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Methyl Transfer in Chemotaxis toward Sugars by *Bacillus subtilis*

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Like amino acids, the sugars glucose and the nonmetabolizable 2-deoxyglucose caused a turnover of methyl groups on the methyl-accepting chemotaxis proteins. These sugars also caused methanol formation on addition. Thus, in contrast to chemotaxis in *Escherichia coli*, taxis to phosphotransferase sugars by *Bacillus subtilis* utilizes the methyl-accepting chemotaxis proteins.

Chemotaxis is the process by which cells travel toward higher concentrations of attractant and lower concentrations of repellent. This process involves cells recognizing changes in their environment and altering their behavior accordingly. Our laboratory is concerned with elucidating the mechanism of chemotaxis in the gram-positive bacterium *Bacillus subtilis*. We have found that despite functional homology between the methyl-accepting chemotactic proteins (MCPs) of *B. subtilis* and *Escherichia coli* (3, 5, 12, 14), there exist many striking differences in the mechanisms (4, 21–23).

In both species, addition of attractant (or removal of repellent) causes counterclockwise flagellar rotation, resulting in smooth swimming. Both species have the ability to adapt to this new ambient concentration of attractant and after a time return to their prestimulus random-walk pattern of smooth swims alternating with tumbles (clockwise flagellar rotation). Addition of repellent (or removal of attractant) conversely causes a period of tumbling, with suppression of any smooth swimming.

It has been observed that in *B. subtilis*, addition of the amino acid aspartate causes an immediate change in the distribution of methyl groups on the three MCP species (21). This methylation pattern is thereafter maintained. No change in the overall extent of methylation is observed. In addition, there is an observed increase in turnover of methyl groups on the MCPs (21). This turnover appears to persist throughout the period of swimming (i.e., the adaptation period). There is also a high rate of methanol generation during the adaptation period, but the methanol is not a direct product of demethylation of the MCPs (21).

There is another fundamental difference between the MCPs of *B. subtilis* and those of *E. coli*. Those in *E. coli* are dedicated to responding to particular attractants or repellents; those in *B. subtilis* do not appear to be. Thus, for instance, Tsr binds serine to mediate its taxis, and Tar binds aspartate to mediate its taxis (19). However, when any amino acid is added to a suspension of *B. subtilis*, a turnover of methyl groups on all MCPs occurs (M. S. Thoelke, J. M. Casper, and G. Ordal, J. Biol. Chem., in press).

In *E. coli*, sugars transported by the phosphotransferase system (PTS) use that system for chemotaxis (1, 13). It is presumed that the chemotactic signal that ensues as the result of arrival of a PTS sugar at the PTS transporter is related to the cascade of phosphoryl transfers that lead to the phosphorylation of the sugar being transported (17). Mutants in MCPs are unimpaired for taxis to glucose and *N*-acetylglucosamine, both PTS substrates (13). The only instance in which a PTS sugar can affect MCP methylation is by binding as an analog of galactose to the galactose-binding protein (7, 9). However, in a way this is nonphysiological, since growth on glucose represses this protein (2). The galactose-binding protein is located in the periplasmic space, which does not exist in *B. subtilis*, so that there is nothing analogous in *B. subtilis*.

Glucose is also transported by the PTS system in *B. subtilis* (18). Not just glucose but many sugars are transported by the PTS system (18), and many are also attractants for *B. subtilis* (16). However, the behavioral response to sugar addition is weak compared with amino acid addition.

Aspartate can cause up to 50-s counterclockwise rotation at saturating concentrations (15). Glucose at 3.2×10^{-3} (10 times the peak concentration as determined by capillary assay [13]) caused approximately 5-s counterclockwise rotation on tethered cells (D. O. Nettleton and G. W. Ordal, unpublished data). Mannitol produced similar times yet elicited excellent chemotaxis on swarm plates (22).

In view of the rather profound differences between chemotaxis in *B. subtilis* and *E. coli*, we wanted to know whether methylation was involved in sugar taxis in *B. subtilis*. Previously, we had determined that mutant strain OI1100, which is unable to methylate MCPs, showed only 0.6% normal chemotaxis to mannitol, as measured in capillary assays (22). As mentioned above, mutants of *E. coli* lacking MCPs show normal taxis to PTS sugars (13).

Effect of sugar on methanol production. We tested the sugar glucose for the ability to induce methanol formation in the wild-type strain (OI1085). Cells were grown in L broth, suspended in chemotaxis buffer (21), and labeled with [*methyl*-^{3}H]methionine (30 μCi/ml) for 10 min at 37°C as described by Thoelke et al. (20). They were placed on a microfilter unit in an apparatus, and buffer containing 10 μM nonradioactive methionine was pumped past the cells and collected in fractions (8, 20). The [^{3}H]methanol was subsequently quantitated (20). Upon exposure to glucose, there was an immediate increase in the rate of methanol formation (Fig. 1a). The increase was much less than the amount induced by amino acids (approximately 2% of the [^{3}H]methanol released upon addition of 0.1 M aspartate in the same assay) (data not shown) but was readily discernible. Upon removal of glucose, no change in the rate was detected.

We also tested the effect of a nonmetabolizable substrate, 2-deoxyglucose. Once again, an increase in methanol production was seen upon addition to the sugar (Fig. 1b). This

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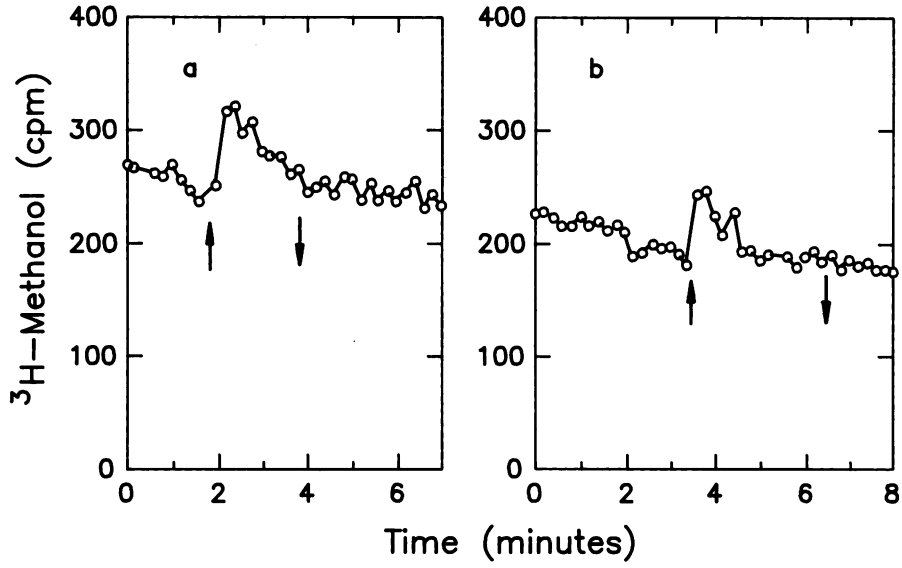

FIG. 1. Flow assay of effect of sugar on methanol production. At the upward arrows, the tubing was transferred to buffer containing sugar solution; at the downward arrows, it was transferred back to buffer lacking sugar. The sugar was glucose (6.4 mM) (a) or 2-deoxyglucose (23 mM) (b).

increase lasted only a short time. Again, no response was seen upon removal of the stimulus.

Effect of sugar on the MCPs. To visualize methylation of MCPs, cells were grown in L broth, suspended in protoplast buffer with lysozyme (1 mg/ml) for 30 min, and then incubated with 10 μM [*methyl*-^{3}H]methionine (10 μCi/ml) for 7 min. Glucose or buffer was then added for 1 min, and the preparation was quick frozen. The samples were solubilized and fractionated by sodium dodecyl sulfate-polyacrylamide (10%) gels (10), and the gels were dried and fluorographed (8, 11). Glucose had no effect on MCP methylation (Fig. 2). There was no difference in the extent of radiolabeling and no alteration in the pattern of the MCPs seen on the fluorogram compared with results for the buffer control.

We next investigated the effect of sugars on the flux of methyl groups on the MCPs. If cells are incubated in [*methyl*-^{3}H]methionine for only 15 s before 5 s of effector exposure, one is able to gain a measure of the rate of replacement of methyl groups on the MCPs (Thoelke et al., in press). In this time course, "hot" groups are replacing "cold" groups, and faster turnover will show up on fluorograms as an increase in label on the MCPs. It was found that, like the amino acids, glucose caused an increased rate of turnover, resulting in increased radiolabeling of the MCPs (Fig. 3). The percent increases over controls for each MCP were 50 (H1a), 41 (H1b), 56 (H2), and 42 (H3). These amounts indicate that turnover occurred fairly equally at all of the transducer proteins. This turnover was not apparent in the conditions of Fig. 2 because the cells had been labeled longer and the labeling had begun to reach a maximum. The turnover would have resulted largely in hot groups replacing other hot groups, not detectable on the fluorogram.

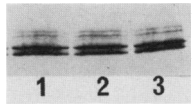

FIG. 2. Fluorogram of effect of sugar on MCP methylation. Lanes: 1, buffer control; 2, glucose (0.32 mM); 3, glucose (3.2 mM).

In this note, we have shown that, like amino acids, the sugars glucose and 2-deoxyglucose cause a flux of methyl groups through the MCPs. Thus, basically there may be a similar underlying mechanism by which chemotaxis toward sugars is carried out. It is not known whether 2-deoxyglucose is transported by a PTS system. Its chemotaxis is fourfold inducible rather than constitutive as is that of glucose, and thus 2-deoxyglucose probably is not just an analog of glucose (16). However, there are some interesting differences between taxis toward these sugars and taxis toward aspartate. First, aspartate causes a change in distribution of methyl groups among the MCPs (6, 21), and these sugars do not. Second, when this change is taken into account, the amount of methyl group turnover on the upper MCP, H1, is small and that on the MCPs H2 and H3 is large (Thoelke et al., in press). For these sugars, the methyl group turnover is fairly uniform on all MCPs. Third, aspartate causes methanol production both when added and when removed (20a). These sugars cause only a small production of methanol when added and none when removed. Generally, for attractants the amount of methanol produced on addition of attractant is larger than that generated on removal, and it is possible that the lack of an apparent removal response for these sugars is due to insensitivity of the assay.

In summary, taxis to glucose and 2-deoxyglucose resem-

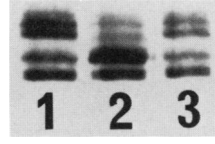

FIG. 3. Effect of sugar on MCP methyl group flux. Cells were grown and washed as for Fig. 1 and prepared as protoplasts as for Fig. 2. While incubating at 25°C, cells were labeled with [*methyl*-^{3}H] methionine (10 μCi/ml) for 15 s before addition of effector. After 5 s of exposure, cells were quick frozen in dry ice-acetone. Samples were prepared for fluorography as for Fig. 2. Effectors: Lane 1, glucose (0.32 mM); lane 2, aspartate (0.25 mM); lane 3, buffer control.

bles taxis to amino acids in causing a turnover of methyl groups on all MCPs and methanol release. The turnover on each MCP is about the same, whereas the turnover caused by amino acids is much less uniform. Perhaps because of the weak signal, no methanol is released on removal of the sugars. Taxis to PTS sugars in *E. coli*, by contrast, does not involve the MCPs. The MCPs can be missing and taxis is unaffected, and PTS sugars seldom affect MCP methylation (13).

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