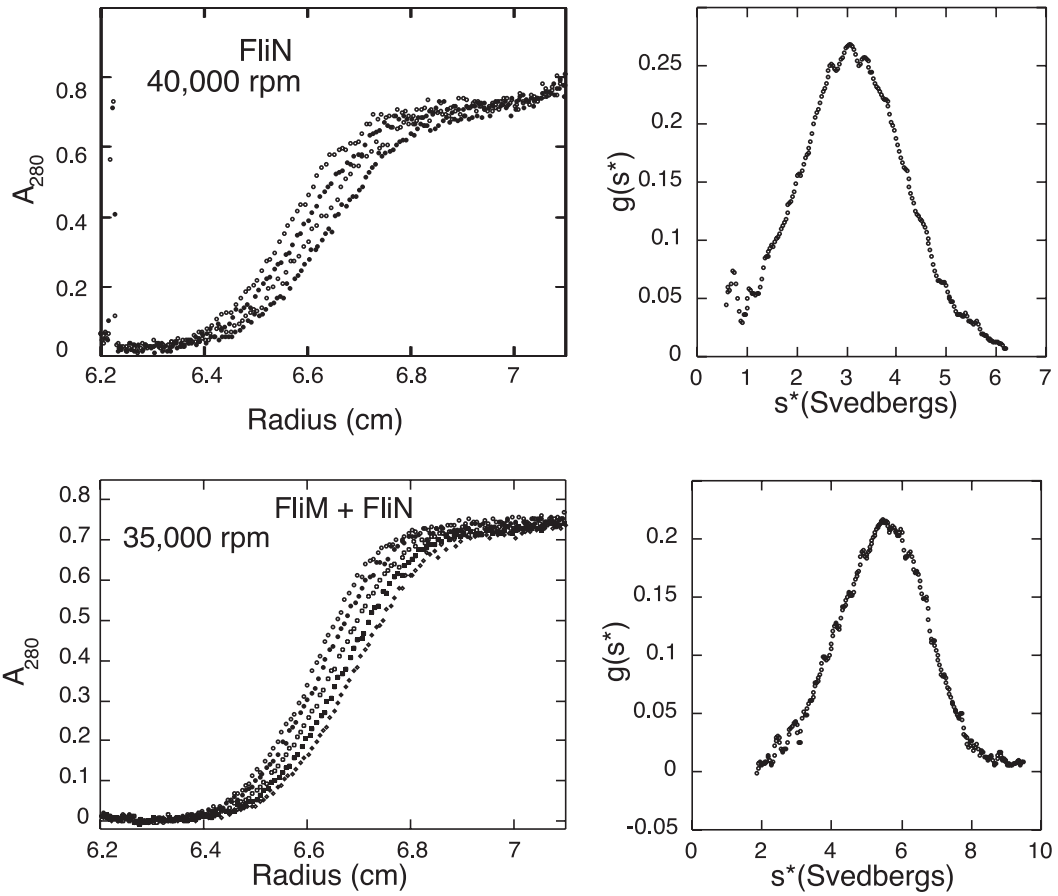

FIG. 7. Velocity sedimentation of *E. coli* FliN (top panel) and the *T. maritima* FliM-FliN complex (bottom panel). Representative absorbance traces are shown on the left, at ~7-min intervals for FliN and at ~11-min intervals for the FliM-FliN complex. The scans were analyzed by the method of Stafford (60) to obtain the distributions of apparent sedimentation coefficients [g(s*)] shown on the right.

in FliN, this makes the hydrophobic patch larger in FliN than in HrcQB (Fig. 10B and C).

The strong conservation of residues that form the hydrophobic patch suggests that it is important for function. To test this proposal, we mutated the *E. coli fliN* gene to replace a well-conserved hydrophobic residue of the patch, Val113, with Asp. This residue corresponds to Val130 in the *T. maritima* protein, as indicated in Fig. 10B (orange). A plasmid encoding the *fliN* mutation was transformed into the *fliN*-null strain DFB223 (63), and motility and flagellation were examined. The mutation caused a severe reduction in swarming in soft agar at 32°C (Fig. 11). Cells taken from the edges of the swarms were highly motile when they were viewed with a microscope but swam smoothly, indicating that there was a CCW bias of the flagellar motors. Cells cultured in liquid medium (tryptone broth, 32°C) showed a delay in the onset of motility compared to wild-type cells, and staining showed that there were fewer flagella. At later stages of growth in liquid, mutant cells were highly motile but aberrantly smooth. The motility impairment was more severe when cells were grown in tryptone broth at a higher temperature. Wild-type cells cultured at 38°C exhibited fair motility at the mid-log phase, whereas most cells of the mutant were immotile and nonflagellate.

DISCUSSION

The flagellar C ring is a fairly large structure (in *Salmonella*, it is 45 nm in diameter and 15 nm high) (19, 68, 79) and contains more than 100 subunits of FliN (31). Sedimentation experiments give clues to the arrangement of FliN dimers in the flagellum. Because *E. coli* FliN forms a stable tetramer and *T. maritima* FliN forms a stable $FliM_1$-$FliN_4$ complex, we propose that an $FliM_1$-$FliN_4$ complex is the building block of the C ring. The copy number of FliM in the flagellum has been estimated to be 34 ± 6 (80, 81), and en face electron micrographs of the C ring show subunit structure with a rotational symmetry of about 34-fold, varying slightly from specimen to specimen (69, 79). A typical C ring should then contain about 34 copies of the $FliM_1$-$FliN_4$ unit and therefore 136 copies of FliN, in fair agreement with an experimental estimate of 111 ± 13 (81).

The crystal structure of $HrcQB_C$ provides a basis for modeling the arrangement of subunits in the FliN tetramer. Patterns of sequence conservation and similar patterns of hydrogen bonding seen in $HrcQB_C$ and the modeled FliN tetramer support the view that the FliN tetramer has an organization similar to that of $HrcQB_C$. An elongated shape for the FliN