Fumarate or a Fumarate Metabolite Restores Switching Ability to Rotating Flagella of Bacterial Envelopes

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Flagella of cytoplasm-free envelopes of Escherichia coli or Salmonella typhimurium can rotate in either the counterclockwise or clockwise direction, but they never switch from one direction of rotation to another. Exogenous fumarate, in the intracellular presence of the chemotaxis protein CheY, restored switching ability to envelopes, with a concomitant increase in clockwise rotation. An increase in clockwise rotation was also observed after fumarate was added to partially lysed cells of E. coli, but the proportion of switching cells remained unchanged.

Bacterial flagella rotate by a motor located at the base of each flagellum (for reviews, see references 5, 8, 13, 14, and 21). The motor is driven by an inward current of protons (31; see references 5 and 16 and references cited therein for evidence that the proton motive force is the driving force of flagellar rotation). Although several models have been proposed for the way by which the rotation is coupled to the proton flux (see, for example, references 15, 17, 18, 25, and 26 and references cited therein), the molecular mechanism of the rotation is still obscure. Even less is known about the molecular mechanism of switching, i.e., the mechanism by which the switch at the base of the motor changes the direction of rotation of the flagella. Understanding of this mechanism is essential for an understanding of the process of chemotaxis, because the direction of flagellar rotation determines the mode of swimming (for recent reviews on chemotaxis, see references 6, 9, 21, and 35).

The default direction of rotation in bacteria such as Escherichia coli or Salmonella typhimurium is counterclockwise (CCW). This was shown both for intact bacteria genetically depleted of chemotaxis proteins ('"gutted bacteria" [38], i.e., bacteria in which chemotactic signaling cannot occur [12, 27, 38]) and for cytoplasm-free envelopes having functional flagella (10, 30). Clockwise (CW) rotation can be restored to gutted bacteria by the intracellular, plasmid-mediated of the chemotaxis protein CheY (7, 34, 38) and to envelopes by the inclusion of CheY, either purified (11, 32) or crude (2). This indicated that CheY interacts with the switch, as had already been implied by second-site suppression analysis (28, 39), and that the interaction is direct (32). One of the major behavioral differences between CheY-containing gutted bacteria and envelopes is that the former display reversals whereas the latter never switch from one direction of rotation to another. The fact that the intracellular composition of chemotaxis proteins is similar in both preparations suggested to us that a cytoplasmic constituent, other than a chemotaxis protein, must be required for switching. Here we report that this constituent may be fumarate or a metabolite of fumarate.

Fumarate was found to restore to a straight-swimming mutant of Halobacterium halobium the ability to reverse direction (23). We examined whether it can restore switching ability to envelopes. An S. typhimurium strain, ST1 (1), wild type for chemotaxis, was grown at 35°C in nutrient broth as described previously (10). Cell envelopes were isolated from this strain by penicillin treatment and subsequent osmotic lysis according to a procedure described previously (30), except that the lysis medium and the flow medium contained 50 mM Tris-HCl (pH 7.9), 5 mM MgSO₄, and 0.1 mM tetraethylpentamine (Tetren). CheY was overproduced by the plasmid pRL22(ΔPvuII), received from P. Matsumura, and purified essentially as described by Matsumura et al. (24) with the modifications described by Barak and Eisenbach (2). The effect of the exogenous addition of fumarate on the rotation of tethered envelopes was dramatic (Fig. 1): there was a shift from a situation in which neither the CCW-rotating nor the CW-rotating envelopes made reversals to a situation in which two-thirds of the CW-rotating envelopes were able to switch (third group of columns). Concomitant with the appearance of switching ability, the proportion of CW-rotating envelopes increased threefold. The intracellular presence of CheY was obligatory for these effects.

If fumarate or a metabolite of fumarate is indeed the cytoplasmic factor required for switching (called hereafter a "switch factor"), there should be a difference between its effect on cytoplasm-free envelopes and its effect on cytoplasm-containing cells. Its exogenous addition to cytoplasm-containing cells should not affect switching, because the switch factor is presumably already present. To test this prediction, we examined the effect of fumarate on semi-envelopes, which are considered to be the cytoplasm-containing cells most similar to envelopes (2). Semi-envelopes are partially lysed cells, prepared from RP1091 (a mutant strain lacking the cytoplasmic chemotaxis proteins because of a deletion of the genes from cheA to cheZ [27]). They are obtained along with envelopes during the regular procedure for isolation of the latter and contain, in addition to the inserted substances, various amounts of the original cytoplasmic constituents (2). Like their precursor bacterial cells, RP1091 semi-envelopes rotate their flagella only in the default direction of rotation, CCW, because of the absence of chemotaxis proteins. However, when they contain inserted CheY, the flagella rotate CW (2) and make reversals (Fig. 2). The mere fact that the insertion of CheY alone to gutted semi-envelopes causes them to switch (Fig. 2) is already indicative that the switch factor is cytoplasmic and is not one of the known cytoplasmic chemotaxis proteins. The addition of fumarate to these semi-envelopes invariably increased the
number of semi-envelopes which could rotate CW up to three- or fourfold, depending on the batch (and with large variability from batch to batch), but, as predicted, neither the fraction of CW semi-envelopes that could switch nor the average reversal frequency significantly changed after the addition of fumarate (Fig. 2). This supports the possibility that fumarate (or a metabolite of fumarate) is indeed a switch factor.

Several factors have been recently found to increase CW rotation in envelopes and semi-envelopes: CheY phosphorylation was found to increase the ability of the protein to cause CW rotation in semi-envelopes by two orders of magnitude (2), and intracellular acetyl adenylate (in the presence of CheY, possibly by way of CheY acetylation) was found to have a similar effect in envelopes (3) as well as in intact bacteria (37). However, neither CheY phosphorylation nor acetyl adenylate had any significant effect on switching. Thus, an increase in CW rotation per se is not sufficient for an effect on switching. Therefore, the effect of fumarate on switching is apparently not the consequence of its effect on CW rotation. It remains to be seen how all these mechanisms, including the mechanism of fumarate function, integrate into a common regulatory mechanism of flagellar rotation.

Here we provided evidence that in E. coli and in S. typhimurium, as in H. halobium, fumarate or a metabolite of fumarate may be a switch factor. The mechanism of function of fumarate is not yet known, but this study suggests that no cytoplasmic constituents (including other cytoplasmic proteins or ATP) other than CheY are essential for its function. Being a natural electron acceptor of the respiratory system (19, 20), fumarate may act as an oxidizing agent or by another mechanism; therefore, it may also serve as the connection point between the metabolic (or energetic) state of the bacteria and the chemotaxis system. Fumarate is commonly present both in H. halobium (23) and in E. coli (36). A halobacterial cell contains about 20,000 molecules of fumarate, most of which are bound to the membrane (23). The intracellular level of fumarate in H. halobium increases upon stimulation by light, possibly as a result of fumarate release from the presumptive membrane-binding protein, with a concomitant increase in the number of reversing halobacterial cells (22). The specificity and the molecular mechanism of the fumarate effect in E. coli or S. typhimurium are yet to be studied.

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