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Nucleation and Crystallization of Polymers and Biopolymers

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The problem of formation of interfaces is treated with special emphasis on nucleation problems in polymers. Factors affecting conformation are discussed, and it is shown, that the nature of the solvent is among the most important. Electron microscopy and laser excited Raman spectroscopy techniques are used in conformational analysis of crystalline biopolymers.

INTRODUCTION

One of the most fundamental of questions concerned with interfaces is how they form in the first place. Nucleation studies seek to resolve the problem of how molecular rearrangement occurs in a phase transition, the end product being a crystal or droplet with its accompanying surface or interface to the surrounding medium. One way of expressing the energy barrier to formation of this new phase relates to the interfacial energy. This lecture is not, however, intended as a review of the theories and experiment of nucleation, which have been thoroughly expounded previously[1], but rather represents an extrapolation to polymeric systems, where the overriding feature is molecular conformation and geometry.

Polymer Science abounds with examples of nucleation problems and processes, many of them under intensive study by the plastics industry. On the other hand, the great strides made in X-ray crystallography of important biological compounds have, to a large extent, been dependent upon the art and science of nucleating and growing reasonable crystals.

Nucleation of Melts

Let us start with a brief consideration of synthetic polymer nucleation. Here we have the choice of studying nucleation from the melt or from solution. Whereas quantitative theory and experiment are fairly well developed for the solidification process, only qualitative data are available for nucleation from solution. However, the latter process does afford us much interesting conformational information.

The cluster or nucleus in a melt is often visualized, for a crystalline polymer, as a chain folded bundle with a characteristic interfacial (free) energy for the fold surface σ_e and the other surfaces σ. The energy required to form such a nucleus is correlated with the critical degree of supercooling which the melt would sustain in the absence of impurities[2]. Equations relating the rate of nucleation, supercooling and interfacial properties of

polymers have been proposed by Hoffman and Lauritzen[2] and will not be detailed here. The Hoffman-Lauritzen theory has been subjected to experimental test in our own laboratory, using the kinetic droplet method, and good accord was found with the general form of the theoretical predictions[3].

Heterogeneous nucleation, *i. e.* the effect of (solid) impurities in catalysing the nucleation process is much more difficult to study and to my knowledge, has only been studied quantitatively for polyethylene on solid alkali halide crystals[4]. The general impression is that interface geometry and energetics are vitally involved.

Nucleation from Solution

Nucleation of synthetic polymers from solution is very difficult to characterize in terms of supercooling, interfacial forces, *etc.* and we are forced to take the tack of gleaning information from the morphology of the product. Usually, if the product is crystalline, very thin, small lamellar crystals are produced of approximate dimensions $1\,\mu \times 1\,\mu \times 100$ Å. Further information on synthetic polymer crystals may be sought from my colleague, Professor Phillip Geil's book on polymer single crystals[5]. Fig. 1 shows an electron micrograph of single crystals of polyethylene grown from xylene solution. Electron diffraction of such crystals shows the molecular axis to be perpendicular to the plane of the major surface and since the molecules are much longer than the crystal is thick, we must assume that the chains are folded with the major plane being the fold surface. Attempts to grow large single crystals of synthetic polymers have been singularly unsuccessful and hence, much of the structural elucidation of such crystals has depended on single crystal electron diffraction in the electron microscope.

Single Crystals of Biopolymers

Although isolated reports of single polypeptide crystals (*e. g.* polyglycine) have been published for some years, it was not until 1964 that Keith and Padden[6] were able to demonstrate electron diffraction which established that the chain arrangement was quite similar to polyethylene. These biopolymer crystals (poly-L-alanine, poly-L-tyrosine, polyglycine, *etc.*) were thin lamellar structures with the peptide backbone (molecular axis) perpendicular to the lamellar surface. Again the molecular length is often much larger than the crystal thickness, thus promoting a folded surface. Such folding has been observed by the Keith and Padden group for α helices, β sheets, D. N. A. fragments and the polyglycine II helix[7,8]. This last named example is of particular interest since the distorted polyglycine II conformation is believed to be the main structural conformation in the fibrous protein collagen. The observation of folded polypeptide conformations automatically leads to the concept that the crystals contain antiparallel helices; *i. e.* in adjacent or near adjacent chains the sequence-N-C-CO- is reversed. This fact explains some of the anomalies which are observed in X-ray fibre diagrams. Ramachandran[9] has, in fact, proposed a complete crystal structure for polyglycine II based on the electron microscope observations.

If we are to understand the mechanism of nucleation of these biopolymers, and eventually of more complex polypeptides, it is necessary to know as much as possible about the conformation or molecular shape of the molecule

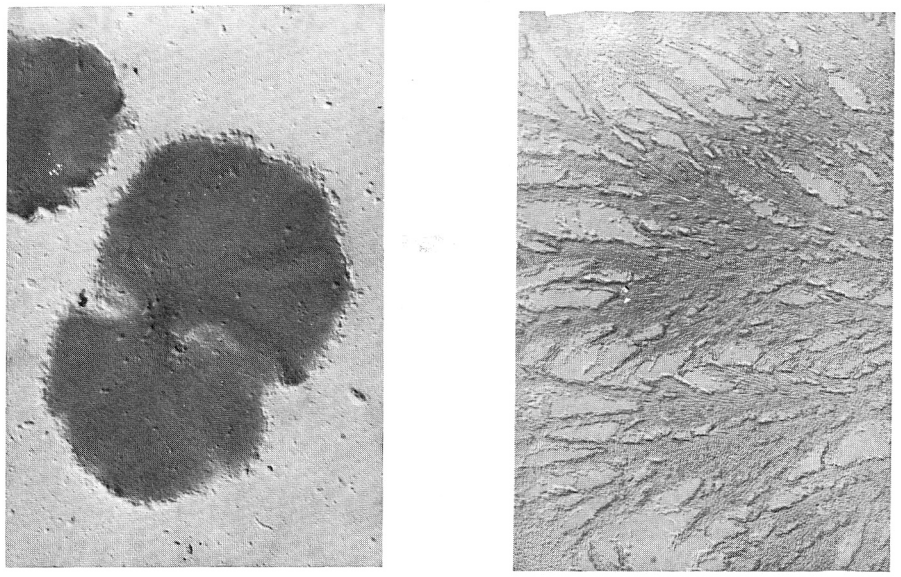

Fig. 3. Electron micrographs of poly-L-proline spherulites *a*) is for poly-L-proline I which is a rigid (right-hand) helix, which does not allow chain folding. The product is poorly crystalline *b*) is poly-L-proline II, and shows clearly the lamellar spherulitic structure.

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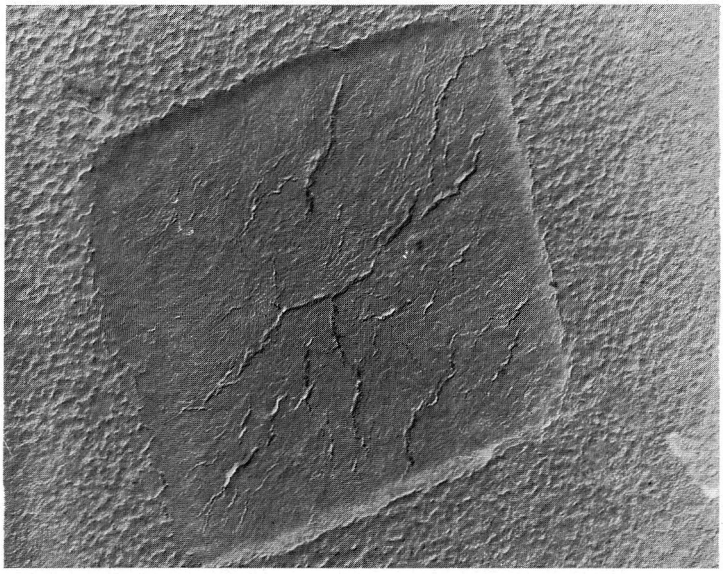

Fig. 4. An electron micrograph of Poly (Gly-Pro-Pro). This polypeptide contains chain folded triple helices lying perpendicular to the plane of the crystal (hedrite).

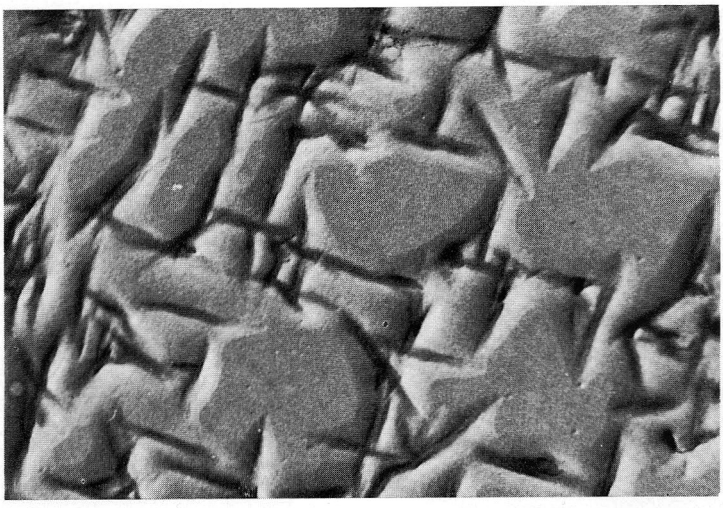

Fig. 5. Epitaxy of POAHP on NaCl. Needle directions are [100], [010] and [320], [230].

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in solution prior to nucleation and the forces which cause it to maintain this shape. In addition, we must know how this conformation changes upon nucleation and what role desolvation plays.

Factors Affecting Conformation

In the early 1950's the recognition of the planarity of the peptide bonds in the molecular skeleton of proteins and the postulate of linear hydrogen bonds led to the, now classical, concept of the α helix. It was felt that the major conformational stability originated from the energetics of hydrogen bonds which were responsible for the intramolecular stabilization of the α helix and intermolecular stabilization of the β sheet conformation. It is now known from detailed calculations emanating from several laboratories[10–12], including our own[13], that the major forces maintaining the conformation of polypeptides are electrostatic (dipolar) and London/van der Waals type. The $>C=O\ldots H—N<$ hydrogen bonds can and do alter the balance of conformational stability in some cases, but there are examples, *e. g.* polyimino acids, where conformation is independent of hydrogen bonding (which is non-existent) — yet quite stable helices are produced. Poly-L-proline, one of these polyimino acids is of particular interest to many workers because of its collagen-like conformation.

In solution it is possible to study the conformation of polypeptides by optical rotatory dispersion and circular dichroism and although these techniques have certain major disadvantages they have been used extensively to study helical character. The α helix tends to be stable in solution provided the solvent is inert or only a weak polar solvent (*e. g.* chloroform). However, in strong acidic solvents such as dichloroacetic acid (D. C. A.) and strong hydrogen bonding solvents, *e. g.* trifluoroethanol (T. F. E.), the intramolecular hydrogen bonding of the α helix is disrupted and the solvent becomes bound at the bonding sites[14]. It is of considerable interest that the poly-α-amino acids tend to precipitate as fibers or as amorphous matter from weak solvents, but form thin lath-like crystals from several of the strong solvents. In some cases where fibers are formed there is considerable evidence that the molecules are already aggregated in solution, *e. g.* the precipitation of poly-γ-benzyl-L-glutamate from ethylene dichloride or other weak solvents. Paracrystalline (liquid crystal) forms are sometimes noted for solutions. More quantitative evidence has emerged from low angle X-ray diffraction of solutions prior to precipitation.

A new technique has been developed in our own laboratory for studying the conformation of polypeptides, both in solution and in the solid state[15]. This method is laser excited Raman spectroscopy. Although Raman spectroscopy is an old technique, the application to biopolymers has only become feasible in the past five years with improved electronics and high intensity Ar^+ coupled lasers. Raman spectroscopy may be readily accomplished in aqueous solutions and is responsive to conformational changes in molecules through certain infrared inactive backbone vibrations. Fig. 2 shows the Raman spectra of poly-L-proline in the solid and in aqueous solution. The only changes occurring are in the 1650 cm^{-1} amide I band, which changes as the solvent becomes hydrogen bonded to the peptide carbonyl group and in the 830 cm^{-1} region where the apolar atoms in the pyrollidine ring change

environment. The residue and skeletal modes below 700 cm^{-1} are identical and the molecule is therefore in the same, or similar, conformation in solid and aqueous solution.

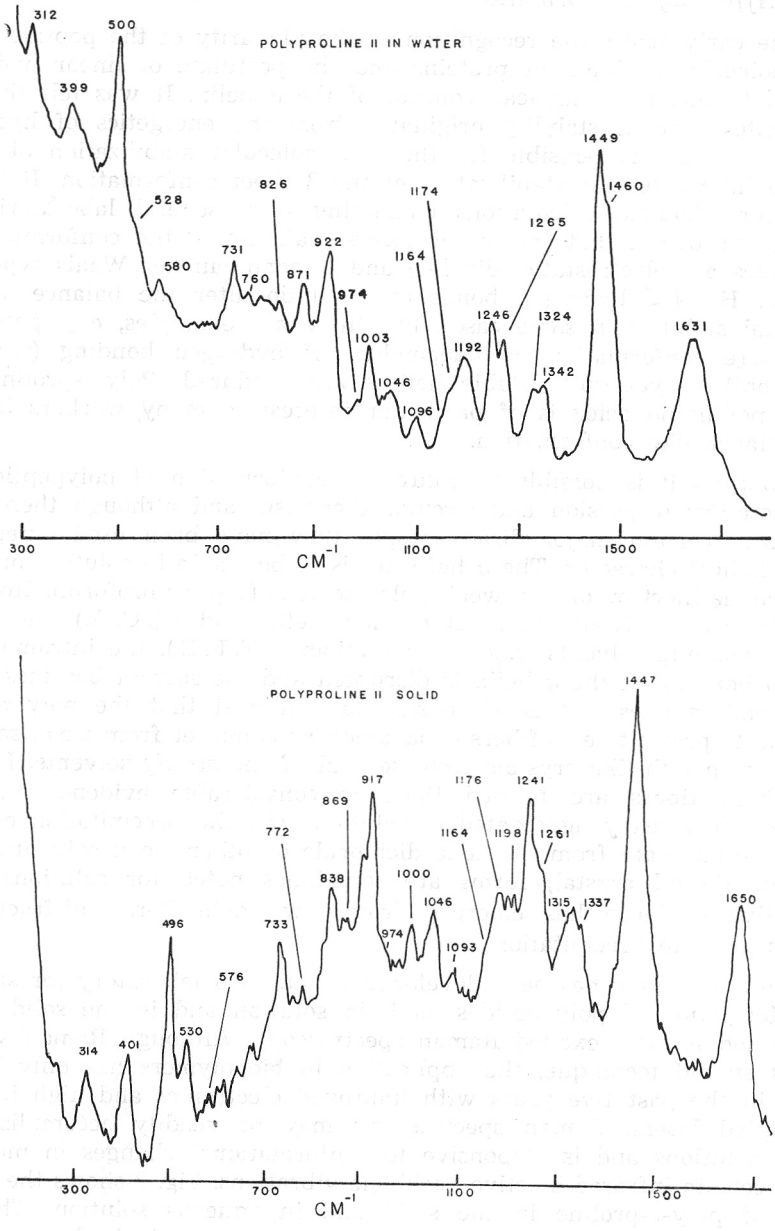

Fig. 2. Raman spectrum of poly-L-proline II in *a*) Aqueus solution and *b*) solid state. The polyproline II structure is a trans, left-hand helix with 3.1 Å residue repeat. There is apparently little or no conformational change on passing from solid to solution state.

Crystallization and Conformation of Polyproline

Poly-L-proline is a particularly interesting material to work with since it undergoes solvent-induced conformational changes from the rigid cis right-handed helix of form I (code named PP I) in weak solvent to the more flexible trans righthand helix of form II (PP II) in polar solvents (including water). If polyproline is caused to precipitate from weak solvents in form I, an amorphous-looking spherulite showing very weak electron diffraction is formed (Fig. 3a). From strong solvents, highly crystalline spherulites are produced (Fig. 3b), and we have shown from electron diffraction that the molecules lie flat on the substrate and perpendicular to the apparent fiber axis[16]. The molecules fold and thus have antiparallel helices.

Thus, it would seem that homopolypeptides only crystallize well if they can form chain folded lamellae. Thus, PP I, which is a rigid chain, cannot fold and chain entaglement produces a material of low crystallinity.

SUMMARY AND PROSPECTS

Now it is understood that most synthetic biopolymer crystals often nucleate and grow into chain folded lamellar crystals of very small size, we can expect progress with more complicated polypeptides. One interesting example is the application to model polytripeptides of the collagen type. Collagen is known to be basically a triple stranded helical structure of predominantly polytripeptides of the form $(Gly\text{-}Pro\text{-}X)_n$. It has been suggested recently[17] that a detailed picture of atomic coordinates for collagen can be derived from X-ray diffraction patterns of poly (Gly-Pro-Pro). Collagen has (in most forms) a parallel strand structure. We have recently crystallized $(Gly\text{-}Pro\text{-}Pro)_n$ from dioxane-water mixtures, producing lamellar crystals as shown in Fig. 4. Although detailed diffraction has yet to be obtained, it seems most likely that the chain folded conformation is prevalent with *antiparallel* chains.

Many new biopolymers are being synthesized in various laboratories as the basis for biomaterials and as models for fibrous proteins. Efforts to nucleate and crystallize these materials will probably follow along similar lines to those expounded here. The possibility of adding nucleating agents should not be overlooked. We have found that it is sometimes possible to force crystallization by using a crystal substrate and the principles of epitaxy. Fig. 5 shows poly-*O*-acetyl-L-hydroxyproline crystallized on potassium chloride. In addition to the possibility of forcing nucleation when it may not normally occur, the use of a crystalline substrate sometimes has the advantage of turning the crystals on edge, so that edge-on diffraction can be obtained. Such is the case with the POAHP crystals shown in Fig. 5.

One of the disadvantages with the electron microscope method is the difficulty of obtaining adequate diffraction patterns. The advent of the new generation high voltage microscopes should help considerably and will possibly herald a new era in electron diffraction and structural studies of biological crystals.

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IZVOD

Nukleacija i kristalizacija polimera i biopolimera

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Jedno od najosnovnijih pitanja teorije površina je kako one uopće nastaju. Cilj je proučavanja pojava nukleacije odgonetnuti problem molekularnih pregrupiranja u faznim prijelazima, u kojima je konačni produkt kristal ili kap tekućine zajedno s površinom ili međufazom koju tvori prema mediju postanka. U ovom radu su prikazani faktori koji određuju nukleaciju biopolimera te faktori koji određuju konformaciju molekularnog skeleta u monokristalima. Spomenuti su rezultati istraživanja kristalizacije i određivanja konformacije poli-L-prolina metodom laserske Raman spektroskopije. Prikazane su i elektronske mikrografije različitih uzoraka poli-L-prolina kao i nekih polipeptida koji sadrže prolin. Metodom forsirane heterogene nukleacije dobiveni su kristali poli-*O*-acetil-L-hidroksiprolina i prikazani njihovi elektronski difraktogrami.

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