THEORY OF POLYMORPHISM IN BACTERIAL FLAGELLA

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Summary Bacteria such as *Escherichia coil* use rotating flagella to swim. Each flagellum consists of a rotary motor, a flexible coupler known as the hook, and a helical filament. The filament undergoes mechanical phase transitions (e.g. from left-handed to right-handed) when subject to external torque. Incorporating the basic properties of the constituent protein subunits, we develop a new effective theory for the flagellar filaments and compute the phase diagram of ground states.

INTRODUCTION

Escherichia coli and *Salmonella typhimurium* have helical propellers called flagella. Each cell has several flagella, driven by rotary motors embedded at random points in the cell wall. A flagellum has three parts: a helical filament ten microns long and fifteen nanometers in diameter, a rotary motor which turns at 100 Hz, and a flexible “hook" joining the filament to the motor. In the absence of external forces, the wild-type filaments are left-handed. When the motors turn counter-clockwise (as viewed from outside the cell), the hydrodynamic forces cause the left-handed helices to bend and wrap around each other, forming a bundle which pushes the cell along. This kind of motion is called a run. In the absence of chemical gradients, the bacteria are observed to execute a series of runs, interspersed with irregular motions with little or no translational motion known as tumbles. The direction of the new run after a tumble is uncorrelated with the direction of the previous run; hence the bacterium traces out the path of a random walk. In the presence of a gradient of a desirable chemical such as sugar, the bacterium measures the local concentration and decreases its probability of tumbling for those runs which happen to lead to higher sugar concentrations. Thus, the bacterium executes a directed random walk, and drifts up the sugar gradient. This behavior is bacterial chemotaxis [1].

The mechanics of the flagellar filaments plays a crucial role in chemotaxis. The tumbles arise when the motors reverse direction from counterclockwise to clockwise. Only a subset of the filaments need to reverse rotation direction to cause a tumble. First, the filaments unwind from the bundle. Then the left-handed, clockwise-rotating filaments undergo a transformation to a right-handed state of the same helical radius but half the pitch. This transformation changes the swimming direction. While the filament is still turning clockwise, there is another transformation to a right-handed state with half the helical radius as the left-handed state. At this point the bacterium has slowed from its typical run speed. Finally, when the motors reverse, the helix reverts to the left-handed state and the cell speeds up to its typical run speed [2].

The changes in helical handedness in the course of chemotaxis, known as polymorphism, are driven by hydrodynamic forces. Polymorphism can also arise from mutations in the amino acid sequence of the protein making up the flagellar filament, and changes in ionic strength or pH. In this paper we report on a new theory for the polymorphism of bacterial flagella. Our theory is an effective, coarse-grained theory based on the properties of the protein subunits of the filament. We predict the phase diagram of ground states (states subject to zero external stress).

FILAMENT STRUCTURE

The flagellar filament is made of a single kind of protein subunit, known as flagellin. The flagellin subunits are organized in eleven protofilaments which are almost longitudinal but gently wind around the surface of the filament. There are two mutant filaments which are straight, one in which the protofilaments have a gentle left-handed winding, and the other in which the winding is right-handed. X-ray experiments have shown that the spacing between the subunits along the protofilament direction in the left-handed state is 5.27 nm, while the corresponding spacing for the right-handed state is 5.19 nm [3]. The helical states are believed to be the result of some of the protofilaments being in the long state, and some in the short state. Imagine a bimetallic strip consisting of two rods glued together with different coefficients of expansivity; heating the strip causes it to bend. If the strip is first twisted and then heated, it will form a helical shape. The shape of the flagellar filaments is thought to arise in an analogous way, with the short protofilaments on the inside of the helical filament, and the long ones on the outside.

PREVIOUS APPROACH

Calladine was the first to make a quantitative theory for polymorphism [4]. He modelled an element of the filament by two initially parallel rigid circular plates connected around their circumference by eleven linear springs. He supposed that some of the springs are short, and some are long, and further assumed that the plates are connected with a twist which increases in regular intervals as the number of short springs increases. By using force and moment balance to calculate

the relative tilt between the plates, he deduced that there were twelve possible states. Two of the states (all short springs or all long springs) correspond to the right- and left-handed straight mutants. The other states are helical, some left-handed, and some right-handed. Calladine's theory gives a simple geometrical explanation for the possible ground states states of the flagella. However, an extended or new theory is required to justify the assumption relating the twist to the number of short springs, to address how identical protein subunits can sometimes be modelled as short springs and sometimes as long springs, and to calculate when the filament will change handedness as a function of applied forces.

EFFECTIVE THEORY BASED ON FRUSTRATED BISTABLE SPRINGS

Our theory for polymorphism builds on the basic structure of Calladine's theory, but adds several essential new features. The elements still consist of rigid disks connected by eleven springs, but now the springs are identical, and each has a double-well potential for extension. These bistable springs represent the subunits and their bonds along the directions of the protofilaments. But there are also bonds between subunits on neighboring protofilaments. We model these bonds by a single spring connecting the centers of the two disks of the element. We introduce the feature of frustration by supposing that the length of the central spring in the absence of external force need not be the same as either of the two stable lengths of one of the bistable springs. One way to partially relieve the frustration is for the plates to spontaneously tilt relative to each other. If successive plates in the filament are attached with fixed twist, then the ground state will be a helix.

To calculate when a helix will form, we minimize the elastic energy. The energy consists of the sum of the eleven double-well potentials for the bistable springs and the potential for the central spring. Since twist resistance of the filament arises mostly from the resistance to stretching of the bonds between neighboring protofilaments (and not the almost longitudinal bonds along the protofilaments), we suppose the central spring also has a torsional resistance. Finally, to allow a change in handedness of the protofilament wrapping from right to left upon extension of the filament, we further suppose the central spring has a twist-stretch coupling. Thus, the potential of the central spring is like that of a true helical spring.

RESULTS AND CONCLUSIONS

To simplify the calculation, we choose a symmetric double-well potential. Although we expect the true potential will be asymmetric, the simplified potential will illustrate the qualitative features. Since the energy is nonlinear, there are more unknown parameters than in Calladine's linear model. Choosing particular fixed values for the nonlinear potential of the bistable springs, we minimize the energy as a function of the parameters of the central spring to find the phase diagram for helical and straight states. We find helical states when the rest length of the helical spring is close to the unstable length (the length corresponding the the maximum of the double-well potential) of a bistable spring. Otherwise, the filament is straight. The greatest contrast with Calladine's theory is that the nonlinear theory predicts a continuous family of ground states, rather then only twelve discrete states.

To conclude, our theory gives a completely self-contained and consistent model for the flagellar filaments, and shows how identical protein subunits can arrange to form a helical (instead of cylindrical) structure in the absence of external forces. Furthermore, it provides a framework for calculating the response of the filament to external forces and torques, such as the critical clockwise rotation speed for the polymorphic transformation from left-handed to right-handed. These experiments are currently underway in several groups, and will be crucial for evaluating the correctness of our theory.

References

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