AMPLIFICATION OF DIVERSE CATALYTIC PROPERTIES OF EVOLVING MOLECULES IN A SIMULATED HYDROTHERMAL ENVIRONMENT

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Abstract. We observed chemical evolution in a mixture of four amino acids, glycine, L-alanine, L-valine and L-aspartic acid, circulated through a flow reactor simulating the thermodynamic conditions of a hydrothermal environment. These monomers form peptides with tertiary structures and potential catalytic functions. The HPLC profile of synthesized oligomers varied with each particular run, but the products were found to separate into distinct clusters when more than one hundred runs were compared statistically. This observation suggests that chemical evolution on the early Earth had stochastic aspects that must be understood in order to develop useful models of prebiotic evolution.

Keywords: amino acids, catalytic reaction, cluster analysis, hydrothermal vents, peptides

1. Introduction

Hydrothermal vents have been proposed as potential sites for the synthesis of complex molecules on the prebiotic earth (Corliss *et al*., 1979; Edmond *et al*., 1982; Russell *et al*., 1988; Shock, 1990; Ferris, 1992). Energy to drive the synthetic reactions was available in the form of heat activation of monomers (Simoneit, 1995; Russel and Hall, 1997; Huber and Wächterhäuser, 1998; Amend and Shock, 1998; Cody *et al*., 2000), followed by rapid quenching in the colder surrounding water to reduce thermal decomposition (Matsuno, 1997). Subsequent recirculation of products into the hot vents could enhance further synthetic reactions if the residence time in the high temperature region was limited (Bada *et al*., 1995). We have already tested this hypothesis by investigating oligomerization of amino acids (Lahav *et al*., 1978; Brack, 1987; Rode and Schwendinger, 1990; Ferris *et al*., 1996) under simulated vent conditions (Imai *et al*., 1999a, b; Ogata *et al*., 2000). We shall then examine chemical evolution in a mixture of four amino acids, glycine (G), L-alanine (A), L-valine (L), and L-aspatic acid (D) in simulated hydrothermal environments. In fact, GADV are those amino acids that can be thought of as an evolutionarily minimal set for making peptides with tertiary structures (Ikehara *et al*., 2002; Hartman, 1995; Knight *et al*., 1999).

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2. Methods

In our flow reactor (Matsuno, 1997; Imai *et al*., 1991a), the reaction solution was injected at a flow rate of 8 mL per min from a 15 mL heated chamber at 200 °C and 24 Mpa. The reactants passed through a nozzle (diameter 0.8 mm) into a larger, cooler chamber at 0 °C and approximately the same pressure. Cycle time for the total volume of fluid (500 mL) was 62.5 min, but with stirring the reactants recycled roughly once per minute. Each run lasted 2 hr and was started at room temperature, requiring approximately 10 min to reach 200 °C. The circulated reaction solution consisted of four amino acids (glycine, alanine, valine and aspartic acid) each at 20 mM in pure water. Ionic salts were not added, but the solution contained trace amounts of iron due to the stainless steel chamber (SUS-316). The reaction products were profiled by HPLC analysis (A195 nm), especially focusing on those products possessing carbonyl groups.

3. Results

An HPLC elution profile is displayed in Figure 1. The peaks clearly suggest that a variety of oligomers were synthesized (Ogata *et al*., 2000). In order to detect possible patterns in the spectrum of products, we quantified each HPLC profile in discrete pairs corresponding to their absorbance amplitude and the elapsed elution time sampled at 12 sec intervals over 20 min periods (Figure 2). In this way a quantitative expression for the entire elution pattern could be represented by one point in a 100 dimensional space spanned by all of the sampled absorbance amplitudes. It was then possible to compare any two individual elution patterns by measuring the distance between the two points representing the two profiles. This arbitrary value was a quantitative measure of the similarity or dissimilarity of the products from any two trials.

Figure 3 shows the distance between profiles of two different runs, both measured at t = 120 min. Dissimilarity of the patterns was estimated by unweighted pair-group method with arithmetric mean or UPGMA (for details, see Sokal and Rohlf (1981)). Dissimilarity between an arbitrary pair of different patterns, each of which is represented by an eigenvector in the 100-dimensional space, is defined as the distance between two vectors. Clustering was observed by group average method, in which a pair of eigenvectors whose distance becomes minimal among all possible pairs is taken to constitute a cluster. The cluster thus obtained was considered to be a newly formed eigenvector in the phase space. By continually repeating the search for clusters in the reduced set of eigenvectors, one cluster was finally generated. It is apparent that there is robust clustering of similar elution patterns.

Figure 4 illustrates several of these distinct clusters. The elution patterns identified with each cluster constituted a local Gaussian frequency distribution that

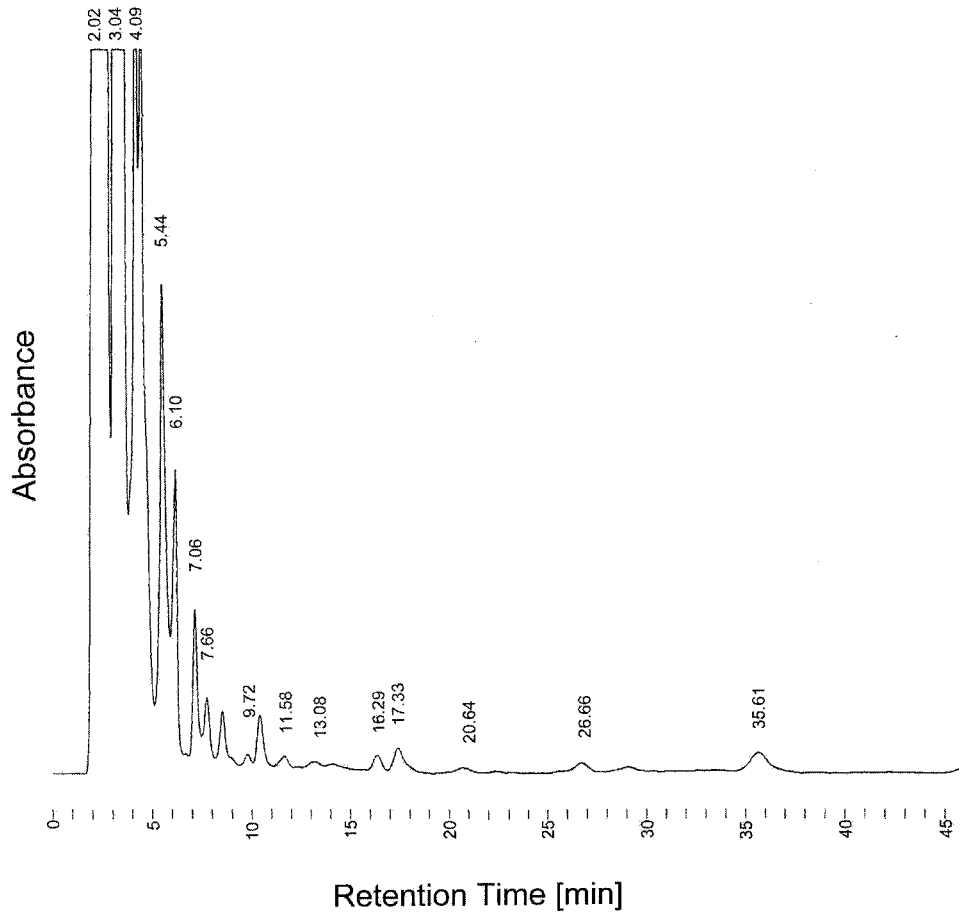

Figure 1. An HPLC profile of products after two-hour trial. All samples were analyzed by YMC hydrosphere C18 column (150 × 4.6 mm ⌀). The mobile phase consists of 50 mM K-phosphate buffer pH = 2.5 and 7.2 mM Na-hexasulfonate. The flow rate of the mobile phase was 1.0 mL min^{-1}. Profile intensity was absorbance at 195 nm.

was independent of the parameters chosen to quantify the elution profiles. The frequency distribution of distances between different intra-cluster elution profiles was distinct from those of inter-cluster patterns. Repeated runs of the experiment generated a Gaussian frequency distribution of multiple peaks instead of a single peak. A single peak would suggest reproducibility of the unique mean value and its standard deviation specified by error distribution. In our analysis, the multi-peaked Gaussian frequency distribution suggested that the chemical evolution toward more complex polymers could not be predicted by simple reaction mechanisms involving condensation of specific amino acids. Instead the products and their clusters varied from one run to the next as though a set of stochastic seed reactions dictated the final groupings of clusters.

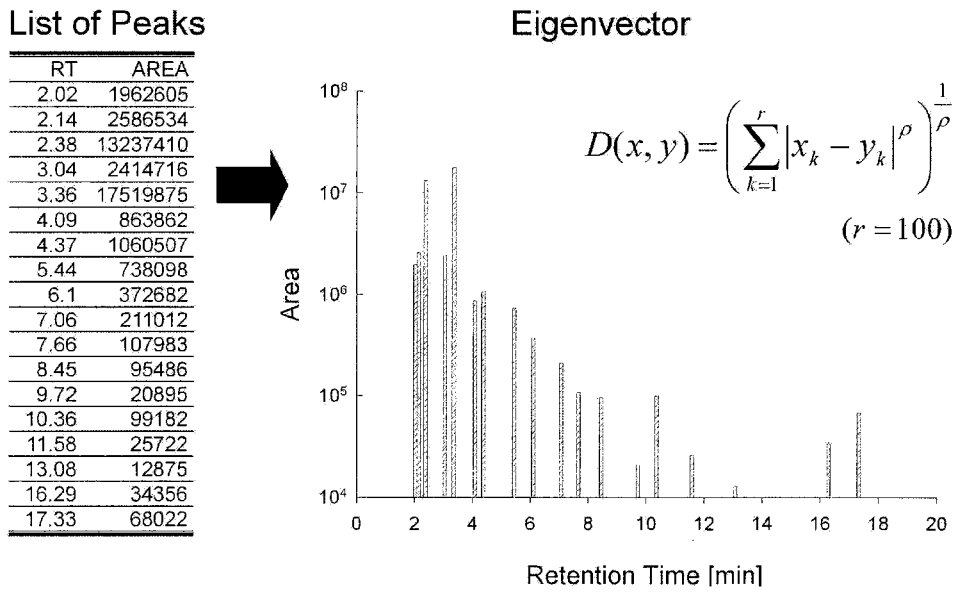

Figure 2. Discrete patterning of an HPLC profile of the products. The intensity of the absorbance along the retention time was discretized at 12 sec intervals for 20 min. Each elution pattern was represented by an eigenvector $\{x_k\}$ $(k = 1, 2, \ldots, 100)$ in a 100 dimensional space, in which x_k referred to the k-th component of the absorbance intensity. Difference between the elution patterns $\{x_k\}$ and $\{y_k\}$ can be calculated by comparing the Minkovski distances $D(x, y)$. The Minkovski parameter ρ could be arbitrary, in which Euclidian distance is recovered at $\rho = 2$.

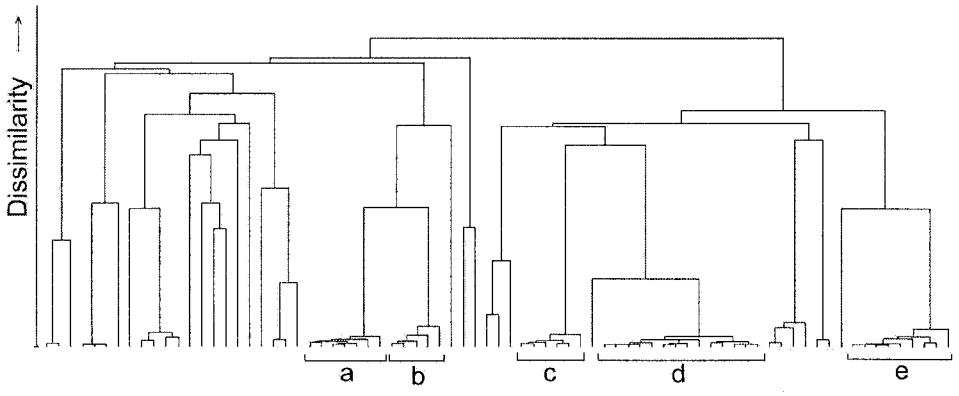

Figure 3. A cluster analysis of the elution patterns at t = 120 min over a hundred independent trials. The clustering was robust to changes in the Minkovski parameter ρ. Five distinct clusters a, b, c, d and e will further be analyzed in Figure 4.

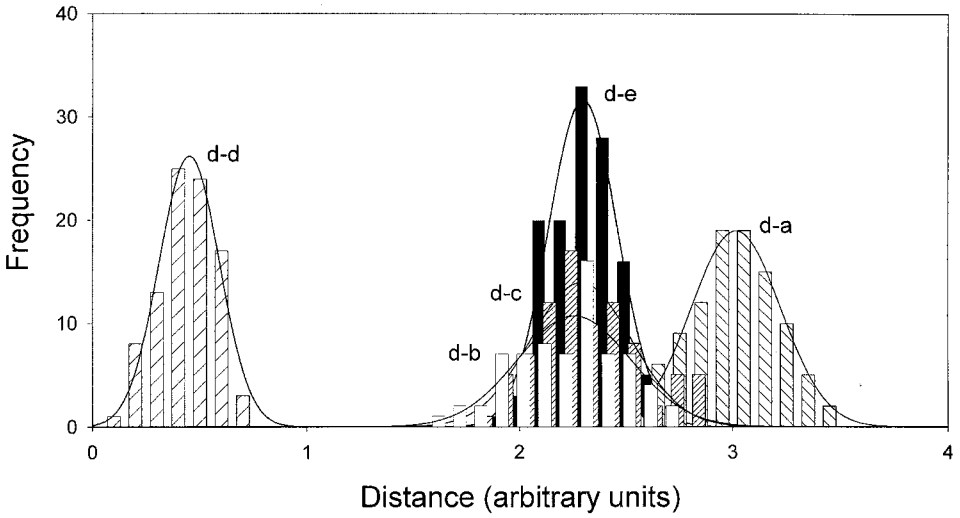

Figure 4. Frequency distribution of the distances between intra-cluster members $d - d$ and between inter-cluster members $d - a$, $d - b$, $d - c$ or $d - e$. Both the frequency distributions of the distances between intra-cluster members and between inter-cluster members followed Gaussian distributions locally. The Gaussian character remained robust to the choice of the Minkovski parameter ρ defining the distance.

4. Discussion

Experiments designed to model prebiotic chemical evolution and to monitor the subsequent development of catalytic functions must meet two requirements. They must first address the fact that the resulting products will be determined by the sum of previous events that may not be experimentally controllable. Secondly, the results should demonstrate at least some degree of reproducibility (Morowitz *et al.*, 1991). In our experiment both the diversification of the product patterns into distinct clusters and the reproducibility of each cluster was due to successive amplification of slight differences in the reaction pathway, perhaps attributable to catalytic function of the developing oligomers (Matsuno, 1982, 1989; Kauffman, 1986).

In summary, a robust clustering of product patterns starting from an inventory of monomers occurred in our simulation of peptide syntheses propagated by a hydrothermal environment. In our model, prediction of latent catalytic functions developing in the flow reactor could not be demonstrated. For example, the catalytic properties of a particular amino acid can change dramatically, depending upon the pre-existing peptide to which it is chemically joined. However, through successive reactions, it is possible to amplify slight differences between molecules so that product clusters are produced. Similar experiments are now in progress utilizing nucleotides to generate oligomers with potential catalytic functions.

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