Liposome-Assisted Selective Polycondensation of α-Amino Acids and Peptides

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Received June 8, 1999
Revised Manuscript Received August 20, 1999

The present paper deals with the question as to whether and to what extent lipidic bilayers can aid the polycondensation of amino acids and peptides. We believe this question to be relevant in the field of peptide/lipid interaction and in particular to prebiotic chemistry.[1,2] We will show that indeed lipidic bilayers can aid the polycondensation, particularly when the starting materials are hydrophobic amino acids or peptides.

Among the pioneering work on the field, Fox and collaborators[3] carried out thermal polycondensation of amino acid mixtures. This was resumed later on and extended by Yanagawa et al.[4] using mixtures of amino acids and phospholipids or fatty acids. Under more conventional terms, the polycondensation of amino acids using the *N*-carboxyanhydride (NCA)[5] method in aqueous solution has been studied by several authors with respect to a prebiotic scenario.[6−11] The activation by NCA is interesting because of the recent claims that NCA−amino acids or their thio equivalents could have been prebiotic compounds.[6,12,13] One should also recall in particular the work by Orgel et al.,[14−16] who were able to reach polymerization degrees up to 55 using glutamic acid. In these studies, clay minerals served as support for the polymerization. However, different kinds of minerals with different chemical and physicochemical properties were needed for different types of amino acids. Orgel et al.[17] were also able to induce the polymerization of negatively charged NCA−amino acids using positively charged micelles in an aqueous solution. In these two cases—clays and micelles—electrostatic interactions were a prerequisite for efficient oligomerization.

A quite different support for the polycondensation of amino acids is the lipidic bilayer of liposomes. In the pioneering work of Fukada et al.,[18] Kunieda et al.,[19] and Ringsdorf et al.,[20−22] the amino acid was generally covalently modified with long alkyl chains (e.g., long-chain esters of glycine). These hydrophobic chains enabled the amino acids to be anchored in an organized way to the liposome, and due to the increased local concentration on the surface, oligomerization was possible. Several variations of this main idea have been published in the literature.[23−27] This kind of polycondensation on liposomes is based on the interaction between amino acid derivatives and the lipidic surface. This type of interaction was proposed to be central in prebiotic chemistry and precellular, chemical evolution.[28−31] The possible relevance of surfactant aggregates to the origin of life at large has been emphasized by our group on the basis of self-reproducing micelles and vesicles.[32−34]

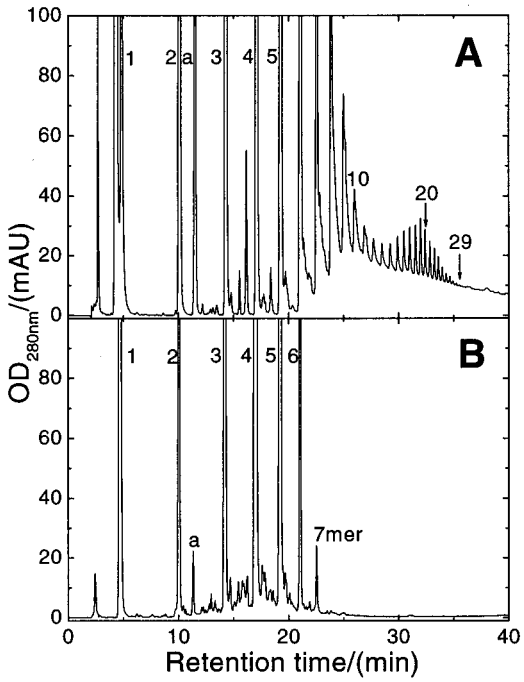

Figure 1. Effect of POPC liposomes on the oligomerization of NCA−Trp in repeated feeding experiments. HPLC absorption chromatogram is shown at 280 nm, where the numbers indicate the degree of oligomerization. Cyclo(−Trp_2−) is represented by the letter a. To 600 μL of a "100 nm liposome" suspension (100 mM POPC) in 0.4 M imidazole buffer pH 6.80 (A) or 600 μL buffer without liposomes (B) was added 80 mM NCA−Trp in 0.4 M imidazole buffer pH 6.80 in three steps, 200 μL each with 4 h intervals. The reaction mixtures were diluted 1: 3 (v/v) (A) with "100 nm liposomes" (50 mM POPC) and (B) with 50% (v/v) ACN after 24 h incubation (in order to solubilize possible precipitate). Finally 50 μL was analyzed by HPLC (HP1050 LC; buffer A, 0.1% TFA; buffer B, 99.9% acetonitrile (ACN), 0.1% TFA; starting with 20% up to 95% B; gradient, 1.875% B/min; 1 mL/min) with a C18 column (Macherey-Nagel, ET 250/4 Nucleosil 100−5). The eluate was examined by a diode array detector (HP1050 DAD). Peaks were identified also by mass spectrometry; see below. The quantification is possible up to H−Trp_{23}−OH (0.04% of initial H−Trp−OH).

The work presented here will focus on the polycondensation of natural amino acids and peptides on liposomes. We would like to address the following questions: (i) Is the membrane able to select among different starting monomers, thus permitting the formation of polypeptide chains with somewhat more specific sequences? (ii) Does the membrane matrix allow the formation of longer, hydrophobic and generally water-insoluble polypeptides, which cannot be obtained in the absence of liposomes in an aqueous solution? Both types of selection are based on the hydrophobic interaction between the bilayer and the amino acids or peptides. In this work, we bring evidence to these two selection effects by utilizing 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) liposomes and two different polymerization techniques: One is based on the NCA polycondensation of L-tryptophan (H−Trp−OH), the other based on the polycondensation of L-tryptophyl-L-tryptophan (H−Trp_2−OH) by using the lipophilic condensing agent 2-ethoxy-1-ethoxycarbonyl-

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10.1021/ma990917m CCC: $18.00
Published on Web 09/24/1999

Table 1. POPC Liposomes-Aided Product Selectivity for the Cooligomerization of Dipeptides, Where All Solutions Were Incubated for 26 h on a Vortex at Room Temperature and Products Were Analyzed as Described Above[40]

products (X,Y: Asp, Glu, or Gly)	% of dipeptide(s) reacted			
	Lip/mix[a]	ref/mix[b]	Lip/Trp_2[c]	ref/Trp_2[d]
	Peptides			
cyclo(−Trp_2−)	0.9	1.6	0.8	0.9
H−Trp_4−OH	43	0.6	62	1.0
H−Trp_6−OH	2.1	0.03	5.9	0.00
H−Trp_8−OH	0.03	0.00	0.3	0.00
cyclo(−Trp−X−)/H−Trp−X−Trp−Y−OH	8.0	13		
H−Trp_3−X−OH/H−Trp−X−Trp_2−OH	7.0	6.4		
	Derivatives			
EtO−CO−Trp_2−OH	11	33	15	93
EtO−CO−Trp−X−OH	22	43		
EtO−CO−Trp_4−OH	1.6	0.1	4.3	0.3
EtO−CO−Trp_6−OH	0.1	0.00	0.7	0.00
rest	4.2	2.5	11	4.8

[a] 5 mM H−Trp_2−OH, 5 mM H−TrpGly−OH, 5 mM H−TrpAsp−OH, 5 mM H−TrpGlu−OH, "100 nm liposomes" (25 mM POPC), 50 mM phosphate, pH 5.90, 2 mM EEDQ, 1.2% v/v ACN. [b] As in footnote *a* but no liposomes. [c] 5 mM H−Trp_2−OH, "100 nm liposomes" (25 mM POPC), 50 mM phosphate, pH 5.90, 2 mM EEDQ, 1.2% v/v ACN. [d] As in footnote *c* but no liposomes.

1,2-dihydroquinoline (EEDQ).[35] In both cases, we will emphasize the difference between the membrane-assisted polymerization and the reference reaction carried out in aqueous solution in the absence of liposomes.

The NCA of H−Trp−OH has been obtained in situ according to a well-established procedure.[36] The comparison of typical polycondensation results obtained from NCA−Trp−OH in a POPC liposome system and in bulk aqueous solution showed that a variety of oligo-Trp's were obtained. The presence of POPC liposomes, however, significantly improved the yields of higher oligo-Trp's, e.g., H−Trp_8−OH and higher. This effect can be enhanced by repeated feeding of NCA−Trp−OH to POPC liposomes. When NCA−Trp−OH was added iteratively the oligomerization degree of the highest oligo-Trp obtained was up to H−Trp_{29}−OH (Figure 1). The solubilization of oligo's-Trp's by the POPC liposomes most likely enables the elongation of the oligomers: After their formation, the short oligo-Trp's remain bound to the membrane surface, due to hydrophobic interactions,[38,39] and the next elongation steps take place on the membrane. In comparison, in the case of repeated feeding carried out in the bulk aqueous solution, where oligomers higher than H−Trp_4−OH tend to precipitate, only the product distribution changes, but not the length of the highest oligomer obtained (H−Trp_7−OH).

Let us consider now the condensation of the dipeptide H−Trp_2−OH by using EEDQ.[40] This hydrophobic, specific reagent for activation of carboxyl residues has been shown to be useful for coupling hydrophobic amines to carboxylic acids,[35] potentially including those buried in a cell's membrane.[41] In the presence of POPC liposomes 29.5% of the initial H−Trp_2−OH has reacted and oligomerized up to the oligo-Trp 8-mer (amount of initial H−Trp_2−OH in H−Trp_4−OH: 21%; H−Trp_6−OH: 1.3%; H−Trp_8−OH: 0.05%), whereas in the aqueous reference system only 16.4% has reacted. In the latter case the side product *N*-ethoxycarbamate EtO−CO−Trp_2−OH dominates (14%) and the oligomerization products are practically absent (H−Trp_4−OH, 0.4%; H−Trp_6−OH, 0.03%).

To test the selection principle for monomers by the membrane, one experiment was carried out starting from a mixture of four dipeptides containing H−Trp_2−OH and the more hydrophilic dipeptides L-tryptophyl-L-glycine (H−TrpGly−OH), L-tryptophyl-L-aspartic acid (H−TrpAsp−OH), and L-tryptophyl-L-glutamic acid (H−TrpGlu−OH). Selectivity toward specific oligo-Trp sequence condensation was observed (Table 1): i.e., out of the 16 theoretically possible tetrapeptides, H−Trp_4−OH makes up about 70% of all the tetrapeptides formed. EEDQ and H−Trp_2−OH most likely are accumulated at the membranes surface due to the hydrophobic effect, resulting in a selective activation of H−Trp_2−OH, followed by its selective oligomerization. H−TrpGly−OH, H−TrpAsp−OH, and H−TrpGlu−OH are not bound by the POPC membrane (tested by equilibrium dialysis experiments; data not shown) and consequently hardly polymerized.

In conclusion, this preliminary work shows that the bilayer membrane of liposomes has the capability for two kinds of selection toward the polycondensation of amino acids and peptides: (i) In the repeated feeding experiment with NCA−Trp−OH, the presence of liposomes leads to oligo-Trp's with polymerization degrees over 20. The elongation to higher water-insoluble oligomers seems to take place only on the membrane bilayer. (ii) Sequence-selective condensation of dipeptides can be achieved on the liposomes by using EEDQ as a lipophilic condensing agent: H−Trp_2−OH is selectively bound to the membrane and activated by the EEDQ which also shows affinity to the lipid bilayer of the liposomes.[41] Therefore, preferential oligo-Trp's are formed out of a pool of four dipeptides: H−Trp_2−OH, H−TrpGly−OH, H−TrpAsp−OH, and H−TrpGlu−OH.

The liposome-aided polycondensation carried out in this work solely makes use of the hydrophobic interactions. In principle, charged membranes could also bind amino acids or peptides on the basis of electrostatic interactions, as shown by Orgel et al.[17] in the case of micelles. The possibility of carrying out polycondensations based on both hydrophobic and electrostatic interactions appears to be challenging. This would in principle allow the formation of chains consisting of different types of amino acids, which is a prerequisite for obtaining polypeptides with functionality. Our future work will be strongly influenced by this idea.

Acknowledgment. The authors thank Prof. Dr. Paul Rys and Andreas Dutly (Chemical Engineering Laboratory, ETH Zurich, Zurich, Switzerland) for their kind instrumental support and Anna Suter from No-

vartis (Basel, Switzerland) for the POPC used in the present work.

References and Notes

(1) Brack, A. *Origins Life* **1984**, *14*, 229−236.
(2) Oró, J. *J. Biol. Phys.* **1994**, *20*, 135−147.
(3) Fox, S. W.; Dose, K. *Molecular Evolution and the Origin of Life*; W. H. Freeman and Company: San Francisco, CA, 1972; pp 134−191.
(4) Yanagawa, H.; Ogawa, Y.; Kojima, K.; Ito, H. *Origins Life Evol. Biosphere* **1987**, *18*, 179−207.
(5) Barlett, P. D.; Jones, R. H. *J. Am. Chem. Soc.* **1957**, *79*, 2159−2197.
(6) Ehler, K. W.; Orgel, L. E. *Biochim. Biophys. Acta* **1976**, *434*, 233−243.
(7) Ehler, K. W.; Girard, E.; Orgel, L. E. *Biochim. Biophys. Acta* **1977**, *491*, 253−264.
(8) Brack, A. *Origins Life Evol. Biosphere* **1984**, *14*, 229−236.
(9) Brack, A. *Origins Life Evol. Biosphere* **1987**, *17*, 367−379.
(10) Hill, A. R., Jr.; Orgel, L. E. *Origins Life Evol. Biosphere* **1996**, *26*, 539−545.
(11) Liu, R.; Orgel, L. E. *Origins Life Evol. Biosphere* **1998**, *28*, 47−60.
(12) Taillades, J.; Beuzelin, I.; Garrel, L.; Tabacik, V.; Bied, C.; Commeyras, A. *Origins Life Evol. Biosphere* **1996**, *28*, 61−77.
(13) Huber, C.; Wächtershäuser, G. *Science* **1998**, *281*, 66−68.
(14) Ferris, J. P.; Hill, A. R., Jr.; Liu, R.; Orgel, L. E. *Nature* **1996**, *381*, 59−61.
(15) Liu, R.; Orgel, L. E. *Origins Life Evol. Biosphere* **1998**, *28*, 245−257.
(16) Hill, A. R., Jr.; Böhler, C.; Orgel, L. E. *Origins Life Evol. Biosphere* **1998**, *28*, 47−60.
(17) Böhler, C.; Hill, A. R., Jr.; Orgel, L. E. *Origins Life Evol. Biosphere* **1996**, *26*, 1−5.
(18) Fukada, K.; Shibasaki, Y.; Nakahara, H. *J. Macromol. Sci.—Chem.* **1981**, *5*, 999−1014.
(19) Kunieda, N.; Watanabe, M.; Okamoto, K.; Kinoshita, M. *Makromol. Chem.* **1981**, *182*, 211−214.
(20) Folda, T.; Gros, L.; Ringsdorf, H. *Makromol. Chem. Rapid Commun.* **1982**, *3*, 167−174.
(21) Laschewsky, A.; Ringsdorf, H.; Schmidt, G.; Schneider, J. *J. Am. Chem. Soc.* **1987**, *109*, 788−796.
(22) Neumann, R.; Ringsdorf, H.; Patton, E. V.; O′Brien, D. F. *Biochim. Biophys. Acta* **1987**, *898*, 338−348.
(23) Shibata, A.; Yamashita, S.; Ito, Y.; Yamashita, T. *Biochim. Biophys. Acta* **1986**, *854*, 147−150.
(24) Shibata, A.; Yamashita, S.; Ueno, S.; Yamashita, T. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 257−261.
(25) Nishikawa, N.; Arai, M.; Ono, M.; Itoh, I. *Chem. Lett.* **1993**, 2017−2020.
(26) Nishikawa, N.; Miyasaka, T.; Ono, M.; Itho, I. J. *Synth. Org. Chem. Jpn.* **1995**, *53*, 275−283.
(27) Li, Z.-C.; He, W.; Li, F.-M. *React. Funct. Polym.* **1996**, *30*, 299−308.
(28) Loomis, F. W. *Four Billion Years*; Sinauer Associates, Inc.: Massachusetts, 1988.
(29) Morowitz, H. J. *Beginnings of Cellular Life*; Yale University Press: London, 1992.
(30) Ourisson, G.; Nakatani, Y. *C. R. Acad. Sci. Paris, IIb* **1996**, *332*, 323−334. Ourisson, G.; Nakatani, Y. *Tetrahedron* **1999**, *55*, 3183−3190.
(31) Oró, J.; Lazcano, A. In *Prebiological Self-Organization of Matter*; Ponnamperuma, C., Eirich, F. R., Eds.; Deepak Publishing: Hampton, VA, 1990.
(32) Bachmann, P. A.; Luisi, P. L.; Lang, J. *Nature* **1992**, *357*, 57−59.
(33) Walde, P.; Goto, A.; Monnard, P.-A.; Wessicken, M.; Luisi, P. L. *J. Am. Chem. Soc.* **1994**, *116*, 7541−7547.
(34) Walde, P.; Wick, R.; Fresta, M.; Mangone, A.; Luisi, P. L. *J. Am. Chem. Soc.* **1994**, *116*, 11649−11654.
(35) Belleau, B.; Malek, G. *J. Am. Chem. Soc.* **1968**, *90*, 1651−1652.
(36) H−Trp−OH in a 0.4 M imidazole buffer pH 6.80 was incubated with a 2.5-fold excess of carbonyldiimidazole (CDI) at 0 °C for 2 min. Yields up to 95% of NCA−Trp−OH and N-[imidazolyl-(1)-carbonyl]tryptophan were obtained, as documented also for other amino acids.[6,7,10,11,14−17] This resulting NCA−Trp−OH solution was mixed 1:1 with an aqueous suspension of "100 nm liposomes" (50 mM POPC) in a 0.4 M imidazole buffer pH 6.80 prepared by the extrusion technique, using for final extrusions polycarbonate membranes with 100 nm pore diameter.[37] Then it was incubated on a shaker (VORTEX-Genie 2, Scientific Industries) for 12 h at room temperature.
(37) Mayer, L. D.; Hope, M. J.; Cullis, P. R. *Biochim. Biophys. Acta* **1986**, *858*, 161−168.
(38) White, S. H.; Wimley, W. C. *Biochim. Biophys. Acta* **1998**, *1376*, 339−352.
(39) Yau, W.-M.; Wimley, W. C.; Gawrisch, K.; White, S. H. *Biochemistry* **1998**, *37*, 14713−14718.
(40) After 26 h incubation of 10 mM H−Trp_2−OH, 2 mM EEDQ in 50 mM phosphate buffer, 1.2% v/v ACN, pH 5.90, at room temperature in the presence of "100 nm liposomes" (25 mM POPC) or reference (without liposomes), 25 μL of the reaction mixture was analyzed by HPLC (HP1100 LC, buffers as above, starting with 30% up to 95% B, gradient: 1.625% B/min, 1 mL/min) using a C18 column (Macherey-Nagel, ET 250/4 Nucleosil 100−5) and a diode array detector (HP1100 DAD) in series with a single quadrupole mass spectrometry detector (HP1100 MSD). Quantification has been performed using absorbance at 280 nm.
(41) Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 6th ed.; Molecular Probes: Leiden, The Netherlands, 1996; pp 72−73.

MA990917M