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Line defects in gel phase lipid monolayers

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We investigate various models of the hydrolysis of gel-phase phosphatidylcholine monolayers by phospholipase A_2 (Grainger et al. (1989) FEBS Lett. 252, 73–82). We assume that the probability of hydrolysis of a given lipid depends only upon how many of its nearest neighbour lipids have already been hydrolysed. We find that the experimental data are consistent with a model in which line defects exist in the gel phase and that lipids on such defects are more easily hydrolysed than the other gel-phase lipids. Based on this model, we calculate the course of hydrolysis of a gel-phase region possessing line defects, and we suggest how such a structure might be made and the model tested. An experiment, similar to that proposed by us, has been carried out by Grainger et al. (1990) Biochim. Biophys. Acta 1023, 365–379). We also calculate the fractal dimension, d_f, of the interface created by the hydrolytic process and show that a measurement of d_f might identify how this process proceeds.

1. Introduction

Recently Grainger et al. [1] demonstrated that hydrolysis of monolayers of gel phase L-α-dipalmitoylphosphatidylcholine (L-α-DPPC) by phospholipase A_2 (PLA_2) offered evidence that lipid interfacial recognition, enzyme aggregation and hydrolytic activity are closely related. This result supported similar proposals of Hazlett and Dennis [2] obtained from observations of PLA_2 aggregation under assay conditions. The method used by Grainger et al. [1] consisted of observing the fluorescence of a lipid probe which was known to partition preferentially into the fluid phase of lipid monolayers. Because of this, gel phase aggregates, which display some of the chiral shapes arising via slow lateral compression of the monolayer [3,4], appear dark. The action of PLA_2 was reflected in the appearance of fluorescing areas which appeared within the dark patches of gel phase lipids as the lateral pressure and temperature were kept constant. Two striking results were: (i) PLA_2 appeared to attack the gel aggregate, initially, only at one point at the interface between gel and fluid phase lipids; (ii) the subsequent hydrolysis of the lipid molecules proceeded in a way that was not obviously isotropic. These observations can be clearly seen in Figs. 2B–G of Ref. 1. The intention of this paper is two-fold: firstly, we advance the hypothesis that the non-isotropic appearance of the hydrolytic process is due to the existence of line defects, such as disclinations, in the plane of the aggregate of gel phase lipids. We will present the results of computer simulations in support of this statement. Secondly, we will predict the time-course of hydrolysis of a gel phase aggregate which contains many such line defects, and we will suggest how such a system could be realized and the prediction tested. After this work was finished we became aware of a recent paper by Grainger et al. [5] in which just such an experiment has been carried out. The effects of PLA_2 upon L-α-DPPC gel phases ([5], Fig. 2) look remarkably similar to the simulations which we present in Fig. 6. In order to quantify the structures which arise, we will also calculate the fractal dimension, d_f, of the interface created by the hydrolytic process, and show that a measurement of d_f could identify some aspects of this process. In the next section we describe the theoretical model and the computer simulation technique and in the third section we present the results.

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2. Theoretical model and computer simulation

The intention of the model is to represent what we assume to be, for the purposes of simulating the hydrolytic process, the essential aspects of a gel-phase lipid monolayer. These are: (a) a sufficiently close-packed structure distributed on a two-dimensional surface which (b) possesses line defect structures. We represent the plane of a gel phase monolayer by a square lattice, the sites of which represent the positions of the lipid molecules. The choice of a square lattice is purely for computational convenience: It is well-known that the choice of lattice does not affect the essentials of cooperative phenomena even at critical points. The boundary of the lattice could be specified so as to mimic the shape of a gel phase structure (see, for example, Refs. 3 and 4), but this is not relevant to our calculations. We classify the lipids into two sets: one set comprises lipids which are located on line defects. The other set comprises all the other lipids in a gel phase, including those which are on the boundary of this phase but which do not belong to the first set. We also identify those line defects which terminate at, or sufficiently close to, the boundary of the gel phase region and we define the positions on the gel phase boundary at which these terminations occur to be the set of points $\{s(j)\}$. We then specify the rules which allow PLA_2 molecules to hydrolyse lipid molecules. These rules are:

(i) The processes must begin at the set of points $\{s(j)\}$.

(ii) PLA_2 molecules can attempt to hydrolyse a lipid molecule only if one of its nearest neighbours has already been hydrolysed.

(iii) We shall consider models of hydrolysis in which the process depends only upon the nearest neighbour environment of a lipid. More complicated models, in which hydrolysis depends upon the structure of more extended environments, will not be considered. Accordingly, each attempt to hydrolyse a lipid molecule is governed by two sets of relative probabilities, $P = \{p(n)\}$ and $P_d = \{p_d(n)\}$, where $n = 0,\ldots,3$. Here, n, is the number of nearest neighbour positions to the lipid under attack by PLA_2, at which lipids have already been hydrolysed and $p(n)$ and $p_d(n)$ are probabilities that a lipid molecule located away from, or on, a line defect, respectively, will be hydrolysed. If all four neighbours to a lipid have been hydrolysed then we assume that this lipid is detached from the gel phase structure, and becomes a fluid phase lipid. In the case that a lipid lies initially on the boundary of a gel-phase

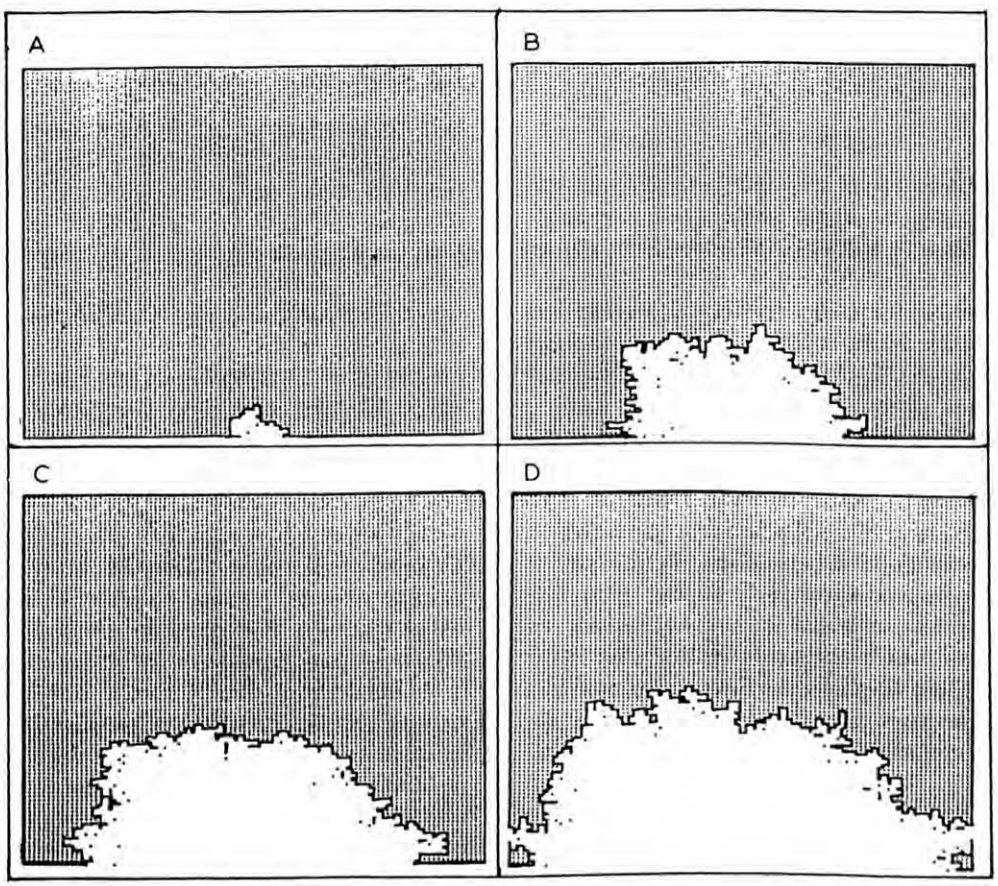

Fig. 1. Model of lipid gel-phase (darker areas) hydrolysis by PLA_2 using set P_1. A: 50, B: 1000, C: 2000 and D: 3000 monte carlo steps.

region we reduce the number of neighbours appropriately in the probabilities.

We shall first study the structures resulting when the gel phase monolayer contains no line defects, using the four sets of relative probabilities, P_1, P_2, P_3 and P_4

$$p_1(0) = 0,\ p_1(1) = p_1(2) = p_1(3) = 1.0 \quad (1)$$

$$p_2(0) = 0,\ p_2(1) = 0.2,\ p_2(2) = 0.9,\ p_2(3) = 0.1 \quad (2)$$

$$p_3(0) = 0,\ p_3(1) = 0.9,\ p_3(2) = 0.1,\ p_3(3) = 0.2 \quad (3)$$

$$p_4(0) = 0,\ p_4(1) = 0.05,\ p_4(2) = 0.1,\ p_4(3) = 0.3 \quad (4)$$

These choices represent processes which attempt hydrolysis in an isotropic way, (Eqn. 1), or in ways which favour a lipid in a particular environment, (Eqns. 2, 3 and 4). Other choices, restricted to properties of the nearest neighbour environment, give essentially the same results. In these four cases we assume that there is only one point, s, on the boundary of the gel phase at which the hydrolysis process can begin.

It seems plausible that the set P_4 is the most physically realistic: the more lipids that have been hydrolysed in the immediate neighbourhood of a lipid, the easier it is for that lipid to be hydrolysed. The intent of studying the effect of these probabilities is to see whether they lead to hydrolysis which eliminates the need to assume the existence of defects.

We shall then consider a case in which a set of line defects meet at one point, s, on the boundary of the model gel phase. We choose P_d to be,

$$p_d(0) = 0,\ p_d(1) = 0.8,\ p_d(2) = 0.85,\ p_d(3) = 0.95 \quad (5)$$

and perform calculations using the sets P_d and P_4 since, as we have argued above, P_4 is the most realistic set. Our intention is to compare the results of the simulations using these five sets of relative probabilities in order to identify the 'best set', which represents what is actually observed in the hydrolytic process.

Finally, we consider the case in which the gel phase region has many line defects meeting its boundary at many points, $\{s(j)\}$ and we perform a computer simulation using the 'best set', of relative probabilities which we have identified as reproducing the experimental observations of lipid hydrolysis by PLA_2.

The computer simulation studies were carried out using a pseudo-random number generator written [6],

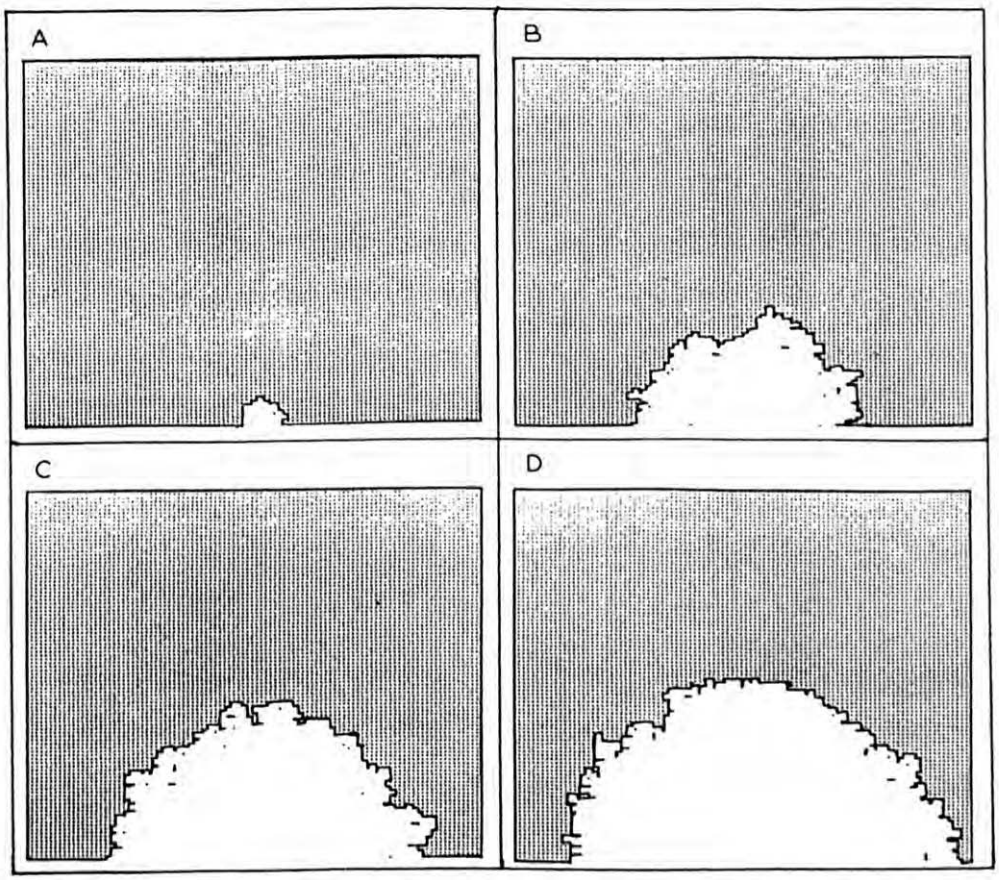

Fig. 2. Model of lipid gel-phase (darker areas) hydrolysis by PLA_2 using set P_2. A: 50, B: 1000, C: 2000 and D: 3000 monte carlo steps.

and previously used, by us. A lipid from the set $\{s(j)\}$, or the lipid at s, is selected and hydrolysed. Thereafter a lipid is selected randomly. If it belongs to the set $\{s(j)\}$ it is hydrolysed. If it does not, then we consider whether it possesses a non-zero probability, p, of being hydrolysed. If $p > 0$ then we select a number $0 \leq R \leq 1$, using the pseudo-random number generator. If $R \leq p$ then that lipid is hydrolysed. Otherwise the lipid is not hydrolysed and we choose another lipid. One monte carlo step consists of one of the choices, described above, involving one lipid.

3. Results

Figs. 1–4 show the results obtained using the sets P_1, P_2, P_3 and P_4 after 50, 1000, 2000 and 3000 monte carlo steps. Here, we show the gel phase as a darker, outlined area and the fluid phase and the regions which have been hydrolysed as light areas. In the measurements of Grainger et al. [1], dark areas represent gel-phase lipids. Single lipids detached from the gel phase by the hydrolytic process become part of the lipid fluid phase. A cluster of more than one lipid, however, has not been assumed to become a number of independent lipids in their hydrocarbon chain's fluid states. Although the interface structures brought about by hydrolysis all appear essentially similar, there are subtle differences which reflects the choices of the probabilities, and this will be dealt with below. It is clear from a comparison of these results with those of Grainger et al. [1], that none of them appear to represent the observations made.

Accordingly, we have created a model gel-phase lipid monolayer containing seven line defects meeting at a common point at the centre of one of the faces. All such defects are shown as lighter lines. We have used the probabilities P_d to model the hydrolysis of any lipid which lies on one of these defects, and P_4 to represent the hydrolysis of other lipids. The results of this are shown in Fig. 5. Here we see the initial arrangement of defects at monte carlo step 0, while subsequent diagrams show the result of hydrolysis after 50, and 1000 to 6000 monte carlo steps. It appears clear that the results shown in Fig. 5 bear a substantial resemblance to those of Grainger et al. [1].

If this model is correct, we can predict what structures would appear if the lipid gel phase, instead of consisting of a structure (or structures) similar to that shown in Fig. 5A, possesses many line defects meeting the edge of the gel phase at many points. These line

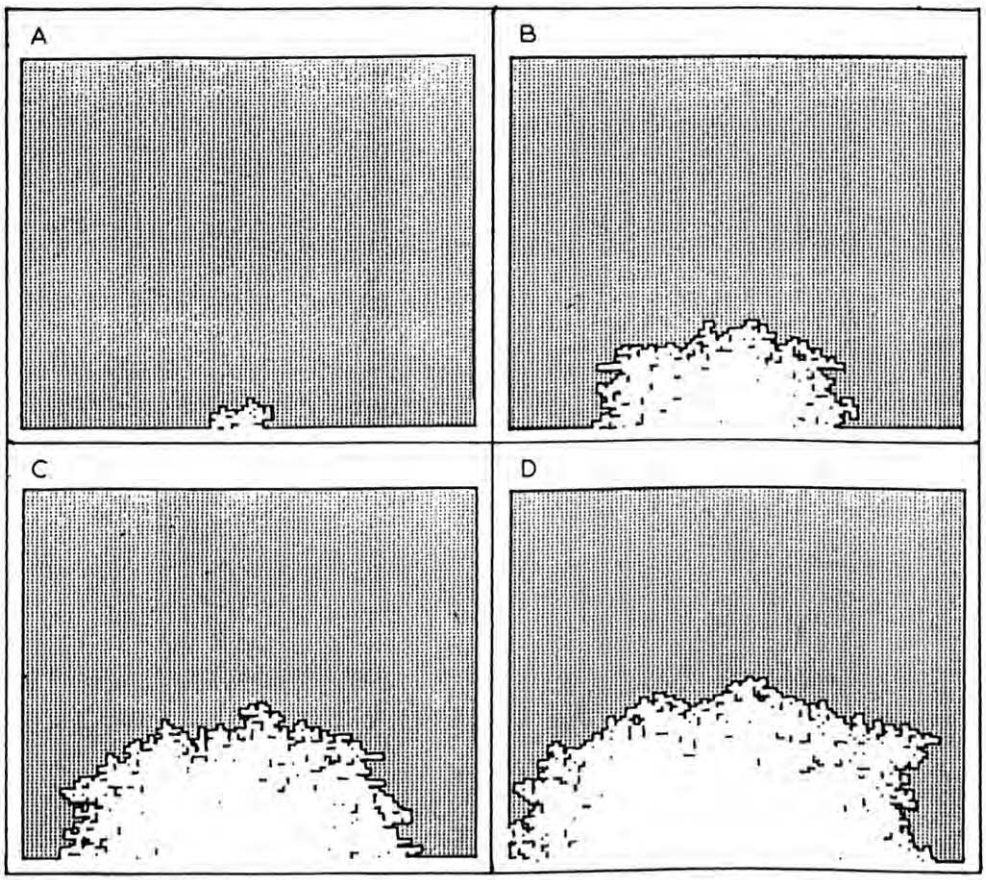

Fig. 3. Model of lipid gel-phase (darker areas) hydrolysis by PLA_2 using set P_3. A: 50, B: 1000, C: 2000 and D: 3000 monte carlo steps.

defects are assumed to possess properties, as far as attack by PLA_2 is concerned, similar to those postulated here. Figs. 6 and 7 show cases for many such line defects which are shown as lighter lines. Our prediction is that PLA_2 would be observed to begin hydrolysis at a number of points on the perimeter, and that relatively large sections will be split off from the main body as hydrolysis proceeds.

Finally, we have analyzed the interfaces of our models which have been hydrolyzed by PLA_2 in order to determine whether their structures are related to the relative probabilities P_n ($n = 1,\ldots,4, d$). We calculated the fractal dimension d_f by measuring the lengths of these interfaces, $L(\epsilon)$, as a function of ϵ, the length of the unit used to measure it, and using the relation [7],

$$L(\epsilon) \approx \epsilon^{1-d_f} \tag{6}$$

Because of the small size of the lattice, the range of ϵ that we used is very small, and these results should be considered as preliminary. It should be noted, however, that the values of d_f obtained differ substantially for the cases described by P_1, P_3 and P_4, and that for cases in which we might expect values of d_f to be the same

TABLE I

Fractal dimensions obtained from analysis of interfaces created by model lipid hydrolysis

Figure	Model	d_f
1C	P_1	1.31
2C	P_2	1.26
3C	P_3	1.40
4C	P_4	1.25
5D	P_d and P_4	1.28
6G	P_d and P_4	1.29

our results differ by 0.04. Fig. 8 shows plots of log ($L(\epsilon)$) against log (ϵ) from analyses of the interfaces created by the hydrolysis of the model lipids, at monte carlo step 2000 shown in C of Figs. 1 (P_1), 2 (P_2), 3 (P_3) and 4 (P_4), in Fig. 5D (P_d and P_4) and at monte carlo step 5000 shown in Fig. 6G (P_d and P_4). The results are shown in Table I.

It should be noted that even our crude analysis gives results for d_f in the last three cases which are close: 1.25, 1.28 and 1.29. This is expected since the effect of P_d is simply to hydrolyse the model lipids at the line defects. However, the fact that we obtain a similar

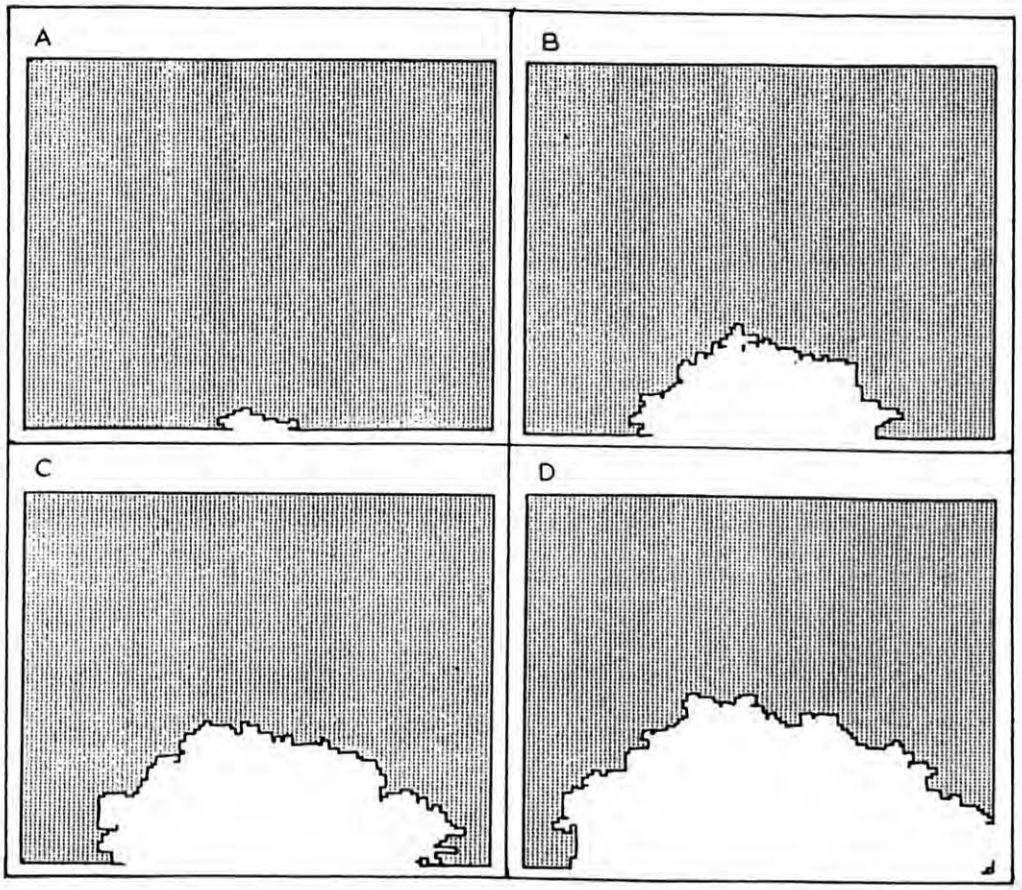

Fig. 4. Model of lipid gel-phase (darker areas) hydrolysis by PLA_2 using set P_4. A: 50, B: 1000, C: 2000 and D: 5000 monte carlo steps.

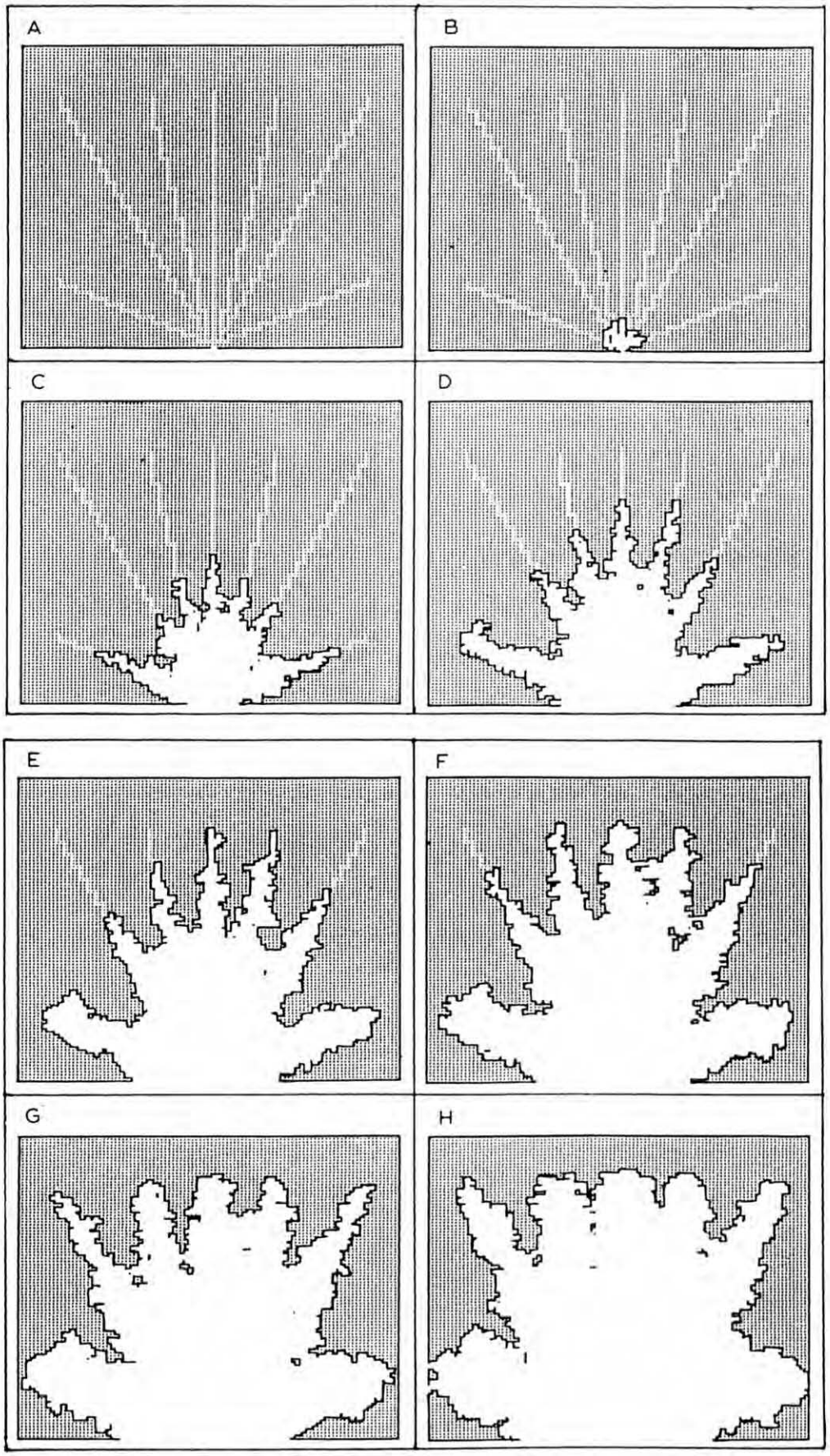

Fig. 5. Model of lipid gel-phase (darker areas) hydrolysis by PLA_2 using sets P_d and P_4. Lighter lines indicate line defects. A: 0, B: 50, C: 1000, D: 2000, E: 3000, F: 4000, G: 5000 and H: 6000 monte carlo steps.

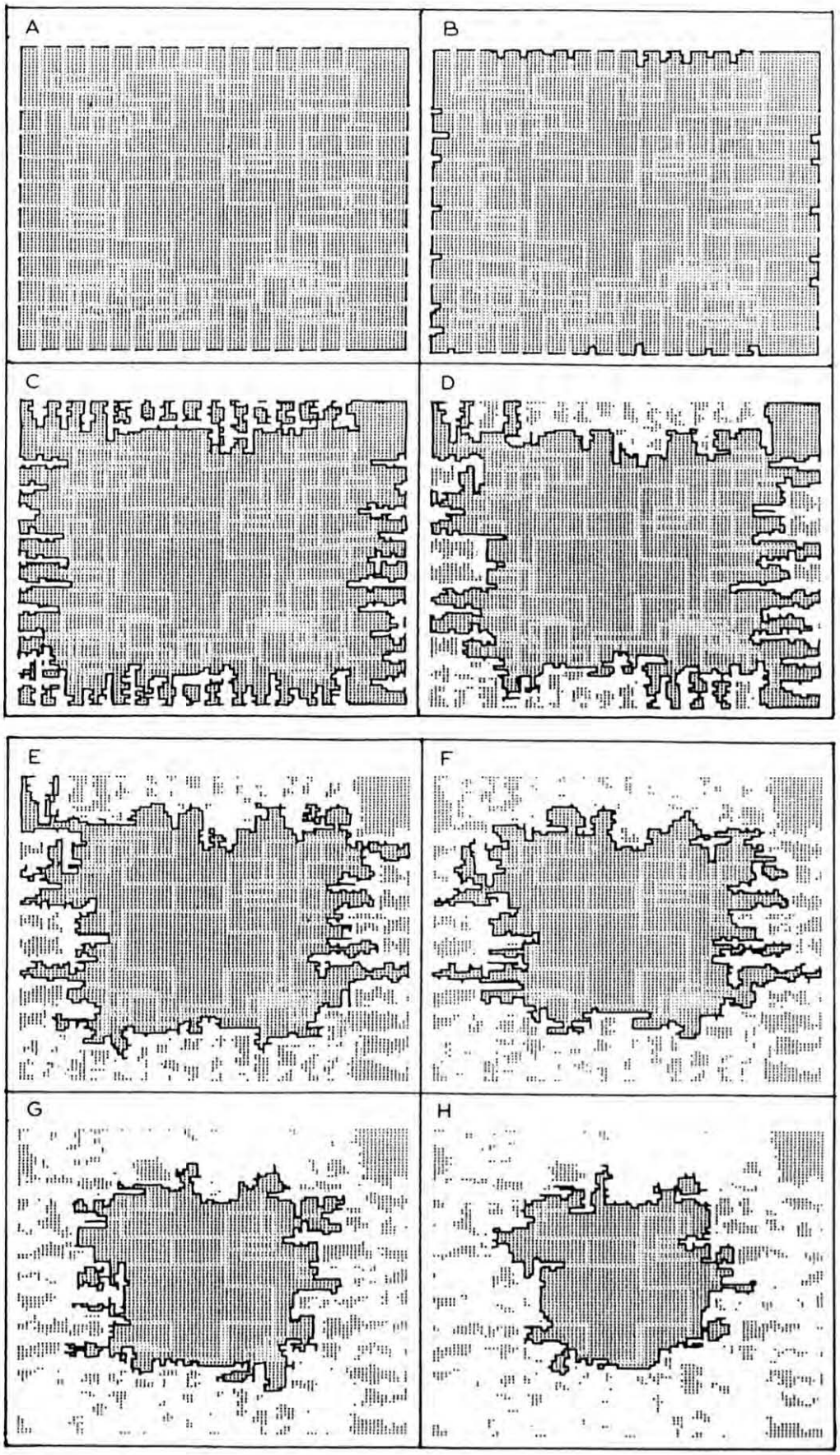

Fig. 6. Model of lipid multi-domain gel-phase (darker areas) hydrolysis by PLA_2 using sets P_d and P_4. Lighter lines indicate line defects. A: 0, B: 50, C: 1000, D: 2000, E: 3000, F: 4000, G: 5000 and H: 6000 monte carlo steps.

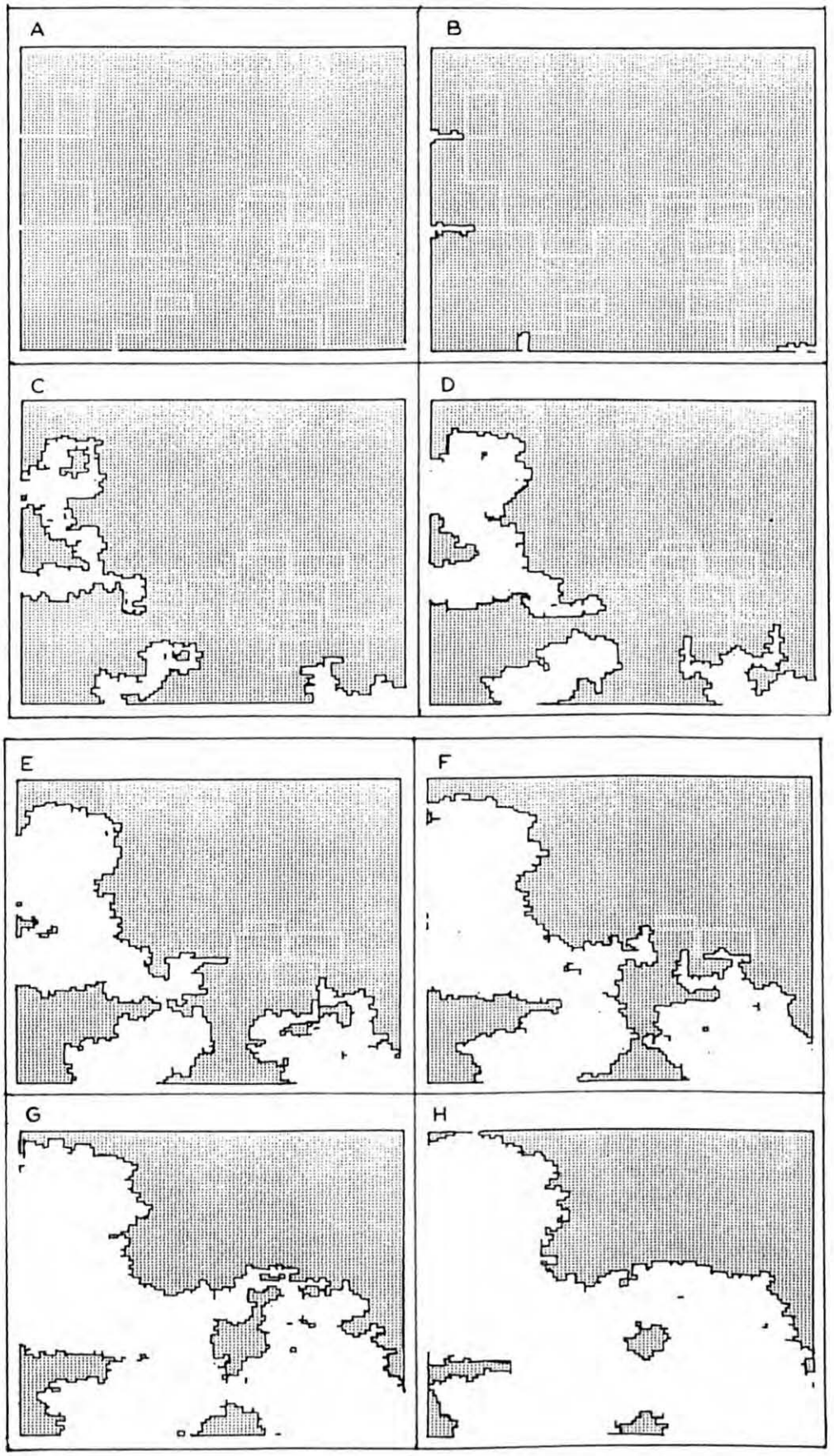

Fig. 7. Model of lipid multi-domain gel-phase (darker areas) hydrolysis by PLA_2 using sets P_d and P_4. Lighter lines indicate line defects. A: 0, B: 50, C: 1000, D: 2000, E: 3000, F: 4000, G: 5000 and H: 6000 monte carlo steps.

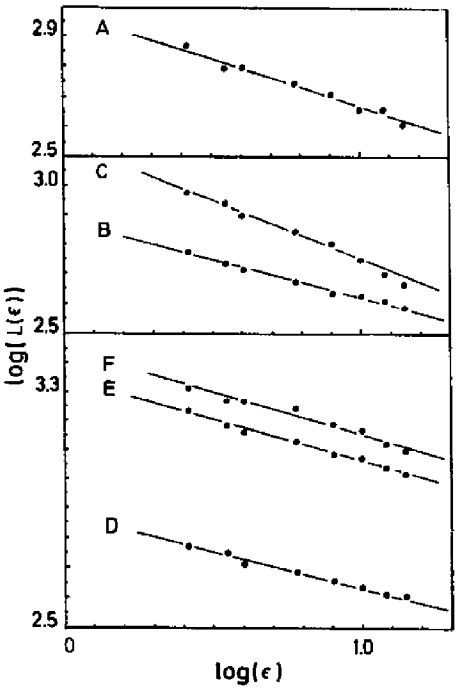

Fig. 8. Plots of $\log(L(\epsilon))$ against $\log(\epsilon)$ for the interfaces shown in C of Figs. 1, 2, 3 and 4 (A to D, respectively), in Fig. 5D (E) and in Fig. 6G (F).

result for the interface of Fig. 2C means that we cannot distinguish between the mechanisms defined by P_2 and P_4.

4. Discussion and Conclusions

We have proposed a model of the large-scale structure of the gel-phase of slowly-compressed phospholipid monolayers, in order to account for the experimental data of Grainger et al. [1]. The model proposes that the gel-phase monolayer possesses line defects which extend over large distances. It is also proposed that PLA_2 can hydrolyse lipid molecules located on the defects more easily than lipids located elsewhere in the gel-phase, unless the lipids are completely surrounded by other lipids of that phase. We have studied a variety of other models without line defects but have found none that reproduced the experimental data. All the models of PLA_2 hydrolysis of lipids propose that the process is controlled by the local environment of the lipid under attack.

We have calculated the fractal dimension, d_f, of the interface created by the hydrolysis of the model lipids, and we have shown that d_f is related to the probabilities $P_1, \cdots, P_4$, which define the hydrolytic process. Accordingly, if the mechanisms for hydrolysis are similar to those proposed here (see next paragraph) so that the interface is self-similar over some range of scale, then a measurement of d_f might yield information about the hydrolytic process.

The results of Grainger et al. [1] are consistent with those of Op den Kamp et al. [8] who studied the temperature-dependence of the PLA_2 hydrolysis of pure and mixed lipid vesicles. They observed local maxima in the percent hydrolysis at phase transition temperatures and in the neighbourhood of phase boundaries. In the case of DLPC-DSPC mixtures, the percent hydrolysis at the solidus boundary was about twice as large as that at the liquidus, and the percent hydrolysed in the fluid phase and two-phase regions were both equal but smaller, by a factor of four, than that at the liquidus phase boundary. An analogous result can be seen in the temperature-dependence of the rate of lipid exchange between vesides of DPPC and DSPC by exchange protein [9], where local maxima in the relative amount of lipid exchanged occurs at the phase transition temperatures. Although it might be argued that these observations can be understood as a consequence of dynamic lateral density fluctuation enhancement at phase transitions, phase boundaries or in narrow two-phase regions ([8], Fig. 7), the time-course of PLA_2 hydrolysis reported by Grainger et al. [1] and its spatial distribution together with the results for our simulations, suggests that lipid hydrolysis and possibly lipid exchange is enhanced by lipid packing defects existing in a gel phase. It is of relevance to note that the existence of line defects, was proposed in order to (successfully) account for the non-horizontal isotherms observed at the low-area end of what is considered by some to be the coexistence region of lipid monolayer isothermal phase transitions [10].

There are a number of questions which can be raised. The first is concerned with the mechanism, proposed by us, by which PLA_2 attacks a lipid molecule. We have assumed that this is determined entirely by the nearest neighbour environment of the lipid under attack. It is possible, however, to develop other criteria which would involve, not only the nearest neighbour (local) environment of a lipid, but a more extended environment which would reflect a larger-scale geometric structure. Thus, for example, it could be postulated that PLA_2 preferentially attacks gel-phase lipids which are located at a gel-fluid interface possessing a small negative (with respect to the gel phase) radius of curvature. It is possible that such an assumption might render our assumption concerning the existence of line defects unnecessary. However, while the existence of extended line defects in a quasi two-dimensional structure is plausible, a mechanism which assumes that PLA_2 will preferentially attack gel-phase lipids located at interfaces with a small negative radius of curvature may be more difficult to justify.

A second question is why PLA_2 appears to attack each macroscopic gel phase at only one point on its

perimeter [1], and then appears to eat its way in from there. Although it is possible that there is only one section of the perimeter at which line defects join it, one might have expected that at least some of the gel phases might have possessed more than one such point.

It has been reported that sufficiently-slowly-compressed monolayer gel phases are chiral and possess ordered molecular electric dipole moments ([11,12] and other references therein). One possibility for coupling between the director order and long-range dipolar order has been described in Ref. 11 (Fig. 9), where the projection onto the plane of the local dipole moments make an angle of $\approx \pi/6$ with the director. We have performed model calculations of the electric potential around a chiral shape with dipole moments assumed to lie in the plane and oriented similar to those of Ref. 11. Our results, however, have lead to no insights as to why one particular point, on the perimeter of a gel phase of lipids, if more than one were available, was selected as the only point at which hydrolysis began and continued.

A third question is related to the proposed line defects themselves: do they exist? One can speculate as to how line defects or domains might arise. Büldt et al. [13] have shown, using neutron diffraction, that PC polar group dipoles lie approximately parallel to the plane of a DPPC bilayer between 28°C and 50°C, the range of temperatures studied. This range encompasses the pre- and main transitions and the fluid phase. In the gel phase, it is plausible that the dipoles are ordered throughout some region [11]. The existence, however, of two such neighbouring regions, with different dipole orientations, could give rise to a line defect, not only because of the dipole–dipole interaction, but also because of packing problems involving lipids with sufficiently different orientations. Such a line defect would be analogous to a disclination in a nematic liquid crystal with the director determined by the orientations of the glyceride backbone and the polar group, and the tilt of the lipid molecule. The question of packing defects has been raised by Sackmann et al. [14] who make plausible that, because of the frustration which can arise in attempting to pack lipid molecules possessing non-equivalent hydrocarbon chains, the density of defects in a gel-phase monolayer could be high.

Finally, the question arises as to how one might create a high concentration of line defects in order to see whether the predictions of Figs. 6 and 7 are correct. One way might be to perform a sufficiently rapid compression so that the nucleation centres do not have sufficient time to relax before a macroscopic gel-phase region is formed. After this work was completed we realized that Grainger et al. [5] had carried out a similar experiment, though they allowed the dendritic structures, formed by the rapid compression (10 $Å^2$/mol per min), to anneal ([5], Fig. 2). Their photographs of the effects of PLA_2 show structures similar to those of Fig. 6.

Acknowledgements

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