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CHARACTERISATION OF PHOTOCHEMICALLY FORMED BIOMIMETIC PHOTOAUTROPHIC SUPRAMOLECULAR ASSEMBLY "JEEWANU", SYNTHESISED IN SUNLIGHT EXPOSED STERILISED AQUEOUS MIXTURE OF SOME INORGANIC AND ORGANIC SUBSTANCES

V. K. GUPTA[1]

Department of Zoology, C.M.D. Post Graduate College, Bilaspur, Chhattisgarh, India

ABSTRACT

Sunlight exposed sterilised aqueous mixture of some inorganic and organic substances showed photochemical formation of protocell-like microstructures, "Jeewanu" (Bahadur & Ranganayaki, 1970). The Cytochemical investigation of Jeewanu showed that they are capable of multiplication by budding, grow from within and show various metabolic activities. They have been analysed to contain various compounds of biological interest viz. amino acids in free as well as in peptide combination, nucleic acid bases, sugars as ribose as well as deoxyribose and phospholipid like material in them. These microstructures can catalyse photolytic decomposition of water utilizing sunlight as a source of energy. Further it was found that hydrogen thus released in the mixture is utilized in the photochemical fixation of molecular nitrogen and carbon dioxide.

In the primitive atmosphere the function properties of molecules led to self-organisation of a specific group of molecules in specific stearic position and transformation of lifeless materials into emergence of earliest energy transducing system similar to Jeewanu. (Bahadur and Ranganayaki, 1970).

KEYWORDS: Jeewanu, Protocell, Microstructures, Cytochemical

One of the most fundamental problems of origin of life is that in primitive atmoshphere how energy transferring systems would have converted sunlight into chemical form (Lipman *et al.,* 1965) postulated that in all cells a tendency exists to convert major part of oxidation-reduction energy into phosphate bond energy. Living organisms photo chemically trap energy by the following two mechanisms.

i. Photochemical reduction of CO_2

ii. Photophosphorylation

The concept of photosynthetic phosphorylation suggested by Arnon *et al.*, 1954, opened up new vistas in the mechanism of conversion of solar energy into energy rich biological compounds and in electron transport phosohorylation.

Energetic Coupling: Chemically intelligible reactions

Several cases of phosphorylation viz. non-phosphorylation (Calvin *et al.*, 1969, Kenyon *et al.*,1969, Wahnedt *et al.*,1967, Broda *et al.*, 1975); inorganic photophosphorylation (Baltcheffsky *et al.*, 1974, Krasnovsky *et al.*,1974); organic photocatalysis (Hayatsu *et al.*,1971, Heinz *et al.*, 1979), have been studied by various workers. The direct photophosphorylation of ADP with iP ^{32} to ATP in aqueous suspension of semiconductors has been reported (Fan *et al.*, 1976, Fan *et al.*, 1978). An extremely sensitive methods for PPi synthesis was suggested by Nyren & Ludin *et al.*, 1990. Bacteriorhodopsin is a light driven photon pump (Oesterhelf *et al.*, 1973). The energy absorbed by them is utilized to drive ATP synthtase which converts ADP and inorganic phosphate into ATP (Danon *et al.*, 1974). The metabolic production of ATP can be viewed as a general mechanism for the coupling of energy yielding end energy requiring process. Coupling of metabolic activities and functional activities. The generation of ATP by chromatophores of photosynthetic synthetic bacteria has been investigated (Frankel *et al.*, 1954). In chromatophores of R.rubrum the driven transport is catalysed by alternating phosphorylating enzymes, the proton translocating PPase, which both are membrane bound (Baltcheffsky *et al.*, 1985). It was observed that final product of energy transfer reactions PPi and ATP respectively are formed at a catalytic site or very close to the outer membrane of the chromatophores (Brook *et al.*, 1985).The concept

[1]**Corresponding author**

of phosphorylation introduced entirely new possibilities based on light energy.

Photosynthetic Apparatus: A Supramolecular Array

In photobiological systems the phtotosynthetic apparatus consist of macromolecules (proteins embedded in a matrix of a bilayer phospholipids membrane (Tien *et al.*, 1974). The early events in photosynthetic energy conversion include phto-induced electron transfer mediated by donor and acceptor moieties. The small organic species are bound to proteins, which in turn are embedded in a lipid bilayer membrane. Thus the photosynthetic apparatus of a plant or other organisms actually comprises a large supramolecular array. In these covalent linkages model the structural role of proteins (Gust *et al.*, 1978). The emergence of chemistry beyond molecules Lehn 1995, has initiated a shift over last 25 years where concept like self-assembly are transferred from biological processes into chemical nanosystems through the medium of syntheses. Construction of natures molecules in the laboratory from atoms or single molecules a process known as "Total Synthesis" (Nicolaou *et al.*,1996). Molecular self-assembly, a concept central to nature's form and functions (Cramer, 1993). Assembly of molecular components to obtain photochemical devices has been studied (Balzani *et al.*, 1991, Balzani *et al.*, 1987). Supramolecular chemistry demonstrates cooperativity at structural and functional level.

At present best –characterised biological motor is ATP synthtase. The synthesis of ATP is based on a proton pump across a membrane. Perhaps the most spectacular molecular machine constructed in recent years is a biomimetic power ATP synthtase to produce ATP (Elsoton *et al.*, 1998). The working mechanisms of key biological machines that involved ATP syntheses have been studied in detail (Stock *et al.*, 1999, Boyer, *et al.*, 1999). Balzani studied Photo induced electron transfer to chemical potential associated with ATP-ADP conversion. It constituted a synthetic biological motor or a biomimetic system (Anna *et al.*, 2002).

The photochemical formation of protocell-like microstructures "Jeewanu" was observed in a sunlight exposed sterilized aqueous mixture of ammonium molybdate, diammonium hydrogen phosphate, biological minerals and formaldehyde (Bahadur *et al.*, 1964, Bahadur *et al.*, 1975, Bahadur *et al.*, 1970). These microstructures have a definite boundary wall and intricate internal structure. They multiply by budding, grow from within by actual synthesis of material and are also capable of showing various metabolic activities (Bahadur *et al.*, 1975). Jeewanu have been analysed to contain a number of biochemicals-like materials in them viz. amino acids which are present in free as well as in peptide combination, nucleic acid bases as purines as well as pyrimidines, sugars as ribose as well as de-oxyribose and phospholipids-like material in them. The presence of urease, esterase, peroxidase and phosphatase –like activities have been detected in the mixture (Bahadur *et al.*, 1970, Bahadur *et al.*, 1963, Singh *et al.,*1975). The esterase and phosphatase-like activities in Jeewanu mixture were also reporte (Bahadur *et al.*, 1970, Briggs *et al.*, 1965). The presence of acid phosphatase-like activity in Jeewanu has been histochemically demonstrated (Gupta *et al.*, 1974). Jeewanu have been also found to contain ferredoxin-like material in them (Rao *et al.*, 1978, Rao *et al.*, 1978). The photochemical reduction of acetylene by Jeewanu, indicated the presence of nitrogenase-like activity in the mixture (Smith *et al.*, 1981). The cytochemical and histochemical investigations of Jeewanu showed that they can be fixed with biological fixatives and can be stained with acidic and basic dyes (Bahadur *et al.*, 1972, Gupta *et al.*, 2013).

It is quite possible that earliest energy transducing systems were possibly a photoautotroph. Therefore an attempt was made to investigate the photochemical formation of protocell-like microstructures Jeewanu (Bahadur *et al.*,1970), under highly precise laboratory conditions.The morphological characteristics of Jeewanu were investigated using optical, electron microscope (SCM & TEM) and Atomic Force Microscope to undertand self-organisation of photoproducts at mesoscopic level and emergence of protocell-like supramolecular assemblies "Jeewanu" in the mixture. Further an attempt was made to study whether the earliest energy transducing systems could utilize energy rich compounds like ATP. A comparative study of high and low mineral mixture of Jeewanu was carried out to find out probable primitive pathways of energy production in the laboratory simulated possible primitive atmosphere.

Experimental

The following two types of Jeewanu mixtures were prepared (Bahadur *et al.*,1970).

- Low Mineral Jeewanu Mixture
- High Mineral Jeewanu Mixture

Method of preparation of Low Mineral Jeewanu Mixture

The following solutions were prepared:-

- 4% Ammonium Molybdate (w/v)
- 3% Di-ammonium hydorogen phosphate (w/v)
- Mineral Solution

It was prepared by dissolving 20 mg each of potassium di-hydrogen phosphate, calcium acetate, sodium chloride, potassium sulphate, magnesium sulphate and 50 mg of ferrous sulphate in 100 ml of distilled water. The salts were added one by one, a new salt was added when one was dissolved completely by shaking.

Ammonium molybdate 50 ml (1 vol.), di-ammonium hydrogen phosphate 100 ml. (2 vol.), mineral solution 50 ml (1 vol.) were mixed in a conical flask. The flask was cotton plugged and sterilized in an autoclave at 15 lb pressure for 30 minutes. After cooling 10 ml (1 vol.) 36% formaldehyde was asceptically added in the mixture. A part of the mixture was taken in a conical flask covered with black cloth was kept as control mixture. The mixtures were exposed to sunlight for 24 hours giving 6 hours exposure each day.

Morphometric characterization of Jeewanu by optical Microscope

A drop of suspension of suspension of mixture was examined under optical microscope at 1500 X. The images obtained were digitally recorded and analysed by Image Analysis Software Pro C provided by Olympus. The various morphometric measurements were taken to characterize the photoproduct synthesized in the mixture. (Figure.1)

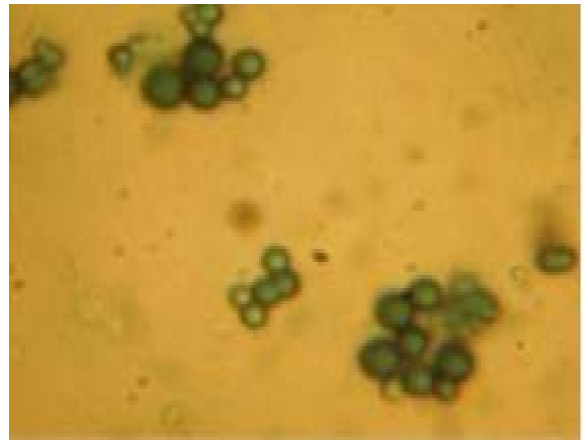

Figure 1: Low Mineral Jeewanu (1500X) showing multiplication by budding and growth from within

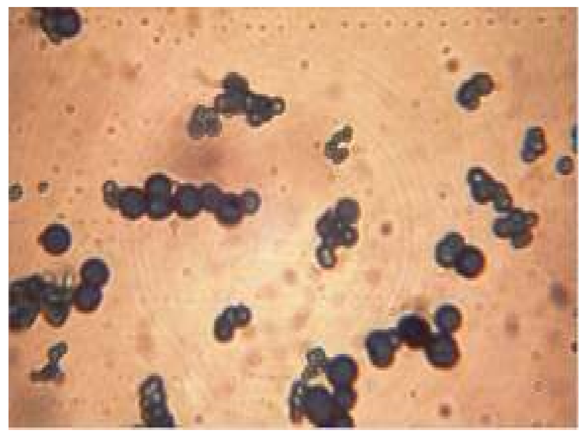

Figure 2: High Mineral Jeewanu (1500X) showing multiplication by budding and growth from within

Scanning Electron Microscopic Study of Jeewanu (SCM)

For scanning electron microscopic observation, the sterilized aqueous suspension of reaction mixture was thoroughly shaken. A drop of suspension containing Jeewanu was placed on a clean microscopic slide and covered with a coverslip. The slides were dried at room temperature. Samples were analysed by scanning electron microscope at National Chemical Laboratory, Pune, India. The specimen were coated with platinum, palladium (80:20) in an Eiko IB-3 ion coater. The slides were examined under scanning electron microscope operated at 20 KV and analysed.

OBSERVATIONS

Scanning electron micrographs of High Mineral Jeewanu revealed that they are spherical in shape. Their size varies from 0.5μm to 3.5μm in diameter. Micrographs taken at high resolution showed that their surface is smooth. Many of the Jeewanu possessed junctions. It appears that junctions were not due to binding of particles to each other but were the result of budding. The differences in the size of particles clearly shows that newer units come from parental unit which is larger in size by budding. Some of the transitional stages of formation of Jeewanu also suggests the occurrence of phenomena of budding in the reaction mixture. (Figure 3)

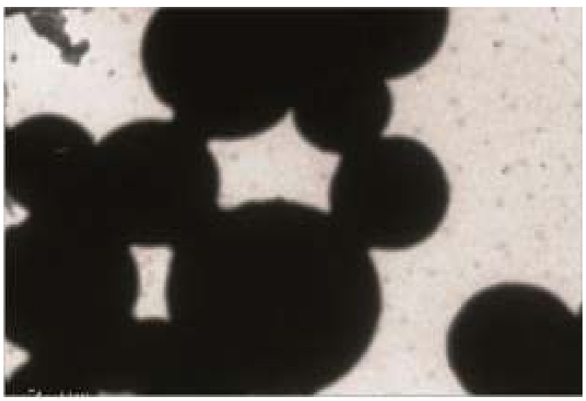

Figure 3: Scanning Election Micrograph of Jeewanu showing multiplication by budding and growth from within

Transmission Electron Microscopy of Jeewanu (TEM)

The experimental mixture of Low mineral Jeewanu (Bahadur *et al.*, 1970) was prepared as discussed above and a part of it was covered with black cloth and was kept as control. The mixtures were similarly exposed to sunlight.The suspension of Jeewanu was filtered and air-dried in vacuum deciccator at room temperature.

The dried samples of Jeewanu were sealed and sent for transmission microscopy at All India Institute of Medical Sciences, New Delhi, India. The routine procedure for preparation of blocks of the sample was followed.

Fixation:

The dried particles of Jeewanu were fixed in 1.5% Glutraldehyde in 0.1 M cacodylate buffer.

Post-fixative treatment:

The Samples of fixed particles were suspended in equal amounts of 2% osmium tetraoxide (aqueous) and 0.1 M sodium cacodylate for post-fixative treatment for 2 hours.

Washing:

After secondary fixative treatment the particles were thoroughly washed in 0.1 M sodium cacodylate buffer.

Dehydration:

The samples were dehydrated in ascending series of acetone and cleared in toluene.

Embedding:

Jeewanu were embedded in araldite using gelatine.

Polymerisation:

The embedded blocks were kept in an over at 50^0c for 24 hours. The temperature is then raised to 60^0c and then blocks were kept for another 48 hours to complete polymerization.

Preparation of ultra-thin sections: stains and staining procedure:

Ultra-thin (60 nm) sections of blocks of Jeewanu were taken. The sections were stretched by exposing them to chloroform. The slides were mounted and examined by transmission electron microscope.

OBSERVATIONS:

The ultra-thin sections of Jeewanu clearly showed the presence of a definite boundary wall and an intricate internal structure at the centre. At some places sections were broken during sectioning may be due to lack of polymerization or less stability of the microstructures.

Atomic Force Microscopy (AFM)

The suspension of Jeewanu mixture was mounted on a glass grid and air dried. The scanning probe Atomic Force Microscopy of the same was carried out at Inter University consortium, Dept. of Atomic Energy, Inodre (M.P.), India.

Preparation of smears of Jeewanu on glass disc for Atomic Force Microscopy:

The suspension of Jeewanu mixture was thoroughly shaken. A drop of suspension of Jeewanu mixture was evenly spread on glass disc and an air-dried preparation of sample at room temperature was made. The surface of one of the discs was made sticky by albumen and then an air-dried smear of the sample was prepared for investigation.

OBSERVATIONS:

AFM contact probe procedure was followed for the scanning of the sample. Three-dimensional image at nano-scale resolution of the sample by AFM was obtained. The various surface characteristics of the sample, viz. surface roughness analysis, grain size analysis was carried out. The Scanning Probe Microscope (SPM) revealed the presence of that surface of particles bears numerous protuberances and thus appears rough. The size of protuberance varied.

Method of preparation of High Mineral Jeewanu Mixture

Following solutions were prepared

- 4.00 gm ammonium molybdate and 12.00 gm di-ammonium hydrogen phosphate were dissolved in 100 ml of distilled water.
- Mineral Solution

It was prepared by dissolving each of 3.00 gm sodium chloride, 0.30 gm calcium acetate, 0.30 gm potassium sulphate, 0.30 gm, magnesium sulphate and 0.50 gm of ferrous sulphate in 100 ml of distilled water.

Solution (i) and (ii) were mixed in a conical flask as a result of which a white precipitate was formed, which was digested in the least quantity of Conc. HCl and by boiling.

After cooling the volume of solution was made up to 300 ml by distilled water.

The mixture was cotton plugged and then sterilized in an autoclave at 15 lb pressure for 30 minutes.

After cooling, 3 vol of the mixture A and 1Volume of 36% formaldehyde were aseptically mixed in a conical flask. Mixture was exposed to sunlight for 2.30 hours and then analysed for the photochemical formation of Jeewanu and for the presence of ATP-ase-like activity.

Method of detection of ATP-ase-like activity in Jeewanu mixture

The standard assay mixture of Jeewanu for detection of ATP-ase-like activity consisted of 200µl of suspension of Jeewanu mixture in 10 micromoles Tris HCl buffer (pH 7.5) supplemented with 5 micromoles of $MgCl_2$. In the first set at the time (t=0) 50 µl (0.5mm) ^{32}P 5'ATP* (I 400000 – 200000 c.p.m) was added to each assay, performed in duplicate.

In the second set of experiment at the time (t=0) 50µl (0.5mm) AMP*was added to the standard assay mixture prepared as described above. The mixtures were then incubated at 37^0C for different periods as described in respective experiments described below.

The incubation reaction was stopped by adding 350 µl of NORITA (20% in 1N HCl) to each incubation medium and after 20 minutes of agitation, the charcoal and Jeewanu were centrifuged at 3000 g for 10 minutes.

All the inorganic material was absorbed on Norita A while ^{32}Pi resulting from ATP hydrolysis remained in the supernatant. Two kinds of control were run concurrently. In one assay labeled substrate was incubated without Jeewanu suspension. While in other assay Norita A and Jeewanu suspension were added simultaneously. Radioactivity of ^{32}Pi was determined in Beckman scintillation counter (LS 7500) in aqueous counting Scintillater liquid ACS. Counting efficiency of control mixture did not exceed 5% of the substrate activity.

Detection of ATP-ase-like activity in High mineral Jeewanu mixture given varying periods of incubation using labeled *ATP and *AMP as substrate.

Table No. 1 showing the periods of incubation and and nano moles of labeled substrate hydrolysed in the mixture (Figure 9)

S. No	Period of incubation in hours	Nano moles of *ATP hydrolysed/ 200 µl of Jeewanu suspension	Nano moles of *AMP hydrolysed/ 200 µl of Jeewanu suspension
1.	1	2.58	Not detected
2.	3	7.56	1.25
3.	6	10.32	2.1
4.	24	27.4	8.1

Inference

- The increase in the amount of ATP and AMP hydrolysed by Jeewanu mixture with the increase in the period of incubation clearly indicates the presence of ATP-ase-like enzyme activity in the Jeewanu mixtures.
- The values of hydrolysis of the substrates suggests that in the Jeewanu mixture ATP seems to be better substrate than AMP.

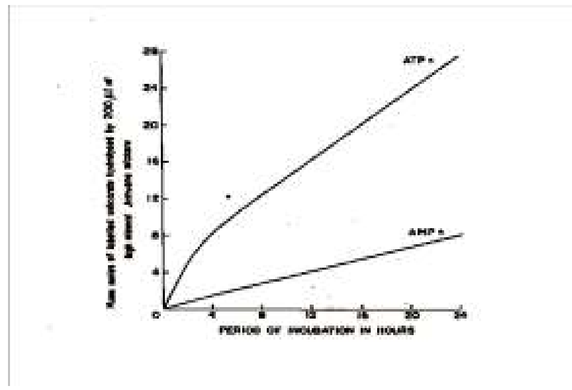

Figure 9: Detection of ATP-ase-like activity in Jeewanu mixture

Comparison of ATP-ase-like activity in Low Mineral and High Mineral Jeewanu mixture

OBSERVATIONS:

Table no.2 showing the periods of incubation and and nano mole of labeled substrate hydrolysed in the low mineral Jeewanu mixture

S.No	Jeewanu Mixture	Exposure to sunlight in hours	Duration of incubation in hours at 4^0c	nano moles of *ATP hydrolysed
1.	High Mineral	2.30	6.0	12 n moles/ 200 µl
2	High Mineral	Mixture was kept in dark at 40^0c for a month	For a month	11.9 n moles/ 200 µl
3	Low Mineral	2.30	6.0	2.88 n moles/200 µl
4	Low Mineral	Mixture was kept in dark at 40^0c for a month	For a month	2.25 n moles/200 µl

Inference

- Hydrolysis of labeled substrate in the Jeewanu mixture shows the presence of ATP-ase-like activity due to photoinduced phenomenon in the mixture.
- High Mineral Jeewanu mixture showed 5 fold more activity than low mineral mixture.
- The presence of ATP-ase –like activity detected in Jeewanu mixture is much stable as activity didn't change even after one month.

Experiment No. 3

The observations take in presence of EDTA mM showed no difference upto 24 hours indicating that ATP is non mm complex M ATP($M=Mg^{2+}$, Ca^{2+} or cation divalent).

In case of control mixtures in labeled substrates was incubated without Jeewanu mixture and in which Norita A and Jeewanu mixture were added no significant differences were noticed. Control mixture did not exceed 5% of the substrate radioactivity.

RESULTS AND DISCUSSION

Irradiated sterilized aqueous mixture of ammonium molybadate, diammonium hydrogen phosphate biological minerals and formaldehyde (Bahadur *et al.*,1970) shows photochemical formation of protocell-like supramolecular assemblies “Jeewanu” having a definite boundary wall and an intricate internal structure. They are capable of showing multiplication by budding, grow from within by actual synthesis of material and show various metabolic activities in them. The optical and electron microscopic studies have clearly revealed that newer smaller units comes from the parental unit by budding. The photochemical formation of Jeewanu is a self-sustaining process and are formed by autocatalytic photochemical transformations mediated by inorganic metal ions and transitional elements present in the mixture. The scanning probe microscopy (SPM) of Jeewanu revealed their structural characteristics at high resolution showing the presence of microstructure in different stages of their formation. The optical microscopic and transmission microscopic studies have shown that Jeewanu are spherical in shape, have a definite boundary wall and an intricate internal structure.

The scanning probe analysis of the sample by atomic force microscopy (AFM) includes mechanical contact force, Vander Waal force, chemical bonding, electrostatic forces at nanoscale.

The remarkable features of scanning probe microscope (SPM) is their ability to view details at atomic and molecular level, thus, increasing our understanding about structural and functional properties of the system.

The various intermolecular forces, viz. electrostatic, Vander Waal forces, decreases with decreasing particle size. Vander Waal force become dominant for collections of very small particles. The intermolecular forces are also dependent on surface topography. The presence of protuberances or asperities result in greater total area of contact between two particles. This increases intermolecular forces of attraction as well as tendency for interlocking and adhesion.

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The transformation of free energy from environment into high energy reactive anhydrides of phosphoric acid is the most fundamental type of biochemical reaction. In the modern cells these phosphate groups are accepted, transported and donated by ADP carrier system. The oxidative phosphorylation of ATP continues in the system (Eakin *et al.,* 1985). Mitchell *et al.,*1979, suggested that ATP-ADP conversion is intiated by light photosensitive pigments to produce a charge separation across a hydrobhobic membrane.

Electrons are transported towards the exterior surface of the membrane surrounded compartment and protons are transported in opposite direction. In the part of the membrane separated from the photosensitive pigment and charge carriers the protons from this gradient flew through a membrane bound ATP-as. The protons drive the ATP-ase reversibility to make ATP from ADP and Pi. ADP and Pi charge separation is of paramount importance in photophosphorylation mechanism (Gust *et al.,*1993).

The transmission electron micrographs of Jeewanu show the presence of a definite boundary wall capable of charge separation. The presence of ferredoxin-like molecules mediated electron transfer reactions in Jeewanu mixture.

In light of chemiosmotic theory (Mitchell *et al.,*1979) it can be postulated that ATP-ase like enzyme is possibly bounded to the boundary wall of Jeewanu which participates in oxidative phosphorylation reaction.

CONCLUSIONS

In primitive atmosphere possibly photosynergistic collaboration of non-linear processes at mesoscopic level led to selforganisation and emergence of supramolecular self-sustaining assemblies similar to “Jeewanu”. The systems chemistry concerning formation of dynamic covalent bonds, quantum mechanical resonance stability force and electromagnetic interactions must have led to spatio-temporal coherence showing a cooperative informational hierarchy between structure and function. The formation of non-covalent bond and spontaneousself-organisation of membrane (Furhop *et al.,*1994) is of much interest. Cairns Smith *et al.,* 1982 postulated that the first photosynthetic systems would have been made of clays such mineral membranes could hold transitional metal ions to catch light and conduct charges as well as inert barrier to separate photoproducts.

It can be said that energy rich compounds like ATP were synthesized in the prebiotic atmosphere by photophosphorylation reactions and were degraded to ADP and Pi with the help of ATP-ase-like enzymes. Energy thus released was utilized various bioenergetic processes. Presence of ATP-ase-like activity in Jeewanu mixture suggests that earliest energy transferring system would have been of jeewanu grade of organization.The free energy is needed to overcome thermodynamic limitations. In prebiotic atmosphere possibly photo-isomerisation and conformational changes initiated novel coherent emergent phenomenon at mesoscopic level, subsequently evolution of common universal ancestor or open chain microstructures possibly similar to Jeewanu showing certain degree of intelligence.

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Conflicts of Interest

"The authors declare no conflict of interest".

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