Analogy between the Magneto-Optical Properties of Fish Light Organ and the Bonghan System

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Abstract: Crystalline and auto-fluorescent structures could arise in acupuncture meridians and fish ducts which react to light, magnetic fields and other treatments. The symbiotic fish light organ which has the specific function of producing light is compared with the thread-like structures found in both mammalian connective tissue and human skin. A motility biosensor based of Fourier spectra and the real-time parameters detection was developed using the light intensity fluctuation spectrometry. Diparamagnetic changes of auto-fluorescent ducts were induced under blue light illumination.

Key words: Bonghan system; magnetic separation; DNA-nanoparticles, acupuncture, fish light organ.

1. Introduction

Physical properties of light producing living systems such as a fish light organ and possible mammalian analogs, the acupuncture system, and Bonghan threadlike structures, could give valuable information about fundamental bio-communication principles in Nature.

Bacteria which colonize the symbiotic fish light organ have come to us from times comparable to the period in which life has existed on the Earth. Being in the state of endospores they are almost immortal. There are certain species of luminous bacteria, which are obligatory symbionts, requiring unique nutritional substances, which exist also as free living forms in the sea. Bacterial aggregations use the ciliary mucus motility to assist in their colonization and they cause mucus secretion through lipopolysacharides, which are components of the outer membrane of gram-negative bacteria.

Some free-living luminous bacteria dispersed in sea water are habitats of the gut tract and the skin surface of marine animals. Despite the physiological diversity among different species of luminous bacteria, the biochemical machineries to produce light are homologous varying only in the reaction kinetics of the luciferase-enzymes; all luminous bacteria are gram-negative microorganisms with flagella facilitating motion. Several symbiotic genes have been identified as essential for the initiation, colonization and persistence of Vibrio fishery in the host squid light organ.

Analysis of particles in a high gradient magnetic field (HGMF) has been applied for magnetic susceptibility measurements and for comparative measurements of diamagnetic biological particles: nucleoprotein granules (DNA containing granules, named Bonghan granules) and phytobacteria cells. A fruitful application of high magnetic fields for study of macromolecules and bio-microparticles used an alignment method combined with detection of phase transitions which allow detection of diamagnetic structures.

The physical-chemical background for the method is related to the concentrating processes of bio-macromolecules, which is also a research subject using colloidal chemistry methods, polarization microscopy, optical methods and dynamical light intensity fluctuation spectrometry. Temperature and concentration phase transitions of a "gel-liquid crystal" are of great importance to study optical, magnetic and morphological properties of the Bonghan system, and are the subject of the present investigations.

2. Methods

The coordinate system was defined for the ferromagnetic cylindrical wire with radius a and saturation magnetization M, with the wire axis parallel to the z direction. The applied magnetic field H is along the x axis. The magnetic susceptibility of particles (with a volume V) with respect to the solution could be obtained from the equation for pondermotive force and drag force:

$$6\pi\kappa_{p} V=1/2(\chi_{p}-\chi_{f})V\cdot \nabla (H\cdot H)$$

where \(\chi_{p}\) and \(\chi_{f}\) are the susceptibilities of the particles and the fluid. The gradient value on the wire surface was \(H\cdot \nabla =5\times 10^{10}\) (Oe/cm). A technique was developed for observing particles shape around an array of wires using reflection and polarization microscopy combined with CCD-camera. DNA-granules by (0.1-2.5 micrometers in diameter, i.e. micro-cells, named Bonghan granules) were obtained from rabbit or mice threadlike structures and analogous fish ducts. The magnetic susceptibilities of single particles, ducts and granules were estimated according to:

$$\chi_{p}=\frac{6\pi \kappa_{p} a^{2} F(r_{f}, r_{p})}{1/H_{0} M_{s} T}$$

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\[ F(r_1, r_2) = \frac{1}{2} (r_1^2 - r_2) K (r_1^2 - r_2^2) K + K \ln \frac{r_1^2 + K}{r_2^2 + K}; \]

\((+1)\) is for paramagnetic capture and \((-1)\) for diamagnetic capture; \(F=74.8\) for \((3.5a-1.25a)\) interval, \(H_0=10^4\) Oe, \(M_s=0.77-10^4\) G; \(K=M_s/2H_0\).

A photo-detector was placed in the optic microscopic image plane, and the light intensity fluctuations of the micro-object were used to study the cell motile activity. This portable device \(^5\) is used to measure visually targeted structures of specimens for their Fourier spectra analysis and dynamical processes documentation in a spectral range from 1 Hz to 1000 Hz and permits motility analysis under high spatial resolution of moving structures up to 0.5 \(\mu\)m.

The photomultiplier tube \(^6\) (PMT; R331-05, Hamamatsu, Japan) was used (by Dr. J.-D. im to obtain a curve presented in the Fig. 8a) for measuring the numbers of emitted photons from the samples and associated data acquisition systems were also attached to the PMT, which was operated at room temperature in a spectral response range of 300 to 650 nm, and the wavelength of maximal response was 420 nm.

### 3. Results and Analysis

Collagen threadlike structures containing nucleoprotein granules have been the object of detailed investigations by Biomedical Physics Laboratory of Seoul National University. In the present study, they showed remarkable diamagnetic and optical anisotropy properties, auto-fluorescence when observed by polarization and fluorescent microscopy (in part of the experiments - in high gradient magnetic field, HGMF). In Fig.1a the freshly extracted collagen tube (Bonghan duct) was attached perpendicular to a wire (30 microns in diameter) and bright granules are seen. Fig.1b shows the collagen dried tube with granules. In Figs.1, c,d,e the fish duct is shown in polarized light and under a blue excitation of an inverted microscope (Olympus, Japan).

![Fig. 1. Threadlike structures containing nucleoprotein granules on 30 micron wire magnetized perpendicular to plane: a - in the solution; b - after drying, c - a fish threadlike structure; d - blue excited auto-fluorescence of same duct as in c; e - part of a fish duct with symbiotic bacteria.](image)

The appearance of the very bright cholesteric crystalline structures from the isotropic ones in HGMF were observed for the macro-cell and the nucleoprotein dense suspensions, and are shown in Fig.2. Fig.3 shows the DNA-fraction which magnetically separated on the wire in the diamagnetic area. This fraction was obtained from the Bonghan granules suspension. The micro-analytic magnetic separation method developed for little amounts of a sample was applied to detect the high concentrations of DNA seen as the bright crystalline structures.

![Fig.2. Aligned liquid crystalline structures near 30-micron wire magnetized by 1T magnetic field: a - granules inside the tissue piece are aligned perpendicularly to the wire axis and the birefringence effect of the crystalline dried sample is seen in polarized light; b - macrocell containing diamagnetic nucleoprotein attached to the wire; c - the granule droplet on the wire which shows the highest brightness intensity and the highest DNA concentration after removing all the protein.](image)

![Fig.3. DNA-granules separation in HGMS from rabbit Bonghan structures. The suspension was observed in the magnetic separation along the magnetized wire after; a - 15 min.; b - 30 min., c - 85 min. The appearance of the cholesteric-smectic phases and screw-like structures, which are known for DNA concentration phase transitions.](image)

The dynamical spectroscopy of intensity fluctuations allowed measurements of light dynamic scattering from samples. The technique is applicable for motility detection of bio-microparticles in suspensions and tissues. Two photodiodes inside the photo-detector were used in order to detect intensity fluctuations at two points, \(r_1\) and \(r_2\), of small objects. When two spatially separated photo-diodes with zero time delay were applied, the parameter of a coherent distance was associated with the average size of the micro-particle (“speckle”). An autocorrelation function of the photocurrent generated by a coherence area was represented by a relation including two temporarily shifted currents induced in a differential scheme with two photodiodes. The light intensity 1 detected by the photodiodes is proportional.
to the electrical vector of electromagnetic wave from light source \( E \) and the intensity correlation function is:

\[
I(t) - I(t + \tau) = I^2(\tau) \exp(-2\tau),
\]

\[
A = (\pi/44) \{1 - \cos \theta^2 \cdot D_1 + \sin^2 \theta \cdot D_2 \}
\]

where \( A \) depends on the translational diffusion coefficients \( D_1 \) along \( x \) and \( y \) axes, \( \theta \) is scattering angle of the beam on a particle, and \( \lambda \) is a wavelength.

![Graphs and images](image)

**Fig. 4.** a - Image of Bonghan duct and BH-granules; b - detected Fourier spectrum of a single Bonghan granule motion; c - the 6-graph design of the photo-detector supplementation used for real-time graphs and temperature shift.

Fig. 4 shows single cell motion graphs and temperature phase transition for Bonghan granules, the phase transition at 33 degrees is seen, it occurred under a fast procedure incubation. Since the dependence “Frequency versus Temperature” did not reflect the signals intensity but only the average frequency from many cells in the visual field, this graph was very convenient to identify the thermo-resistance. The graph shows a reversible character of the curve during the cooling of the suspension while visually only 3-5% of the cells continued to move. The critical temperature at only one point was repeatedly observed indicating the homogeneity of the sample. Temperature phase transitions of a gel - liquid crystal in cellular membrane are the reason for the maxima in temperature dependences of optical, magnetic and morphological properties.

While the fish light ducts showed green-blue autofluorescence (Fig. 5), the skin vessels and rabbit tissues observed under UV-blue-microscopy study revealed porphyrin fluorescence with intense blue and red bands, seen both in polarized and non-polarized illuminations, emitted by the membrane walls surface (Figs. 6 and 7). Due to identified proporphyrin IX produced inside tissues by mitochondria and, possibly, by BH-granules, the single thread-like structures emitted a blue light (as it was seen in a blue filter) with a very high intensity and a delay time of 1-5 sec. Concentration of the porphyrin was successfully done by applying the high gradient magnetic fields (Fig. 6). The fluorescence substance and structures had paramagnetic susceptibility with respect to the solution, under blue light, while before the blue illumination the ducts indicated strong diamagnetic properties, crystalline phase transitions, aggregation and alignment in magnetic fields. The difference may be a result of increased free radical generations of super-oxide during photo-chemical reactions.

![Graphs and images](image)

**Fig. 5.** a - The fish light organ; b - the organ during flashing; c - the organ 1 sec. after flashing ended; the autofluorescence decay process was detected.

Illumination of the endogenous proporphyrin with blue light induced division of the fist cells and synchronous photoproducts generation. The method of photo-dynamics in tissue threadlike structures can be a sensitive indicator of skin growth processes emitting spontaneous ultra-weak UV radiation which is then amplified by the porphyrin photoinactivation with superoxide chain reactions and due to the porphyrins produced by mitochondria (and sometimes, in human skin and fish, by colonizing bacteria). It was reported that protoporphyrin IX biosynthesis is regulated differently in different tissues revealing the tissue specificity: the localized protoporphyrin IX fluorescence and photosensitization was observed following the local administration of 5-Aminolevulinic Acid (ALA) to adenocarcinoma of the sebaceous gland, actinic keratoses, areas of psoriasis, skin abrasions, carcinoma of the lungs, breast, parotid. The fluorescence emission and excitation spectra of tissue protoporphyrin IX showing a strong affinity for membrane lipids might be sensitive biosensor of an intensive heme synthesis within structures containing mitochondria and numerous nuclei. Little amounts of extracted tissue threadlike structures were appeared to reveal crystal properties and diamagnetic characteristics different to surrounding tissues. They contained nucleoprotein granules with a strong diamagnetic susceptibility and showed a diamagnetic alignment and an autofluorescence in high gradient magnetic fields. In order to detect the kinetics of autofluorescence two shutters for the PMT and the light
source were operated with a mechanical system and an air compressor. After illuminated by opening the shutter for the light source, measurement of delayed luminescence was started at 117±10 msec after cutting off the light (Fig. 8a).

Fig.6. Magnetic separation of vessels (threadlike structures) using the micro-analytical HGMS (high gradient magnetic separation); a - the diamagnetic capture (the experiment done in Fig.1a); b - the paramagnetic capture; c - experimental magnetic capture of threadlike structures seen in the inverted microscope for a paramagnetic vessel under a blue light (385, 405, 420 nm excitation wavelengths) with autofluorescence effects; d - the magnified image shown in c of the auto-fluorescent effect of a paramagnetic Bonghan duct in the dark.

Fig.7. Human skin threadlike structures which reveal the photodynamic effect; a - human skin duct in polarized image; b - autofluorescence without any additions under blue excitation in the dark.

The decay process of the white and blue light excited autofluorescence is shown in Fig.8 for the measurements using a biophoton device (a) and fluorescent microscopy (b).

4. Conclusions

Crystalline structures arise in acupuncture meridians and react to light, magnetic fields and other biotherapy treatments. Autofluorescent threadlike structures were observed having crystalline diamagnetic aggregates, i.e. Bonghan granules, which were analogous to phytobacteria colonization and their shining in fish light organs.

Fig.8. Kinetic curves of a long decay process of auto-fluorescence observed with a - a biophotonic device in the measurement of delayed luminescence (done by Dr.Kim); and b - with blue illumination of the inverted microscope during a decay process over 0.5, 1.2 and 2.0 sec after the 0.3 sec flashing.

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