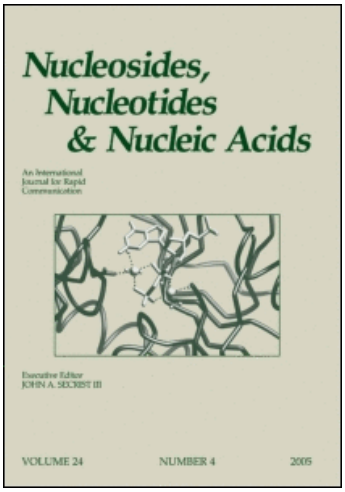

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Bergstrom, Donald E. , Abrahamson, Julie K. and Chan, Michael Y.-M.(1991) 'Periodic-Catastrophic Transport of an Adenosine 5'-Monophosphate Amantadine Conjugate Through a Model Liquid Membrane', Nucleosides, Nucleotides and Nucleic Acids, 10: 1, 685 — 688
To link to this Article: DOI: 10.1080/07328319108046573
URL: http://dx.doi.org/10.1080/07328319108046573

NUCLEOSIDES & NUCLEOTIDES, 10(1-3), 685-688 (1991)

Periodic-Catastrophic Transport of an Adenosine 5'-Monophosphate Amantadine Conjugate Through a Model Liquid Membrane

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Abstract

Adenosine 5'-phosphor(adamantyl)amidate (**5**), an analog derived by linking the antiviral drug amantadine to 5'-AMP is transported through a model membrane system in a discontinuous periodic-catastrophic fashion. The system was composed of a glass cell containing two aqueous buffer phases separated by a chloroform layer. A more lipophilic, but structurally related derivative, adenosine 5'-phosphor(n-decyl)amidate (**3**) showed linear transport in the same system. Less lipophilic substances, including 5'-AMP and adenosine 5'-phosphor(morpholidyl)amidate (**2**) did not show transport. It is hypothesized that the periodic-catastrophic transport is a result of the collective activity of amidate **5** at the interface between the first aqueous interface and the chloroform layer. The time between catastrophic events is thought to be a reflection of the time necessary for molecular organization at the interface. The phenomenon is a new example of molecular organization in a system far from equilibrium leading to a repetitive dynamic process.

Results and Discussion

We have discovered that apparently certain structural features can profoundly influence the way in which molecules cross a model membrane barrier. One compound in our study, adenosine 5'-phosphor(adamantyl)amidate (**5**) shows transport that is both catastrophic and periodic, while the other derivatives show apparent pseudo-first order kinetics. The simplest system for measuring transport rates, and yet which can be extrapolated to more complex lipid bilayers, is a model membrane constructed of two aqueous phases separated by an immiscible organic layer such as chloroform. The rates of transport of the nucleoside/nucleotides from one aqueous phase to another through a chloroform organic phase can be determined by measuring changes in UV absorbance.

When the three C-5 substituted nucleosides **6-8** were allowed to migrate from aqueous phase 1 to aqueous phase 2, the increase in absorbances for each compound was linear (pseudo-first order kinetics) over the time span of the experiment (1 to 5 hr). The respective relative rates of transport for **6**, **7**, and **8** were 1: 7 : 39 . In contrast to the C-5 alkyluridines the transport behavior of the phosphoramidates of similar lipophilicity (as measured by extraction coefficients) was far less consistent.

The most interesting compound was adenosine 5'-phosphor(adamantyl)amidate (**5**), which in most experiments (Fig. 2) showed periodic-catastrophic transport. We have deemed the observed transport "catastrophic" because it shows behavior typical of systems far from equilibrium which reach bifurcation points that lead to sudden changes in the state of the system.[1] The transport is periodic with multiple catastrophic events typically

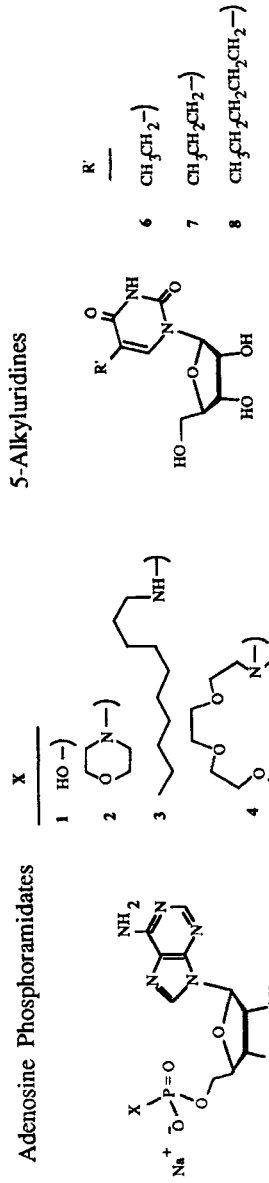

Figure 1

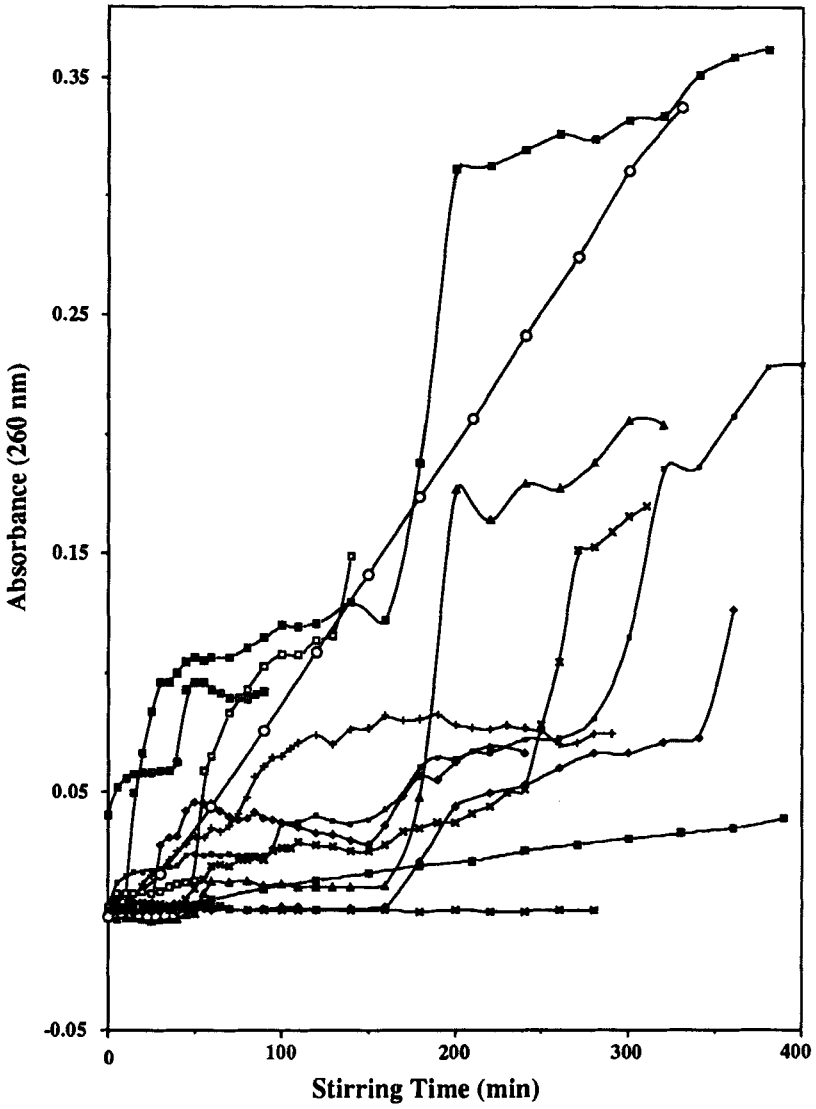

Figure 2

The absorbance in aqueous phase 2 was plotted versus the stirring time of the system for adenosine 5'-phosphor(adamantyl)amidate initially placed in aqueous phase 1 at a concentration of 0.0005 M. Ten separate experiments are shown. Typically multiple catastrophic events occured during each experiment. For comparison, experiments with adenosine 5'-monophosphate (1), which gave virtually no transport (-x-), and with adenosine 5'phosphor(n-decyl)amidate (3), which resulted in nearly linear transport (-o-), are shown. The curves connecting the points were generated by a Steinman interpolation routine.

occurring within the 300 min time span of most experiments. From the molar absorptivity of **5** (ε = 12,900) and the volume of aqueous phase 2 (3.0 mL) the number of molecules per 0.0001 AU can be calculated to be (0.003 L)(1/12,900 M^{-1})(0.0001 AU)(6.02 x 10^{23} molecules-$moles^{-1}$) = 1.4 x 10^{13} molecules/0.0001 AU. From this data we can determine how many molecules are transported in any given transition. For example, the transitions with A = 0.012 ± 0.001 (six transitions in this range were observed in ten experiments) result from transport of 1.7 x 10^{15} molecules. Since we believe that interface absorption may be a prerequisite to catastrophic transport, it is of interest to compare this number to the number of molecules potentially absorbable on the interface between aqueous phase 1 and the chloroform. Measurements of CPK models suggests a minimum surface area of about 70 $Å^2$ / molecule (0.7 nm^2). The number of molecules capable of packing into a monolayer at interface 1 (12.8 cm^2) would theoretically be about (12.8 x 10^{14} nm^2) / 0.7 nm^2 = 1.8 x 10^{15}. This number is strikingly similar to that calculated above for the 0.012 AU transition.

How does adenosine 5'-phosphor(adamantyl)amidate (**5**) organize in order to cross the interface? Studies on structural requirements for molecular association would suggest that **5** is not a good candidates for micelle, reverse micelle or liposome formation.[2] The lipophilic portion of the molecule can not obtain the critical length (cf. three dimensional representation of **5** in Fig 1) necessary for packaging into any of these forms. Also osmotic coefficient data[3] on adenosine suggests that very little association between purine rings would occur in aqueous solution [K = 4.5 (m^{-1})] at the concentrations employed in our experiments. Association between molecules could be enhanced at an interface since orientation of the molecules at the interface might place the adenine moieties in appropriate positions for base stacking to occur.

We speculate that the following occurs: 1) In a slow step **5** is randomly absorbed at the interface between aqueous phase 1 and the chloroform with the adamantyl moiety projecting into the chloroform layer and the ionic phosphoramidate group projecting into the aqueous phase. 2) At some point an interface packing limit is obtained and the systems becomes unstable. The shape of the molecules implies that packing interactions between them could lead to stress in the form of surface curvature. Eventually crowding of additional molecules into the monolayer may lead to either collapse of the monolayer discharging **5** into the chloroform phase in an unknown aggregated form or to higher dimensional structures (e.g. layers folding back on themselves) which remain at the interface. 3) Driven by the osmotic pressure difference between the aqueous phases, **5** migrates through the chloroform layer to interface 2. 4) The molecules are absorbed at interface 2. 5) Molecules diffuse from the interface into aqueous phase 2. Transport across the second interface may or may not occur as aggregates. The low concentration of 5 in aqueous phase 2 and its high solubility in water may favor rapid diffusion into the aqueous phase 2.[4]

References and Notes

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[4] The effects of the unstirred boundaries at the interfaces (T. J. Pedley, *Quart. Rev. Biophys.* **16**, 115 (1983)) on our simplified model are unknown. Concentration gradients at these boundaries are possible and this could certainly play a role in the way in which molecules become organized at the interface. We have also neglected effects due to specific chloroform interactions with phosphoramidate **5**, a problem which should be addressed in future studies.