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— SHORT COMMUNICATION —

# Was the first molecular replicator on the primitive Earth an informational amyloid? EGGSVVAAD, a prebiotically plausible peptide, spontaneously forms amyloid assemblies

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To test the amyloid world theory of the origin of life, we constructed a nonapeptide, EGGSVVAAD, composed of the six most abundant amino acids produced in prebiotic synthesis experiments, and incubated the nonapeptide in aqueous solution at temperatures likely to have existed on the primordial Earth. The synthetic peptide spontaneously formed polymorphic fiber networks, including amyloid fibrils exhibiting typical green birefringence in polarized light after Congo-red staining and measuring 4.6 to 8.5 nm in diameter. The demonstration of spontaneous amyloid formation of a prebiotically plausible short peptide provides experimental support for the idea that the first biomolecular entities on the early Earth might have been structurally stable β-sheet-rich amyloid entities possessing both replicative and informational (prion-like) characteristics.

**Key words:** origin of life, amyloid, prions, prebiotic chemistry, replicator.

## INTRODUCTION

The origin of life on Earth has remained an enigma. Given the informational and catalytic properties of ribonucleotides, the concept of a primordial RNA world (Barbieri, 1985; Gilbert, 1986) has gained wide popularity. It has, however, been difficult to explain the emergence of stable, meaningful and self-replicative RNA under the assumedly harsh conditions on the primitive Earth. Electrical discharges, cosmic radiation and high temperatures are all known to decompose/denaturate polyribonucleotides (Larralde *et al*., 1995).

Amino acids and short peptides, in contrast to nucleotides, are easily produced under a variety of prebiotic conditions (Brack, 2007). On mineral surfaces, peptides up to 55 monomers long may be formed (Ferris *et al*., 1996). Such peptides, as well as shorter ones, may adopt functional structures (Isaac *et al*., 2001). Within the origin-of-life frame, the peptide/protein model is, however, like the RNA model, problematic with respect both to the stability of the native protein structure and the issue of information transfer under the harsh early Earth conditions.

In contrast to natively folded polypeptides, the amyloid fold has some extraordinary structural and functional properties (Chiti & Dobson, 2006; Maury, 2008; Wiltzius *et al*., 2009; Eichner & Radford, 2011; Hammarström *et al.*, 2011) that makes it unique with respect to the quest for the origin of life: (i) it is highly stable, resisting both high temperatures, ultra-violet and ionizing radiation, (ii) it self-propagates by a seeded nucleated growth mechanism in which monomers or oligomers are added to the growing protofiber; even peptides as short as three to 11 mer long, may self-assemble into amyloid-like structures (Maury *et al*., 1994; Hamley, 2007). Importantly, a given sequence may give rise to conformational variability

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which may, then, selectively, be passed on to daughter molecules in a conformation-specific manner and (iii) the β-sheet amyloid conformation is, according to the present view, the basis for the strain specificity and transmissibility of prions. Based on these, and additional arguments including the accumulating evidence of protein encoded inheritance related to the amyloid fold (Wiltzius *et al*., 2009; Wickner *et al*., 2010), we advanced the idea that peptide-based β-sheet amyloids were the first self-propagating and information-processing biomolecules that evolved on the primitive Earth (Maury, 2009a). Here, we present experimental data supporting that hypothesis.

## MATERIALS AND METHODS

To test the amyloid world hypothesis (Maury, 2009a) we constructed a nonapeptide, EGGSVVAAD, composed of the six most abundant amino acids (G, glycine; A, alanine; D, aspartic acid; E, glutamic acid; V, valine and S, serine, in decreasing order) that have been produced in various experiments simulating abiotic conditions, including those on atmospheric discharges, hydrothermal vents and ice dust (Van der Gulik *et al*., 2009). These amino acids have also been detected in carbonaceous meteorites (Botta *et al*., 2002).

98.5% pure EGGSVVAAD-amide (as the acetate salt, CASLO Laboratory ApS, Lyngby, Denmark), stored at –20˚C, was used in all experiments. The purity of the peptide was determined by high-pressure liquid chromatography and mass spectrometry. The lyophilized peptide was dissolved in water at concentrations of 5 mg $ml^{-1}$ or 10 mg $ml^{-1}$ and used fresh or after storage at –20˚C. Samples were incubated at room temperature (23.0-24.5˚C) for 24 hrs; at 40˚C for 24 hrs after 60 min preincubation at room temperature; and at 60˚C for 24 hrs without or after 60 min or 24 hrs preincubation at room temperature. Amyloid formation was also tested after peptide incubations at room temperature for 14 days. After incubation aliquots were taken for Congo-red staining and polarization microscopy and for electron microscopy.

For ultrastructural studies, specimens were thawed (samples were stored at –20˚C before testing) and absorbed on freshly glow discharged girds ($1 \times 10^{-1}$ mbar air, 25 mA, 2 min in an EMTECH K 100X glow discharge unit, Agar Scientific LMT, Essex, England) for 1 min, stained with 2% potassium Tungsten phosphate (pH 6.5) for 30 sec and air dried. The grids were examined in a JEOL 1400 TEM (JEOL, Tokyo, Japan) electron microscope operated at 80 kV. Electron micrographs were taken by a side-mounted TEM digital camera Morada (Olympus-Soft Imaging Solution, Munster, Germany).

## RESULTS AND DISCUSSION

Following incubation of EGGSVVAAD in aqueous solution, a large network of fibers of variable length and morphology was seen on electron microscopy (Fig. 1A, C and D), including amyloid-like fibrils exhibiting typical green birefringence in polarized light after Congo-red staining (Fig. 1B), and measuring 4.6 to 8.5 nm in diameter (Fig. 1C). Amyloid was formed following incubation at 24˚C, 40˚C, and 60˚C for 24 hrs, and also from fresh peptide solutions as well as from solution that had been kept frozen for varying times. Incubation of the test peptide at room temperature for 14 days resulted in spontaneous amyloid formation as judged by Congo-red staining and polarization microscopy.

The demonstration of spontaneous polymorphic amyloid formation of a prebiotically plausible short peptide provides experimental support for the amyloid world theory of the origin of life on the primordial Earth (Maury, 2009a). The nano-ordered cross β-sheet structure of amyloid represents a protein-folding state with a low energy arrangement of the polypeptide chains. Amyloidogenesis occurs at submicromolar concentrations and is characterized by the for-

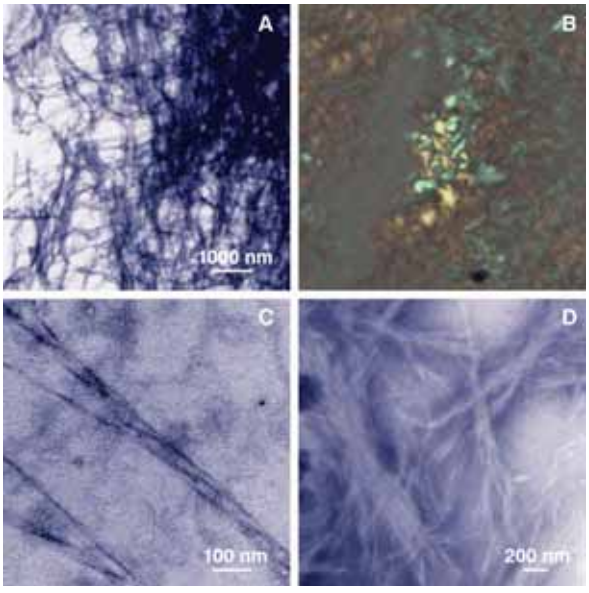


FIG. 1. Amyloid fibril formation of EGGSVVAAD. A: Fibrils formed at 60˚C, after 24 hrs incubation (5 mg $ml^{-1}$); B: Congo red staining and polarization microscopy; C: Fibrils formed at 24˚C after 24 hrs incubation (10 mg $ml^{-1}$); D: Fibrils formed at 60˚C after 24 hrs incubation (10 mg $ml^{-1}$).

mation of β-strands oriented perpendicular to the long axis of the fibers (Chiti & Dobson, 2006; Eichner & Radford, 2011). Amyloid is traditionally associated with a number of pathological conditions in humans, including prion diseases, but recent studies have demonstrated the existence of functional amyloid as well. Beneficial amyloids have been found in a wide range of organisms from pro- and eukaryotic microorganisms to mammals with functions as diverse as biofilm formation, scaffolding, epigenetic control of polyamines, regulation of melanin synthesis, peptide hormone storage, and information transfer (Fowler *et al*., 2007; Maji *et al*., 2009; Maury, 2009b; Wiltzius *et al*., 2009; Wickner *et al*., 2010). In fact, amyloid formation seems to be generic propensity of polypeptides and the amyloid β-fold an evolutionary highly conserved primordial structure.

From a prebiotic view, amyloid possesses a number of extraordinary characteristics that make it a very likely candidate for being the first informational molecular replicator on the early Earth. These characteristics include structural stability, self-replication, variability, information transfer, and compartmentalization (Claussen *et al*., 2003; Chiti & Dobson, 2006; Maury, 2008; Wiltzius *et al*., 2009; Wickner *et al*., 2010; Eichner & Radford, 2011). The stability of the amyloid fold, which is maintained by hydrogen bonds, van de Waals forces and electrostatic polarization, is essential: it resists both high temperatures and radiation. Another important feature is the auto-assembling nature of the amyloid fibrils: they self-propagate their specific cross-β conformation by adding monomers/oligomers to their growing protofiber ends. Both packing and segmental polymorphisms have been identified, the former is encoded by alternative packing arrangements of the β-sheets formed by the same segment and the latter by distinct β-sheets built from different segments (Wiltzius *et al*., 2009). The process occurs in a random fashion in which several polymorphic forms may be produced from a given sequence. Importantly, the conformational variability may then, under certain conditions, be selectively passed on to daughter amyloid entities in a conformation-specific and enduring mode (conformational 3D self-replication). A similar mechanism is also the basis for strain specificity and transmissibility of prions (Wiltzius *et al*., 2009; Wickner *et al*., 2010).

The amyloid model fulfils two basic requirements of a primordial chemical evolution model, namely, (i) the structural components can be produced randomly from compounds present in the presumed primitive environment, and (ii) the components are sufficiently stable to allow spreading in that environment. The molecular amyloid evolution system can be considered from a Darwinian perspective, too: the system is self-replicative and it produces variants of which the environmentally fittest ones and those with the fastest production rates are likely to be selected to become the most populated molecular entities in the presumptive preRNA world.

For a prebiotic molecular system to be able to increase in complexity, compartmentalization is essential. The self-propagating amyloids possess the intrinsic propensity to form fibrillar and tubular networks that can act as scaffolds (Hamley, 2007) for the β-sheet assemblies themselves and for other molecules, such as ribonucleotides (Liu *et al*., 2008) and lipids (Domanov & Kinnunen, 2008). Noteworthy, the scaffolding properties of amyloid are exploited by extant organisms: e.g. microbes use amyloid as part of the matrix in biofilm formation, and silk and fish moths utilize it for protecting oocytes from environmental hazards (Iconomidou *et al*., 2000; Barnhart & Chapman, 2006).

Prions, apart from being infective agents in humans, represent also one form of functional β-sheet aggregates (Wickner *et al*., 2010). The mechanism of prion propagation is strikingly similar to that of amyloid, and the prion structure has been highly conserved during evolution. Importantly, marked homology exists between the amino acid sequences of common prions and the sequences preferentially formed in the salt-induced peptide reaction (Rode *et al*., 1999), a reaction believed to have occurred under prebiotic conditions.

In conclusion, we show that a prebiotically plausible nonapeptide, EGGSVVAAD, in aqueous solution, spontaneously forms polymorphic fiber networks with amyloid-like characteristics at temperatures likely to have existed on the early Earth (Owen *et al*., 1979). This finding provides the first experimental support for the amyloid world theory of the origin of life suggesting that the primeval biomolecular entities on the primitive Earth might have been structurally stable prion-like β-sheet rich amyloid entities possessing both replicative and informational characteristics.

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