Structural biology investigation of EMF effect on insulin in solution

LIU Yahui[1,2,#] CHEN Yongbin[3,#] XU Chunyan[1] XU Shenglong[3] SUN Bo[1]
ZHOU Huan[1] SUN Lihua[1] YU Feng[1] TANG Lin[1]
GUO Guozhen[3,*] HE Jianhua[1,*]

[1]*Shanghai Institute of Applied Physics*, *Chinese Academy of Sciences*, *Shanghai 201800*, *China*

[2]*Graduate University of Chinese Academy of Sciences*, *Beijing100049*, *China*

[3]*Department of Radiation Medicine*, *Fourth Military Medical University*, *Xi'an 710032*, *China*

Abstract It was reported that the α-helix of protein molecules could be destroyed when they were exposed to the electromagnetic field (EMF) and finally the signal transduction could be affected. To study this effect, one signal molecule, insulin, was exposed to electromagnetic fields at different combinations of the field strength, repetition rate and exposure time. For the first time, structural biology approach was used to detect the EMF effect. The results of a series of measurements on the interaction of electromagnetic fields with insulin *in vitro* are described. Under our experimental conditions, no effects of electromagnetic field exposure were observed on the molecular conformation.

Key words Electromagnetic field, Insulin, Macromolecular crystallography, Signal transduction

1 Introduction

The environments nowadays are filled with various kinds of electromagnetic fields (EMFs), and concerns have been growing over the EMF effect on human health. It was found in 1967 that high strength EMFs (60–70 T) increased the activity of catalase while decreased the activity of L-glutamic dehydrogenase[1].

However, humans are not generally exposed to high strength EMFs. Actually, since Goodman *et al*. reported in 1983 that weak pulsing EMFs increased the transcription process in dipteran salivary gland cell[2], low frequency EMF biological effects on living systems has been extensively studied. Most studies focused on changes in physiologic parameters of cells cultured *in vitro*, such as secretion of growth factors, proliferation, synthesis[3], mitotic cycle[2,4], cytosolic Ca^{2+} concentration[5] and HSP70 protein levels[6].

The cell membrane plays a key role in mediating cell signal transduction events, and it was supposed to be the target of EMF[7-14]. Zhou J L *et al*[14], Verma S P *et al*[15], Li X *et al*[16], Yan Z *et al*[17], George I *et al*[5] and Levin M *et al*[4] suggested that this might be a result of EMF-induced structural changes in signal molecule such as insulin, and the contact with the receptor binding in the membrane might be influenced, or the receptor protein structure might be changed. Thus, a series of biological effects at cellular levels were produced. However, it is still a controversy whether such a mechanism even exists. Some authors argued that it was improbabl for an EMF in such a low energy to cause a direct damage to DNA and other macromolecules[18].

Insulin is a typical peptide hormone, which is used almost in all serum-free medium as growth factor in cell cultures. This study was aimed at determining whether the signal molecular insulin between cells was affected by exposing to low frequency EMF. To our knowledge, structural biology approach was used for the first time in detecting the radiation effect. Unlike Raman spectroscopy used in most previous studies,

Supported by National Natural Science Foundation of China (No.10774155, No.30901215, No.30972430, No.30870576, No.60971055, No. 31000389) and Grant from Shanghai Institute of Applied Physics, Chinese Academy of Sciences (No. O95501C061).

Who contribute equally to this article

* Corresponding author. *E-mail address:* kangchu@fmmu.edu.cn (GUO Guozhen), hejianhua@sinap.ac.cn (HE Jianhua)

Received date: 2011-02-23

which may be affected by environment conditions, crystallography are straight forward and accurate.

2 Experimental

2.1 Protein preparation

All reagents and chemicals were of at least analytical quality and obtained from Sigma-Aldrich (Stockholm, Sweden) unless otherwise indicated. Insulin (CAS NUMBER: 11070-73-8) was dissolved in a buffer consisting of 0.1 mol·L^{-1} $Na_2CO_3^-$ $NaHCO_3$ at pH =10.1, with the final concentration of 2 mg·mL^{-1}. The insulin solution was plated in 96 Well Micro plate (greiner bio-one Cat.-No. 650901) at 100 μL per well.

2.2 Exposure conditions

In the present study, electromagnetic pulses (EMPs) were used. However, since it is not yet clear in which way the influence of the EMP exposure is related to the dose (exposure time, total energy provided to the system), the samples were exposed to EMPs at 4 kV/m and 70 kV/m, the repetition rate was 100 Hz or 10 kHz. The insulin samples to be exposed to EMPs were divided into 7 groups: One sham group and 6 groups of different exposure times (0.5, 1 and 4 h for 10 kHz EMP exposure; 5, 10 and 50 min for 100 Hz EMP exposure). The sham group, as the control, was kept in a separate room under identical conditions.

The EMP was generated by a spark gap pulse generator which was devised by Northwest Nuclear Technology Research Institute, Xi'an, Shanxi Province, China. Fig.1 shows the waveform of EMP we used.

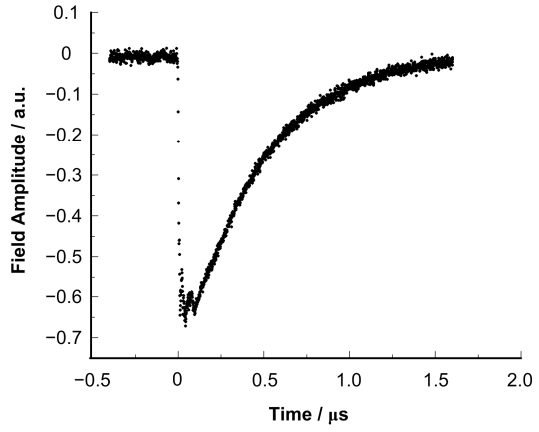

Fig.1 Electromagnetic pulse wave form used in this work.

2.3 Structure analysis

Insulin, an intercellular signal molecule, may be an important target of EMFs. Zhou J L *et al*[14], Li X *et al*[16] and Yan Z *et al*[17] suggested that by exposing insulin to EMFs, content of the secondary structure of the alpha helix might decrease. As a result, the insulin might change biological activity with reduced affinity of the peptide hormone for the receptor.

We used circular dichroism spectroscopy (CD) to observe the secondary structure. The measurements were carried out on a Chirascan spectrometer (Applied Photophysics Limited, UK) at 18°C. The instrument, quipped with a microcomputer, was pre-calibrated with (+)-10-camphorsulfonic acid. The measurements were taken in the far UV region (260–190 nm) using insulin concentration of 0.15 mg/mL. Spectra of the insulin were recorded in 100 nm/min in a response time of 1 s. The sham group were also evaluated. Each spectrum was the average of five scans.

Since the spectrometer measured average content of the secondary structure of insulin, it may be difficult to note structural changes of specific amino acids which affect *in vivo* biological function. Then, we crystallized insulin for X-ray diffraction on beamline 17U at Shanghai Synchrotron Radiation Facility.

The insulin was concentrated in a buffer consisting of 100 mmol·L^{-1} Na_2CO_3-$NaHCO_3$ at pH =10.1. Insulin at a final concentration of 15 mg·mL^{-1} was crystallized using the hanging-drop vapour-diffusion method at 291 K. After condition screening, the following crystallization condition was identified: a hanging drop containing 1-μL protein solution, 1-μL reservoir solution (0.1 mol·L^{-1}NaCl, 0.1 mol·L^{-1} BICINE pH=9.0, 20% v/v polyethylene glycol monomethyl ether550). After a week, the crystal appeared. The crystal were soaked in a cryoprotectant solution (0.1M NaCl, 20% glycerol, 0.1 mol·L^{-1} BICINE pH 9.0, 20% v/v polyethylene glycol monomethyl ether550) and flash-frozen in liquid nitrogen. The XRD data were collected in ~1.5-Å resolution and processed using HKL-2000[19]and CCP4i[20].

3 Results and discussion

Figure 2 shows the output of the circular dichroism we used to detect the second structural change of three EMF-exposed insulin groups (others were not shown). In the middle of Fig.2 there is the control group line, from which the lines of the EMF-exposed insulin groups vibrate up and down. CDNN software (a third-party program that is included as a courtesy on the installation disk of the machine, with the permission of its author) was used to calculate the secondary structure of insulin. The results were shown in Table 1. The content of the Helix and Random coil varied from 1% to 3%. Thus it is concluded that the variation in the secondary structure of insulin cannot be regarded as significant. There is not sufficient evidence to assert that the secondary structure was influenced by the applied EMP.

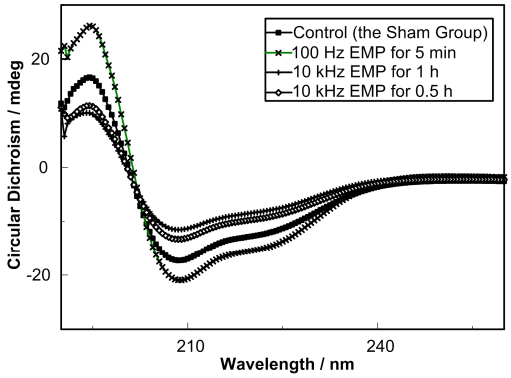

Fig.2 Output of the circular dichroism spectroscopy.

Table 1 Content of the second structure of insulin (%)

Groups	Helix	R.C.*
Control (the sham group)	18.2±0.91	42.2±2.11
A (10 kHz EMP, 0.5 h)	17± 0.85	42.9±2.15
B (10 kHz EMP, 1 h)	18.4±0.92	41.7±2.08
C (100 kHz EMP, 5 min)	17±0.85	43±2.14
D (100 kHz EMP, 10 min)	18.4±0.92	41.7±2.08
E (100 kHz EMP, 50 min)	17±0.885	40±2.025
F (10 kHz EMP, 4 h)	17±0.85	42.9±2.15

*R.C. represents the Random Coil.

Beautiful crystals (see Fig.3a as a typical one) were obtained. The ribbon diagram in Fig.3(b) shows two protein models: the sham group and the group exposed to 100 Hz EMP for 50 min. The two models were overlapped each other. This kind of ribbon diagram was done with the other protein models. No decrease in the content of Helix, nor increase in the content of Random coil, was observed. And no significant difference was found between the EMF-exposed groups and the control group.

Since certain structural features and the more highly conserved amino acids, which form the receptor binding surface or maintain its structural topology, play a key role in contact with the receptors, they are worthy of specific discussion and comparative analysis. Three regions of the A-chain (there is two chains in insulin: A and B), residues 1-3, 12-17, and 19 are of central importance to insulin structure and function. Similarly the critical region in the B-chain is spatially contiguous to the conserved A-chain region and spans residues 8–25[21]. The importance of the three insulin disulfides to insulin function was well established[22].

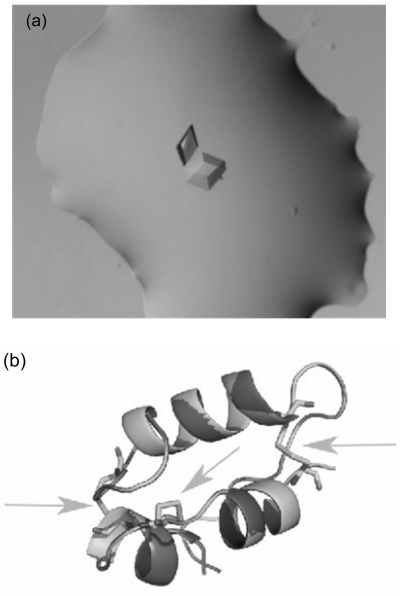

Fig.3 Crystal of insulin exposed to 100 Hz EMP for 50 min (a) and ribbon diagram of insulin (b). The control (in dark grey) and the EMP-exposed (in light grey) overlapped each other. The disulfide bonds are indicated by the arrows.

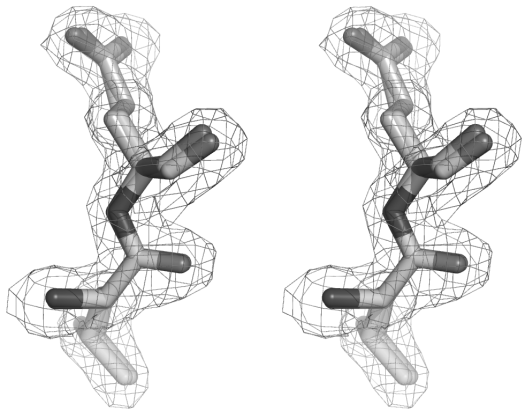

Fig.4 Stereoview of A16-17 from the control and Group F (100 Hz EMP for 50 min). The $2F_o$-F_c map is set to 1 σ.

We compared all the important amino acid models between control and other groups in Fig.4 (A16-17, others were not shown), and found no significant differences in conformational parameters for amino acids. Fig.3(b) shows clearly that there are three disulfide bonds in the two models and none was affected by the EMP.

In our opinion, the EMFs affect the living system through interactions between the fields and the positive or negative charges which were contained in the ions and molecules. It is well known that a changing magnetic field generates an electric field, and a changing electric field produces a magnetic field. They are described by the Maxwell's equations. For a stationary magnetic field, there is no induced electric field. The force on these charges due to the electric field E is $\boldsymbol{F}=q\boldsymbol{E}$, while that due to the magnetic field $\boldsymbol{B}$ is $\boldsymbol{F}=q\boldsymbol{v}\times\boldsymbol{B}=qv\boldsymbol{B}\cdot\sin\theta$. There are other forces: the viscous drag of $h\boldsymbol{v}$ (Stockers Law) and the random force of molecular bombardment (Brownian motion), $M(t)$. All these forces form $\boldsymbol{F}$ by Eq.(1):

$$\boldsymbol{F} = -h\boldsymbol{v} + M(t) + q(\boldsymbol{E} + \boldsymbol{v}\times\boldsymbol{B}) \qquad (1)$$

where h is the friction coefficient for the viscous drag; and $M(t)$ is a fluctuating force, of which the average value is zero. If $\boldsymbol{v}$=o, there is no magnetic force. Since $\boldsymbol{v}$=sqrt($3kT/m$), where k is the Boltzmann constant, T is temperature, and m is mass of the element, the velocities at the normal biological temperatures are very low. Finally the magnetic force may be ignored. So in experiments on living systems using EMF, the electric field is the important parameter.

It is puzzling that S P. Verma [15]had reported that a static MF could get effective structural changes in poly-L-lysine, since the static MF could not have significant Lorentz force acting on the molecular. Their samples were exposed to a static MF (~50 mT) during the recording of the Raman spectra which showed some obvious changes from the control.

In our measurements we found no effect of EMP on insulin, whereas Yan Z *et al* and Chen S H *et al.*[17,23] found altered conformation of insulin molecular by electric fields (peak strength E_p=1 V·m^{-1}) exposure for 30 minutes. Similarly, they detected the conformation changes by the Raman Spectrum and Fluorescent Spectrum. As is well known, Raman spectroscopy and fluorescent spectroscopy for protein and nucleic acid structure analysis have notable advantages. But the introduction of any substance on the test system will bring some degree of pollution which will result of errors in the analysis. Maybe, that's why they had got some structural changes.

Although we found no effect of EMF on the insulin conformation, we do not think that the induced time-varying electric field does not have any effect on proteins. There are studies showing effects on the cell proliferation and cell cycle-related end points[24–28]. We suggest that this could be a result of the relative magnitudes of the forces. The membrane proteins under physiological conditions can be treated as a charged particle. In Eq.(1), we can see that for sufficiently large $\boldsymbol{E}$, the $\boldsymbol{F}$ will be large enough and this could result in the redistribution of membrane proteins since the membrane is mobile. On the other hand, the signaling molecules (and ligands) between cells, e.g. insulin, could be mobile under the forces. The two aspects may affect the binding of extracellular signaling molecules (and ligands) to cell-surface receptors. However, since the signal transduction starts with a signal to a receptor, and ends with a change in cell function, the cell proliferation and cell cycle-related end points may be changed[3].

4 Conclusion

This study under our experiment conditions provided no support for structural change of insulin exposed to EMP. We assume that not their structural changes but their motions (both the signal molecular and the receptor in the membrane) under the forces resulted in the cellular function changes.

References

1 Haberdit W. Nature, 1967, **213:** 72–73.

2 Goodman R, Bassett C, Henderson A. Science, 1983, **220:** 1283–1285.

3 Goodman E M, Greenebaum B, Marron M T. Int Rev Cytol, 1995, **158:** 279–338.

4 Levin M, Ernst S. Bioelectromagnetics, 1995, **16:** 231–240.

5 George I, Geddis M S,Goodman R. J Cell Physiol, 2008, **216:** 816–823.

6 Reinbold K A, Pollack S R. Bioelectromagnetics, 1997,

18: 203–214.

7 Merla C, Liberti M, Apollonio F. Mircrowace Theory Tech, 2010, **3:** 691–698.

8 Malka N. Halgamuge, Bertil R R. Environ Eng Sci, 2009, **26:** 1473–1480.

9 Karabakhtsian R, Broude N, Shalts N. FEBS Lett, 1994, **349:** 1–6.

10 Lindstrom E, Berglund A, Mild K H. FEBS Lett, 1995, **370:** 118–122.

11 Luciana D, Luigi A. Miron, 2005, **36:** 195–217.

12 Simko M, Mattsson M O. J cell biochem, 2004, **93:** 83–92.

13 Massot O, Grimaldi B, Bailly J M. Brain Res, 2000, **858:** 143–150

14 Zhou J L, Li C L, Yao G D. Bioelectromagnetics, 2002, **23:** 339–346.

15 Verma S P, Goldner R B. Bioelectromagnetics, 1996, **17:** 33–36.

16 Li X, Chen S D, Wang L Y. Spectrosc Spect Anal, 2001, **21:** 406–408.

17 Yan Z, Chen S D, Qiao D J. Spectrosc Spect Anal, 2008, **28:** 1343–1347.

18 Mannerling A C, Simko M, Mild K H. Radiat Environ Biophy, 2010, **49:** 731–741.

19 Otwinowski Z, Minor W. Method Enzymol, 1997, **276:** 307–326.

20 Bailey S. Acta Crystallogr D, 1994, **50:** 760–763.

21 Mayer J P, Zhang F,DiMarchi RD. Biopolymers, 2007, **88:** 687–713.

22 Murrayrust J, Mcleod A N, Wood S P. Bioessays, 1992, **14:** 325–331.

23 Shi C, Chang W H, Chu P. J Bone Miner Res, 2002, **17:** M238.

24 Chen G, Upham B L, Sun W. Environ Health Perspect , 2000, **108:** 967–972.

25 Harris P, Lamb J, Heaton B. Electromagn fields, 2002, **2:** 3–3.

26 Korzhsleptsova I L, Lindstrom E, Mild K H. FEBS Lett , 1995, **359:** 151–154.

27 Lange S, Richard D, Viergutz T. Radiat Environ Biophys , 2002, **41:** 131–137.

28 Verheyen G R, Pauwels G, Verschaeve L. Bioelectromagnetics, 2003, **24:** 160–164.