JOURNAL OF BACTERIOLOGY, Dec. 1988, p. 5765–5770
0021-9193/88/125765-06$02.00/0

Vol. 170, No. 12

Inhibition of Cell-Cell Interactions in *Myxococcus xanthus* by Congo Red

JUDY W. ARNOLD AND LAWRENCE J. SHIMKETS*

Department of Microbiology, University of Georgia, Athens, Georgia 30602

Received 28 March 1988/Accepted 12 September 1988

The function of molecules associated with the cell surface may be determined by examining the phenotype of cells treated with inhibitors specific to these cell surface molecules. This strategy was used to examine the function of the major Congo red receptor of the myxobacterium *Myxococcus xanthus*, which has a developmental cycle that involves social interactions among cells. A class of social motility mutations (A^+ S^-), known as *dsp*, may inhibit the same subcellular component as Congo red because the phenotype of wild-type cells which had been treated with Congo red resembled in several ways the phenotype of the Dsp mutants. First, Congo red inhibited agglutination of wild-type cells, whereas Dsp cells were incapable of agglutinating, even in the absence of Congo red. Second, Congo red inhibited fruiting body formation by wild-type cells and reduced the yield of myxospores. Untreated Dsp cells were unable to form fruiting bodies and produced few myxospores. Third, Congo red reduced the rate of wild-type gliding motility to a level comparable to that of untreated Dsp cells, but did not inhibit the A motility of Dsp cells. Finally, binding studies showed that Dsp cells lacked the major Congo red receptor. Wild-type cells bound Congo red with an apparent association constant of 2.4×10^5 M^{-1}, while Dsp cells bound it with an apparent association constant of 8.5×10^3 M^{-1}. Binding of Congo red to wild-type cells was saturated in less than 10 min and was reversible when excess Congo red was removed. These results suggest that the Congo red receptors are controlled by the S motility system and that these receptors are involved in cell cohesion, social motility, and fruiting body formation.

Myxococcus xanthus is a gram-negative soil bacterium with a primitive form of multicellular development that is dependent on gliding motility. Under conditions of high cell density, nutrient depletion, and a solid surface, vegetative cells migrate toward discrete foci and form macroscopic mounds that develop into fruiting bodies (3). Vegetative cells glide slowly, leaving slime trails composed of polysaccharide, lipid, and protein in their wake (4). Gliding cells are close to each other, forming a coherent swarm which migrates as a unit, although individual cells are able to leave the group. The swarm moves in a coordinated manner to feed on other bacteria and soil detritus during vegetative growth.

Genetic studies have provided evidence for two nearly independent systems controlling the behavior of gliding cells known as A (adventurous) and S (social). Single mutations in the S system result in A^+ S^- cells that are less likely to move in groups (5, 6). Single mutations in the A system result in A^- S^+ cells that can glide only if they are close, making them unable to venture away from the group as single cells. Only double mutants (A^- S^-) and those with a mutation in the *mgl* locus are completely nonmotile. Social motility is required for several types of multicellular behaviors, including fruiting body formation (5) and rippling (22). S motility requires cell contact, since A^- S^+ cells that are separated by one cell length or more are nonmotile (10), suggesting that the mechanism(s) by which cells attach to one another may be an important aspect of S motility. Indeed, when an agglutination assay is used as a measure of cohesion, A^+ S^+ and A^- S^+ cells are cohesive, whereas A^+ S^- cells are weakly cohesive or noncohesive (20, 21). Perhaps the most extreme example of A^+ S^- mutants are the Dsp (dispersive) mutants, which do not agglutinate, do not migrate in large swarms, and do not form fruiting bodies (21).

This work arose out of an effort to examine the cell surface with chemical probes that might be specific to receptors involved in social behavior. Certain dyes such as Congo red, Calcofluor white, and Leucophor have been shown to bind bacterial extracellular polysaccharides (2, 16, 24). Congo red binding has been used to supplement morphological criteria for the separation of myxobacteria into taxonomic groups (17). However, the function of the Congo red receptor has never been described, and it is the focus of this work. This paper shows that Congo red blocked the development of wild-type cells of *M. xanthus*, causing them to behave like A^+ S^- Dsp cells, in three independent assays that measured gliding motility, fruiting body formation, and cell cohesion. Furthermore, binding studies suggest that wild-type cells have a class of Congo red receptors which are absent in Dsp cells. Therefore, the Congo red receptor may be necessary for agglutination, S motility, and fruiting body morphogenesis.

MATERIALS AND METHODS

Bacterial cells and growth conditions. *M. xanthus* cells were grown in CTT broth and plated on CTT agar (5) at 32°C. To enhance cell dispersal during growth of liquid cultures, the cells were shaken at 325 rpm in a New Brunswick G24 environmental shaker. Strain DK1622, the wild type, and the A^+ S^- strains DK3470, DK3473, and DK3482 were constructed and furnished by D. Morandi and D. Kaiser. The genotypes of these and other strains used in this study are listed in Table 1. In order to obtain a wild-type strain isogenic with DK3470 (*dsp-1693* linked to Tn*5* insertion ΩDK1407), strain LS308 was constructed by using the generalized transducing myxophage Mx8cp2 (15). Phage were grown on strain DK3470 in liquid lysates (18). The lysates were UV-irradiated at 1,500 μW/cm^2 for 60 s and used to transduce DK1622 to kanamycin resistance, with screening for wild-type motility. Mx8 antiserum was added to the transduction mixture before plating.

* Corresponding author.

TABLE 1. Congo red-staining characteristics of gliding motility mutants

Motility phenotype	Strain	Relevant genotype	Congo red staining
$A^+ S^+$	DK1622	Wild type	+
	LS308	Wild type, Tn*5* ΩDK1407	+
$A^- S^+$	DK2608	*cglC-1* Tn*5* ΩDK1903	+
	DK2616	*cglB-2* Tn*5* ΩDK1932	+
$A^+ S^-$	DK3470	*dsp-1693* Tn*5* ΩDK1407	−
	DK3473	*sgl-3163* Tn*5* ΩDK3163	−
	DK3482	*tgl-1* Tn*5* ΩDK3482	−

Inhibition of agglutination by Congo red. Cells were grown to 100 Klett units (red filter), centrifuged at 11,400 × *g* for 5 min at 4°C, washed in 10 mM MOPS (morpholinepropanesulfonic acid, pH 6.8), suspended in 1 ml of 10 mM MOPS–1 mM $MgCl_2$–1 mM $CaCl_2$ (agglutination buffer) to a density of 200 Klett units (red filter), and added to 1 ml of agglutination buffer containing a series of solutions of Congo red (Sigma Chemical Co.), giving a final concentration of 5×10^8 cells per ml. The suspensions were incubated without shaking at 26°C, and the absorbance at 625 nm was measured at 6-min intervals in a Beckman DU-40 spectrophotometer. The rate of change in absorbance units with time was calculated by the sum of the least-squares method. Each point is the average of data from five experiments.

Congo red-binding studies. The kinetics of Congo red association were determined in a 5-μg/ml solution of Congo red in agglutination buffer. The cell suspension, at a density of 5×10^8 cells per ml, was incubated at 26°C. A portion was withdrawn at intervals and sedimented at 11,400 × *g* for 5 min at 4°C, and the absorbance of the supernatant was measured at 500 nm to determine the amount of Congo red unbound. The amount bound was calculated as the difference from the amount in the original solution to which the cells were added.

To determine the kinetics of dissociation, cells which had been treated with Congo red (5 μg/ml) for 40 min were resuspended in agglutination buffer at a concentration of 5×10^8 cells per ml. At intervals, 1.8-ml cell samples were sedimented in a Brinkman Eppendorff centrifuge, and the pellet was resuspended in 1 ml of agglutination buffer. Buffer-saturated phenol (1 ml) was added to extract the Congo red, and the mixture was inverted gently for 2 min. After separation of the phases, the A_{500} of the phenol phase, which contained the Congo red, was measured to determine the amount remaining bound at each time point.

The apparent association constant (k_a) was determined from binding studies with 5×10^8 cells per ml in agglutination buffer with a series of concentrations of Congo red. After addition of the cells to the Congo red solutions, the samples were incubated at 26°C for 40 min. Then the cells were pelleted at 11,400 × *g*, washed by suspension in agglutination buffer, and finally resuspended in agglutination buffer. Phenol was added to extract the Congo red as described above, and the A_{500} for both the extracted pellet and the supernatant of the samples was measured to determine the amounts of Congo red bound and left unbound. Each point is the average of four experiments, with two replicates in each experiment. The k_a for Congo red with each cell type was determined by the method of Klotz (11).

Swarm expansion assay. Swarm expansion was measured by the method of Kaiser and Crosby (10) except that CF (clone fruiting) agar was substituted for motility agar. Liquid cultures were grown to a density of 100 Klett units, harvested, and suspended to a calculated density of 1,000 Klett units in 10 mM MOPS buffer. This culture was diluted to 500, 250, 100, and 50 Klett units in 10 mM MOPS buffer, the final density being calculated from the dilution factor. Norit (carbon particles) for position markers was added to each dilution of cells at 10 mg/ml final concentration. Three 7-μl drops of each mixture were placed on CF agar plates and incubated at 30°C for 30 min; then the plates were kept at room temperature (26°C) for the assay. The same procedure was used for a duplicate set of CF agar plates containing 5 μg of Congo red per ml.

At six sites chosen randomly around the periphery of each drop of cells, the distance from the edge of the Norit particles to the edge of the swarm was measured (in micrometers) for each culture by phase contrast microscopy at 30-min time points for 3.5 h. An arithmetic average of the six measurements was plotted as the distance moved versus time for each initial cell density. The expansion rate (micrometers per minute) for each density was the slope of the line obtained by a least-squares fit for each such plot. Thus, each spreading rate was based on 36 measurements.

Inhibition of myxospore and fruiting body formation. Developing cells were prepared by the submerged-culture technique (12). Cultures were grown in CTT broth (5) to 70 Klett units and then diluted to 2.8 Klett units. An 8-ml amount of this suspension was placed in a petri plate (50 by 100 mm) and incubated at 32°C for 20 h. The liquid was then decanted, and the culture was washed twice by adding 8 ml of distilled water, which was then decanted. An 8-ml amount of development buffer (10 mM MOPS) [pH 6.8], 1 mM $CaCl_2$ 1 mM $MgCl_2$, and 0.1% pyruvate) or development buffer containing either 5 or 25 μg of Congo red per ml was added to a series of plates. After incubation for 48 h at 32°C, the plates were examined for the presence of characteristic fruiting bodies with a Wild dissecting microscope and then photographed. Myxospores were harvested by decanting the liquid and scraping the material from the bottom of the plate with a razor blade into a centrifuge tube, sedimenting the cells at 11,400 × *g* for 5 min, and suspending the pellet in 1 ml of TMP buffer (10 mM Tris [pH 7.6], 8 mM $MgSO_4$, 1 mM KPO_4). The samples were treated twice for 30 s each with an ultrasonic probe (Heat System Ultrasonic, Inc.) at 40 μW, which lyses vegetative cells but not myxospores (23), and myxospores were counted in a Petroff-Hausser counting chamber with a Leitz phase-contrast microscope.

Growth and viability with Congo red. Cells grown in CTT broth to a density of 50 to 100 Klett units were diluted to a density of about 20 Klett units in fresh CTT broth containing Congo red. These cell suspensions were incubated at 32°C with shaking, and changes in turbidity were monitored for three generations in a Klett-Summerson colorimeter. Samples of the suspensions before and after the experiment were streaked on CTT agar to check cell viability. Morphological examination of the treated cells was made by phase-contrast microscopy.

RESULTS

Inhibition of agglutination by Congo red. Congo red is a strong inhibitor of the agglutination of *M. xanthus*. Wild-type cells typically agglutinate in about 40 min in the presence of divalent cations (20). The rate of agglutination decreased sharply as the Congo red concentration increased (Fig. 1). At 7.2 μM (5 μg/ml) Congo red, agglutination did not occur during the course of the assay. Dsp mutants ($A^+ S^-$) are incapable of agglutinating (20), even in the absence

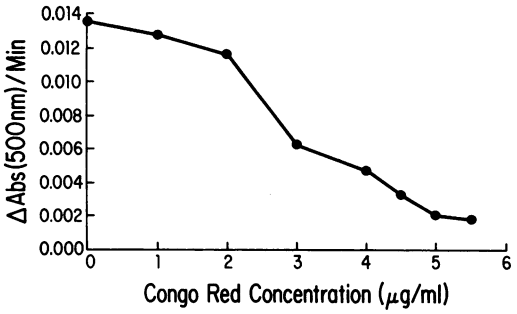

FIG. 1. Inhibition of wild-type agglutination by Congo red. Reactions were carried out as described under Materials and Methods. Each point is the average of data from five experiments. The rate of change in A_{500} units with time was calculated by the sum of least-squares method with the absorbance readings taken at 6-min intervals for 5 h.

of Congo red, and in this respect Congo red caused the wild type to behave like the Dsp mutants.

Inhibition of swarm expansion. The swarm expansion assay measures the rate of gliding away from a fixed point on the edge of a colony (10). The rate of swarm expansion is determined by the activity of two nearly independent motility systems, known as A and S, as well as the cell density (10). Swarm expansion of wild-type and Dsp cells was measured at different densities with and without Congo red in the agar. At each density, the spreading rate was determined from a plot of zone width versus time. Each spreading rate dictated a single point for the graph of rate versus cell concentration (Fig. 2).

The rate of swarm expansion for the wild type depended on the initial cell density, rising from 0.26 μm/min at a density of 100 Klett units to 0.9 μm/min at a density of 1,000 Klett units. At low densities, the rate of spreading increased proportionally with the density before leveling off at high densities. Dsp cells were much less cell density dependent than wild-type cells but achieved a maximal rate of motility of only 0.65 μm/min, significantly less than the wild type. Congo red inhibited the maximal rate of swarm expansion for the wild type to about 0.65 μm/min at 1,000 Klett units, the same rate observed with untreated Dsp cells, and it did not further inhibit the rate of expansion for Dsp cells, which is due solely to A motility. Thus, Congo red appears to specifically decrease the rate of S-directed swarm expansion. However, it did not change the cell density dependence observed with wild-type swarm expansion.

After 120 min on CF agar plates containing 5 μg of Congo red per ml, red spots about 50 μm in diameter appeared throughout the wild-type colony. When examined by phase-contrast microscopy, the spots were observed to contain bright red extracellular material around clusters of cells. Subsequent observation of these plates after 24 and 48 h showed that the extracellular material surrounding the fruiting bodies was stained. Slime trails deposited by gliding wild-type or Dsp cells were not stained red. Dsp cells did not localize the stain to any particular region of the field.

Inhibition of myxospore and fruiting body formation. Wild-type cells which are suspended in growth medium and dispensed into empty petri plates will settle to the bottom and adhere to the plastic, forming a thin layer of cells. When the growth medium is removed and replaced with buffer, rapid and synchronous development is induced (12). Wild-type cells which were prepared by this submerged-culture technique were induced to develop and produced fruiting bodies which contained myxospores (Fig. 3A). Cultures of Dsp cells did not adhere to the bottom of the petri plate during growth in CTT broth and were removed along with the buffer during decantation. Wild-type cells which were incubated in buffer containing Congo red (5 μg/ml) formed fruiting bodies which were less compact than those of the wild type (Fig. 3B), but there was no reduction in the number of myxospores produced. In buffer with 25 μg of Congo red per ml, fruiting bodies were not formed (Fig. 3C), and there was a 67% reduction in the number of myxospores present. Congo red treatment mimics the effect of the Dsp mutation with regard to fruiting body formation and sporulation. On CF agar, Dsp cells are unable to fruit and produce only 1% of the wild-type levels of spores (21). Fruiting body formation is dependent on the S motility system (7), and it appears likely that Congo red inhibits development by interfering with S motility. These concentrations of Congo red did not greatly inhibit the rate of growth in CTT broth. The generation times of wild-type cells in CTT broth (5.8 h) varied little whether Congo red was added at 5 μg/ml (5.4 h) or 25 μg/ml (6.1 h). Because there was no evidence of distortion of cell morphology or loss of cell viability (data not shown), it seems unlikely that Congo red is acting as a metabolic poison.

Binding of Congo red. Since the previous experiments suggested that Congo red acts as a specific inhibitor of S motility, one might expect to observe the Congo red receptor on S^+ cells but not on S^- cells. Colonies with social motility (A^+ S^+ or A^- S^+) stained bright red when CTT agar plates were flooded with an 0.01% aqueous solution of Congo red (Table 1). Dsp colonies and those of other A^+ S^- mutants did not become red. Since obvious differences were observed, binding studies were initiated to assess quantitatively the affinity of Congo red for wild-type and Dsp cells.

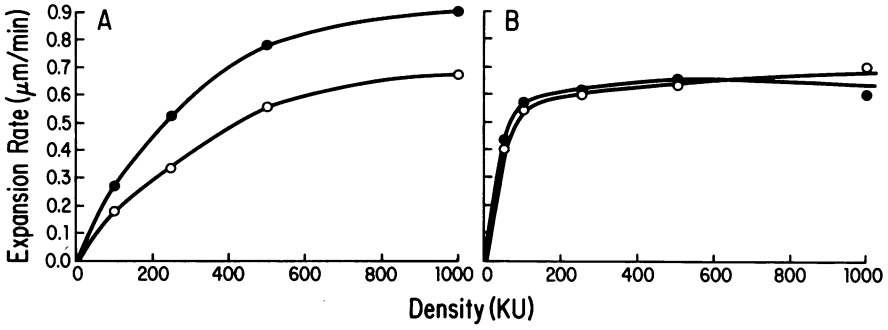

FIG. 2. Swarm expansion of wild-type (A) and Dsp (B) cells. CF agar plates with (○) and without (●) Congo red (5 μg/ml). KU, Klett units.

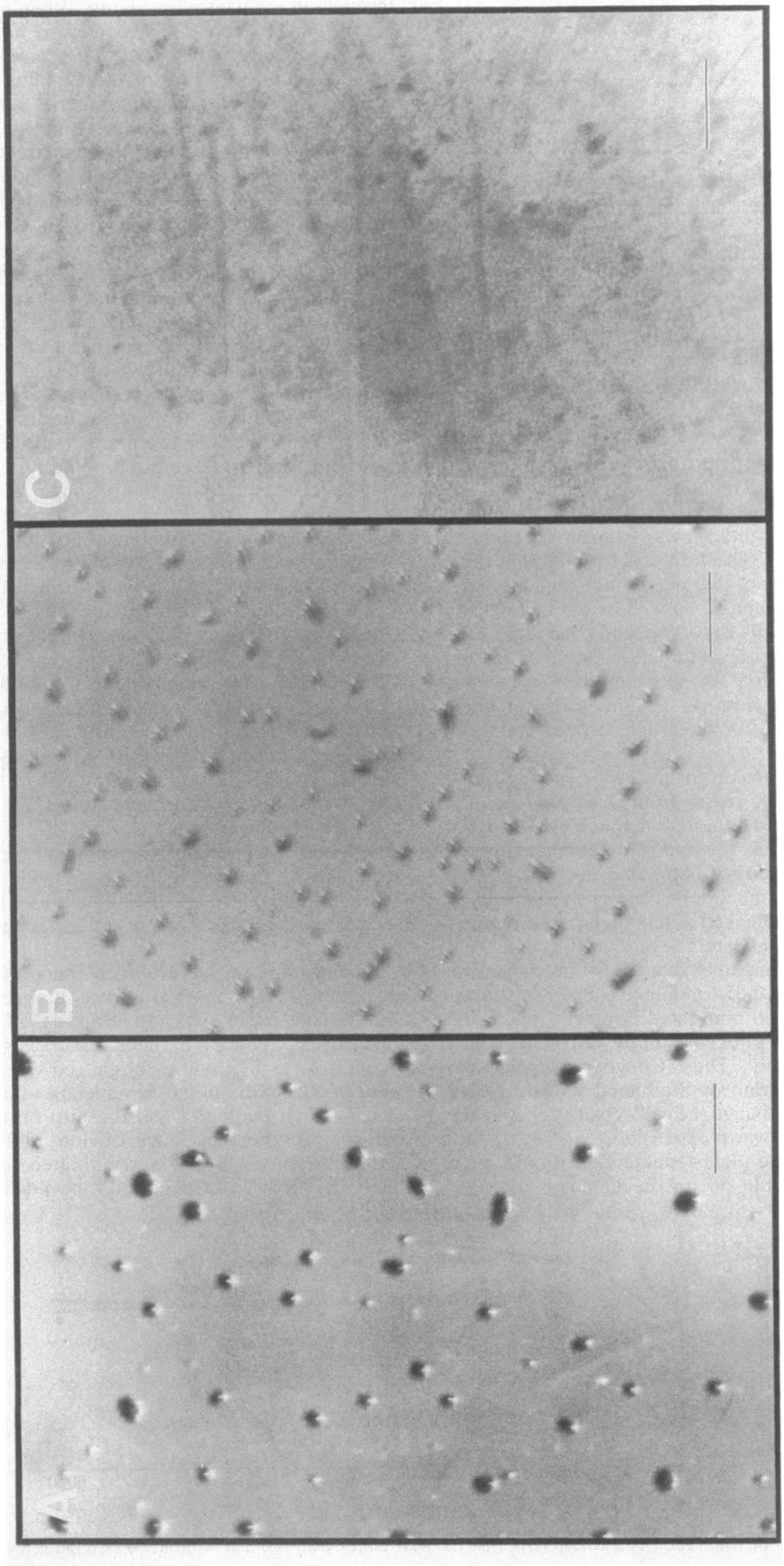

FIG. 3. Fruiting body formation by wild-type cells in submerged culture with no (A), 5 μg (B), or 25 μg (C) of Congo red per ml. Bars, 400 μm.

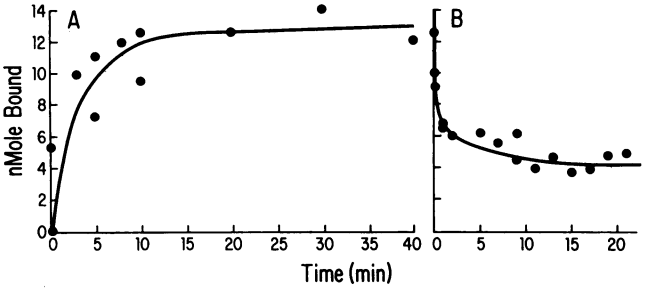

FIG. 4. Kinetics of association (A) and dissociation (B) of Congo red with wild-type cells at a density of 5×10^8 cells per ml.

To determine the time necessary to establish equilibrium, wild-type cells suspended in a solution of Congo red were harvested at intervals, and the amount of Congo red associated with the cells was determined by the decrease in Congo red in the solution to which the cells were exposed. Saturation occurred in less than 10 min, after which the amount bound to the cells remained constant for at least 40 min (Fig. 4A). Binding was reversible, with similar kinetics. After 40 min in Congo red, cells were resuspended in agglutination buffer lacking Congo red, and the amount associated with the cells was determined by extracting Congo red from the cell pellet with phenol. A new equilibrium of about 4 nmol was established in less than 10 min (Fig. 4B). Dissociation of Congo red from the cells also restored agglutination (1).

The apparent association constant (k_a) was determined from a series of binding studies in different concentrations of Congo red. The amount associated with the cells was determined following phenol extraction of the cell pellet. The amount left unbound was the amount left in the solution to which the cells were exposed. The k_a was determined by the method of Klotz (11) according to the equation $(1/r) = (1/k_a)(1/[\text{Congo red}]) + (1/n)$, where [Congo red] is the concentration of Congo red free at equilibrium (in moles/liter), n is the number of binding sites per cell, $1/r$ is the number of cells per mole of Congo red bound, and k_a is the apparent association constant of Congo red. The x intercept is $-k_a$, and the y intercept is $1/n$.

While both wild-type and Dsp cells bound Congo red, wild-type cells bound much more at each concentration tested (Fig. 5). The linearity of the plot for the wild type indicated identical and independent (noncooperative) binding sites for Congo red for which the k_a and n remained constant at each concentration. The 30-fold difference in the

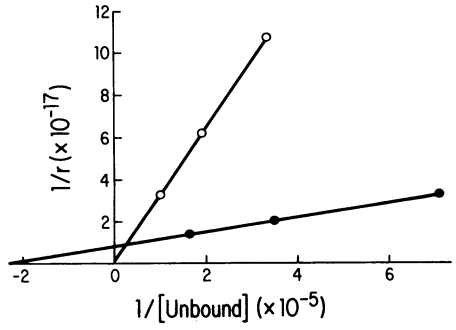

FIG. 5. Binding of Congo red with wild-type (●) and Dsp (○) cells. Cells were prepared for the binding studies as described in Materials and Methods. Each point is the average of four experiments with two replicates each.

TABLE 2. Association of Congo red with wild-type and Dsp cells

Strain	Congo red bound[a] (μg/10^9 cells)	Molecules/ cell[a]	Binding sites/ cell[b]	K_a (M^{-1})
LS308 (wild type)	3.5	3.0×10^6	4.5×10^6	2.4×10^5
DK3470 (Dsp)	1.1	9.5×10^5	2.1×10^7	8.5×10^3

[a] Cells were suspended in a 5-μg/ml solution of Congo red at a density of 5×10^8 cells per ml as described in Materials and Methods.

[b] The number of Congo red-binding sites per cell was calculated from the association constants, as described by Klotz (11).

k_a between the wild type (2.4×10^5 M^{-1}) and the Dsp mutant (8.5×10^3 M^{-1}) was a strong indication that the wild type had a class of Congo red receptors which were absent in the mutant. Although the wild-type receptors had a fairly weak affinity for Congo red, the dye was a very strong inhibitor of agglutination, which may be due in part to the large number of receptors. From the k_a, the actual number of receptors on a cell was calculated to be 4.5×10^6, assuming that one receptor binds one molecule of dye (Table 2). At a concentration of 5 μg/ml, about 2/3 of the receptors were occupied, and this was apparently enough to inhibit agglutination.

The linearity of the plot for Dsp cells also indicated identical and independent binding sites for Congo red where k_a and n are constants. The Dsp receptor for Congo red had a much lower affinity for the dye than the wild type, suggesting that it has a different chemical composition (Table 2).

DISCUSSION

All myxobacteria are descended from a single ancient line and share a number of properties, including cohesion, gliding, and multicellular morphogenesis (19). Cataloging of the 16S RNA oligonucleotides has suggested that the myxobacteria more recently diverged into two groups (14), and these two groups can be distinguished from each other by Congo red staining, cell morphology, morphogenesis, and swarm behavior (17, 19). One group, which does not bind Congo red, has stout vegetative cells with blunt, rounded ends that undergo little shape change during morphogenesis. They have characteristic swarms and do not move in multicellular waves known as ripples. The other group, which binds Congo red and includes the genus *Myxococcus*, has rod-shaped vegetative cells with tapered ends that change their shape considerably during morphogenesis. These species swarm in distinctly different patterns and form ripples. The strong correlation of Congo red binding with these other taxonomic indicators and the preservation of this trait during the evolution of one group suggests that the Congo red receptor has an important biological function.

The colony-staining technique was used to examine a variety of motility mutants of *M. xanthus*, and the results suggested a strong correlation between Congo red binding and motility genotype. Cells with social motility, A^+ S^+ or A^- S^+, bound Congo red, whereas cells lacking social motility, A^+ S^-, did not (Table 1). Social motility has been found to regulate a number of processes, including cell agglutination (21), fruiting body formation (7), and swarm expansion (10). Therefore, one might expect the Congo red receptor to be involved in each of these three processes. This was tested by comparing the phenotype of wild-type cells which had been treated with Congo red with that of

untreated Dsp (S^-) and untreated wild-type (S^+) cells. In each case Congo red caused the wild type to behave like a Dsp mutant. First, Congo red was a potent inhibitor of wild-type agglutination. Dsp cells are incapable of agglutinating even in the absence of Congo red, and A^+ S^- cells in general have weak agglutinating activity (20). Second, Congo red reduced the ability of wild-type cells to form fruiting bodies and reduced the yield of myxospores. In comparison, Dsp cells are unable to form fruiting bodies and form fewer myxospores than the wild type (21). About 2/3 of all A^+ S^- mutants are unable to develop (6). Third, Congo red inhibited the optimal rate of wild-type swarm expansion to the level observed with the Dsp mutant. A^+ S^- cells tend to have an optimal rate of expansion of about 0.6 μm/min (10). Congo red reduced the rate of wild-type swarm expansion to 0.65 μm/min and did not inhibit Dsp swarm expansion, which occurs solely by A motility. Thus, treatment of wild-type cells with Congo red renders them phenotypically similar to the Dsp mutants by all three criteria. These results indicate an essential role for the Congo red receptor in social behavior and development.

Binding studies have suggested that wild-type cells have a Congo red receptor which is absent in Dsp mutants; the k_a for Congo red binding to the wild type is 30 times higher than that to the mutant. While the chemical nature of the receptor (13) is unknown, an extracellular polysaccharide (predominantly galactosamine and *N*-acetylgalactosamine) which binds Congo red has been isolated from the unrelated gliding bacterium *Flexibacter columnaris* (formerly known as *Chondrococcus columnaris*) (8). The *M. xanthus* Congo red receptor also appears to be extracellular in that Congo red stained material surrounding cells and fruiting bodies. In a companion paper (1), the surfaces of wild-type and Dsp cells were examined by electron microscopy, and an extracellular matrix which is elaborated by wild-type but not Dsp cells was found (1). Furthermore, the study shows that Congo red interferes with the production of this matrix.

ACKNOWLEDGMENTS

This work was supported by grants PCM 8351306 and DCB 8312425 from the National Science Foundation and by the Georgia Power Company.

LITERATURE CITED

1. **Arnold, J. W., and L. J. Shimkets.** 1988. Cell surface properties correlated with cohesion in *Myxococcus xanthus*. J. Bacteriol. **170**:5771–5777.
2. **Colvin, J. R., and D. E. Witter.** 1983. Congo red and Calcofluor white inhibition of *Acetobacter xylinum* cell growth and of bacterial cellulose microfibril formation: isolation and properties of a transient, extracellular glucan related to cellulose. Protoplasma **116**:34–40.
3. **Dworkin, M.** 1972. The myxobacteria: new directions in studies of procaryotic development. Crit. Rev. Microbiol. **2**:435–452.
4. **Gnosspelius, G.** 1978. Myxobacterial slime and proteolytic activity. Arch. Microbiol. **116**:51–59.
5. **Hodgkin, J., and D. Kaiser.** 1977. Cell-to-cell stimulation of movement in nonmotile mutant of *Myxococcus*. Proc. Natl. Acad. Sci. USA **74**:2938–2942.
6. **Hodgkin, J., and D. Kaiser.** 1979. Genetics of gliding mobility in *Myxococcus xanthus* (Myxobacterales): genes controlling movements of single cells. Mol. Gen. Genet. **171**:167–176.
7. **Hodgkin, J., and D. Kaiser.** 1979. Genetics of gliding motility in *Myxococcus xanthus* (Myxobacterales): two gene systems control movement. Mol. Gen. Genet. **171**:177–191.
8. **Johnson, J. L., and W. S. Chilton.** 1966. Galactosamine glycan of *Chondrococcus columnaris*. Science **152**:1247–1248.
9. **Kaiser, D.** 1979. Social gliding is correlated with the presence of pili in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **76**: 5952–5956.
10. **Kaiser, D., and C. Crosby.** 1983. Cell movement and its coordination in swarms of *Myxococcus xanthus*. Cell Motil. **3**:227–245.
11. **Klotz, I. M.** 1946. The binding of organic ions by proteins. J. Am. Chem. Soc. **68**:1486–1490.
12. **Kuner, J., and D. Kaiser.** 1982. Fruiting body morphogenesis in submerged cultures of *Myxococcus xanthus*. J. Bacteriol. **151**: 458–461.
13. **Lillie, R. D.** 1982. H. J. Conn's biological strains, 9th ed., p. 53–55 and 147–148. The Williams & Wilkins Co., Baltimore.
14. **Ludwig, W., K. H. Schleifer, H. Reichenbach, and E. Stackebrandt.** 1983. A phylogenetic analysis of the myxobacteria: *Myxococcus fulvus*, *Stigmatella aurantiaca*, *Cystobacter fucus*, *Sorangium cellulosum*, and *Nannocystis exedens*. Arch. Microbiol. **135**:58–62.
15. **Martin, S., E. Sodergren, T. Masuda, and D. Kaiser.** 1978. Systematic isolation of transducing phages of *Myxococcus xanthus*. Virology **88**:44–53.
16. **Matthysse, A. G.** 1983. Role of bacterial cellulose fibrils in *Agrobacterium tumefaciens* infection. J. Bacteriol. **154**:906–915.
17. **McCurdy, H. D.** 1969. Studies on the taxonomy of the Myxobacterales. I. Record of Canadian isolates and survey of methods. Can. J. Microbiol. **15**:1453–1461.
18. **Orndorff, P., E. Stellwag, T. Starich, M. Dworkin, and J. Zissler.** 1983. Genetic and physical characterization of lysogeny by bacteriophage Mx8 in *Myxococcus xanthus*. J. Bacteriol. **154**: 772–779.
19. **Reichenbach, H., and M. Dworkin.** 1981. The order myxobacterales, p. 328–355. *In* M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel (ed.), The procaryotes, vol. 1. Springer-Verlag, Berlin.
20. **Shimkets, L. J.** 1986. Correlation of energy-dependent cell cohesion with social motility in *Myxococcus xanthus*. J. Bacteriol. **166**:837–841.
21. **Shimkets, L. J.** 1986. Role of cohesion in *Myxococcus xanthus* fruiting body formation. J. Bacteriol. **166**:842–848.
22. **Shimkets, L. J., and D. Kaiser.** 1983. Induction of coordinated cell movement in *Myxococcus xanthus*. J. Bacteriol. **152**:451–461.
23. **Sutherland, I. W.** 1976. Ultrasonication—an enrichment technique for microcyst-forming bacteria. J. Appl. Bacteriol. **41**: 185–188.
24. **Thomashow, M. F., J. E. Karlinsey, J. R. Marks, and R. E. Hurlbert.** 1987. Identification of a new virulence locus in *Agrobacterium tumefaciens* that affects polysaccharide composition and plant cell attachment. J. Bacteriol. **169**:3209–3216.