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RESEARCH ARTICLE

Detection of Thiamine Pyrophosphatase-like activity in Minimal Protocell-like Microstructures 'Jeewanu'

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ABSTRACT

Irradiated sterilised aqueous mixture containing ammonium molybdate, di-ammonium hydrogen phosphate, biological minerals and formaldehyde showed the photochemical formation of self-sustaining, minimal protocell-like microstructures 'Jeewanu'. They have an ordered structural configuration and capable of showing some properties of biological orders viz. multiplication by budding and growth from within. The presence of Thiamine pyrophosphatase-like activity have been histochemically detected in Jeewanu, the abiogenic protocell-like microstructures. In primitive prebiotic atmosphere possibly an alternative mechanism of photophosphorylation must have existed which might have led to evolution of bioenergetics processes and emergence of earliest living systems.

Keywords: Jeewanu, self-sustaining, protocell, abiogenic, microstructures

INTRODUCTION

The emergence and evolution of life and metabolic pathways in plausible prebiotic atmosphere represented crucial steps in molecular and cellular evolution. It was suggested that the primordial molecules imposing a progressively stronger selective pressure, that led to increasing ability of self-assembly and self-organization of molecules, that synthesizing those complex molecules whose used in increasing complexity and complex metabolic cycles and steps (Fani, 2012). The heterotrophic hypothesis (Horowitz, 1945) for the origin of life suggested that abiotically synthesized molecules with energy content would have been involve in the construction of their own component, maintain their self-organization and the downhill-chain reaction or proto metabolic cycles (Pascal *et al.*, 2011). It was also suggested that protocells have the ability to capture energy from the environment to initiate catalyzed heterotrophic growth directed by heritable genetic information in the polymers (Deamer and Weber, 2010).

Previous researchers on the origin of life argued that the energy sources were required to produce the small molecules of life viz. amino acids, nucleobases, amphiphils and synthesis of biologically relevant polymers and some sort of metabolism liberating the energy which is necessary for the various activities for the aggregation and self-assembly of molecules (Bahadur and Ranganayaki, 1981).

Overview of contemporary energy sources

In plausible prebiotic experiments scientists used various energy sources to synthesize some compounds of biological interest.

The high energy sources viz. ultraviolet light, atmospheric electric discharges and geochemical electric energy have the potential to serve as monomer for assembled into random polymers and small molecules synthesis (Chyba and Sagan, 1922; Deamer and Weber, 2010). Thermal energy was used in the amino acid polymerization (Fox and Harada, 1958), adenylic acid synthesis (Usher and Mchale, 1976) and oligonucleotide synthesis (Verlander and Orgel, 1974; Usher, 1977). Mooner and Oro, (1987) suggested that geochemical conditions were the potential prebiotic sources of hydrocarbon derivatives synthesis.

Certain kinds of energy could have driven from chemical reactions related to origin of life. DeDuve, (1991), Huber and Wachtershauser (1998) and Huber *et al*., (2003) have proposed that sulphur chemistry was a source of chemical energy related to origin of life. Maurel and Orgel (2000) and Zepik *et al*. (2007) have incorporated sulphur chemistry in their research.

Pyrophosphate bond energy could have been a significant source of chemical energy leading to the origin of life. Phosphorylation reactions must have been incorporated in primitive metabolic pathways (Deamer and Weber, 2010). Baltscheffsky (1996) reported that the pyrophosphate is also an energy source, used by a photosynthetic bacterial species Rhodospirrilum *rubrum*. It was assumed that all the monomers required for the synthesis of major polymers of life and inorganic phosphates were available in the prebiotic environment for the next stage of increasing complexity (Deamer and Weber, 2010).

Thiamine pyrophosphate functions as a coenzyme vital to tissue respiration. It is required for the oxidative phosphorylation of pyruvate to form acetyl-coenzyme A, providing entry of oxidizable substrate into the Kreb's cycle for the generation of energy. It is also a coenzyme for 'transketolase' which functions in the pentose phosphate pathways, an alternative pathway for glucose oxidation. Pyrophosphate bond energy could have been a significant source of energy in the reaction leading to origin of life (Deamer and Weber, 2010). The dephosphorylation of the thiamine pyrophosphate was demonstrated by Pratt (1954).

The study of origin and evolution of bioenergetics and primitive metabolism in the plausible prebiotic atmosphere created much interest to know the simpler way in which energy could have been captured from the environment and made available for primitive version of metabolism and polymer synthesis. Due to the absence of appropriate oxygen, the primitive bioenergetics is limited to anaerobic source of energy (Deamer and Weber, 2010). Hull (1960) and Bahadur (1964a) suggested that light energy is a most abundant and primary energy source for the formation of biogenic molecules and first form of life.

Bahadur and Ranganayaki (1970) synthesised self-sustaining abiogenic protocell-like microstructures 'Jeewanu' in sterilised aqueous mixture of some inorganic and organic substances using sunlight as a source of energy. The size of Jeewanu varies from 0.2µ to 3.0 µ in diameter (Bahadur, 1964b). They have a distinct boundary wall and intricate internal structure (Gupta, 1976; Upadhyaya, 1977). They show properties of growth, multiplication and metabolic activities (Bahadur and Ranganayaki, 1970). They have been analyzed to contain various materials of biological interest as amino acids that are present in free as well as in peptide combination (Bahadur and Ranganayaki, 1970; Bahadur *et al*., 1963; Briggs, 1965), nucleic acid bases purines as well as pyrimidines (Bahadur and Ranganayaki, 1970; Ranganayaki *et al*., 1972), sugars as ribose as well as deoxyribose (Bahadur *et al*., 1963), RNA-like material (Gupta and Rai , 2013) and phospholipids-like material (Singh, 1975) in them. The presences of various enzyme-like activities viz. phosphatase, ATPase, ester-ase and nitrogen-ase (Bahadur and Ranganayaki, 1970; Bahadur *et al*., 1963; Singh, 1973; Bahadur and Gupta, 1984) have been observed in the Jeewanu mixture. The EPR spectra of Jeewanu showed the presence of ferredoxin-like material in them (Rao, 1978).

An attempt was made to study histochemical localisation of Thiamine pyrophosphatase-like activity in abiogenic, protocell-like microstructures Jeewanu with a view to understand the nature of origin and evolution of proto-metabolic pathways and primitive alternative source of energy, necessary for self-assembly of molecules and increasing complexity of protocell-like microstructures with properties of biological orders, in plausible prebiotic atmosphere.

The Eranko and Hasan method (1954) was used for histochemical localisation of Thiamine pyrophosphatase-like activity in photochemically formed abiogenic protocell-like microstructures Jeewanu (Bahadur and Ranganayaki, 1970).

METHODS AND MATERIAL

Method of preparation of Jeewanu (Bahadur and Ranganayaki, 1970)

The following solutions were prepared –

1. 4 % Ammonium molybdate (w/v)
2. 3 % Di-ammonium hydrogen phosphate (w/v)
3. Mineral solution -

It was prepared by dissolving 20 mg. each of potassium di hydrogen orthophosphate, sodium chloride, magnesium sulphate, potassium sulphate, calcium acetate, manganous sulphate and 50 mg. of ferrous sulphate in 100 ml. of distilled water. Each salt was dissolved one by one; a salt was added when previous salt was dissolved completely.

The above solutions were taken in a sterilised conical flask, cotton plugged and were sterilised in an autoclave at 15 lb. pressure for 30 minutes.

After cooling, 4% ammonium molybdate solution (1 volume), 3% di-ammonium hydrogen phosphate (2 volumes) and mineral solution (1 volume) were mixed in a sterilised conical flask. 36% Formaldehyde (1 volume) was aseptically added in the above solution.

A part of above solution was covered with several folds of thick black cloth and was kept as control. All the solutions were exposed to sunlight for photochemical formation of protocell-like microstructures Jeewanu.

Methods of preparation of smears of Jeewanu

- The sunlight exposed mixture was shaken thoroughly and a drop of suspension of Jeewanu mixture was taken out with the help of a sterilized glass rod on clean microscopic glass slide.
- The drop of suspension was homogeneously spread and covered with micro cover glass and named 1.
- Four smears of Jeewanu were similarly prepared and named 2 to 5 and were used for histochemical localisation of thiamine pyrophosphatase -like activity.

Method of preparation of incubating media

The incubating media was prepared as given below –

Lead nitrate $(PbNO_3)2$	-	60 mg.
0.005 M-acetate buffer (pH 5.0)	-	45.0 ml.
5% thiamine pyrophosphatase	-	5.0 ml.

Above solution was incubated at 37^0C for 24 hours. The above solution was filtered twice and used as an incubating media.

Incubation

The smears no. 2 to 4 was incubated in above incubating media at 42^0C for 5-60 minutes. The smear no. 5 was incubated without substrate. It was used as control.

Preparation of slides

The smears no. 2 to 5 were treated as follows –

- The slides were rinse in 1% acetic acid.
- The slides were blotted and dried at 60^0C for 5 minutes.
- The slides were covered with a thin film of celloidin.
- The slides were developed in dilute yellow ammonium sulphide for 2 minutes.
- The slides were washed with distilled water.
- The slides were mounted with glycerin jelly and examined under optical microscope.

OBSERVATIONS

- The mixture kept as control revealed negative results for photochemical formation of Jeewanu. (Fig. no. 1.)
- The optical microscopic observations of smear no. 1 showed the presence of spherical, blueish coloured microstructures Jeewanu, having double walled boundary and intricate internal structure. (Fig. no. 2)
- Optical microscopic observations of slides no. 2 to 4 showed the presence of brownish-black patches inside the boundary wall of Jeewanu. (Fig. no. 3) The particles of 24 hours of exposures showed better result.

- The control slide no. 5 showed negative result for histochemical localisation of thiamine pyrophosphatase like activity. (Fig. no. 4)

RESULTS AND DISCUSSION

The presence of brownish black deposits or patches inside the boundary wall of Jeewanu showed the histochemical localisation of thiamine pyrophosphatase like activity.

Natural selection can only work on entities that have three basic features: information, metabolism and membrane, where metabolism must include the capability of producing all cellular structures, as well as energy (ATP) from external sources (Hevia *et al.*, 2008).

It can be said that energy rich compounds like-ATP were synthesised in the prebiotic atmosphere by photo-phosphorylatin reaction, where degradatation of ADP and pi were completed with the help of ATP-ase-like enzyme. The phosphatase like activity helped in the liberation of energy for metabolism and physiological activities of protocell like microstructures.

The protocell-like model Jeewanu is photochemically synthesised by using weakest source of energy the sunlight. They have a definite membrane and capable of showing small amount of metabolic reactions.

Fig. 1

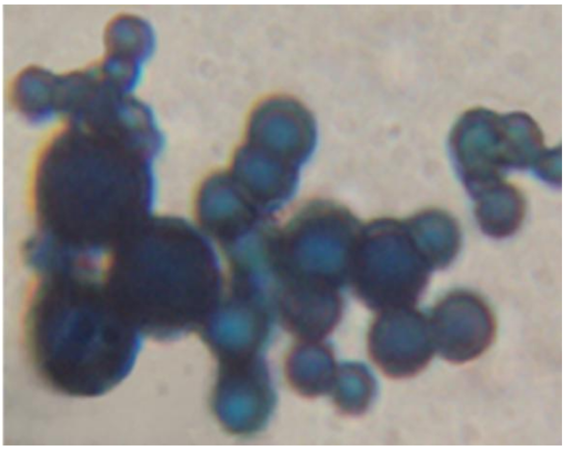

Fig. 2

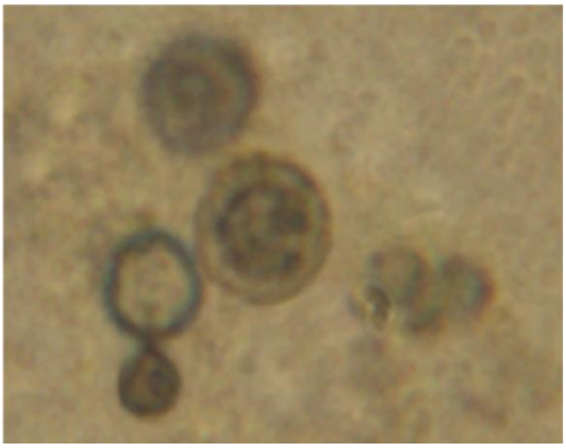

Fig. 3

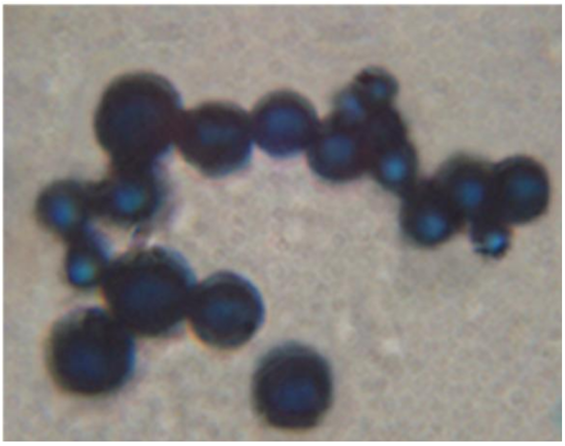

Fig. 4

Fig. 1: Control mixture of Jeewanu, showing negative results for photochemical formation of Jeewanu.
Fig. 2 : Micrograph of Jeewanu of 24 hours exposure, showing definite boundary wall intricate internal structure (1500X)
Fig. 3: Micrograph of Jeewanu showing histochemical localisation of Thiamine pyrophosphotase-like activity inside the boundary wall of Jeewanu (1500X).
Fig. 4: Control slide of Jeewanu showing negative results for histochemical localization of Thiamine pyrophosphotase-like activity (1500X)

The presence of Thiamine pyrophosphatase-like activity in the Jeewanu shows the existence of alternative pathways of energy production in the plausible prebiotic atmosphere. Jeewanu are capable of generating organic material as well as producing an energy rich compound (ATP) necessary for biosynthetic processes (Bahadur and Ranganayaki, 1970).

Membrane encapsulation is a ubiquitous in biology and is thought to have been an essential aspect of the earliest cell-like structure (Szostak *et al*., 2000). The presence of limiting boundary wall of Jeewanu possibly played a significant role in charge separation and redox transformations.

The presence of Thiamine pyrophosphatase-like activity in protocell-like model Jeewanu provide the alternative mechanism of energy transduction.

In primitive atmosphere possibly self-assembly and polymerization reactions initiated the synthesis of energy rich compounds by photophosphorylation, may reveal evolution of bioenergetics pathways and emergence of earliest living system.

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