Regulation of *Bacillus subtilis* Macrofiber Twist Development by D-Cycloserine

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The effect of D-cycloserine on the establishment of twist states in *Bacillus subtilis* macrofibers was examined. Macrofibers produced in the presence of the drug differed in twist compared with those produced in its absence. The degree of twist alteration was dependent on the concentration of D-cycloserine in the growth medium. Macrofibers of different twist states representative of the entire twist spectrum from tight left-handedness to tight right-handedness were produced in strains FJ7 and C6D in four different ways: by control of the concentration of L-alanine, magnesium sulfate, or ammonium sulfate in the growth medium or by control of the growth temperature. The structures so produced were used to determine the effect of D-cycloserine on twist establishment starting from different twist states throughout the twist spectrum. In all but one case, twist resulting from growth in the presence of D-cycloserine was further towards the left-hand end of the twist spectrum than that produced in its absence, the exception being the unusual left-handed twist states produced in strain C6D and the closely related RHX 11S at high L-alanine concentrations described here. Studies of the interaction between D-cycloserine and D-alanine both used alone and used independently with the other twist-modifying systems (temperature, magnesium sulfate, and ammonium sulfate) revealed that changes in twist resulting from D-cycloserine were always in the opposite direction from those resulting from D-alanine. This antagonism suggests that the biochemical mechanism of twist regulation involves the metabolism of peptidoglycan, particularly reactions involving D-alanine or the dipeptide D-alanyl-D-alanine. The possibility that peptidoglycan cross-linking is involved is discussed.

During the growth of *Bacillus subtilis* macrofibers, processes occur that result in the production of a particular twist state by all the fibers in the culture. This state represents one of many possible states, ranging from tight left-handedness to tight right-handedness, that can be achieved by a single macrofiber-producing strain. Biomechanical studies of macrofiber structure and growth dynamics previously showed that all of the cells growing in a single macrofiber assemble their cell surfaces according to the same geometrical plan (6, 8, 10), and thus the production of a particular macrofiber twist state corresponds to the assembly of cell surfaces in a particular conformational state. The factors that govern the determination of macrofiber twist state must therefore be concerned at a molecular level with the regulation of cell wall assembly in individual cells.

The facts that very low levels of penicillin G block the production of macrofibers but not cell growth, that lysozyme digestion of live macrofibers leads to changes in twist state termed relaxation motions prior to breakdown of the fibers and liberation of spheroplasts, and that production of macrofibers in the presence of D-alanine results in different twist states from those grown in its absence all suggested that peptidoglycan is involved in the determination and maintenance of macrofiber twist states (5, 14, 18). This contention is supported by results reported here indicating that D-cycloserine, a specific inhibitor of D-alanine metabolism and consequently of peptidoglycan synthesis, at concentrations below those resulting in arrest of growth, affects the development of macrofiber twist states (12). Specifically, changes in macrofiber twist produced by D-cycloserine were always in the opposite direction from those produced by D-alanine. In all but one case, the direction of twist modification resulting from growth in D-cycloserine was towards the left-hand end of the twist spectrum.

In the course of studying the interactions between D-cycloserine and other systems of twist regulation, including D-alanine, new information was also obtained about the D-alanine system. In strain C6D, two helix hand inversions were found to arise as a result of increasing concentrations of D-alanine. Based on these observations, a convention has been adopted to describe the entire twist spectrum that satisfies all the information currently available on twist regulation in all strains. The interactions between different systems of twist modification reported here suggest that a single biochemical target concerned with peptidoglycan assembly may be involved in the establishment of twist states.

**MATERIALS AND METHODS**

**Strains.** The macrofiber-producing *B. subtilis* 168 strains used in this investigation are shown in Table 1. Information given for each strain for the response patterns to D-alanine and D-cycloserine are discussed in the Results section. Media and growth conditions. The complex medium TB consisted of (in grams per liter of water): Bacto tryptose (Difco Laboratories), 10; Bacto beef extract (Difco), 3; NaCl, 5. The medium was supplemented with uracil (20 \( \mu \text{g/ml} \), final concentration) and thymine (20 \( \mu \text{g/ml} \), final concentration). The medium referred to as T consisted of 10 g of Bacto tryptose (Difco) per liter of H\(_2\)O (9). Standard protocols were followed for production of macrofibers (7). Effects of twist-modulating compounds on the development of twist were assessed by comparison of the qualitative static twist of structures produced in six-well tissue culture plates. Each well contained 5 ml of growth medium plus a different volume of a highly concentrated stock of the compound being tested for twist-modulating activity. The range of concentrations present in each six-well plate generally spanned from that without effect on the development of twist
TABLE 1. Properties of *Bacillus subtilis* macrofiber-producing strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>d-Ala Response</th>
<th>d-Cyclo Response</th>
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<tbody>
<tr>
<td>C6D</td>
<td>pyrA metB divIV-B1 fha fibB</td>
<td>L-R-L&lt;sub&gt;m&lt;/sub&gt; TB</td>
<td>R-L TB, 2 x 10&lt;sup&gt;-3&lt;/sup&gt; M d-ala + 2 x 10&lt;sup&gt;-4&lt;/sup&gt; M d-cyclo</td>
</tr>
<tr>
<td>RHX 11S</td>
<td>pyrA metB divIV-B1 fha fibB</td>
<td>L-R-L&lt;sub&gt;m&lt;/sub&gt; TB</td>
<td>R-L TB, 2 x 10&lt;sup&gt;-3&lt;/sup&gt; M d-ala + 2 x 10&lt;sup&gt;-4&lt;/sup&gt; M d-cyclo</td>
</tr>
<tr>
<td>2C8</td>
<td>dal-1 lyt-2 aroI906</td>
<td>L-R-L&lt;sub&gt;m&lt;/sub&gt; E TB</td>
<td>R-L TB, 2 x 10&lt;sup&gt;-3&lt;/sup&gt; M d-ala + 2 x 10&lt;sup&gt;-4&lt;/sup&gt; M d-cyclo</td>
</tr>
<tr>
<td>635B</td>
<td>pyrA metB divIV-B1 fha fibB</td>
<td>L-R-L&lt;sub&gt;m&lt;/sub&gt; E TB</td>
<td>R-L TB, 2 x 10&lt;sup&gt;-3&lt;/sup&gt; M d-ala + 2 x 10&lt;sup&gt;-4&lt;/sup&gt; M d-cyclo</td>
</tr>
<tr>
<td>FJ7</td>
<td>metC3 lyt-2</td>
<td>L-R-L&lt;sub&gt;m&lt;/sub&gt; E TB</td>
<td>R-L TB, 2 x 10&lt;sup&gt;-3&lt;/sup&gt; M d-ala + 2 x 10&lt;sup&gt;-4&lt;/sup&gt; M d-cyclo</td>
</tr>
<tr>
<td>A734</td>
<td>purB6 lyt-2</td>
<td>L-R-L&lt;sub&gt;m&lt;/sub&gt; E TB</td>
<td>R-L TB, 2 x 10&lt;sup&gt;-3&lt;/sup&gt; M d-ala + 2 x 10&lt;sup&gt;-4&lt;/sup&gt; M d-cyclo</td>
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<td>1913</td>
<td>pro leuA8 hisB Tn917 cry lacZ&lt;sup&gt;*&lt;/sup&gt;</td>
<td>L-R-L&lt;sub&gt;m&lt;/sub&gt; E TB</td>
<td>R-L TB, 2 x 10&lt;sup&gt;-3&lt;/sup&gt; M d-ala + 2 x 10&lt;sup&gt;-4&lt;/sup&gt; M d-cyclo</td>
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<tr>
<td>84A</td>
<td>metC3 lyt-2</td>
<td>L-R-L&lt;sub&gt;m&lt;/sub&gt; E TB</td>
<td>R-L TB, 2 x 10&lt;sup&gt;-3&lt;/sup&gt; M d-ala + 2 x 10&lt;sup&gt;-4&lt;/sup&gt; M d-cyclo</td>
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<td>L-R-L&lt;sub&gt;m&lt;/sub&gt; E TB</td>
<td>R-L TB, 2 x 10&lt;sup&gt;-3&lt;/sup&gt; M d-ala + 2 x 10&lt;sup&gt;-4&lt;/sup&gt; M d-cyclo</td>
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<td>R-L TB, 2 x 10&lt;sup&gt;-3&lt;/sup&gt; M d-ala + 2 x 10&lt;sup&gt;-4&lt;/sup&gt; M d-cyclo</td>
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<tr>
<td>PS5</td>
<td>metC3 lyt-2</td>
<td>L-R-L&lt;sub&gt;m&lt;/sub&gt; E TB</td>
<td>R-L TB, 2 x 10&lt;sup&gt;-3&lt;/sup&gt; M d-ala + 2 x 10&lt;sup&gt;-4&lt;/sup&gt; M d-cyclo</td>
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*<sup>*</sup>The temperature was 20°C in all cases. Abbreviations: L, left-handed; R, right-handed; L<sub>m</sub>, upper left-handed; E, coiled individual filaments; R<sub>m</sub>, tight right-handed; L<sub>r</sub>, very tight right-handed; L<sub>r</sub>, left-handed; 0, no twist; d-ala, d-alanine; d-cyclo, d-cycloserine; TB, a complex medium (see Materials and Methods); T, a tryptone medium.

RESULTS

**Influence of d-cycloserine on establishment of macrofiber twist.** The presence of d-cycloserine during the production of macrofibers of many different strains resulted in structures with different twist from those produced by the same strain in the absence of the drug (Table 1). Two response patterns were found. In the majority of cases, structures that arose in the presence of d-cycloserine were further towards the left of the twist spectrum than those produced in its absence. In two cases the opposite was true. Strains C6D and RHX 11S, when grown in the presence of high concentrations of d-alanine plus d-cycloserine, produced macrofibers further towards the right of the twist spectrum than those produced when only a high concentration of d-alanine was present. To understand this unusual response, it is necessary first to examine the way in which these strains respond to high concentrations of d-alanine, for unlike other strains studied previously, C6D and RHX 11S can produce left-handed structures under two very different conditions, as shown below. A summary of the d-alanine response patterns is shown in Table 1.

**Influence of d-alanine on the establishment of twist in C6D macrofibers.** In previous studies it was shown that a change in twist towards the right-hand end of the twist spectrum resulted from the addition of d-alanine to the growth medium and that the magnitude of the change was proportional to the concentration of d-alanine (14). This relationship was known to hold for many macrofiber-producing strains, including C6D, a strain that grows as left-handed structures at 20°C in TB medium, in contrast to most strains, which produce right-handed structures under these conditions. When C6D macrofibers were cultured in higher concentrations of d-alanine than previously used, instead of the expected right-handed structures they were found to produce very tight left-handed macrofibers. This observation led to the findings shown in Fig. 1. Change of twist in the rightward direction occurred only over a particular range of d-alanine concentrations, between 0 and about 15 mM. Concentrations of d-alanine above 15 mM resulted in a progressive leftward shift in twist. The two transitions corresponding to the concentrations at which 0 twist arose were found to be at about 2 and 23 mM. Below 2 and above 23 mM d-alanine,
left-handed macrofibers were produced. For purposes of discussion, the two left-handed forms will be distinguished by referring to those produced at below 2 mM D-alanine as left (L), whereas those produced at above 23 mM D-alanine will be designated upper-left (L\_u).

In contrast to D-alanine, neither \( L \)-alanine (Fig. 1) nor the dipeptide \( D \)-alanyl-\( D \)-alanine exhibited any twist modulation activity when present during growth of \( \text{C6D} \) macrofibers. The latter was examined over the range of 8.7 to 175 mM. It is not certain, however, whether the dipeptide can be transported into \( B \). \textit{subtilis}. A temperature-conditional \( D \)-alanine ligase mutant was not able to grow at the restrictive temperature in the presence of \( D \)-alanyl-\( D \)-alanine, suggesting that it cannot be taken up or used if transported at all (1).

The \( D \)-alanine response pattern of strain \( \text{C6D} \), consisting of transitions from left to right to upper-left (L \_R \_L \_u), differs from that of most other strains studied previously (Table 1). A second transition to upper-left-handedness has been found thus far only in two additional strains, RHX 11S, closely related to \( \text{C6D} \), and 2C8, a strain that requires \( D \)-alanine for growth. In most other strains, the response to concentrations of \( D \)-alanine above that resulting in maximum right-handedness consisted of the production of highly coiled helical filaments (Fig. 2) that resemble the structures described by Tilby (15).

**Influence of \( D \)-cycloserine on the establishment of macrofiber twist.** Macrofiber twist states produced in the presence of various concentrations of \( D \)-cycloserine were examined with three kinds of strains: (i) those that undergo two transitions (L \_R \_L \_u) as a function of \( D \)-alanine concentration (\( \text{C6D} \)), (ii) those that can achieve the full twist spectrum as a function of temperature (\( \text{F7} \) and \( \text{A734} \)), and (iii) mutants of \( \text{F7} \) that are restricted to only a single helix hand at all temperatures (right-handed strains \( \text{S4A} \) and \( \text{S10A} \) and left-handed strains \( \text{PS5} \) and \( \text{PS6uB} \)). The results obtained with strain \( \text{C6D} \) are shown in Fig. 3. Left- and right-handed twist states of \( \text{C6D} \) were produced both by temperature and by the concentration of \( D \)-alanine in the growth medium. Right-handed forms were converted to left-handedness by \( D \)-cycloserine. Left-handed structures produced at 48°C (or 20°C; data not shown) did not respond to \( D \)-cycloserine by change of twist. In contrast, upper-left-handed structures were converted to right-handedness by the drug, as indicated by the two examples in Fig. 3. Not all of the curves shown extended over the full concentration range of \( D \)-cycloserine used because macrofibers of different twist (or culture conditions associated with their production) resulted in different sensitivity to growth inhibition by the drug. When less than two mass doublings of the input inoculum took place in the presence of \( D \)-cycloserine, the resulting structures were considered inadequate for the determination of twist.

The pattern of response to \( D \)-cycloserine observed in strains \( \text{F7} \) and \( \text{A734} \) was similar to that found in strain \( \text{C6D} \). In the case of \( \text{F7} \), left- and right-handed structures of different degrees of tightness were produced by growth in different media (TB and T) and by addition of either magnesium or ammonium sulfate to the growth medium. Right-handed forms became left-handed when grown in subinhibitory concentrations of the drug, whereas left-handed forms did not change twist states at any concentration of \( D \)-cycloserine examined up to that which caused growth inhibition (Fig. 4). The kind of response to \( D \)-cycloserine observed in strain \( \text{A734} \) were very similar to those found with strain \( \text{F7} \) grown in the presence of magnesium. \( \text{A734} \) is a derivative of \( \text{F7} \) that produces tight right-handed structures in the absence of magnesium. The twist state of \( \text{F7} \) can be adjusted to that of \( \text{A734} \) by addition of magnesium ions, as shown in Fig. 4 (in the absence of \( D \)-cycloserine). Such fibers appear to be equivalent to those of \( \text{A734} \) in terms of the degree to which they change twist as a function of the concentration of \( D \)-cycloserine in the growth medium.

The leftward influence on twist production by \( D \)-cycloserine was also found when mutants restricted to either left- or right-handedness were examined. The two left-handed strains studied, \( \text{PS5} \) and \( \text{PS6uB} \), failed to show any twist modification in response to the drug (Fig. 5). Both right-handed strains were shifted towards the left when grown in \( D \)-cycloserine, but neither was able to achieve left-handedness prior to growth inhibition (Fig. 5). In all of the cultures examined, except as noted in the figure legends, the growth temperature was 20°C and the growth medium used was TB.

**Interactions between \( D \)-cycloserine and other systems of twist modification.** The relationship between the \( D \)-cycloserine influence on twist development and other systems that affect the establishment of twist is shown for strains \( \text{C6D} \) and \( \text{F7} \) in Fig. 6. The conventions adopted were based on the results obtained with strain \( \text{C6D} \). This strain undergoes two transitions (from left- to right- to left-handedness) as a function of either \( D \)-alanine concentration (Fig. 1) or temperature (9). In both cases the changes in twist that spanned the second transition (from right- to left-handedness) involved a decrease from maximum right-handedness to zero twist and then increasing left-handed twist until a maximum was achieved. These findings may be conveniently illustrated if the twist spectrum is depicted as a circle with maximum left-handedness and right-handedness opposed 180° and two regions of zero twist located 90° from each maximum (see the legend to Fig. 6). Concentric circles surrounding the twist spectrum are used to represent the effects of each twist modulator. In Fig. 6a these correspond to the effects of \( D \)-alanine, \( D \)-cycloserine, and temperature, respectively. Starting with maximum left-handed \( \text{C6D} \) macrofibers, increasing concentrations of \( D \)-alanine caused a reduction in

![ALANINE CONCENTRATION (mM)](image)

**FIG. 1.** Effect of alanine on the establishment of \( B \). \textit{subtilis} macrofiber twist. \( \text{C6D} \) macrofibers were cultured in TB medium at 20°C in the standard manner. \( D \)- or \( L \)-alanine was added at the concentrations indicated. Twist state values were assigned as described in the text.
FIG. 2. Phase-contrast micrographs of highly coiled structures produced by growth in a high concentration of D-alanine (A) and of a normal macrofiber for comparison (B). (A) F37 cultured at 20°C in T medium containing 30 mM D-alanine. Bar, 50 μm. (B) A734 macrofiber cultured at 20°C in TB medium. Bar, 200 μm.
twist and then eventually right-handedness. The direction taken around the circle could be either clockwise or counterclockwise. Of the two routes, the clockwise route has been adopted by convention. All other directions shown are based on interaction studies and this convention. Circular representation of the twist spectrum should not be interpreted as indicating that continuously increasing concentrations of twist modulators can drive changes of twist repeatedly around the circle without limit. It is meant rather to indicate only the direction of change from any given starting point on the spectrum brought about by increasing concentrations of a twist-modulating factor. There are of course limits to the degree of change possible with each twist-modulating factor.

The connecting arrows shown in Fig. 6 represent the conditions used for examination of interactions between two systems of twist modulation. The arrowheads lie on a circle corresponding to the modulator that was used to influence the twist of macrofibers initially produced by the system from which the connecting arrow originates. For example, C6D structures produced at 20°C were close to maximum left-handedness. When D-alanine was added, twist changed in the direction indicated by the D-alanine circle pointed to by the connecting arrowhead. Figure 6a indicates that all of the twist modulation effects observed, including the change from upper-left-handedness to right-handedness, produced by D-cycloserine (Fig. 3) followed a single pattern in which the influence of D-alanine and that of D-cycloserine were always in opposite directions.

The conventions and format adopted in Fig. 6a have been applied to the data obtained with strain FJ7, as shown in Fig. 6b. In this case, the magnesium and ammonium ion systems were also used to establish twist states that were then challenged by D-alanine and D-cycloserine. The results were always consistent with the basic observations found initially with C6D. The directions of twist modulation by D-alanine and D-cycloserine were always opposite. That portion of the
DISCUSSION

The results reported here concerning the influence of D-cycloserine, and here and elsewhere concerning the influence of D-alanine, on the establishment of twist states in B. subtilis macrofibers suggest that assembly of peptidoglycan during cell growth is a key element, if not the essential biochemical component, controlling the establishment of twist states. Although twist states were measured from macrofiber structures, there is much evidence from biomechanics studies that such states reflect directly the geometry of cell wall assembly in the individual cells that constitute macrofibers. The fact that a given strain can produce macrofibers of many different twist states ranging from tight left- to tight right-handedness indicates that the cell wall must be able to assume a corresponding spectrum of conformational states. It now seems likely that during the assembly of peptidoglycan into cell wall, reactions involving D-alanine or the dipeptide D-alanyl-D-alanine take place that influence the geometry of the cell surface in ways affecting the rate of helical turning per unit of length increase of the individual cells and eventually the twist state of macrofibers. This conclusion stems from the fact that both D-cycloserine and D-alanine influence the establishment of twist states.

The response to D-alanine, generally a rightward shift in twist proportional to concentration, is shown here to be dependent on strain and absolute concentration. C6D, a strain genetically distant from most macrofiber strains currently studied (those derived from FJ7 or based on the lyr-2 mutation from FJ7) clearly displays a D-alanine concentration optimum for production of maximum right-handedness, suggesting that the biochemical reaction(s) concerned with twist establishment is sensitive to the pool size of D-alanine or a product derived from D-alanine, such as the D-alanyl-D-alanine dipeptide. Other strains that do not undergo the second transition to left-handedness at high D-alanine concentrations (unless additional magnesium is present), such as FJ7, nevertheless grow with drastically altered structure at these concentrations, indicating again an influence on the biochemical reaction concerned with twist establishment.

D-Alanine has been found in four different macromolecules that are components of the cell surface: peptidoglycan, teichoic acid, lipoteichoic acid, and two membrane proteins of unknown function (3, 11, 12). The regulation of macrofiber twist states by D-cycloserine reported here suggests that the metabolism of D-alanine concerned with peptidoglycan synthesis is most likely to be the means by which D-alanine influences twist establishment. The relevant biochemical pathways in which D-alanine can participate are conversion to L-alanine, conversion to D-glutamic acid, and ligation to produce the dipeptide D-alanyl-D-alanine, all resulting in products that become directly incorporated into peptidoglycan. The enzymes catalyzing the first and third of these reactions, a racemase and ligase, are the targets of D-cycloserine. Thus, blocking the production of L-alanine or the dipeptide from D-alanine affects the establishment of twist states. It seems likely that blocking formation of the dipeptide is the significant factor for the following reasons: addition of either L-alanine or D-glutamic acid to macrofiber cultures did not affect twist states, and a macrofiber strain, 2C8, that is genetically defective in the racemase and thus requires D-alanine for growth responded to D-alanine by appropriate changes in twist. Thus, the size of the D-alanine or D-alanyl-D-alanine dipeptide pool(s) appears to be involved in regulation of cell wall geometry during growth.

The summary of interaction studies shown in Fig. 6

FIG. 6. Interactions between D-alanine and D-cycloserine and with other systems that influence the establishment of twist states in macrofibers. (a) Results obtained with strain C6D. The twist spectrum is shown as a circle in the center, ranging from maximum left-handedness to maximum right-handedness. Effects of D-alanine, D-cycloserine, and temperature are shown in concentric circles. Interactions between two systems are shown by connecting arrows. Arrows point in the direction of twist modification resulting from increasing values of the twist-modulating factor. Arrows pointing to dots represent conditions in which no change of twist resulted from addition of the modulator. (b) Results obtained with strain FJ7. Details as in panel a. Twist modulation by magnesium and ammonium sulfate are also shown. Conventions concerning the direction of twist modulation obtained from studies of C6D macrofibers were followed in plotting the FJ7 results as well.
indicates that D-cycloserine and D-alanine behave as antagonists to each other with respect to twist establishment and also with respect to the temperature, magnesium, and ammonium systems of twist regulation. The D-alanine–D-cycloserine antagonism is precisely the same as that previously found for these compounds with respect to growth inhibition (13), suggesting that the same biochemical mechanism may be involved in twist regulation at subinhibitory concentrations as in growth inhibition. The fact that there is a consistent response to these agents regardless of the initial twist state or the means used to achieve that twist state suggests that a single mechanism underlies twist establishment in all systems.

Although the metabolism of D-alanine affected by D-cycloserine inhibition is concerned with the synthesis of the pentapeptide precursor to peptidoglycan synthesis, the D-alanine pool itself is available for use in teichoic acid and lipoteichoic acid esterification and presumably also for attachment to the membrane proteins mentioned earlier. Manipulation of the D-alanine pool size by addition of either D-alanine or D-cycloserine does not guarantee that peptidoglycan synthesis itself controls establishment of twist states. Nevertheless, it seems likely that the peptidoglycan pathway is indeed the critical one for the following reasons. If the size of the D-alanine pool is made very large by growing macrofibrers in high concentrations of the compound, the size of the dipeptide D-alanyl-D-alanine pool should also increase proportionally. Under such conditions, the addition of D-cycloserine would not be expected to cause much change in the size of the D-alanine pool, since two of the reactions which drain D-alanine from this pool would be blocked, but would cause a reduction in the size of the dipeptide pool. Assuming for a moment that the dipeptide pool size was actually the factor controlling twist establishment, then the x-axis of Fig. 1 could be relabeled dipeptide concentration, and the changes produced by D-cycloserine would result in exactly the effects on twist that were found. The same reasoning may be applied to all cases shown in Fig. 6 involving D-alanine–D-cycloserine interactions. The predicted pool size variations of the dipeptide therefore appear to be better suited to a model in which twist establishment is controlled by a reaction sensitive to an optimum concentration of an effector than do the predicted pool size variations of D-alanine itself. Direct addition of the dipeptide to macrofibrers failed, however, to alter twist, presumably due to the inability of the cells to transport the compound.

Unlike the inhibition of D-alanine transport caused by D-cycloserine in Escherichia coli, in Bacillus subtilis D-cycloserine was found to enhance rather than reduce the rate of transport of D-alanine in cells incubated in the inhibitor long enough for the appearance of the so-called inducible resistance to D-cycloserine to be expressed (2). No reduction in the rate of D-alanine transport was found in cells during the interval between the addition of D-cycloserine and the appearance of resistance. Therefore, when the establishment of twist was examined in the presence of both D-alanine and D-cycloserine, the effects of the transport of one on the other do not seem to be a plausible explanation for the results. Rather, variations on the pool size of D-alanine and the dipeptide resulting from increased transport when cells are grown in the presence of D-alanine, coupled to inhibition of the racemase and ligase when D-cycloserine is present, appear to be the most likely mechanisms involved.

Reactions concerned with peptidoglycan synthesis that might be sensitive to compounds such as D-alanine or the dipeptide are those involving cross-linking and enzymes such as the carboxypeptidase and transpeptidase (12). D-Amino acids were previously shown to inhibit cell growth by interference with peptidoglycan synthesis (16). The targets were later shown by in vitro studies to be carboxypeptidases required for eventual cross-linking (17). In related work, Tuomanen and Tomasz reported that subinhibitory concentrations of D-amino acids, including D-alanine, protected E. coli against lysis and cell growth inhibition by a variety of β-lactam antibiotics (17). They suggested that the D-amino acids might exert their effect by binding to penicillin-binding proteins and thus preventing access of the antibiotics to their targets. The concentration range in which they obtained protection was precisely that corresponding to right-handedness in macrofibrers shown in Fig. 1. Although this may be purely fortuitous, the possibility exists that a common mechanism is involved, particularly in light of preliminary observations indicating that right-handed macrofibrers are more resistant to growth inhibition by a number of antibiotics than are left-handed ones of the same strain. These observations, currently being further documented, will be reported in detail elsewhere.

All of the results described here and elsewhere concerning establishment of macrofibrer twist states are compatible with a model in which twist states correspond to conformational states of the cell wall polymers determined by insertion of new polymer into the wall during growth. Peptidoglycan cross-linking reactions could be of central importance. Under optimum conditions, a wall conformational state corresponding to maximum right-handedness would be assembled. Deviations from the optimum in one direction would lead in most strains to the entire twist spectrum ranging to tight left-handedness, whereas the ramifications of deviations from the optimum in the other direction would depend on genetic and other factors. In some strains such as C6D, again the entire twist spectrum would be achieved. In others, highly coiled structures similar to those described by Tilby (15) would arise. Once twist states were established, other factors such as charge interactions in the wall could contribute to their maintenance. The production of macrofibrer twist states would trace directly to the conformational states of the individual cell surfaces by deformation mechanisms described previously (6). In this way molecular events in the cell wall would be amplified to macroscopic dimensions in macrofibrers.

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LITERATURE CITED