BioSystems, 17 (1985) 275–280
Elsevier Scientific Publishers Ireland Ltd.

PROTEINOID MICROSPHERES MORE STABLE IN HOT THAN IN COLD WATER

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(Received September 18th, 1984)
(Revised version received March 14th, 1985)

The occurrence of organisms of primitive appearance near submarine hydrothermal vents has indicated sea-floor conditions that are like those under which proteinoid microspheres are produced in the laboratory. Experimental examination of the question of whether some proteinoid microspheres might be stable in hot water has revealed proteinoids that are soluble in cold water but precipitate on heating. Unanswered questions are discussed.

Keywords: Hot water origins; Hydrothermal vents; Microspheres; Proteinoid.

Introduction

Recent studies have focussed attention anew on the possibility that living organisms arose and arise in hydrothermal vents in the sea-floor (Corliss et al., 1981; cf. Stetter, 1984). This inference raises questions about the thermal stability of such units.

Although Corliss et al. have inferred a common geological matrix for their organisms and for the proteinoid microspheres made in the laboratory under similar conditions (Fox and Dose, 1977), the closeness of the evolutionary relationship of metabolic and other capabilities yet needs to be investigated. A related question is whether any of the polymers from which the microspheres organize can yield microspheres that are more stable in hot water than in cold. This question is answered affirmatively in the work reported here. Such unusual solubility is also of interest in the technology of proteinoids.

Clues to composition of a thermal protein that would assemble to microspheres most stable in hot water were available from occasional observations in this laboratory and from analyses of thermophiles by Oshima (1972). Oshima found a high proline content. The polymer chosen for study here was made from the amino acids asp:glu:lys base:leu:pro in the molar proportions of 1:2:3:4:2, respectively.

Materials and methods

Proteinoid RS VII-36

The polymer was made by mixing 13 g aspartic acid, 29 g glutamic acid, 55 g lysine base, 52 g leucine and 23 g of proline. The mixture was dissolved in 50 ml water by heating, the water was boiled off in about 1 h, and the residual amino acid melt was then heated at 195°C for 24 h, followed by 235–240°C for 4 h, all done under a N_2 blanket. To the reaction mixture was added 400 ml water, the mixture was refluxed 1 h, and the aqueous layer was decanted hot. The residue was an oily mass that hardened on cooling; it represented the predominant part of the product.

Since temperatures of 235°C are above those that have usually been employed in thermal polymerization, a polymerization was carried out mainly in order to compare influences on composition. The same proportions of five amino acids were used as in the earlier reaction. In one case, the temperature was 185°C

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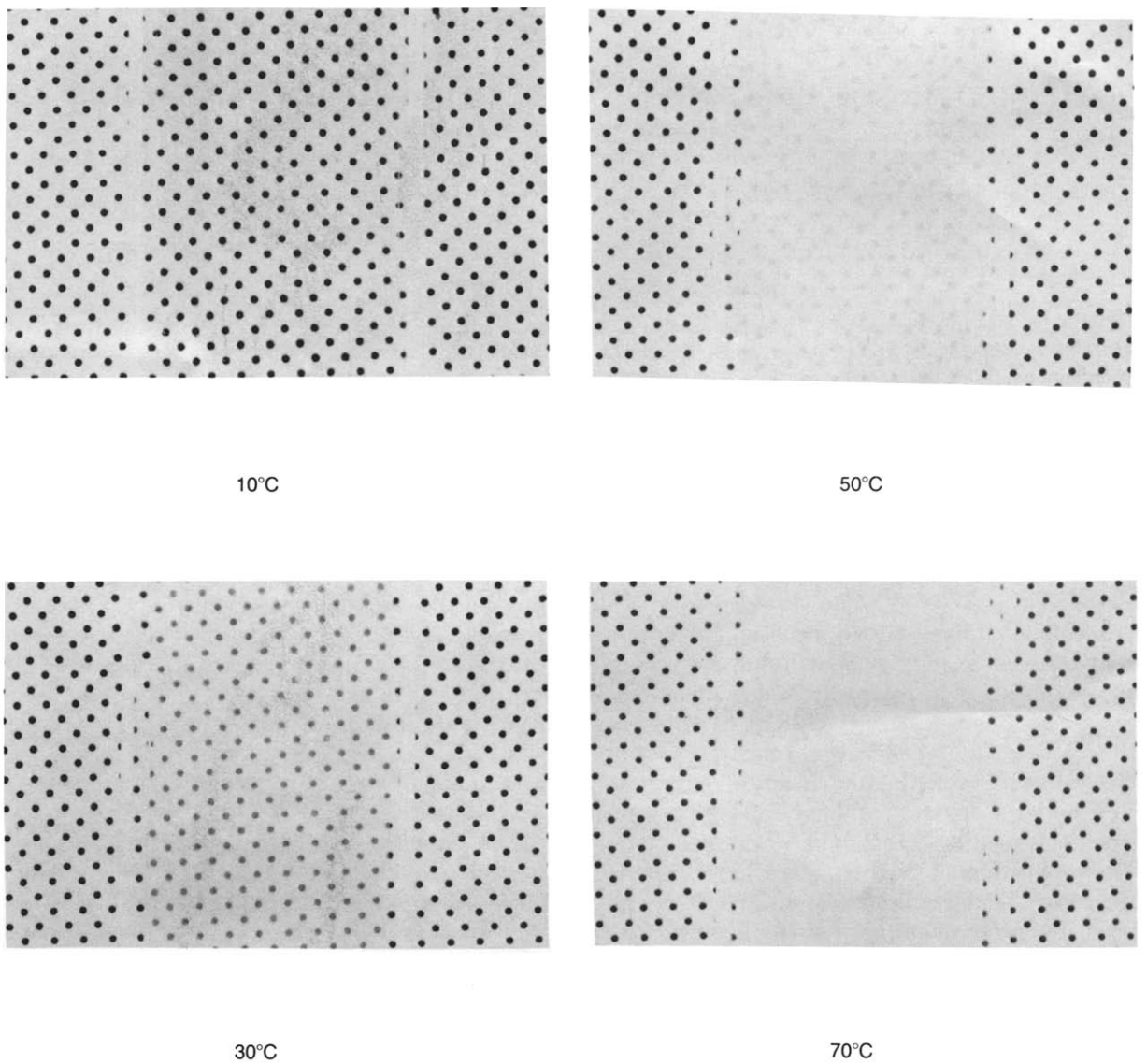

Fig. 1. Visual estimation of turbidity of microsphere suspension at pH 7.0 (artificial pond water). Transparency of suspensions at various temperatures, as explained in text. In those cases in which dots can be fully seen, proteinoid is fully dissolved.

for 8 h, whereas in the other it was 200°C for 24 h followed by 240°C for 4 h. The major part of the product was a spongy mass, in contrast to the oiliness of the higher-temperature polymer.

Analysis of proteinoid

Samples of 5–7 mg of polymer were hydrolyzed in 5 ml of 6 N HCl in sealed tubes at 105°C, and then dried in a desiccator. The

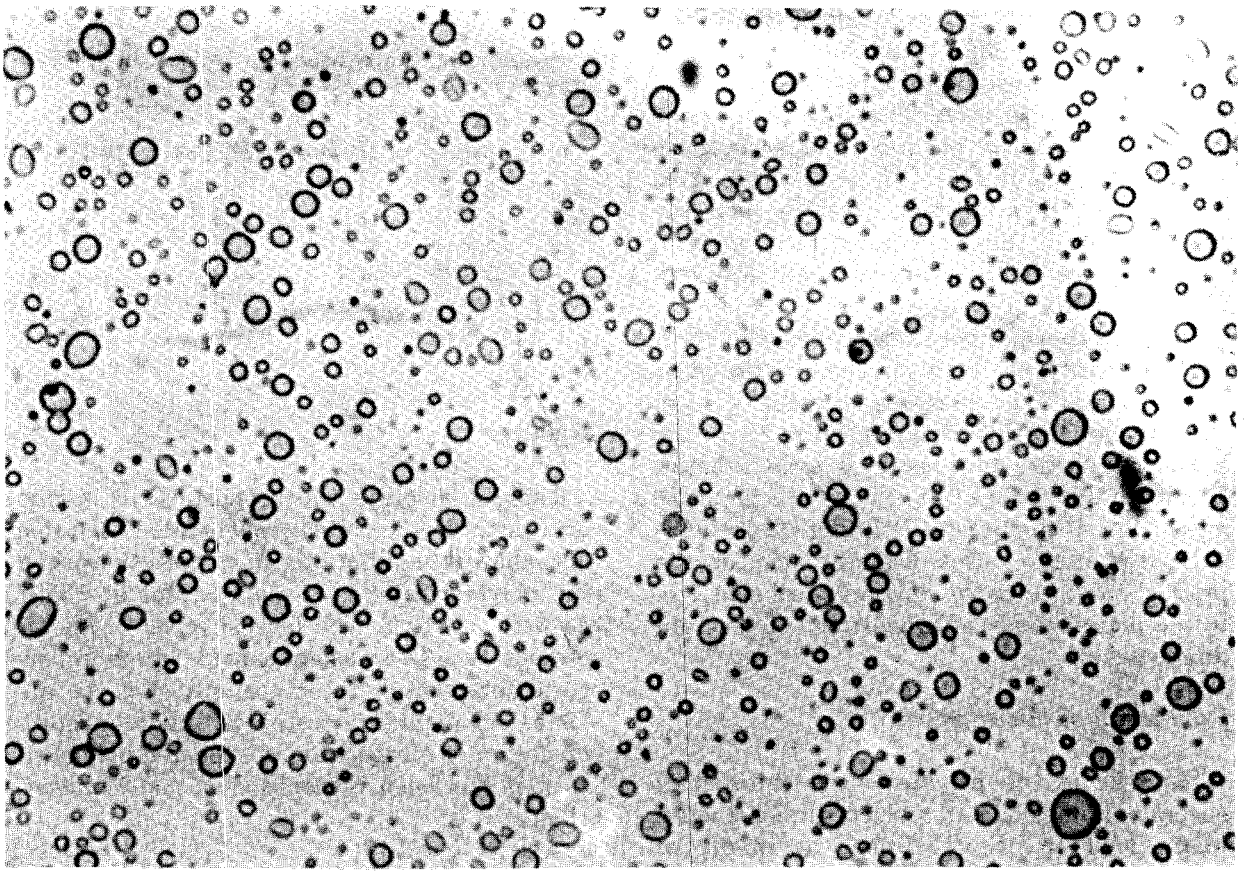

Fig. 2. Microspheres of p(asp, glu, leu, lys, pro) at temperatures elevated from room temperature, at which polymer was in solution.

amino acids were analyzed by use of a Waters Associates high performance liquid chromatography assembly using fluorescence detection with *o*-phthalaldehyde (Umagat et al., 1982) and dansylation for estimation of proline (Tapuhi et al., 1981).

These OPA analyses were performed on hydrolyzate dissolved in borate buffer (pH 9.5) and treated with OPA in methanol/borate buffer stabilized by ethanethiol. The vial was shaken for 1 min and the solution injected into a Rainin Microsorb Short-One C_{18} column on a Waters Associates HPLC, comprising two solvent delivery pumps, solvent programmer and fluorescence detector, with use of 0–100% methanol for 55 min at 1.5 ml/min. The companion solution was 0.05 M sodium acetate adjusted to pH 6.6, with 1% tetrahydrofuran (THF) added.

The dansyl derivatives were made on 50 μl of the hydrolyzate dissolved in lithium carbonate solution (40 mM adjusted to pH 9.5) by reaction with 50 μl of dansyl chloride in acetonitrile (1.5 mg per ml), all heated to 60°C for 10 min. The product was fractionated on a Brownlee RP-300 column for 4 min at 10% B and then for 26 min at 10–95% B at 4.0 ml/min. Solvent A was formic acid and acetic acid at pH 9.6 while solvent B was 35% propanol-2 and 65% solvent A.

The 1.0% of unknown is calculated on the basis of color value equivalent to that of leucine. It represents unidentified material that reacted with OPA. That the proportions of amino acids in the polymer differ from those in the reaction mixture is well-known (Fox, 1980). Such disproportion was, in fact, the first indication that the thermal polymerization of amino acids is non-random (Fox and Harada, 1958).

In many thermal polymerizations, the glu > asp or the ratio of the two in the mixture prior to reaction is 1:1 but reverses to asp > glu in the polymer. In this case glu/asp is 2/1 in the reaction mixture but becomes even greater, 7/1, in the polymer. The results are in rough agreement with those of Rohlfing (1967). It is evident that the ratios of amino acids in the product to those in the polymers are sensitive to the actual range of proportions of reactant amino acids.

Production of microspheres

Samples of the polymer were agitated in several volumes of water. Such samples, when maintained in the coldroom overnight at 1–4°C gave clear yellow solutions. On warming of the cool solution, microspheres were seen to precipitate.

Consistent effects were seen in both directions. For the experimental results recorded in Fig. 1, the microspheres were formed at 80°C in a glass cuvette of 1 mm thickness. The temperature was then cooled to 4°C in an ice-bath, raised to 70°C, cooled to 4°C, raised to 60°C, etc. down to 20°C. The temperatures were raised and lowered only for experimental verification of solubility. The temperature changes have no other significance. These experiments were done at pH 7.0 in artificial pond water. Essentially the same results were obtained at pH values of 3.0, 5.0 and 9.0. The fields of dots are photographed only to permit evaluation of turbidity.

A preparation of the microspheres is seen in Fig. 2.

TABLE 1

Comparison of two temperature regimes in effect on polymer composition (mol%)

	Reaction Mixture	185°C/8 h	200°C/24 h + 240°C/4 h
Asp	8.3	4.6	2.5
Glu	16.7	18.3	18.2
Leu	33.3	32.6	27.8
Lys	25.0	22.2	25.5
Pro	16.7	22.3	26.0

Results

Table 1 displays the proportions of amino acid in the principal polymer made. The table also shows the difference in composition when the polymerization was carried out at 185°C as well as 240°C. The largest effect of higher temperature is on aspartic acid, which undergoes substantial decomposition (Fox et al., 1955). An extensive statistical study of reproducibility of composition in repeated polymerizations has been reported (Fox and Windsor, 1984).

Discussion

The possibility that living organisms originated in hot water was early, or first, suggested by Copeland (1936) from his studies of the biosystematics of thermophilic blue-green algae in the Yellowstone area. Copeland was able to trace ancestry of cold-water algae back to thermophiles but could find no ancestors for the hot-water type. Copeland inferred that, "The morphologically and nutritionally most primitive organisms include the *Myxophyceae*, and their notable incidence in thermal habitats suggests the probability of the origin of living organisms in the thermal waters."

Involvement of the lithosphere as well as the hydrosphere was suggested for ocean-floor

fissures (Fox, 1957) or hydrothermal vents (Mueller, 1972). In fact, Mueller reported organic microspheres from a Precambrian exposure of southwest Africa. After reviewing the hydrothermal vents and the conditions used in the laboratory for the production of simulated protocells (Fox and Dose, 1977), Corliss et al. (1981) stated, "The physical and chemical conditions of those experiments are found in a natural environment which has been present in the oceans since they first formed, in hot springs associated with submarine volcanism."

Corliss' suggestion of an ancestral relationship of some kind of proteinoid microsphere to hydrothermal vent organisms (Corliss et al., 1981) is paradigmatically consistent with the selfordering principle identified in proteinoid experiments (Fox, 1984; Ho and Saunders, 1984), the probability of early reverse translation (Melius, 1982) in turn supported by the anabolic capabilities of the microspheres (Fox and Nakashima, 1980), and by the fact that the microspheres possess simultaneously catalytic variety (Rohlfing and Fox, 1969; Dose, 1984), endogenously generated order of monomers to yield informational polymers (Hartmann et al., 1984), and membrane lipid quality (Kuhn, 1976; Przybylski et al., 1982). These primordial qualities in proteinoid could have served as precursors to organismic proteins, cellular polynucleotides, and modern lipids respectively (Stratten, 1984) without a need for separate generation of three evolved types of molecule.

A special conceptual virtue of microspheres stable in hot water is that such permits visualizing faster reactions in early metabolism promoted by temperatures higher than are generally common in organisms today.

Objections have been raised to the concept of reactions above 150°C in the geological realm due to the assumption that it is a "rare process" (e.g. Miller and Orgel, 1974). While temperatures above 150°C are used for polymerization in the laboratory, they are not required (Lehninger, 1975; Rohlfing, 1976). Temperatures such as 65°C are sufficient for polymerization of amino acids (Rohlfing, 1976); in nature (Osterberg and Orgel, 1972) 65°C is not rare. For that matter, temperatures above 150°C are also not rare on today's Earth (Waring et al., 1965). The hydrothermal vents are appropriate locales for production of proteinoid microspheres, as suggested by Corliss et al. (1971). Bleeding of water vapor from magma in the throats of hydrothermal vents would have provided hypohydrous conditions in which polypeptidic magma low in water content could have formed and then been ejected into a hydrous environment (Fox, 1957). That even low-temperature proteinoid will form microspheres has been established experimentally (Rohlfing, 1976).

The microspheres found in southwest African localities in the vicinity of fumaroles, hot springs, and other hydrothermal vents have been interpreted as fossilized proteinoid microspheres indicating the ease of artificial fossilization of microspheres (Francis et al., 1978) and by other related assessments (Fox et al., 1983; Fox, 1984). That polymerization reactions of amino acids could occur in nature was shown earlier by carrying out the transformation on lava (Fox, 1964).

Although back-extrapolation is extremely valuable for understanding primitive organisms (Copeland, 1936; Schopf, 1976), it is not relevant to assembly processes in protocells and their immediate evolutionary extension; those require experimental retracement from yet earlier precursors (Fox, 1975).

Acknowledgement

This work was supported by a grant from Mr. David Rose, a contract with the National Foundation for Cancer Research, and by the National Aeronautics and Space Administration Grant No. NGR10-007-008. Contribution No. 373 from the Institute for Molecular and Cellular Evolution.

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