base sequence. These base-sequence-dependent fluctuations may be specific for protein recognition or drug binding.

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STRUCTURAL STUDIES OF BEE MELITTIN

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INTRODUCTION

The question of how proteins refold in passing from an aqueous phase to an amphipathic environment such as a membrane is being addressed by a structural study of bee melittin. Melittin is the toxic, main protein of bee venom, and has been shown by others to integrate into natural and synthetic membranes and to lyse a variety of cells. This function is presumably related to its unusual sequence. Except for charges at the N-terminus and at lysine 7, the first 20 residues are largely apolar. In contrast, the last six residues contain four charges and two polar residues. The entire sequence of melittin is (1):

NH_2–Gly Ile Gly Ala Val Leu Lys Val Leu Thr Thr Gly Leu Pro Ala

Leu Ile Ser Trp Ile Lys Arg Lys Arg Gln Gln–$CONH_2$.

RESULTS

Two crystal forms of melittin have been grown from aqueous ammonium sulfate solutions (2), and both are suitable for structural studies at high resolution. Both crystal forms contain two melittin polypeptide chains (each of molecular weight 2,840) in the asymmetric unit. Since melittin is a tetramer in aqueous solution, this shows that it contains at least one twofold axis of symmetry.

The unit cell dimensions of the orthorhombic form II are convenient for diffractometry measurements, and data have been collected for the native protein to 2.0 Å resolution and for five heavy atom derivatives, four of them to 2.8 Å resolution and the other to 3 Å resolution.

Heavy atoms have been located in three of the derivatives, and their positions and occupancies have been refined at 3.5 Å resolution. On the basis of these preliminary parameters, an electron density map has been calculated at 6 Å resolution.

The unit cell dimensions and symmetry of the form I crystals are convenient for intensity measurements by the rotation method, and film data have collected for the native protein to 2.5 Å resolution. These were reduced and used in the calculation of a native Patterson map. This map displays vectors on the Harker section at $W = 0.5$ that are compatible with noncrystallographic twofold axis nearly parallel to c.

DISCUSSION

The 6-Å electron density map of the form II crystals leads to several preliminary conclusions. The melittin tetramer is characterized by 222 (D_2) symmetry. The molecular twofold axis coincides with the crystallographic twofold along a. The molecule is ~42 Å long in this direction. Along b the molecule extends ~40 Å and along c it extends roughly 25 Å. Noncrystallographic twofold axes are approximately parallel to b and c.

Molecular 222 symmetry permits each highly charged C-terminus to be positioned in space, far from the positive charges of the C-termini of the other three chains. This would be expected for a highly soluble, yet relatively apolar, peptide such as melittin: the positive charges attract water dipoles and repel the positive charges on other melittin tetramers.

The connectivity of the polypeptide is not certain at 6 Å resolution. One possibility for the structure of a monomer is that of a bent rod, with the two segments forming an angle of roughly 140° with each other. The total length of a bent rod is roughly 40–45 Å. In this

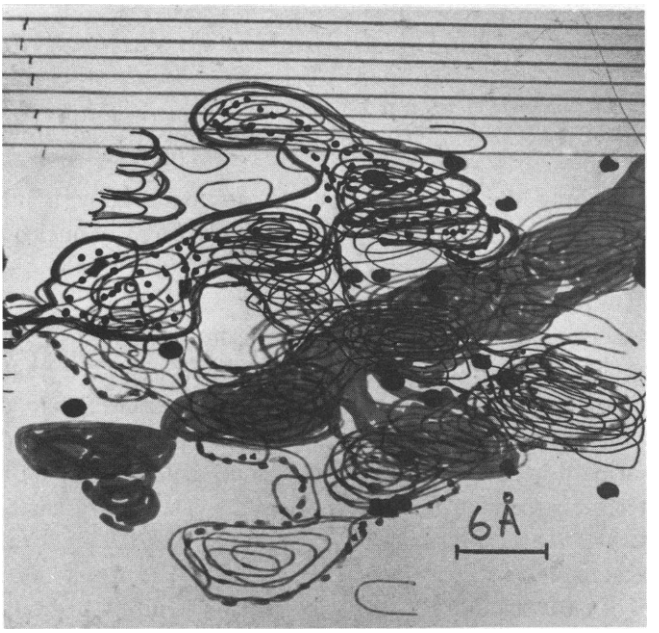

Figure 1 Form II melittin at g Å resolution, sectioned perpendicular to c and viewed 30° away from c from the direction of b. Two upper chains are visible, one solid and one stippled. These are related to each other by a noncrystallographic twofold axis. One of the two lower chains is outlined by dashes. Large dots indicate heavy atom positions.

interpretation, segments from two such rods run antiparallel to each other, and are related by the crystallographic twofold axis to the same segments of the other two chains. These four portions of polypeptide chain in the center of the molecule are in close contact with each other and are presumably composed of the nonpolar regions of the molecule (Fig. 1).

In detergents and nonpolar solvents, melittin is a monomer (e.g., reference 3). In going from tetramer to monomer, it seems likely that nonpolar side chains are exposed to the solvent. Thus the amphipathic properties of melittin may be governed in part by its tetrameric structure of 222 symmetry, and in part by the tetramer-monomer equilibrium.

The shape of the melittin molecule as displayed in the 6-Å electron density map of form II is consistent qualitatively with the unit cell dimensions of the form I crystals.

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NUCLEOSOME AND DNA-PROTEIN CONDENSED STRUCTURES IN SOLUTION FROM FLOW BIREFRINGENCE AND INTRINSIC VISCOSITY

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Highly sensitive streaming birefringence measurements combined with intrinsic viscosity are used to characterize the shape anisometry and optical anisotropy of nucleosomes over a range of salt concentration >30 mM KCl and of structures obtained by the condensation of high molecular weight DNA with polylysine.

These measurements appear useful for several reasons. (*a*) Both streaming birefringence and intrinsic viscosity are hydrodynamic properties based upon the rotational diffusion of macromolecular particles and hence are inherently more sensitive to details of particle anisometry than are hydrodynamic properties based upon translational diffusion. (*b*) An established body of both hydrodynamic and continuum dielectric optical theory is available with which to interpret streaming birefringence results. The theory has been tested experimentally in various ways, and appears to be adequate, at least for highly anisotropic systems such as DNA (Oriel and Schellman, 1966; Harrington, 1970). Extinction angles (i.e., mean orientation angles of particles in a velocity gradient) are entirely hydrodynamic properties, and hence can be interpreted through the rotational coefficient to characterize particle anisometry and to estimate absolute dimensions. The ratio of Maxwell coefficient to intrinsic viscosity is proportional to the absolute particle anisotropy. (*c*) The high optical anisotropy of DNA relative to that of associated protein permits certain details of tertiary structure and