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Histochemical characterization of protocell-like supramolecular assemblies "Jeewanu", synthesized in a irradiated sterilized aqueous mixture of some inorganic and organic substances

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Abstract

The present study shows the occurrence of autoreplicative protocell-like model "Jeewanu" for the origin of life in the possible prebiotic atmosphere. The Jeewanu has been prepared in the laboratory in a sunlight exposed sterilized aqueous mixture of some inorganic and organic substances(Bahadur and Ranganyaki;1970). An attempt has been made to study histology of these artificially prepared particles with acidic and basic dyes. The investigation revealed that Jeewanu has orderly structural organization with metabolic characteristics. In prebiotic atmosphere possibly energy transducing systems similar to Jeewanu existed which had an ability to convert solar energy into useful forms.

Keywords : Jeewanu, Protocell, Supramolecular assemblies, Abiogenesis, Origin of life , Chemoton model.

Introduction

One of the most fundamental problems of origin of life is to know of functional cells in prebiotic times (Maynard 1986 &, 1987; Dawkins 1976). The origin of life presumably occurred by self-assembly of organic compounds on the prebiotic earth into encapsulated molecular systems capable of catalyzed polymer synthesis. Such a self-assembly might possess a membrane bound self reproducing molecular cell systems (Deamer, 2002). The self assembly of molecular systems within a variety of cell-sized has been extensively studied by workers in the field (Deamer 1997, Segre *et al*., 2001). It is suggested that mineral surfaces have an important role into pre-cellular evolution. Life began as a series of reactions resembling metabolism. The autocatalytic pathways were established perhaps on mineral surfaces in aqueous phases as suggested by Bernal (1967). Pinto *et al.* (1980) was of the opinion that endogenous synthetic stages need to be investigated in detail. Overtime the systems became increasingly complex to the point that self reproducing polymers may be synthesized with cellular compartments in course of time

(Wachtershäuser,1988, Cody 2000). Prior to the evolution of biochemical machinery the growth and division of simple primitive cells (protocells) must have been driven by environmental factors (Zhu *et al*., 2012). Self assembly processes can produce supramolecular complex structures with certain properties of living state. Such structures are able to capture energy available in the environment and initiate primitive reactions associated with metabolism, growth and replication (Deamer,2007). The self assembly and replication of membrane, the nature of potential polymers and the nonenzymatic template directed copying nucleic acid sequences postulated formation of vesicles by various scenarios of self-organization (Hanczyc Szostak, 2004; Orgel,2004; 2006;Eschenmoser, 1999). The synthesis of amino acids might be initiated by spark discharge in a mixture of reduced gases (Miller, 1953). The abiogenesis of organic compounds on the early earth became central point in the postulation for the origin of life (Willis and Bada, 2000). Life has been defined as a chemical system capable of Darwinian evolution (Joyce and Orgel, 1998). Prior to evolution of biochemical machinery the growth and simple primitive cells (protocells) must have been driven by environmental factors (Zhu *et al*., 2012).

Laboratory model of protocell systems should be helpful in modeling various hypotheses of origin of life. Assuming if protocell reproduction can be achieved and the adaptive innovations are possible in the spontaneous evolution it will then be simple chemical system. The nature of such adaptations may provide clues as how modern cells evolved from their earliest ancestors (Zhu *et al*., 2012).

Chemical evolution would have produced primitive self maintaining chemical systems with rudimentary mechanisms of energy transduction but without (replication and mutable) records. We call such systems as "Infrabiological Systems" (Szarthmáry, 2005).

Several concepts of models of cells have been produced to test various theories for the origin of cellular life (Cavalier-Smith, 1987; Luisi, 1998; Szostak *et al.*,2001; Pohorille and Deamer, 2002).

"Chemoton model" of living system was given by Gánti (1971). He emphasized combination of a metabolic cycle and a membrane was also proposed called a self reproducing microsphere (Gánti, 2003). In contrast, a protocell-like entity with a boundary and template replication but no metabolic system was conceived earlier (Szostak, 2001). Based on Chemoton concept protocells have been visualized as the functional integration of metabolism, containment and information processing (Rasmussen *et al*., 2003). The photochemical, formation of protocell-like microstructures "Jeewanu" in a laboratory simulated prebiotic atmosphere capable of showing multiplication by budding, growth from within by actual synthesis of material and various metabolic activities has been reported (Bahadur *et al.,* 1963;1964; 1966;1967; 1970; 1975;1980). Jeewanu have been analysed to contain a number of compounds of biological interest viz. amino acids in free as well as in peptide combination (Bahadur *et al.,* 1954; 1961;1965; 1970; Briggs;1965; Maurya;1977), sugars as ribose as well as deoxyribose (Bahadur et al. 1963; 1964; 1966; 1967; 1970), nucleic acid, bases as purines as well as pyrimidines (Bahadur *et al*., 1970, 1972, Ranganayaki *et al.,*1976), phospholipids (Bahadur et al., 1970; Singh, 1975) and ferredoxin -like material (Rao *et al*., 1978; Bahadur *et a*l., 1980) in them. The presence of various enzyme like activities viz., phosphatase, ATP-ase, esterase (Briggs, 1965; Bahadur *et al*., 1970; Singh, 1973; Gupta 1980), nitrogenase (Smith *et al.,* 1975; Bahadur *et al*., 1980) have been also been detected in Jeewanu mixture. It was found that under certain specific conditions Jeewanu can catalyse photolytic decomposition of water utilizing sunlight as a source of energy. Further it was observed that H_2 thus released is utilized in photochemical fixation of nitrogen, and carbon dioxide. These findings were confirmed using C^{14}, N^{15} and D_2O (Smith *et al* ; Bahadur et al., 1980). Gáinti (2003) discussed that Jeewanu posseses a promising configuration similar to protocell-like model. Therefore, an attempt has been made made to investigate histological characteristics of Jeewanu to characterize their structural organization. The present investigation is aimed to acertain the possible nature of earliest living system on the earth.

Materials and Methods

The high mineral Jeewanu were prepared by the method as described by Bahadur and Ranganyaki (1970) and Verma (1980). The histochemical localization of acidic material in the Jeewanu was studied by Eosin (Baker, 1969) while the basic materials were stained with by Gentian Violet (Baker, 1969) and Methyl Green (basic dye) (Brachet, 1944; Pearse,1961). The histochemical localization of RNA-like activity was studied by Pyronin Y staining technique (Brachet, 1944; Pearse,1961). The staining procedure employed in the present study is routine procedure.

The High Mineral Jeewanu mixture was exposed to sunlight and the photochemical formation of Jeewanu was studied at regular intervals under high power and oil immersion (1500x) of optical microscope.

The ingredients of the solution for the preparation of Jeewanu are as follows :

Solution (i) :

Ammonium molybdate 4.00 gm and diammonium hydrogen phosphate 12.00 gm were dissolved in 100 ml of distilled water.

Solution (ii) :

Mineral solution : It was prepared by dissolving the following salts in 100 ml of distilled water. Each salt was added when one salt was dissolved completely.

NaCl	3.00 gm
$Ca(CH_3COO)_2$	0.30 gm
K_2SO_4	0.30 gm
$MgSO_4$	0.50 gm
$FeSO_4$	0.50 gm

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

After cooling, the volume of the solution was made up to 300 ml by distilled water. Mixture was cotton plugged and then sterilized in an autoclave at 15 lb pressure for 30 minutes.

After cooling, 3 volume of above mixture and 1 volume of 36% formaldehyde were aseptically added in a conical flask.

A part of mixture was kept in dark as control. Another part was taken in a separate conical flask, cotton plugged and covered with black cloth and was kept as control.

The mixtures were exposed to sunlight for varying periods of exposure as per requirement of the experiment. The control mixtures covered with black cloth were examined for the formation of photoproducts at regular intervals.

Observations

The Jeewanu mixture kept in dark as control showed negative results. It is colourless and did not show any initiation of photochemical reaction.

The mixture after exposure to sunlight immediately became bluish in colour. The intensity (bluish) of colour increases on further increasing the exposure time of sun light. The photochemical formation of Jeewanu becomes visible under high power of microscope even in few minutes (2-5 minutes) of exposure to sunlight.

Morphologically, Jeewanu are spherical in shape and exhibit bluish colour. The microscopic (1500x magnification) observation of Jeewanu showed that they became yellowish in colour with a definite boundary wall and possesses intricate internal structures. The number of Jeewanu formed increases in number on further exposure to sunlight. The size of Jeewanu forms varies from 0.5μ to

3.5μ in diameter (Fig. 1 & 2)

With Gentian Violet staining, the central structure of high mineral Jeewanu presence of basic material-like substance. In some particles bluish colour was seen diffused in the peripheral region (Figs 3 & 4).

The microscopic examination of slides under high power and oil immersion (1500 X) showed that extra central region of High Mineral Jeewanu was stained pink showing the localization of acidic structure in the extra central region.

With Methyl Green (basic dye) the central structure ofl Jeewanu take intense green stained and diffused greenish colouration was also seen in the peripheral region showing the presence of a basic material-like substance in the Jeewanu mixture (Figs 5 and 6).

The bright red colour of Pyronin Y was diffused throughout the High Mineral Jeewanu showed the presence of RNA like activity in the mixture (Figs 7 &8).

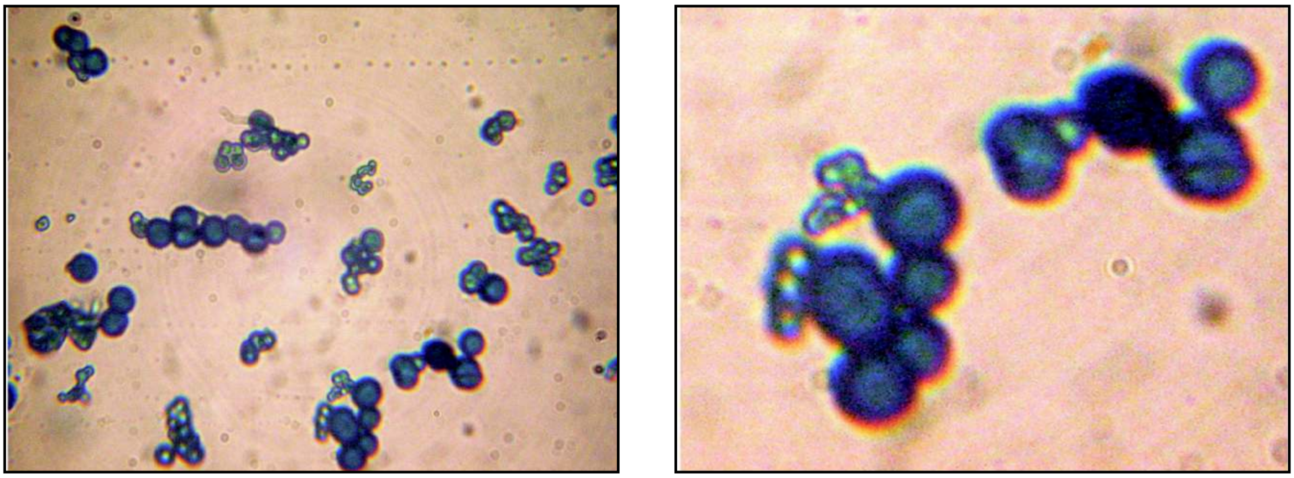

Fig 1 & 2 : Micrograph showing photochemical formation of high mineral Jeewanu showing multiplication by budding and growth from within under optical microscope (1500 X)

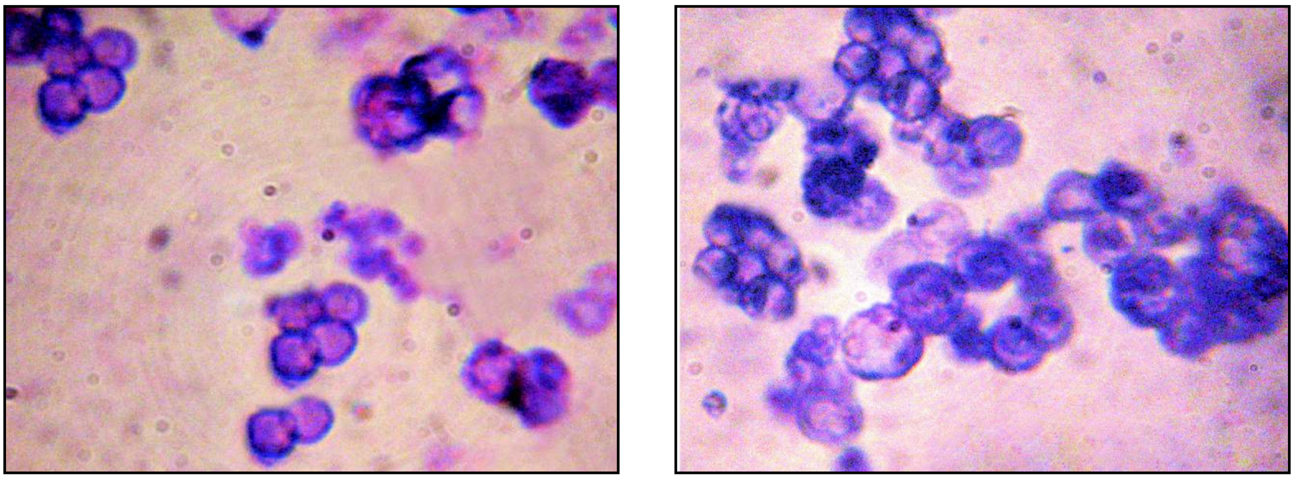

Fig 3 & 4 : High mineral Jeewanu stained by Gentian Violet © showing histochemical localization of acidic in the central region

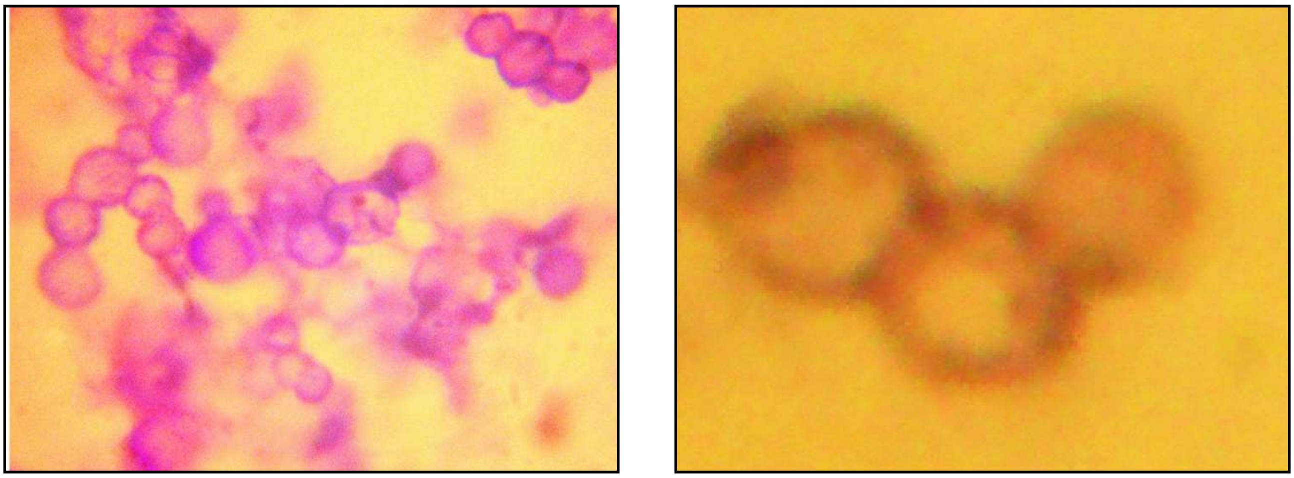

Fig 5 & 6 : High mineral Jeewanu stained by Eosin (micrograph c, d) showing histochemical localization of basic material in the extracentral region

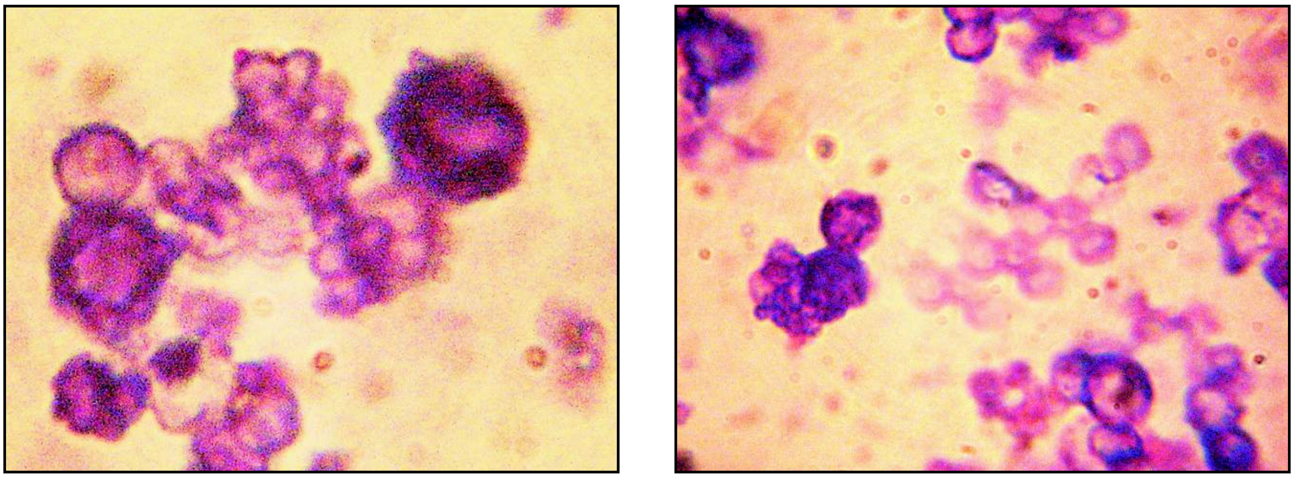

Fig 7 & 8 : High mineral Jeewanu stained by Pyronin Y (micrograph c,d,) showing histochemical localization of RNA-Like activity diffused throughout the micro structure

Discussion

One of the major challenges is to artificially synthesize protocell which can ensemble organic and inorganic substances into self-reproducing system of life (Szostak *et al.,* 2001; Hanczyc *et al*.,2004). Eigen and Shuster (1979) described certain organic molecules which can spontaneously organize into larger structures. Haldane (1928) and Oparin (1957& 1968) gave theory of coacervation of colloidal material as the beginning of growth material characterization of life. Molecular evolution refers to the ultimate formation of molecules by chemical transformation of substances with the condition that earliest living systems were madeup of the same material of which the present day living system is madeup of (Bahadur, 1964 & 1966). Bahadur postulated that first forms of metabolism arose inside closed compartments and became more complex later. At certain concentration additional molecules no longer dissolve but instead begin to associate into small aggregates called “micelles” (Bahadur, 1967). The abiogenesis of biogenic materials viz. amino acids, nucleic acid bases, sugars, phospholipids have been discussed for the origin of life. He has formed photochemically self- sustained protocells- like suprarmolecular assemblies called Jeewanu responsible for the origin of life. The present study is in conformity to the observations of Bahadur (1964 & 1966) that sunlight exposed sterilized aqueous high mineral mixture of ammonium molybdate, diammonium hydrogen phosphate, biological minerals and formaldehyde confirms photochemical formation of protocell-like molecular associations.They have a definite boundary wall and intricate internal structure. Their size varies from 0.5 μ to 3.5 μ in diameter. It is also held that formation of Jeewanu is strictly an outcome of a photochemical reactionin the possible prebiotic atmosphere.

The present cytochemical studies of Jeewanu with basic dyes Gential Violet, Methyl Green and Pyronin Y showed the presence of acidic material-like substance in the central region. Staining with Pyronin Y suggested that RNA-like activity is possibly diffused throughout High Mineral Jeewanu. The histochemical staining of High Mineral Bahadur et al Jeewanu with acidic and basic stains showed that Jeewanu possesses an ordered structural organization. The present investigation confirms the observations of Bhadur *et al*. (1970, 1980, 1972,1973, 1976) that RNA monomers are more readily photochemically synthesized in Jeewanu mixtureas The also supported the view that primitivity of RNA in the possible primitive atmosphere (Joyce and Orgel, 2006). The microscopic examination of Jeewanu, the autoreplicative protocell-like abiogenic microstructure shows that it possesses a definite ordered structure (Bahadur,1975; Bahadur and Gupta, 1972; Gupta,1980; Gupta,2002).

All cellular life today incorporates two processes – (a) self-assembly; and (b) directed assembly. The directed assembly involves the formation of covalent bonds by energy dependent synthetic reactions and require a coded sequence; while spontaneous self-assembly occurs when certain compounds interact through non-covalent, hydrogen bonds, electrostatic forces and non-polar interactions to form closed membrane bounded micro-environment (Pohorille and Deamer, 2002). It is also suggested that it is possible that in the primitive atmosphere there may nonlinear collaboration of photo-redox transformations at mesoscopic level possibly led to emergence of supramolecular assemblies similar to Jeewanu, showing properties of biological order, viz. multiplication by budding, growth from within by actual synthesis of material and metabolic activities. It may be postulated that earliest energy transducing systems were possibly a photoautotroph similar to Jeewanu which had an ability to convert solar energy into useful forms.

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