Self-assembly of rodlike particles in two dimensions: A simple model for collagen fibrillogenesis

John Parkinson,* Karl E. Kadler, and Andy Brass
School of Biological Sciences, University of Manchester, 2.205 Stopford Building, Oxford Road, Manchester M13 9PT, United Kingdom
(Received 8 February 1994)

A model has been developed to study the aggregation of rodlike particles in two dimensions using diffusion-limited aggregation. The model was also extended to examine the aggregation of rods, with binding rules similar to those seen with collagen molecules. The model showed that the aggregates that were formed from the rods had an elongated, fibrillar structure. When the ways in which the rods could adhere to each other were restricted, to model the interactions observed in collagen, a preference for short overlaps between the particles in the aggregate was observed, reflecting the predilection for tip growth in diffusion-limited aggregation. Small changes in the rules of aggregation were found to have a marked effect on the resultant aggregate morphology.

PACS number(s): 82.20.Wt, 87.15.By

I. INTRODUCTION

The generation of supramolecular structures via the self-assembly of proteins is fundamental to the development of shape and form in biological systems. Understanding such aggregation processes is therefore important if we are to gain insights into the development and organization of biological tissues in health and disease.

In biological systems it has been possible to use simple physical models to study aggregation processes in which the number of possible attachment sites for a newly accreting particle is a constant with respect to the size of the aggregate. This maintenance of binding sites during assembly greatly simplifies the theoretical analysis. Such models have been used to study processes such as the assembly of microtubules [1], viral coats [2], actin microfilaments [3], and amyloid plaques [4]. However, biological self-assembly processes such as collagen fibrillogenesis, in which the number of available binding sites is dependent upon the size of the aggregate, have been less amenable to investigation.

Collagen is one of the major components of tissues such as skin, cartilage, and ligaments where it occurs as fibrils in the extracellular matrix (ECM). The fibrils are used by the body to provide a structural scaffold for other molecules in the ECM and to give tissues such as ligaments structural strength in tension. The collagen fibrils form by self-assembly of collagen molecules. Collagen is a long, rod-shaped molecule made up of three polypeptide chains in a triple-helical conformation. Normal collagen fibrils are long tapered cylinders in which the collagen molecules (1.5 nm in diameter $\times$ 300 nm in length) lie in near-parallel array, all point in the same direction, and are regularly staggered by integrals of D (where $D = 234$ residues, or 67 nm) with respect to the long axis of the fibril.

Collagen fibrillogenesis is particularly interesting for theoretical study because it is possible to grow collagen fibrils *in vitro* that have a morphology similar to that seen in the tissue, suggesting that collagen fibrillogenesis is a self-assembly process in which the basic information for generating the fibrillar shape is contained within the collagen molecule itself [5–7]. Algorithms such as diffusion-limited aggregation (DLA) have been developed to study simple aggregation processes in which the number of binding sites available grows with the size of the aggregate, for example dendrite growth, viscous fingering and the formation of gold colloids. In this paper we have used DLA on a two dimensional square lattice to study the morphology of aggregates formed from rods with interaction rules similar to those predicted to be used by collagen. The DLA algorithm has been chosen because of the similarities between the *in vitro* growth model (with the constant creation of small numbers of mature collagen molecules) and the DLA growth mechanism (in which particles are constantly released from a surface distant from the growing aggregate). The model described here could be readily modified to study the self-assembly of other rodlike molecules such as fibrillin into microfibrils [8] and fibronectin into filaments [9].

II. MODELS AND METHODS

The DLA simulations were based on the two dimensional lattice growth model described by Witten and Sander [10], and were performed on a square lattice with a lattice constant of 1. A rod was described in the model as a linear series of connected points on the lattice parallel to the x axis. Collagenlike molecules were modeled using rods of length 18 where the D-period length was four, giving a rod length of $4.5D$ periods. Staggers of 0D, 1D, 2D, 3D, and 4D were simulated by overlapping rods by 0, 4, 8, 12, and 16 lattice constants, respectively (see Fig. 1).

Collagen molecules have a distinct structure along their length which give them a polarity and limits the number of ways that the molecules can adhere to each other (see Fig. 1). Two models have therefore been examined for studying the aggregation of rodlike particles in

*Author to whom all correspondence should be addressed.

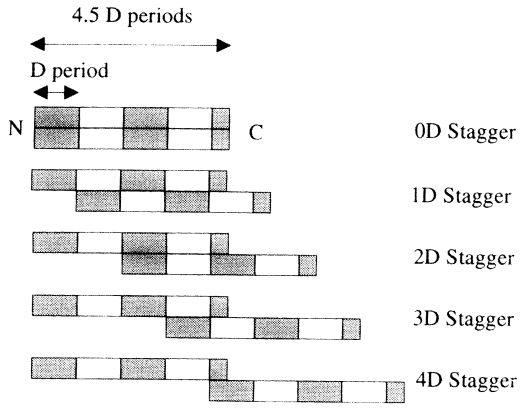

FIG. 1. The model particle is 4.5D periods long. Electron microscopy has demonstrated that collagen molecules adhere to each other in very specific ways, all pointing in the same direction and staggered by integrals of D (where $D = 234$ residues, or 67 nm) with respect to the long axis of the fibril. Therefore there are five specific ways in which collagen can adhere to each other, denoted as the 0D, 1D, 2D, 3D, and 4D staggers, depending on the length of the overhang.

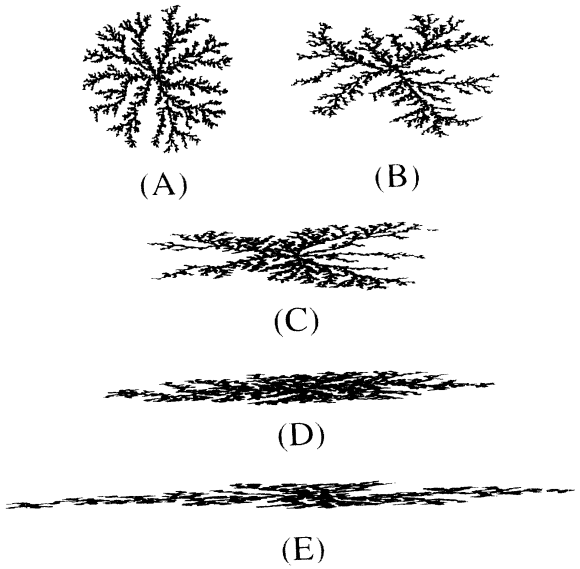

FIG. 2. Aggregates showing the effect of using different sizes of rods. (A) rods of length 1. (B) rods of length 2. (C) rods of length 5. (D) rods of length 10. (E) rods of length 20.

which (i) the rodlike particles were symmetric and could adhere to each other anywhere along their length, and (ii) the rodlike particles were asymmetric (length 4.5D) and could only adhere if the overlap was an integral of D. No rotational motion of the rods was included in the DLA procedures, as collagen molecules always attach parallel to each other.

Initially, the seed particle was placed in the middle of the two dimensional square lattice. An additional particle was then placed at random at a fixed large distance from the surface of the growing aggregate. This was done by generating an ellipse with an aspect ratio similar to that of the aggregate, and releasing the particle from the surface of this ellipse. Particles were only allowed to move within the space enclosed by the ellipse surface. The released particle then underwent a random walk to adjacent lattice sites until it contacted the growing aggregate. For simulations using collagenlike interactions, the incoming particle was only added to the aggregate if it was in one of the allowed sticking conformations [i.e., staggered an integral number of D periods with respect to the rod(s) with which it made contact]. For other simulations any contact automatically resulted in the rod being added to the aggregate. If the incoming rod did not stick to the aggregate, it was reflected away from the surface in a random direction. In some experiments, noise reduction [11,12] was accomplished by allowing a site to become occupied only after it had been visited N times, where N varied between 1 and 50. Fractal dimensions were calculated using a box counting method [13]. Final aggregates typically contained 10 000 rods.

III. RESULTS

A. The fibrillar shape is an intrinsic property of rod self-assembly

To investigate how the length of a rod affected the morphology of an aggregate, DLA simulations were run for symmetric rods of lengths varying between 1 and 20 which stuck on initial contact. As the length of the rod was increased, the morphology of the aggregate varied from highly branched dendritic structures to ones that exhibited an asymmetric near-fibrillar shape (see Fig. 2). Aggregates formed from rods of size 1 exhibited the typical Witten-Sander morphology characterized by dendritic branching [Fig. 2(A)]. The fractal dimensions of these aggregates were in the range 1.68–1.76 and were similar to those calculated by Meakin [14]. As the rod length was increased to 20 there was a corresponding flattening and elongation of the aggregate formed [Figs. 2(B)-2(E)], associated with this was a gradual increase in the fractal dimension of the aggregate to ~ 1.85. The aggregates formed by the different length rods had distinct morphologies. This could most clearly be seen in the distribution of holes in the aggregates (where a hole was defined as an enclosed empty space): with increasing rod length the size of the holes increased but the number of holes decreased.

B. Short overlaps predominate in DLA models of rod self-assembly

We noted in initial experiments that aggregates formed using the asymmetric rods which allowed only combinations of 0D, 1D, and 2D staggers (i.e., excluding 3D and 4D staggers) grew as ribbons in which monomers were aligned in register (Fig. 3). This type of growth occurs because collagen molecules are 4.5D periods long. The final 0.5D period of the molecule sterically hinders the creation of additional binding; therefore, for models excluding 3D and 4D binding the number of available binding sites in the aggregate does not increase with aggregate size. In contrast, aggregates which included 3D and/or 4D staggers, thereby enabling an increase in the number

of available binding sites as the size of the aggregate grows, were fibrillar in shape. Aggregates formed using exclusively 4D staggered interactions were longer and more compact than those produced using other stagger rules. Figure 4 shows that in aggregates generated using 0D, 1D, 2D, 3D, and 4D staggers, the 4D stagger occurred more often than any other type of stagger. No significant differences in morphology were seen between aggregates produced allowing 0D, 1D, 2D, 3D, and 4D sticking, and those produced in which the rods could adhere anywhere along their length.

C. Noise reduction has a marked effect on the morphology of the aggregate formed

Previous models of DLA, e.g., simulation of snow flake formation, have shown that introduction of noise reduction into a simulation emphasises the underlying symmetries of the system. In this system, noise reduction was implemented by insisting that a particle could not stick to the aggregate at a given site until that site had been encountered either one (no noise reduction), ten, or 50 times. Figure 5(A) shows a typical aggregate composed of 10 000 monomers and grown using 0D, 1D, 2D, 3D, and 4D staggers without any noise reduction. Figures 5(B) and 5(C) demonstrate that with increasing levels of noise reduction, aggregates became increasingly more dense and asymmetrical. In addition, branching occurred mainly in one direction, reflecting the polarity of the binding sites on the rod. Simulations were also with noise reduction for rods of length 18 which could stick anywhere along their length (i.e., symmetric) to investigate the influence of the underlying asymmetry of the collagenlike particles on aggregate morphgology. For these simulations it was noted that branching occurred at both ends of the growing fibril [Fig. 5(D)]. Figure 4 shows that the 4*D* stagger remains predominant in aggregates formed using various noise reduction algorithms.

IV. DISCUSSION AND CONCLUSIONS

In this paper we have performed a preliminary analysis of the aggregation behavior of rodlike particles in two dimensions as a first step in describing collagen fibrillogenesis. The creation of a simple two dimensional model of aggregation allows us to explore the physics involved in such processes. By gaining some understanding of the mechanisms involved, such a model can give us useful insights into real three dimensional systems. Here we are

FIG. 3. An aggregate composed of 500 particles using only 0D, 1D, and 2D staggers. The exclusion of 3D and 4D staggers leads to the formation of a ribbon like morphology.

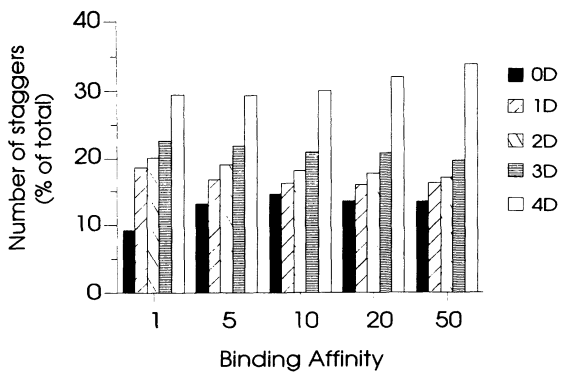

FIG. 4. Graph showing how the numbers of each type of bond varies as the binding affinity increases for aggregates composed of 10 000 particles using 0D, 1D, 2D, 3D, and 4D staggers as a function of binding affinity, where the binding affinity refers to the number of times a site must be encountered before an incoming particle will stick to it.

able to derive some inferences about the nature of collagen fibril formation from a simple two dimensional model of rod aggregation. The model is found to show several features consistent with experimental results. It was initially noted that an increase in the length of the rods led to a corresponding elongation and change in topology of the aggregate. These changes were consistent with one of the basic features of DLA, i.e., that new particles preferentially accrete to the tips of an aggregate [10]. A similar phenomena has been observed in collagen fibrillogenesis: using time-lapse light microscopy it has been shown that collagen fibrils *in vitro* grow from pointed tips [7]. Limiting the kinds of overlap available to newly accreting monomers had dramatic effects on the morphology of the final aggregate. Ribbonlike aggregates resulted when 3D and 4D staggers were excluded and the number of available binding sites remained constant. However, long and fibrillar aggregates resulted when 3D and/or 4D staggers were included. 4D staggers (i.e., short overlaps between adjacent monomers) predominated in these aggregates. Thermodynamic considera-

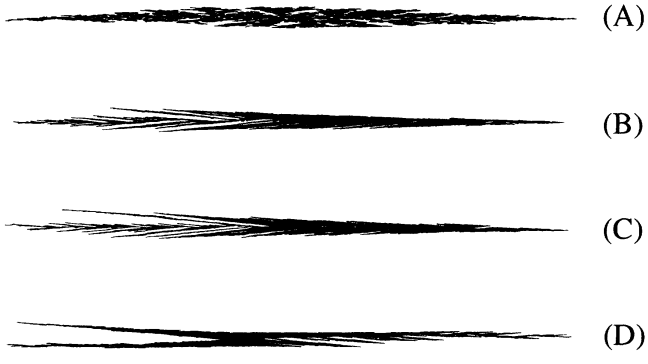

FIG. 5. Diagram showing the effect of altering the binding affinity of incoming particles on aggregates composed of 10 000 particles using 0D, 1D, 2D, 3D, and 4D staggers. (A) Binding affinity of 1. (B) Binding affinity of 10. (C) Binding affinity of 50. (D) Binding affinity of 50 with the particles allowed to stick anywhere along their length.

tions predict that the most abundant type of interaction between two collagen molecule should be the 1D stagger [15]. However, electron microscopy [16,17] studies suggest that 4D staggers predominate. The conclusions from the DLA model, that 4D staggers are more abundant than 1D staggers, is consistent with a model of fibril assembly in which the kinetics of monomer addition are more important in determining bond interactions than simple thermodynamic considerations.

The introduction of noise reduction into the growth model radically altered the morphology of the aggregate. Consistent with previous studies of DLA of point particles [12], sites for the addition of rods on the growing aggregate were shielded by overhanging branches and were less likely to grow than exposed sites. With the addition of noise reduction to the system, concealed sites are even less likely to grow. Hence, with noise reduction, small fjords in the structure were rapidly propagated to large chasms, leading to the formation of highly branched structures. The structures created with a high level of noise reduction did show morphologies dependent on the symmetry of the aggregating particle. Aggregates formed using the $4.5D$ period long asymmetric particle predominantly branched in one direction, whereas those formed with the symmetric particle branched in two directions [Figs. 5(C) and 5(D)].

Interestingly, fractal-like fibrils have been observed *in vitro* when abnormal, overglycosylated collagen containing a single amino acid substitution of gly$\alpha 1(I)$ 748 to cysteine assembled into fibrils [18]. It is known from experiment that overglycosylated collagen molecules assemble into fibrils at a slower rate than normal collagen molecules [18,19]. The bulk of the free energy for collagen-collagen interactions comes from hydrophobic protein-protein interactions, and so increased glycosylation will reduce this entropic contribution to binding. Overglycosylated collagen molecules would therefore be expected to have a lower binding affinity to the growing fibril than normal collagens, presumably resulting in a slower assembly process. The results from the noise-reduced aggregate suggest that changing the binding probability for a rod to adhere to the growing aggregate (as would be the case in overglycosylated collagen molecules) can indeed change the aggregate morphology and give rise to a more highly branched structure.

Several conclusions can be derived from this work. First, the fibrillar shape is an inherent property of the rodlike shape of the aggregating particle. Second, short overlaps of the rods predominate in the aggregates. Third, noise-reduction schemes can have a marked effect on the morphology of the final aggregate. Finally, the detailed structure of the aggregating rod only plays a minimal role in determining the final aggregate morphology.

ACKNOWLEDGMENTS

J.P. would like to thank the Wellcome Trust for financial support. K.E.K. is a recipient of a Wellcome Trust Grant in Basic Biomedical Science (No. 19512).

[1] M. Dogterom and S. Leibler, Phys. Rev. Lett. **70**, 1347 (1993).
[2] P. J. G. Butler, J. Gen. Vir. **65**, 253 (1984).
[3] C. Frieden, Am. Rev. Biophys. Chem. **14**, 189 (1985).
[4] S. J. Tomski and R. R. Murphy, Arch. Biochem. Biophys. **294**, 630 (1992).
[5] D. F. Holmes, J A. Chapman, D. J. Prockop, and K. E. Kadler, Proc. Natl. Acad. Sci. USA **89**, 9855 (1992).
[6] K. E. Kadler, Y. Hojima, and D. J. Prockop, J. Biol. Chem. **260**, 15 696 (1987).
[7] K. E. Kadler, Y. Hojima, and D. J. Prockop, Biochem. J. **268**, 339 (1990).
[8] C. M. Kielty, C. Cummings, S. P. Whittaker, C. A. Shuttleworth, and M. E. Grant, J. Cell Sci. **99**, 797 (1991).
[9] D. F. Mosher, Curr. Op. Struct. Biol. **3**, 214 (1993).
[10] T. A. Witten and L. M. Sander, Phys. Rev. Lett. **47**, 1400 (1981).
[11] J. Nittman and H. E. Stanley, J. Phys. A **20**, L1185 (1987).
[12] M. T. Batchelor and B. I. Henry, J. Phys. A **26**, 3431 (1993).
[13] B. B. Mandelbrot, *The Fractal Geometry of Nature* (Freeman, New York, 1983).
[14] P. Meakin, Phys. Rev. A **27**, 604 (1983).
[15] D. G. Wallace, Biopolymers **32**, 497 (1992).
[16] N. P. Ward, D. J. S. Hulmes, and J. A. Chapman, J. Mol. Biol. **190**, 107 (1986).
[17] R. Fleischmajer, J. S. Perlish, and T. Faraggiana, J. Hist. Cytochem. **39**, 51 (1991).
[18] K. E. Kadler, A. Torre-Blanco, E. Adachi, B. E. Vogel, Y. Hojima, and D. J. Prockop, Biochemistry **30**, 5081 (1991).
[19] A. Torre-Blanco, E. Adachi, A. M. Romanic, and D. J. Prockop, J. Biol. Chem. **267**, 4968 (1992).

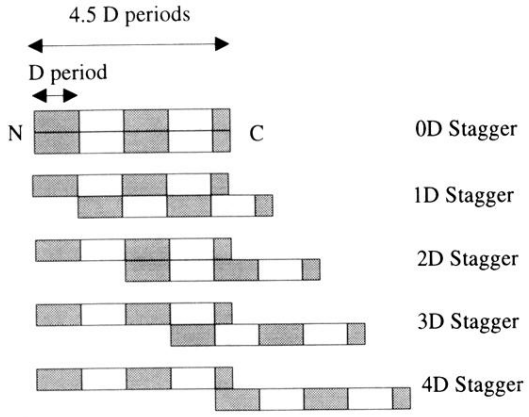

FIG. 1. The model particle is $4.5D$ periods long. Electron microscopy has demonstrated that collagen molecules adhere to each other in very specific ways, all pointing in the same direction and staggered by integrals of D (where $D = 234$ residues, or 67 nm) with respect to the long axis of the fibril. Therefore there are five specific ways in which collagen can adhere to each other, denoted as the 0D, 1D, 2D, 3D, and 4D staggers, depending on the length of the overhang.