Acta Cryst. (1994). D**50**, 569–571

Mesoscopic Theories for Protein Crystal Growth

BY J. T. W. M. TISSEN, J. G. E. M. FRAAIJE, J. DRENTH AND H. J. C. BERENDSEN

BIOSON Research Institute and Laboratory of Biophysical Chemistry, University of Groningen, Nijenborgh 4, 9747 *AG Groningen, The Netherlands*

(*Received* 10 *September* 1993; *accepted* 26 *November* 1993)

Abstract

A computer-simulation method is proposed for studying the hydrodynamic interactions of rigid protein molecules. It is a combination of Stokes dynamics and continuum hydrodynamics. The Stokes equations of motion for the protein molecules, the creeping-flow equation for the solvent together with the no-slip boundary conditions give a complete representation of the system. The resulting three-dimensional boundary-value problem can be rewritten in a two-dimensional form (without any loss of information) considering the surfaces of the particles only. Then, by solving the equations on discrete surface elements, the so-called mobility matrix is determined in which all hydrodynamic interactions are included. Finally, after calculation of the conservative forces and the stochastic force, the new velocities of the protein molecules can be determined. The simulation method can be applied to arbitrary particle shapes. It can also handle arbitrary flow fields, and the effects of applying a flow field to the system can be studied. From analysis of the trajectories, information can be gained on the kinetics and thermodynamics in the early stages of the crystallization process.

Introduction

A large number of experiments are performed in order to obtain a better insight into the mechanisms underlying the protein crystallization process. In addition to these experiments computer simulations can be of great value. They can provide additional information where experimental techniques fail and they can help the interpretation of the experiments. In this paper we propose a method to study the physico-chemical aspects of protein crystallization. The central idea is that the only force acting over a length scale comparable with the size of the protein molecules is the hydrodynamic interaction. This is very important when considering steering effects in the process of nucleation. All other forces such as electrostatic and van der Waals interactions are much more short-ranged and extend only over a few Å under high-salt conditions. Considering protein crystallization, dimensions of millimeters (crystal sizes) and minutes are common, whereas in simulation methods such as molecular dynamics the nanometer and picosecond are used. For the simulation of protein crystallization one has to try to operate somewhere between these macroscopic and microscopic descriptions. It is in this mesoscopic domain that we will perform our simulations.

In the molecular-dynamics method all interactions are calculated on an atomic scale. For particles in a solution this becomes a tremendous task as the total number of degrees of freedom is very large. Application of stochastic simulations such as Brownian dynamics (BD) (Ermak & McCammon, 1978) reduces the computational effort considerably for such systems. Numerous BD simulations have been performed on biological systems (see *e.g.* Dickinson, 1985). However, the method is not very well suited to study protein crystallization as it uses models for the hydrodynamic interactions which assume a very symmetrical particle shape and which collapse for small interparticle distances. For the crystallization process the exact shape of the molecules is very important as well as the events occurring at small interparticle separations. Therefore, we propose a different simulation method which handles the hydrodynamic interactions properly so that the early stages of protein crystallization can be studied.

The outline of this paper is as follows. First, the theory will be briefly explained. The next section deals with the solving of the boundary value problem and gives a schematic view of the simulation procedure. Finally, the possible applications of the method will be discussed.

Theory

To study protein crystallization the movements of the protein molecules resulting from the forces acting on them must be followed. In order to reduce the computer time these molecules will be considered to be rigid. For the velocities of the viscous, incompressible fluid, the creeping flow (linearized Navier–Stokes) equation holds,

$$\nabla^2\mathbf{u} = \frac{1}{\eta}\nabla p, \tag{1}$$

together with the continuity equation,

$$\nabla\cdot\mathbf{u} = 0. \tag{2}$$

Here, $\mathbf{u}$ is the fluid velocity, η the fluid viscosity, and p the hydrostatic pressure. p is related to the pressure tensor Π.

For the motion of a rigid particle in this fluid the Langevin equation holds

$$M\cdot\frac{\mathrm{d}\mathbf{V}}{\mathrm{d}t} = \mathbf{F}_{\mathrm{h}} + \mathbf{F}_{\text{non-h}}, \tag{3}$$

with $\mathbf{V}$ a column vector of dimension 6 containing the translational and angular velocities of the particle [the particle having six degrees of freedom (x, y, z, φ_1, φ_2, φ_3)]. The particle and fluid velocities are related by the no-slip boundary conditions through which it is assumed that the relative tangential velocity component of fluid in contact with the rigid particle's surface is taken as zero. M is a generalized mass/moment of inertia matrix of dimension 6×6. $\mathbf{F}_{\mathrm{h}}$ and $\mathbf{F}_{\text{non-h}}$ are six-dimensional (hydro- and non-hydrodynamic) force–torque vectors.

The non-hydrodynamic forces may come from electrostatic interactions, van der Waals interactions, hydrogen bonding, gravitational forces, *etc.* The hydrodynamic force exerted by the surrounding fluid on the particle is given by

$$\mathbf{F} = \int_{\text{particle}} \Pi\cdot\mathrm{d}S. \tag{4}$$

The torque experienced by the particle may be obtained in a similar way (Happel & Brenner, 1973).

For a low Reynolds number the particle inertia in (3) may be neglected as compared to the viscous forces. With

$$\mathbf{F}_{\mathrm{h}} = -\mathbf{R}\cdot\mathbf{V} + \mathbf{F}_{\text{stoch}}, \tag{5}$$

this leads to solving for the velocities

$$\mathbf{V} = \mathbf{R}^{-1}\cdot\{\mathbf{F}_{\text{stoch}} + \mathbf{F}_{\text{non-h}}\}. \tag{6}$$

Here, $\mathbf{R}$ is the resistance matrix and its inverse is the mobility matrix. The stochastic part of the hydrodynamic force comes from the continuous bombardment of the protein molecules by the solvent particles. Note that inverting the matrix $\mathbf{R}$ consumes the most time, growing as the third power of the number of particles; computing $\mathbf{R}$ grows only as the square of the number of particles.

Thus, with knowledge of the mobility matrix $\mathbf{R}^{-1}$ and the non-hydrodynamic and stochastic forces $\mathbf{F}_{\text{stoch}}$ and $\mathbf{F}_{\text{non-h}}$, the velocities (and positions) of the protein molecule can be calculated for a large number of time steps, thus yielding the trajectories of the molecule. For many-particle systems the procedure can be derived analogously.

Method

The crucial step in the calculations is finding the solution of the no-slip boundary conditions. A new numerical technique is the boundary integral equation method (see Weinbaum, Ganatos & Yan, 1990). An advantage of this method is the reduction of the original three-dimensional boundary-value problem for the velocity field to a two-dimensional problem (without any loss of information).

The Stokes problem can be rewritten as a sum of so-called single- and double-layer potentials (Ladyzhenskaya, 1969), whereby the stress force is distributed over the particle's surface. The local stress force density $\mathbf{f}(\mathbf{x})$ exerted by the fluid on the surface of the particle is then to be determined, satisfying the boundary conditions.

The resulting surface integral equations can be solved numerically, and this transforms them into a linear system of algebraic equations. This can be done by dividing the particle's surface S_p into M elements $\Delta_m (m = 1,2,\ldots,M)$ all of which are small relative to S_p and over which the components of $\mathbf{f}$ may, for the purposes of the integral equations, be considered constant and equal to their value at the centre of the element. The particle's surface can be described by discrete surface elements by using triangulation techniques (Juffer, Botta, van Keulen, van der Ploeg & Berendsen, 1991).

The integral equations are satisfied at the centres $x^{(m)} (m = 1,2,\ldots,M)$ of each element, thereby yielding (Youngren & Acrivos, 1975):

$$\frac{1}{4\pi}\sum_{j=1}^{M} f_k[\mathbf{x}^{(j)}]\iint_{\Delta j}\left\{\frac{\delta_{ik}}{r_{x^{(m)}y}} + \frac{[x_i^{(m)} - y_i][x_k^{(m)} - y_k]}{r^3_{x^{(m)}y}}\right\}\mathrm{d}S_y$$

$$= -U_i[\mathbf{x}^{(m)}] - \frac{3}{2\pi}$$

$$\times\iint_{S_p}\frac{[x_i^{(m)} - y_i][x_j^{(m)} - y_j][x_k^{(m)} - y_k]n_j(\mathbf{y})U_k(\mathbf{y})}{r^5_{x^{(m)}y}}\,\mathrm{d}S_y. \tag{7}$$

Here, $\mathbf{U}$ is the known fluid velocity, $\mathbf{x}$ and $\mathbf{y}$ coordinates, i, j, k directions, $r_{xy} = |\mathbf{x}-\mathbf{y}|$, δ_{ik} the Kronecker delta, $\mathbf{n}$ a vector normal to the surface element, and $\mathrm{d}S_y$ indicates that the integration is with respect to the point $\mathbf{y}$. Here, the usual Cartesian tensor-summation convention is adopted and unit viscosity is assumed.

The above form a linear system of $3M$ equations in the $3M$ unknowns $f_k[\mathbf{x}^{(m)}]$. It can be solved numerically using suitable integration and matrix-inversion techniques. From the A_{ik} the mobility matrix can be derived and using (6) the trajectories of the protein molecule can be calculated.

Summarizing, a schematic representation of the simulation procedures is as follows.

(1) Describe the atomic positions and orientation of the protein molecules.

(2) Triangulate the particles' surfaces.

(3) Solve the boundary value problem using equation (7).

(4) Determine the mobility matrices.

(5) Calculate the non-hydrodynamic and stochastic forces acting on the molecules.

(6) Calculate the new velocities using equation (6).

(7) Calculate new positions and orientation of the protein molecules.

(8) Go to step 3. Perform the iterations for *NSTEPS* time steps.

Discussion

The simulation method can be applied to arbitrary particle shapes. It can also handle arbitrary flow fields, and the effects of applying a flow field to the system can be studied. Furthermore, the effects of changing the temperature and the solvent type can easily be investigated.

From analysis of the trajectories information can be gained on the kinetics and thermodynamics in the early stages of the crystalllization process. For instance, by letting two protein molecules interact, thereby moving around and over each other, nucleation kinetics can be studied, and also, the formation of trimers, tetramers, ..., *n*-mers. And finally, given a description of the shape of the crystal surface, attachment of a protein molecule on the crystal can be followed. This can contribute to a better understanding of the underlying mechanisms.

The help of E. F. F. Botta and F. W. Wubs of our mathematical department is gratefully acknowledged. This study is supported by SRON (Netherlands Space Research Foundation).

References

DICKINSON, E. (1985). *Chem. Soc. Rev.* **14**, 421–455.

ERMAK, D. L. & MCCAMMON, J. A. (1978). *J. Chem. Phys.* **69**, 1352–1360.

HAPPEL, J. & BRENNER, H. (1973). *Low Reynolds Number Hydrodynamics*. Englewood Cliffs, NJ: Prentice-Hall.

JUFFER, A. H., BOTTA, E. F. F., VAN KEULEN, B. A. M., VAN DER PLOEG, A. & BERENDSEN, H. J. C. (1991). *J. Comput. Phys.* **97**, 144–171.

LADYZHENSKAYA, O. A. (1969). *The Mathematical Theory of Viscous Incompressible Flow*. New York: Gordon & Breach.

WEINBAUM, S., GANATOS, P. & YAN, Z.-Y. (1990). *Annu. Rev. Fluid Mech.* **22**, 275–316.

YOUNGREN, G. K. & ACRIVOS, A. (1975). *J. Fluid Mech.* **69**, 377–403.