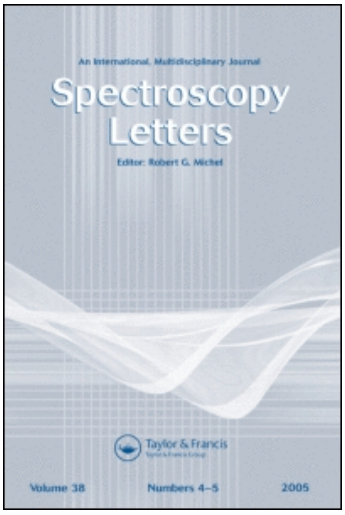

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Fourier Transform Infrared Spectroscopic Studies on the Effect of Cyclic Adenosine Monophosphate on the Secondary Structure of Human Erythrocyte Membrane Proteins

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To cite this Article Li, Yan-Mei , Wei, Ming and Zhao, Yu-Fen(1998) 'Fourier Transform Infrared Spectroscopic Studies on the Effect of Cyclic Adenosine Monophosphate on the Secondary Structure of Human Erythrocyte Membrane Proteins', Spectroscopy Letters, 31: 7, 1537 — 1545
To link to this Article: DOI: 10.1080/00387019808001658
URL: http://dx.doi.org/10.1080/00387019808001658

SPECTROSCOPY LETTERS, 31(7), 1537-1545 (1998)

FOURIER TRANSFORM INFRARED SPECTROSCOPIC STUDIES ON THE EFFECT OF CYCLIC ADENOSINE MONOPHOSPHATE ON THE SECONDARY STRUCTURE OF HUMAN ERYTHROCYTE MEMBRANE PROTEINS

Key Words: FT-IR, Secondary Structure of Membrane Protein, cAMP, cGMP, ATP

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Abstract: In this paper, the study on the effect of cAMP on erythrocyte membrane proteins by FTIR, deconvolution and curvefitting was reported. It was found that cAMP affects the secondary structure of membrane proteins by changing random and β-turn regions to the α-helix segments. The regulation of cAMP has a best concentration region, during which cAMP has the strongest regulating function. Meanwhile, cGMP and ATP has a negative effect on membrane proteins' secondary structure comparing to cAMP.

Introduction

The secondary structure of membrane proteins and signal peptides present within a lipid matrix is of considerable interest at the present time[1]. The

activity of membrane proteins affects the bio-process of the cell. It was also reported that cyclic adenosine monophosphate, ie, cAMP, has been implicated in the regulation of cellular metabolism by many hormones other than steroids and has been postulated as playing a role in regulating the cell cycle[2] and transmembrane channels[3]. It was discovered that cAMP controls the activity of many proteins[4,5]. But it is not very clear how cAMP affects the biomembrane proteins, especially their secondary structure. In the present paper, the effect of cAMP level on the secondary structure of human erythrocyte membrane proteins was reported. Considering that there exists the antagonistic effect among the levels of cAMP, cGMP and ATP, the effects of the mixture cAMP-cGMP and cAMP-ATP were also reported. Fourier Transform IR and computeraided analysis (eg. deconvolution, curvefitting)[7,8] were used to study the effect of cAMP on human erythrocyte membrane proteins.

Materials and methods

Preparation of human erythrocyte membrane see literature[9].

Infrared spectroscopy:

Fourier transform IR spectra were recorded on a Perkin-Elmer System 2000 Micro-FTIR Spectrometer assisted by a Perkin-Elmer Co. Gram Research Software. Samples were examined on a CaF_2 plate. Either 100 scans were recorded, 4 cm^{-1} resolution and signal averaged.

Secondary structure determination:

The main features of the procedure were described by Byler and Susi[10]. Overlapping infrared bands were resolved by Fourier deconvolution and Fourier derivation and band-fitting analysis was performed using established procedures[11].

Results and Discussion

The secondary structure of the proteins was determined from the shape of amide I band which is conformation sensitive. Besides its important conformational sensitivity, the amide I band was located in a region of the infrared spectrum often free of other bands[12], allowing the study of membrane proteins. It was therefore intrinsically simpler than amide II band. For these reasons, we have focused our attention on the analysis of amide I band in the rest of this paper.

Prior to curve fitting, a straight base line passing through the ordinates at 1700 cm^{-1} and 1600 cm^{-1} was subtracted. The spectrum between 1700 cm^{-1} and 1600 cm^{-1} was also normalized between absorbances of 0 and 1. A first least-squares iterative curve fitting was then performed with Lorentzian bands[13]. The input limited number of Lorentzian bands of given frequency for this first curve fitting were chosen by our program as follows: 1691 cm^{-1}, 1680 cm^{-1}, 1666 cm^{-1}, 1658 cm^{-1}, 1650 cm^{-1}, 1641 cm^{-1}, 1630 cm^{-1}, 1622 cm^{-1} and 1613 cm^{-1}. These frequencies were chosen because they often appear in deconvoluted spectra of proteins. The areas of all bands assigned to a given secondary structure were then summed up and divided by the total area. According to the literature data[1,8,14] and our expariment, the frequency limits used were empirically determined and were as Table I. 3_{10}-helix has the similar structure with standard α-helix, than it was combined into the fraction of α-helix. Nonstandard helix is a kind of irregular helix (or coil), than it was combined into the fraction of random structure[1,14].

Effect of cAMP levels on the secondary structure of membrane proteins

The procedure described to evaluate the secondary structure of membrane proteins yielded a distribution of: 24.9% α-helix, 17.7% β-sheet, 27.0% β-turn and 31.2% random. After adding 10^{-5} M cAMP to the pure membrane-

TABLE I
Assigment of the FTIR Bands

Frequence(cm^{-1})	Secondary Structure
1690	β-turn
1678	β-sheet
1664	β-turn
1655	3_{10}-helix[1,14]
1649	α-helix
1642	Random
1630	Nonstandard helix[1,14]
1620	β-sheet
1612	β-sheet

protein system, an increase in α-helix content accompanied by a corresponding decrease in β-turn and random conformations was detected. The increase or decrease was obvious, eg. the fraction of α-helix increased about 21% and random decreased about 27%. The increase of β-sheet was also detected (see Table II, Figure I). It seemed that cAMP affected the secondary structure by changing some β-turn and random structures (mainly random structure) to the α-helix and β-sheet structures (mainly α-helix).

But it was found that cAMP didn't produce a dose-dependent increase in the fraction of α-helix. When the cAMP level reached 10^{-3}M ~ 10^{-4}M, a loss of α-helix content was detected. It seemed that the effect of cAMP's level on membrane proteins secondary structure was non-monotonic.

The experimental results indicated that the existence of cAMP affected the secondary structure of membrane proteins. Generally, it caused an increase of α-helix content and a decrease of β-turn and random. The change of β-sheet content was not very great. At the concentration of about 10^{-3} M~10^{-4} M, the effect of cAMP was the greatest. When the cAMP levels were larger than this

TABLE II

Effect of cAMP levels on the Secondary Structure of Membrane Proteins

cAMP level (M)	Reference	10^{-5}	10^{-4}	10^{-3}	10^{-2}
α-helix(%)	24.1	30.1	38.3	38.5	34.1
β-sheet(%)	17.7	22.9	18.2	21.9	18.7
β-turn(%)	27.0	24.2	18.1	16.2	20.5
Random(%)	31.2	22.8	25.4	23.4	26.7

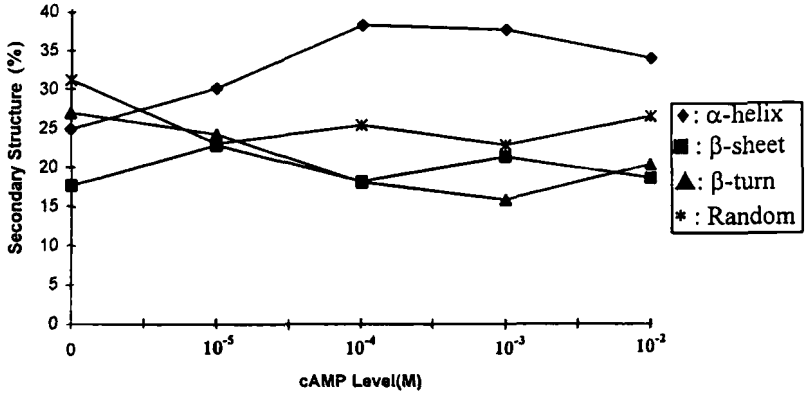

Figure I. Effect of cAMP Levels on the Secondary Structure of Membrane Proteins

extreme concentration, the effect of cAMP was not so strong. It can be concluded that the effect of cAMP on membrane proteins is, at least in part, by changing random and β-turn regions to the α-helix segments and the regulation of cAMP has a best concentration region, in which cAMP has the strongest regulation function. This might be one way that cAMP controls the activity of many proteins(or enzymes).

Effect of cAMP-cGMP mixture

cGMP is known to have the different function comparing to cAMP during bio-process. Goldbery[6] and his co-workers have studied the function of cAMP

and cGMP in the regulation of cellular metabolism. He considered that the dynamic equilibrium between cAMP and cGMP is more important than the single cAMP levels or cGMP levels. Based on this view and some other experimental facts (unreported results in our group), the effect of cAMP-cGMP mixtures were also studied. The results were listed in Table III.

It can be seen from the results that when the amount of cAMP was much more than that of cGMP, ie. in higher cAMP/cGMP ratios, the effect of cAMP-cGMP mixture was almost the same with the effect of pure cAMP. ie. it caused an increase of α-helix and a decrease of β-turn and random. The change of β-sheet was still not very notable. But when the amount of cGMP increased and reached higher levels, ie. in lower cAMP/cGMP ratios, the mixture gave negative effect compared to pure cAMP. ie. it caused a decrease of α-helix and an increase of β-turn and random. When the ratios of cAMP/cGMP were in the region of 3:1~1:3, the mixtures had no obvious effect on the secondary structure of membrane proteins. It can be seen that cGMP has a negative function on the effect of the secondary structure of membrane proteins compared to cAMP. During the life process, the activity of some proteins might be regulated by the dynamic equilibrium of cAMP and cGMP.

Effect of cAMP-ATP mixture

It can be said that ATP is the source of cAMP. When cAMP was needed, some ATP can be converted into cAMP with 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 secondary structure of membrane proteins was also studied (Table IV).

The results indicated that ATP had a negative effect on membrane proteins' secondary structure compared to cAMP. ie. during the existence of large

TABLE III

Effect of Different cAMP/cGMP Ratios on the Secondary Structure of Membrane Proteins

cAMP/cGMP*	Reference	5:1	3:1	1:3	1:5
α-helix(%)	29.5	39.1	30.0	31.3	25.0
β-sheet(%)	20.4	19.4	21.7	20.9	19.1
β-turn(%)	21.5	15.6	20.5	21.2	25.3
Random(%)	28.6	25.9	27.8	26.6	30.6

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

TABLE IV

Effect of Different cAMP/ATP Ratios on the Secondray Structure of Membrane Proteins

cAMP/ATP*	Reference	Pure cAMP	3:1	1:3	Pure ATP**
α-helix(%)	37.7	45.8	34.8	31.0	25.8
β-sheet(%)	17.0	11.8	17.1	17.0	16.4
β-turn(%)	21.0	20.9	22.8	23.6	27.2
Random(%)	24.3	21.5	25.3	28.4	30.6

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

**: ATP's concentration: 10^{-4}M

amount of ATP, some α-helix regions would be changed to β-turn and radom obviously. An increase in the amount of ATP resulted in the increase in the change of protein conformation. When ATP was converted into cAMP, the effect on the secondary structure was also changed. During the life process, the secondary structure of proteins and then even the activity of the proteins might be controlled by adjusting the ratio of cAMP/ATP. ie. switch on or off the change from ATP to cAMP.

Conclusion

cAMP affects the secondary structure of membrane proteins by changing random and β-turn regions to the α-helix segments. The regulation of cAMP

has a best concentration region (10^{-3}M ~ 10^{-4}M), in which cAMP has the strongest regulation function.

cGMP and ATP have negative effect on membrane proteins' secondary structure compared to cAMP. During the life process, the activity of some proteins might be regulated by the dynamic equilibria between cAMP and cGMP or cAMP and ATP.

ACKNOWLEDGEMENT

The authors would like to acknowledge the financial support from the National Science Foundation and Tsinghua University.

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Date Received: April 22, 1998
Date Accepted: June 16, 1998