Nanophtonics and Consciousness: An Anesthetics-Luciferase Appruoch

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Abstract: - In this paper we present results of experimental research of luciferase-anesthetic interaction in vitro. To characterise secondary structure of luciferase we used circular dichroism (CD), while to characterise luciferase-anesthetic interaction we have used scanning tunneling microscope (STM). Understanding molecular mechanism of anesthesia may illuminate biological consciousness. Because the week, non-chemical process based on charge-dipole and dipole-dipole interactions are responsible for photonic swich we believe that nanophotonics and molecular optical networks under determined conditions, in future may lead to new type of information machines based on artificial consciousness.

Key-words: - nanophotonics, luciferase, general anesthesia, consciousness, quantum computing,

1 Introduction

There are three main steps in information machines (computers) evolution according to Man-Machine interacion. [1] The first step encompasses *hand technology*, and is represented by today's computers with which we comunicate trough the keyboard-by hend. The second generation of information machines includes those which will be *automatic intelligent* one, capable of movement in space and time and capable of adapting to their environment, relized on the principles of intelligence brain functions. The first generation of such machines has already been bulit utilizing artificial neural networks and fuzzy logic. The third step will be based on *artificial state of consciousness*. Bearing in mind that starting point of artificial conciousness will be knowlege of biological consciousness modeling, molecular mechanisam of anesthesia is very important topic.

Despite extensive research investigating the cellular and molecular mechanisms of general anesthesia, the fundamental questions, why anesthesia produces unconsciousness and what is the common mechanism throw which various anesthetic agens produce unconsciousness, remains unanswered. According to literature data, a number of theories on consciousness propose that a fundamental part of the neural substrate for consciousness is likely to involve thalamocortical-corticothalamic loops [2]. Thus, these theories would seem to predict that a specific consequence of anesthetic-induced unconsciousness might be change in functional thalamocortical activity. Findings of animal as well as finding of human studies examining anesthetic-induced changes in evoked potential recordings suggest that the primary basis of anesthesia may be the blocking or disruption of biophotonics sensory information processing throw the thalamus [3]. Furthermore, regional thalamic functional activity suppression was found during halothane general anesthesia, and a correlation link between a person's level of consciousness and their level of thalamic functioning at various doses of propofol anesthesia was demonstrated in humans [4]. Direct *in vitro* demonstration of halothane's ability to hyperpolarize thalamic parafascicular nucleus neurons showed that a hyperpolarization block of thalamocortical neurons might be mechanistically related to the loss of consciousness seen during halothane anesthesia [5]. However, *in vivo* human results revealed that a relative decrease of thalamus activity accompanies halothane anesthesia, but a number of other areas, such as the basal forebrain and cerebellum, were also noted to be affected by halothane and isofurane [6]. The molecular mechanism of general anesthesia has been a longstanding enigma. The direct measure of anesthetic binding to protein is

complicated by weak affinity and therefore rapid kinetics. Kin Chan and coworkers characterized the binding of halothane as multisite and low affinity and mioglobin or cytochrom C as strictly nonspecific [7].

Luciferase enzymes are a group of photo emitting proteins found in luminescent bacteria and fireflies, which, when activated by flavins (e.g. NADH) or ATP generates photons. In both whole cells and membrane - free purified preparations, luciferase light emission has been shown to be extremely sensitive to inhibition by general anesthetic gas molecules, n-alkanes, and n-alcohols. Accordingly, luciferase has been considered as a model system for anesthetic mechanism supportive of a direct protein site of action [8, 9]. Whether anesthetic bind to the activated or inactive enzyme, and whether they cause a conformational change or prevent one remain uncertain.

Firefly luciferase from Photinus pyralis (EC 1.13.12.7) is 62 kD monomeric protein that, widely known because of its numerous applications. This enzyme emits a burst of light (yellow-green light λ_{max} = 560 nm) when mixed with ATP and luciferin in the presence of oxygen. Luciferase is a monooxygenase, which catalyzes the oxidation of luciferin in the presence of ATPMg to generate light, according to following scheme, generally proposed:

Luciferase + luciferin + ATPMg ↔
Luciferase-Luciferyl-AMP + PPi

Luciferase-Luciferyl-AMP+O2→
Luciferase + Oxyluciferin + CO2 + light

2 Problem Formulation

The aim of our research was to investigate luciferase-anestetic interaction in vitro.

Firefly luciferase (Photinus pyralis EC 1.13.12.7) was purchased from Sigma.To characterise luciferase and its interacion with anesthetics we have used two methods scanning tunneling microscopy and circular dichroism.

For scanning tunneling microscopy imaging one drop of solution containing firefly luciferase Photinus pyralis EC 1.13.12.7 with or without ethanol was smeared onto a gold surface. Luciferase samples on mentioned surfaces were placed in air at room temperature on the sample stage of Nanoscope II (Digital Instruments, 135 Nogal drive, Santa Barbara CA 93110). Mechanically sharpened, 80/20 platinum /iridium tip where negatively biased with respect to the sample. Typical operating conditions for firefly luciferase sample imaging was 150 mV and 1 nA in a constant current mode with high and low pass filtering.

Circular dichroism (CD) measurements were done with a JASCO spectropolarimeter, model J-500A. CD spectra were recorded between 180 nm and 250 nm in 0.1 nm steps and 4 sec integration time. The CD spectra were obtained in a 0.2 mm cell at room temperature. For each sample 15 spectra and baselines were collected and averaged. A firefly luciferase Photinus pyralis EC 1.13.12.7 solution (0.5 mg/ml peptide concentration, pH 7.5) with or without ethanol (3.7 %) was submitted for circular dichroism measurements. The secondary structure prediction was determined by the method of Provencher SW and Glockner J method [9].

3 Problem Solution

An STM image of firefly luciferase subunit on gold substrate is shown in Figure 1. Dimensions of scanning area are 15.5nm x 15.5nm. The shape and size of observed structure match luciferase monomer with clearly visualized domains and subdomains within protein molecule. This molecular architecture of firefly luciferase determined by STM is in agreement with three–dimensional structure prediction as described by Kumita et al. [10]. Shape and internal changes of luciferase exposed to ethanol are shown in STM image in Figure 2. The STM imaging of luciferase exposed to ethanol, shows direct observation of molecular interaction of alcohol and protein.

Scanning tunneling microscopy (STM) can image conducting and semi conducting surfaces with atomic resolution. In STM, a piezo-controlled single atom tip approaches a surface with a bias voltage. As the tip near to within a few angstroms, electron tunneling

between tip and surface commences; this tunneling current is used in a servo feedback to maintain a constant height of the tip as it scans the surface. Thus, topographic surface map becomes visible. STM of biomolecules is more difficult because of poor conductivity, stability etc, however, optimal sample preparation resolves this problem

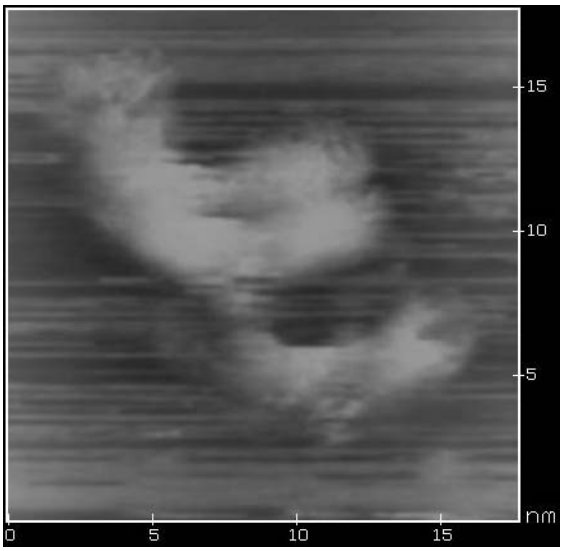

Figure 1. STM image of firefly luciferase Photinus pyralis. Internal structure (domains and subdomains) of luciferase protein subunit is visible.

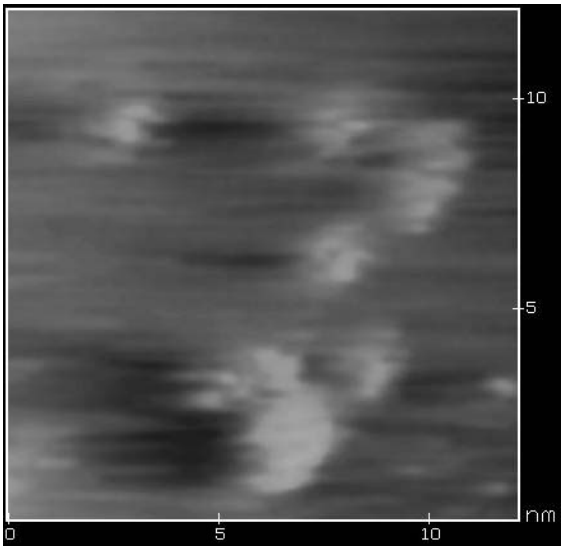

Figure 2. STM image of firefly luciferase *Photynus pyralis* after ethanol exposure. Molecular shape changes as well as internal structural changes are evident.

Firefly luciferase Photynus pyralis
12% α–helix 63% β–sheet 25% random coil (25% β–turn)
Firefly luciferase Photynus pyralis + ethanol
26% α–helix 53% β–sheet 22% random coil (10% β–turn)

Table 1. Prediction of secondary protein structure of firefly luciferase without and with ethanol

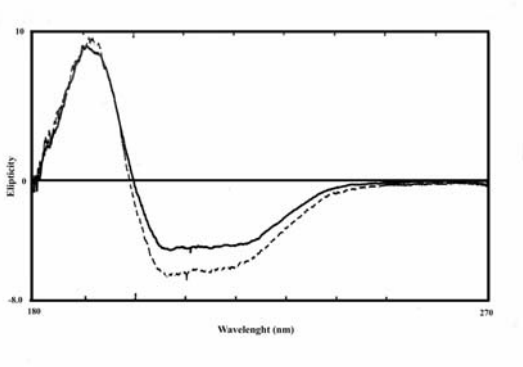

Figure 3. Circular dichroism (CD) spectrum of firefly luciferase Photynus pyralis without (continuous line) and with ethanol (broken line). Each spectrum is the averaged result of fifteen spectra and subtracted baselines.

The circular dichroism spectra of firefly luciferase *Photinus pyralis* in the presence or absence of ethanol demonstrated that alcohol induce conformational transitions of luciferase (Figure 3). These conformational changes are confirmed by protein secondary structure prediction as described by Provencher and Glockner [9]. The estimation of firefly luciferase secondary structure from circular dichroism spectra shows that ethanol induces α–helix and unordered structure formation (Table 1). This finding is in agreement with well-known mechanisms of alcohol effects upon proteins and peptides [11]. Computer simulation of anesthetic quantum effects on bacterial luciferse indicate that nanophotonics may be point of departure of future consciousness machines [13]

4 Conclusion

Understanding molecular mechanism of anesthesia may illuminate biological consciousness. The week, non-chemical process based on van der Waals forces (charge-dipole and dipole-dipole interactions) in luciferase-anesthetic interaction are responsible for photonic swich or "consciousnes-unconsciousness" state. Nanophotonics and molecular optical networks in future may lead to new type of information machines based on molecular artificial consciousness.

References:

[1] Koruga,D.,Neuromolecular Computing, *Nanobiology*, Vol.1, No.1:5-25, 1992

[2] Llinas, R., Ribary, U., Contreras, D., & Pedroarena, C., The neuronal basis for consciousness, *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, Vol.353, 1998, pp.1841- 1849.

[3] Angel, A., Central neuronal pathways and the process of anesthesia, *British Journal of Anesthesia*, Vol.71, 1993, pp.148-163.

[4] Fiset, P., Paus, T., Daloze, T., Plourde, G., Meuret, P., Bonhomme, V., Hajj, Ali, N., Backman, S.B., & Evans, A.C., Brain mechanisms of propofol-induced loss on consciousness in humans: A positron emission tomography study, *Journal of Neuroscience*, Vol.19, 1999, pp.5506-5513.

[5] Sugiyama, K., Muteki, T., & Shimoji, K., Halothane-induced hyperpolarization and depression of postsynaptic potentials of guinea pig thalamits neurons in vitro, *Brain Research*, Vol.576, 1992, pp.97-103.

[6] Alkire, M.T., Pomfrett, C.J., Haier, R.J., Gianzero, M.V., Chan, C.M., Jacobsen, B.P., & Fallon, J.H., Functional brain imaging during anesthesia in humans: effects of halothane on global and regional cerebral glucose metabolism, *Anesthesiology*, Vol.90, 1999, pp.701-709.

[7] Chan, K., Meng, Q.C., Johansson, J.S., & Eckenhoff, R.G., Low-Affinity Analytical Chromatography for Measuring Inhaled Anesthetic Binding to Isolated Proteins, *Analytical Biochemistry,* Vol.301, 2002, pp.308-313.

[8] Ueda, I., Suzuki, A., Irreversible phase transition of firefly luciferase: contrasting effects of volatile anesthetics and myristic acid, *Biochim Biophys Acta*, Vol.1380, 1998, pp.313-319.

[9] Ueda, I., Suzuki, A., Kamaia, H., Do Anesthetics Acts by Competitive-Binding to Specific Receptors-Phase Transition of Firefly Luciferase, *Toxicology Letters*, Vol.101, 1998, pp.405-411

[10] Provencher, S.W. & Glockner, J., Estimation of Globular Protein Secondary Structure from Circular-Dichroism, *Biochemistry*, Vol.20, 1, 1981, pp.33-37.

[11] Kumita, J.R., Jain, L., Safroneeva, E., & Woolley, G.A., A Cysteine-Free Firefly Luciferase Retains Luminescence Activity, *Biochemical and Biophysical Research Communications*, Vol.267, 2000, pp.394- 397.

[12] Hirota, N., Mizuno, K., & Goto, Y., Group Additive Contributions to the Alcohol-Induced α-helix Formation of Melittin: Implication for the Mechanism of the Alcohol Effects on Proteins, *Journal of Molecular Biology,* Vol.275, 1998, pp.365-378.

[13] Louria,D.E.,Koruga,D.L.,Lahozbeltra,R., Hameroff,S,. Computer Simulation of Anesthetic Quantum Effects on Bacterial Luciferase, *Biophysical Journal*, Vol.66, Iss2,p. 389, 1994.