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ORIGINAL ARTICLE

Histochemical localisation of RNA-like material in photochemically formed self-sustaining, abiogenic supramolecular assemblies 'Jeewanu'

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ABSTRACT

Sunlight exposed sterilised aqueous mixture of some inorganic and organic substances showed the photochemical formation of self-sustaining, supra-molecular assemblies 'Jeewanu'. The microscopic observation (Optical, SCM and TEM) showed that they possess an ordered structural configuration. They are capable of showing various properties of biological order viz. multiplication by budding, growth from within and metabolic activities in them. The histochemical investigation of Jeewanu showed the presence of RNA-like material. The Abiogenesis of RNA-like substances in Jeewanu mixture supports RNA world theory suggesting its primitively.

KEYWORDS

Jeewanu, Self-sustaining, Supramolecular, Abiogenesis, Protocells

INTRODUCTION

The emergence of the first cell on the Earth was the culmination of long history of prior chemical and physical process (Schrum *et al*., 2010). The construction of protocell-like model with properties of biological order is significant in the study of origin of life. The physical and chemical conditions for the synthesis of a functional biostructures have been discussed by various workers (Deamer *et al*., 2006).

In the study of prebiotic chemistry various abiogenic molecules viz. adenine (Oro, 1961), guanine (Ponnamperuma, 1965), cytosine (Sanchez *et al*., 1966), deoxyribose sugar (Ponnamperuma, 1965), nucleotides (Orgel, 1994a), and poly-nucleotides (Schramm *et al*., 1962) have been reported. Cytosine, uracil and pyrimidine ribo-nucleotideds can be synthesised earlier under plausible prebiotic conditions (Miller, 1955; Powner *et al*., 2009).

Joyce and Orgel (2006) suggested that during early Earth stage RNA plays a central role in both inheritance and catalysis. RNA might have come first and catalyzed all necessary reactions necessary for survival and replication (Gilbert, 1986; Joyce and Orgel 1993; Orgel, 1994b). It was suggested that RNA being more potent then DNA (Joyce, 1989; Smith and Szathmary, 1995).

A protocell required a self-replicating nucleic acid (Johnston, 2001). The genetic polymer of vesicle promotes faster protocell self-replication to growth and division (Chen *et al*., 2004).

The photochemical formation of autoreplica-tive, protocell-like model Jeewanu in a laboratory simulated plausible prebiotic atmosphere was observed (Bahadur and Ranganayaki, 1970).

Jeewanu have been analyzed for the presence of amino acids in free as well as peptide combination (Bahadur and Ranganayaki, 1970; Bahadur *et al*., 1963; Briggs, 1965), nucleic acid bases as purines and pyrimidines (Bahadur and Ranganayaki, 1970; Ranganayaki *et al*., 1972), sugars ribose as well as deoxyribose (Bahadur *et al*., 1963), and phospholipids-like material (Singh, 1975) in them.

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The presences of various enzyme-like activities viz. phosphatase, ATPase, esterase, and nitrogenase have been detected in Jeewanu mixture (Bahadur and Ranganayaki, 1970; Bahadur *et al*., 1963; Singh, 1973; Bahadur and Gupta, 1984).

Therefore an attempt was made to characterize the morphological characteristics and histochemical localization of RNA-like material in the abiogenic, protocell-like microstructures Jeewanu reported by Bahadur and Ranganayaki (1970).

MATERIALS AND METHODS

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 dihydrogen orthophosphate, sodium chloride, magnesium sulphate, potassium sulphate, calcium acetate, manganous sulphate and 50 mg. of ferrous sulphate in 100 ml. of distilled water.

4% ammonium molybdate (1vol.), 3% di-ammonium hydrogen phosphate (2 vol.), mineral solution (1 vol.) were taken in a sterilized conical flask. The flask was cotton plugged and sterilized in an autoclave at 15 lb. pressure for 30 minutes. After cooling 1 vol. of 36%formaldehyde was aseptically added in this mixture.

The mixture was cotton plugged. The total bulk of mixture was divided in to eight parts and all the mixtures were exposed to sunlight for different periods of exposure (viz. 0.30, 1.0, 4.0, 8.0, 16.0, 24.0, 32.0 hours) for respective periods.

A part (5 ml.) of the mixture was covered with several folds of black cloth and kept as control. All the mixtures were similarly exposed to sunlight for periods mentioned above.

Method of preparation of air dried smears

Photochemically formed Jeewanu the abiogenic microstructures were separated by filtration from experimental mixture and air dried at room temperature. The particles were fixed in 0.25% CrO_3 (aqueous) till bluish colour of particles was bleached (Bahadur *et al*., 1980). The fixed microstructures were thoroughly washed with distilled water. A drop of suspension of fixed particles was homogeneously spread on a clean glass slide and air dried.

Histochemical localization of RNA-like material

The slides were stained with 0.25% Pyronin-Y (basic stain) (Pearse, 1961) for varying periods as given below.

Table 1: Showing duration of fixation and staining of Jeewanu of different periods of exposure

Sr. No.	Jeewanu of different exposures (In hours)	Duration of fixation with 0.25% CrO3 (aqueous) (in hours)	Duration of staining with Pyronin-Y (In hours)
1.	0.30	18.00	5.00
2.	1.00	24.00	3.30
3.	4.00	48.00	2.00
4.	8.00	72.00	1.40
5.	16.00	96.00	1.30
6.	24.00	120.00	1.20
7.	32.00	132.00	1.00

The slides were thoroughly washed in distilled water, dehydrated in ascending series of ethyl alcohol, cleared in xylol and mounted with DPX.

OBSERVATIONS AND RESULTS

The microscopic examination of Jeewanu revealed that they are spherical in shape, blueish in colour, their size varies from 0.5 µ to 3.5 µ in diameter. They have been found to show heterogeneous structural configuration showing presence of a definite boundary wall and intricate internal structure. (Fig. 1, 2, 3).

Staining of Jeewanu with pyronin Y (Pearse, 1961) showed the presence of bright red colour diffused throughout the particles indicated histochemical localization of RNA-like material. (Fig. 4, 5, 6, 7).

The microstructures of lower periods of exposure (0.30 to 8.0 hours) showed feeble red colouration and took longer duration for staining. The microstructures of higher periods of exposure (16.0 to 32.0 hours) showed intense bright red coloration diffused throughout the particles and took relatively lesser duration for staining.

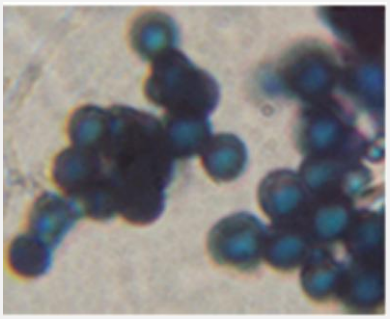

Fig.1

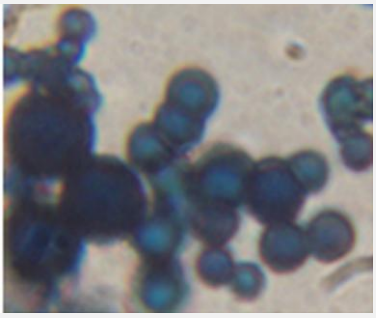

Fig.2

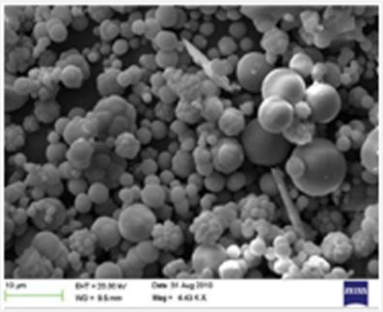

Fig. 3:

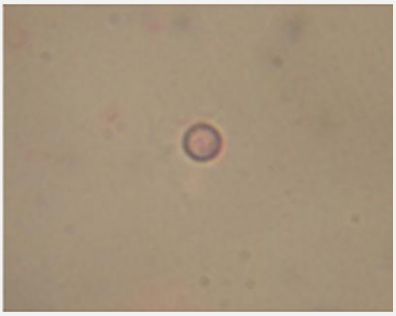

Fig.4

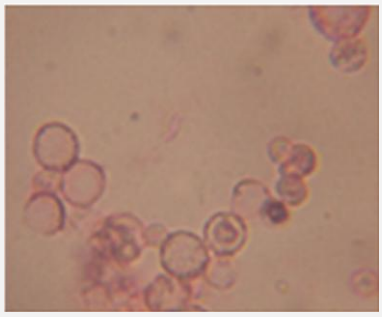

Fig.5

Fig. 6

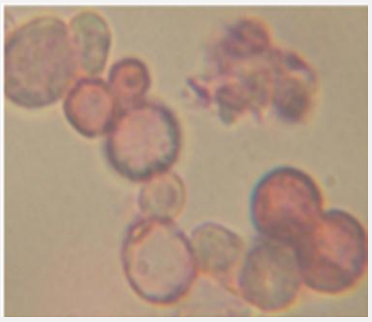

Fig. 7

Fig.1 & 2:
Micrographs showing morphological characteristics of Jeewnu showing multiplication by budding and growth within, (1500x)

Fig.3:
Scanning Electron micrograph of Jeevanu showing multiplication by budding and growth within

Fig. 4 to 7: Micrographs of Jeewanu stained with Pyronin Y Showing histochemical localizasation of RNA-Like material diffused throughout the microstructures (1500x)

DISCUSSION AND CONCLUSION

RNA world hypothesis (Joyce and Orgel 1993) argues that RNA must have come before in the first protein, RNA was the first autonomous self-replicating system or it was a derivative of an earlier system. It was found to be a primordial molecule. Various workers reasoned the primitivity of RNA monomers based on following assumptions.

a) The nucleic acid bases viz. cytosine , uracil and activated pyrimidine ribonuleotides can be synthesized earlier under plausible prebiotic conditions (Miller, 1955; Powner *et al*., 2009).
b) In prebiotic experiments uracil required less energy 130^0C for their formation (Fox and Harda, 1961).
c) RNA has been capable of independent life forms (Atkins, *et al*., 2006).

Histochemical localization of RNA-like material in protocell-like microstructures 'Jeewanu' produced in laboratory simulated possible prebiotic atmosphere suggests primitivity of RNA-like material and supports RNA world theory (Joyce and Orgel, 1993).

The presence of RNA-like material in abiogenic protocell-like microstructures 'Jeewanu' reported by Bahadur and Ranganayaki (1970) was histochemically localized on staining with pyronin Y. The diffused RNA-like activity throughout the particles suggests the transient phase of self organization of biogenic monomers and formation of polymeric substances showing its primitivity in the possible prebiotic atmosphere.

The abiogenesis of nucleic acid bases in an irradiated sterilized aqueous mixture of some inorganic and organic substances is significant in the study of origin of life. In prebiotic atmosphere

possibly RNA monomers formed by photochemical transformations were self-organised to form a polymeric material similar to RNA. The catalytic potentiality of RNA suggests its primitivity and significant role in genetic take over during chemical evolution. The presence of RNA-like activity in Jeewanu mixture shows that self-replicating molecules similar to RNA played a significant role in the emergence of living system.

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