that the function of cAMP has an optimum region. In this region, cAMP has the greatest effect on the biomembrane.

3.2 Effect of cAMP-cGMP

The effects of cAMP-cGMP mixture in different ratios on the membrane phospholipid fluidity were investigated. The concentration of cAMP was kept constant (10-4M). The results (Table III) showed that generally when the amount of cAMP was larger than that of cGMP in the mixture, the effect of the cAMP-cGMP mixture on the membrane phospholipid fluidity was great. The mixture had almost the same effect as pure cAMP. When the amount of cGMP increased, the effect of the mixture became smaller and even had the opposite effect (e.g. the changes in the ratios of I720/I770). It can be seen that cGMP has a negative function on the effect of the membrane phospholipid fluidity compared to cAMP. Goldbery has studied the function of cAMP and cGMP in the regulation of cellular metabolism. He considered that the co-operation between cAMP and cGMP was more important than the single cAMP levels or cGMP levels [12]. During the life process, there might exist dynamic equilibrium between cAMP and cGMP. This dynamic equilibrium might be one of the ways that control the biomembrane phospholipid.

3.3 Effect of cAMP-ATP

It can be said that ATP is the source of cAMP. When cAMP is needed, some ATP can be changed into cAMP under the help of enzymes. During life process, cAMP and ATP are also in a dynamic equilibrium. The equilibrium point might also affect the bio-process. Based on this point of view, the effect of cAMP-ATP mixture on the fluidity of membrane phospholipid was also studied (Table IV).
The results indicated that pure ATP had no notable effect on the membrane phospholipid fluidity. But ATP affected the function of cAMP on the phospholipid fluidity. Generally it weakened the function of cAMP. The dynamic equilibrium between cAMP and ATP is also very important during life process.

TABLE III. Effect of Different cAMP/cGMP Ratios on the Membrane Phospholipid Fluidity

cAMP/cGMP*	Pure Membrane	5 : 1	3 : 1	1 : 1	1 : 3	1 : 5
I_{720}/I_{770}	0.61	0.29	0.48	0.49	0.60	0.87
I_{2890}/I_{2850}	0.52	0.91	1.05	1.04	1.06	1.20
I_{1130}/I_{1090}	1.58	1.97	1.59	1.82	1.84	1.68

*: The concentration of cAMP was kept constant (10-4M)

TABLE IV. Effect of Different cAMP/ATP Ratios on the Membrane Phospholipid Fluidity

cAMP/ATP*	Pure Membrane	Pure cAMP	3 : 1	1 : 1	1 : 3	Pure ATP**
I_{720}/I_{770}	0.98	0.76	0.97	0.93	0.90	1.05
I_{2890}/I_{2850}	0.95	1.20	1.35	0.95	0.35	1.00
I_{1130}/I_{1090}	1.24	1.05	1.79	1.54	1.44	1.33

*: The concentration of cAMP was kept constant (10-4M)
**: ATP's concentration: 10-4M

4. Conclusion

Any change in the level of cAMP has great effect on the fluidity of membrane phospholipid. In general, at low cAMP levels, the polar parts become more mobile and less ordered. At high levels, cAMP has the opposite influence on their mobility. But generally cAMP ------ no matter how concentrated they are ------ produces the long nonpolar chains in higher gauche configuration fraction. The effect of cAMP levels on the packing of the long nonpolar chains is not very notable. Its effect on long chain ends is non-negligible.

The effect of cAMP-cGMP mixture is complicated. When the amount of cAMP is larger than that of cGMP in the mixture, the cAMP-cGMP mixture has almost the same effect as that of pure cAMP. When the amount of cGMP increases, the effect of the mixture becomes smaller and even had the opposite effect. This is due to the negative function of cGMP on the membrane phospholipid compared to cAMP.

Pure ATP has no notable effect on the membrane phospholipid fluidity. But ATP weakens the function of cAMP.

ACKNOWLEDGMENT

The authors would like to thank the National Natural Science Foundation of China and Tsinghua University for financial support.

REFERENCES

1. Y.M.Li, X.H.Wang, Y.F.Zhao, J.Y.Yu, M.Q.Wan and P.Zeng, J. Biol. Phys. 1993; **19**: 85
2. Y.M.Li and S.S.Liao, J. Tsinghua Uni. 1990; **30**: 54

3. S.C.Coheen, L.J.Lis, O.Kucuk, M.P.Westerman and J.W.Kauffman, J. Raman Spect. 1993; **24**: 599
4. S.P.Verma and D.F.H.Wallach, Biochim. Biophys. Acta 1976; **436**: 307
5. R.B.Mikkelsen, S.P.Verma and D.F.H.Wallach, Proc. Natl. Acad. Sci. USA 1978; 75: 5478
6. S.P.Verma and D.F.H.Wallach, Proc. Natl. Acad. Sci. USA 1976; **73**: 3558
7. M.W.Rooney, S.Yachnin, O.Kucuk, L.J.Lis and J.W.Kauffmann, Biochim. Biophys. Acta 1985; **820**: 33
8. G.A.Morrill, F.Schatz, A.B.Kostellow and J.W.Poupko, Differentiation 1977; **8**: 97
9. D.J.Barlow and J.M.Thornton, J. Mol. Biol. 1988; **201**: 601
10. H.DeGrazia, J.G.Harman, G.S.Tan and R.M.Wartell, Biochem. 1990; **29**: 3557
11 G.A.Morrill, K.Doi, J.Erlichman and A.B.Kostellow, Biochim. Biophys. Acta 1993; **1158**: 146
12. N.D.Goldbery, Advanced in Cyclic Nucleotide Research. Ravea Press(New York) 1975; **5**: 307
13. D.F.H.Wallach, S.P.Verma and J.Fookson, Biochim. Biophys. Acta 1979; **559**: 153
14. B.P.Gaber and W.L.Prticolas, Biochim. Biophys. Acta 1977; **465**: 260
15. H.Akutsu and Y.Kyogoku, Biochim. Biophys. Acta 1986; **854**: 213
16. J.L.Lippert, R.M.Lindsay and R.Schultz, Biochim. Biophys. Acta 1980; **599**: 32
17. A.T.Tu, Raman Spectroscopy in Biology, 1982; John Wiley & Sons
18. K.C.Lin, Sheng Li Ke Xue Jing Zhang 1985; **16**: 84

Date Received: November 23, 1998
Date Accepted: December 17, 1998