Hypothesis

SELF-ELECTROPHORETIC LOCOMOTION IN MICROORGANISMS: BACTERIAL FLAGELLA AS GIANT IONOPHORES

Peter MITCHELL
Glynn Research Laboratories, Bodmin, Cornwall, England

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Introduction

Some years ago, it was suggested that certain micro-organisms might swim through aqueous media by a self-electrophoretic type of mechanism; and it was pointed out that the propulsive function of bacterial flagella might be attributable to a specialized role in the cyclic ion-conduction process of self-electrophoresis rather than to a mechanochemical lashing action [1]. This matter now appears to deserve further consideration because the protonmotive and associated ionophoretic functions of bacterial membranes have begun to be better understood [2, 3] and recent observations suggest that bacterial flagella have a microtubular structure [4–6] and are inserted through the plasma membrane by means of a specialized basal segment [7, 8]. The main object of this paper is therefore to draw attention to the possibility that bacterial flagella may be greatly elongated ionophores that specifically conduct H^+ ions or other cations between the outer medium and the cytoplasm, thus causing bacterial locomotion by an electrophoretically operated kind of hydrodynamic jet propulsion.

General principle and efficiency of self-electrophoretic locomotion

The general principles of self-electrophoretic locomotion are somewhat complex because the direction of streaming of the water over the surface of the organism, or over the surface of its specialized organs of locomotion, would depend not only on the ionophoretic potential gradient that was developed tangential to this surface, but also on the net displacement of ions in a normal direction from this surface into the neighbouring mobile aqueous medium, which gives rise to the so-called double electrical layer with its characteristic zeta (ζ) potential difference [9]. However, in spite of the complexities arising from the interplay between these two separate factors, the mechanism of self-electrophoretic locomotion can be easily explained in terms of simple analogies [1]. As the publication [1] in which this elementary explanation was originally given may not be readily accessible to many readers, I reproduce the relevant part of it, as follows.

"The surfaces of most living organisms are negatively charged, and in media of salinity within the normal range of survival of most micro-organisms, the movable water within a few Å of the surface carries a corresponding excess of positive ions. For this reason, when an electrical potential gradient is applied to a suspension of cells in a physiological medium the water close to the surface of the cells (containing the excess positive charge) streams towards the negative electrode and the cells move towards the positive electrode. If the two electrodes were attached to the opposite ends of an organism the water would still stream over its surface towards the negative end, and the organism would glide through the water positive end foremost .... The passage of an electric current in an aqueous solution implies the movement of ions. The mechanism that I am suggesting therefore amounts to the use of a stream of ions passing within the organism in one direction and over its surface in the other, much as a caterpillar track is used for the locomotion of a tank. The negative charge on the surface of the organism acts as a guide which keeps the train of positive ions near the surface of the cell, just as the track

of a tank must be guided in the appropriate position in order to move the vehicle along. The metabolism of the organism is supposed to provide the free-energy necessary to cause the secretion of positive ions at one end of the cell and their absorption at the other. It would, of course, be possible for the effective movement of the positive ions inside the cell to be replaced by the movement of electrons on some electron-conducting structure in the cell, or to be transferred to an appropriate position outside by means of such a structure, and it is tempting to suggest that certain types of flagella might play such a part; but this detail is irrelevant to the general formulation of the idea."

As discussed previously [1], assuming an electrophoretic mobility in the usual range for negatively charged biological materials (1 to 5 μm/sec per V/cm electric potential gradient), an electric potential gradient of 10 V/cm (or 1 mV/μm) would give the observed velocity of bacterial locomotion of some 10 to 50 μm/sec. At this electric potential gradient, the estimated electrical energy dissipation in the usual salt media indicates that only a small percentage of the available metabolic energy would be required to produce the observed velocities of bacterial locomotion by the self-electrophoretic type of mechanism [1].

Specific self-electrophoretic locomotion mechanisms

Since this general idea was originally formulated, it has been found that the redox chain system in the plasma membrane of certain bacteria acts as a source of proticity (the protonic analogue of electricity), which flows through the proton-conducting aqueous media on either side of the membrane [2, 3]. Thus, the proposed self-electrophoretic mechanism of locomotion could be simply achieved if the flagella were proton conductors with a negative surface charge, as illustrated in fig. 1, A and B. Diagram A illustrates the suggested circulation of H^+ as it would occur if the medium contained no acid–base buffers or cations other than H^+. According to this scheme, the outward translocation of H^+ across the plasma membrane is attributed to respiratory or ATPase activity [2, 3], the passage of H^+ back through the flagellum is attributed to H^+-specific facilitated diffusion through the central channel of the flagellum, and the locomotion of the

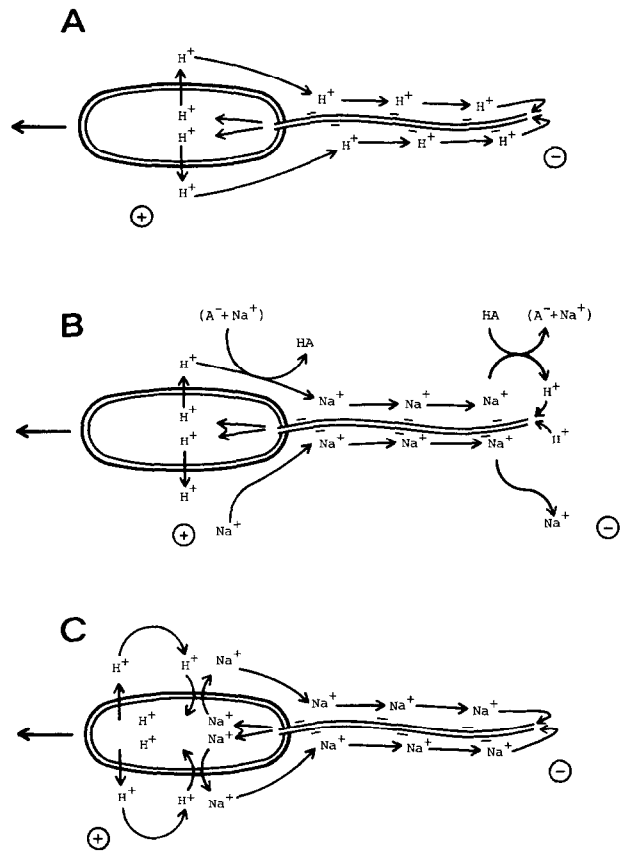

Fig. 1. Suggested mechanisms of self-electrophoretic bacterial locomotion. Protons are translocated outwards through the plasma membrane by the respiratory chain or ATPase system. The micro-tubular flagellum has a negative surface charge. In A and B, the flagellum is a specific H^+ conductor. In C, proton translocation is transformed to Na^+ translocation by an H^+/Na^+ antiporter system in the plasma membrane, and the flagellum is a specific Na^+ conductor. The organism is driven to the left by the stream of water pulled to the right over the flagellum by the H^+ or Na^+ ions moving down their electrochemical potential gradient to the right.

organism to the left is attributed to the net negative charge of the outer surface of the flagellum, which guides the H^+ ions over the flagellum and causes the water to stream to the right. In practice, owing to the relatively high concentration of acid–base buffers, most of the proton current in the usual media would not be carried by free H^+ ions (or H_3O^+ ions), and ion circulation would not be as simple as indicated in

diagram A of fig. 1. For example, if the predominant buffer acid and cationic species in the medium were AH and Na^+, respectively, the acid–base and ion-exchange reactions shown in the upper part of diagram B would occur. The simplified lower part of this diagram illustrates that the organism would move to the left because the electrical polarity and the ion-exchange processes initiated by the H^+ translocation would cause the secondary migration of Na^+ ions and the streaming of water over the flagellum to the right, as in case A. However, a loss of efficiency would occur inasmuch as Na^+ would tend to accumulate at the tip of the flagella because it is not directly withdrawn there.

Diagram B of fig. 1 suggests the interesting variation, shown in diagram C. In this case the proton current generated by respiratory or ATPase activity is supposed to be transformed into a Na^+ ion-current by a H^+/Na^+ antiporter in the membrane (see [2, 3]), and the central channel of the flagellum is supposed to facilitate the diffusion of Na^+ rather than of H^+. There are, of course, other analogous possibilities, and these include the possibility that the cation specificity of the central channel of the flagella and the specificity of the corresponding $H^+/cation^+$ antiporter system or systems might be comparatively broad. It is also conceivable that the specificity of cation diffusion back through the flagella might not be uniform over the length of the flagella, and the cation current (and thus motility) could, for example, be regulated by the specificity or velocity of cation diffusion in the basal or terminal segment of the flagella. The latter could play a part in the regulatory processes of bacterial chemotaxis which, as discussed recently by Kalckar [10], may involve a connection between specific substrate binding and the speed and/or net direction of bacterial locomotion. This might be particularly significant because there appears to be some relationship between the substrate-binding proteins and proton-linked substrate uptake [11].

The diagrams in fig. 1 are not intended to do more than illustrate the general principle of the proposed self-electrophoretic mechanism of locomotion. They show only a short terminal flagellum, which is not drawn to scale. A similar principle of locomotion would be applicable to bacteria with peritrichous or other multiple distributions of flagella, once the movement of the body of the organism caused the flagella to be orientated predominantly in one direction. As pointed out before [1], a similar principle of locomotion might also be applicable to certain microorganisms that lack flagella; but this aspect of self-electrophoresis is beyond the scope of the present brief paper.

Experimental implications of the self-electrophoretic locomotion hypothesis

It is useful to consider this hypothesis because it suggests certain new ways of exploring experimentally the structure–function relationships of bacterial flagella. The purified protein of bacterial flagella, known as flagellin [4–6], possesses no ATPase or other recognised enzyme activity. Therefore experimental approaches may be required that are different from those explored so successfully in the case of the actomyosin type of system* with its linear ATPase motor [12] – as indicated by the following suggestions.

The question of the possible micro-tubular structure of bacterial flagella [4–6] may be experimentally compared with the question of the ionophoretic properties of pore-like polypeptide antibiotics, such as gramicidin [14, 15]. The specific inhibitory effects of various synthetic and naturally occurring ion-conducting agents on bacterial motility [16–18] may possibly indicate an intimate involvement of the membrane system in the generation of the motive power of flagella, as Harold [3] has pointed out; and the present hypothesis may help to rationalise and encourage the further pursuit of this valuable experimental approach to the mechanism of bacterial motility.

The isoelectric point of flagellin in salt media is below pH 5 [19], and this agrees with the self-electrophoretic requirement that the surface of flagella must carry a net negative charge in the usual media in order to drive the bacteria body-foremost. It follows that treatments that would reverse the sign of net charge of the surface of the flagella, without otherwise damaging the bacteria, should reverse the direction of streaming of ions and of water over the surface of the

* Unless, perhaps, the basal segment of the helical flagellum were driven by a rotatory ATPase motor, like the screw of a ship [13].

flagella, with obvious consequences for bacterial locomotion.

Monotrichous bacteria orientated in the same direction in an appropriate system should cause the development of a metabolically-dependent electric potential gradient in the medium if, as suggested by the self-electrophoretic locomotion hypothesis, the flagella function as giant ionophores.

The experimental exploration of possibilities such as those suggested above should help to promote a better understanding of the mechanism of bacterial locomotion, even if only by definitely eliminating the hypothesis of locomotion discussed in this paper.

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