Effects of Inhibition of the B Subunit of DNA Gyrase on Conjugation in *Escherichia coli*

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Antagonism of the DNA gyrase B subunit in the donor bacterium by coumermycin or thermal inactivation inhibited transfer of plasmid R64*drd-11*. Coumermycin also inhibited Hfr transfer, with kinetics after drug removal suggesting that transfer resumed from the point of inhibition, in contrast to inhibition with nalidixic acid, after which transfer reinitiated from the origin of transfer.

DNA gyrase introduces negative superhelical twists into closed circular DNA and is necessary for bacterial DNA replication (reviewed in reference 10). This enzyme is composed of two A subunits, the targets of nalidixic acid, and two B subunits, the targets of coumermycin A1. A requirement for DNA gyrase in bacterial conjugation has been

The effects of antagonism of the gyrase B subunit on conjugation have not been evaluated. We studied the role of the DNA gyrase B subunit in plasmid and Hfr conjugation with coumermycin and a thermosensitive *gyrB* mutant. The kinetics of resumption of Hfr conjugation after removal of coumermycin and nalidixic acid were also compared.

TABLE 1. *Escherichia coli* K-12 strains and plasmids

Strain or plasmid	Genotype	Phenotype[a]	Source (reference)
Strains			
NI747[b]	*thr-1 leu-6 ilvD malB lacY thy thi-1*	MIC = 5 µg of Cou/ml	M. Gellert (11)
NI748[b]	NI747 *gyrB* (Cou[r])	MIC = 75 µg of Cou/ml	M. Gellert (11)
CT31	NI748 derivative	Nal[r]	This study
N99[b]	*galK2*		M. Gellert (8)
N4177[b]	N99 *gyrB41*(Ts)		M. Gellert (8)
CT32	N4177 derivative	Ks[r]	This study
CT34	N99 derivative	Ks[r]	This study
CT36	N99 derivative	Nal[r]	This study
Hfr Cavalli	Hfr *metB1 relA1 spoT1* lambda[−]	MIC = 4 µg of Nal/ml and 5 µg of Cou/ml	B. Bachmann (12)
AB1133	F^{−} *thr-1 leuB6 proA2 his-4 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 supE44* lambda[−]	MIC = 4 µg of Nal/ml and 5 µg of Cou/ml	B. Bachmann (12)
CT46	AB1133 *gyrB* (Cou[r])	MIC = 4 µg of Nal/ml and 75 µg of Cou/ml	This study[c]
CT47	AB1133 *g yrA* (Nal[r])	MIC = 512 µg of Nal/ml and 5 µg of Cou/ml	This study[c]
CT48	Hfr Cavalli *gyrA* (Nal[r])	MIC = >20 µg of Nal/ml	This study[c]
CT49	Hfr Cavalli *gyrB* (Cou[r])	MIC = >30 µg of Cou/ml	This study[c]
JC6256	*lac trp malA* lambda[r]	MIC = 10–15 µg of Cou/ml	J. Maule (2, 3)
CSH50	*ara* Δ(*lac-pro*) *rpsL thi supD+*		B. Kline (17, 18)
CSH50-1	CSH50 derivative	MIC = >20 µg of Cou/ml	This study
Plasmids			
R64*drd-11*		Tet[r] Str[r] IncIα derepressed for transfer	G. Jacoby (13)
F' *lac*			J. Maule (2, 3)

[a] MIC determined by agar dilution; Cou, coumermycin A1; Ks, kasugamycin; Nal, nalidixic acid; Tet, tetracycline; Str, streptomycin.
[b] Strains containing R64*drd-11*.
[c] *gyrA* and *gyrB* mutations were selected independently in this study by using nalidixic acid and coumermycin, respectively.

inferred from studies in which nalidixic acid or related compounds inhibit plasmid or chromosomal transfer (5, 6, 7, 9, 12, 14). However, nalidixic acid may act in some cases by means other than simple antagonism of enzymatic activity (15).

The bacterial strains and plasmids used are listed in Table 1. Drug-resistant recipient bacteria were selected on Luria (17) agar containing either coumermycin A1 (20 µg/ml), kasugamycin (200 µg/ml), or nalidixic acid (20 or 100 µg/ml). *gyrB* (83-min) mutations were confirmed by P1 *vir* cotransduction (17) with *dnaA* (83 min), and *gyrA* (48-min) mutations were confirmed by cotransduction with *glpT* (49 min) (4).

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TABLE 2. Effects of coumermycin A1 on transfer of plasmid R64drd-11

Donor strain[a]	No. of transconjugants/100 donors with coumermycin at (μg/ml)[b]:		% Drug-free control[c]
	0	15	
NI747 gyrB+	1.4 ± 0.50	0.16 ± 0.084	9.7 ± 3.1
NI748 gyrB (Cou^r)	0.52 ± 0.07	0.16 ± 0.065	28 ± 9.3

[a] Recipient strain CT31 gyrB (Cou^r).

[b] All values are mean ± standard error of the mean of five separate experiments.

[c] $P = 0.055$ by paired Student's t test; values determined as the mean and standard erorr of the mean of the values calculated separately for each of five experiments.

Conjugation was carried out in Luria broth. Coumermycin (or dimethyl sulfoxide diluent in controls) was added or the temperature was shifted immediately before or at the time of mixing donor and recipient strains in a ratio of 1:9. After incubation for 30 min, mating pairs were mechanically disrupted for 10 s (16). Transconjugants were selected with tetracycline (10 μg/ml), and counterselecting agents included streptomycin (100 μg/ml), kasugamycin (200 μg/ml), or nalidixic acid (20 μg/ml). Transfer was expressed as number of transconjugants per number of donors at the end of the incubation. In general, the decrease in viable donor bacteria in drug-treated mixtures relative to the control mixtures was <50%.

Hfr interrupted matings were performed as described elsewhere (12). Twenty minutes after mixing of donors and recipients, either nalidixic acid (20 μg/ml) or coumermycin (10 μg/ml) was added to flask A and diluent was added to flask B of a pair of mixtures. Thirty minutes later, samples from flask A and B were each diluted 1,000-fold into drug-free broth (A-1, B-1) and broth containing drug at the same concentration (A-2, B-2). The time of gene entry was extrapolated from the graph of numbers of recombinants versus time.

Transfer of plasmid R64drd-11 was inhibited by coumermycin (15 μg/ml) (Table 2). Inhibition was less in a gyrB (Cou^r) donor bacterium than in a gyrB+ donor. Substantial inhibition remained, however, when donor and recipient strains were both gyrB (Cou^r). One possible explanation is that coumermycin might also inhibit plasmid transfer by mechanisms in addition to gyrase antagonism. Coumermycin (20 μg/ml) also inhibited by 80% transfer of plasmid F' lac from JC6526 to CSH50-1 (data not shown) and by >99% transfer of Hfr Cavalli (Fig. 1). Thus, inhibition of conjugation was not restricted to R64drd-11.

To circumvent possible confounding drug effects, the effect of temperature on transfer of R64drd-11 in gyrB(Ts) strains was determined (Table 3). Transfer from a gyrB(Ts) donor decreased 13-fold at an elevated (nonpermissive) temperature, but that from a gyrB+ donor decreased only 33%. The ratio of transfer frequency at high versus low temperature differed for gyrB+ and gyrB(Ts) donors about 10-fold, suggesting that a functioning DNA gyrase B subunit in the donor was important for normal transfer of R64drd-11. The residual 7% transfer seen at the nonpermissive temperature in the gyrB(Ts) mutant might result from leakiness of the gyrB(Ts) mutation. Also noteworthy in Table 3 is the fivefold difference in transfer frequency between the gyrB+ and gyrB(Ts) donors at the permissive temperature, further supporting an involvement of the gyrase B subunit in the
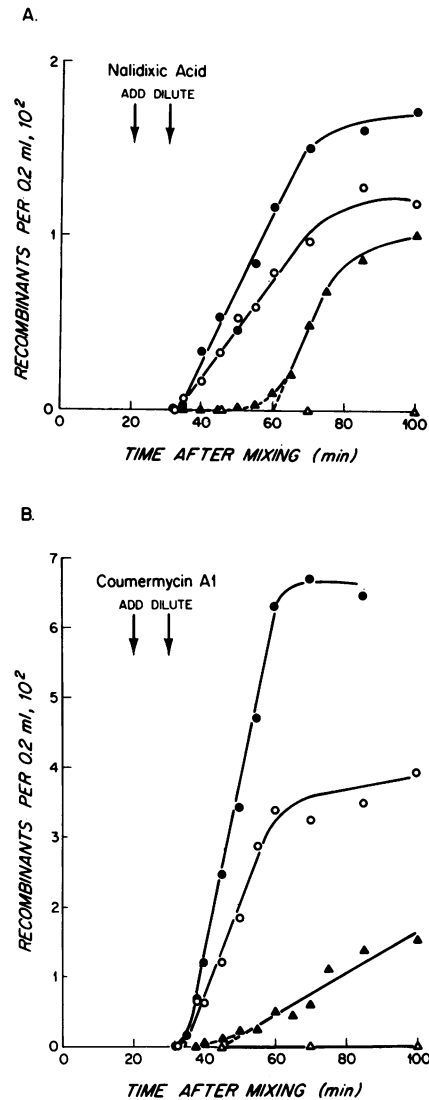

FIG. 1. Kinetics of reversibility of nalidixic acid (A) or coumermycin (B) inhibition of bacterial conjugation. (A) Hfr Cavalli X CT47 gyrA (Nal^r); nalidixic acid added at 10 μg/ml. (B) Hfr Cavalli X CT46 gyrB (Cou^r); coumermycin added at 20 μg/ml. Donors and recipients were mixed at $t = 0$ min, and the mixtures were divided into four portions. Drug was added to two portions of the mixtures at $t = 20$ min; at $t = 30$ min the portions were diluted 1,000-fold, one into broth containing drug (△) and the other into drug-free broth (▲). Diluent was added to the other two portions at $t = 20$ min; at $t = 30$ min, these portions were diluted 1,000-fold into fresh broth (●) or broth containing 1,000-fold dilution of drug (○). Samples were plated at intervals, selecting for Arg+ Str^r recombinants. - - - -, Extrapolation to the time of first entry of the argE marker.

donor in R64drd-11 transfer. The 100-fold difference in transfer frequencies of R64drd-11 in the N99 (Table 3) and NI747 (Table 2) genetic backgrounds was independent of the gyrB mutations and was not studied further.

The role in conjugation of the DNA gyrase B subunit in the recipient bacterium was less clear (Table 3). The ratio of transfer frequency at high and low temperatures differed only threefold in the gyrB(Ts) mutant and its parent. Thus, R64drd-11 transfer is more sensitive to the action of the gyrase B subunit in the donor than in the recipient bacterium.

TABLE 3. Effects of thermal inactivation of the gyrase B subunit
on transfer of plasmid R64drd-11

Strains		No. of transconjugants/10,000 donors at incubation temp of:		40°C/30°C (%)
Donor	Recipient	30°C	40°C	
N99 gyrB⁺	CT36 gyrB⁺	0.20 ± 0.10ᵃ	0.15 ± 0.11ᵃ	67 ± 15ᵇ
N4177 gyrB(Ts)	CT36 gyrB⁺	0.93 ± 0.38ᵃ	0.073 ± 0.046ᵃ	7.3 ± 2ᵇ
N99 gyrB⁺	CT34 gyrB⁺	0.32 ± 0.10ᶜ	0.57 ± 0.20ᶜ	220 ± 66ᵈ
N99 gyrB⁺	CT32 gyrB(Ts)	0.34 ± 0.10ᶜ	0.20 ± 0.04ᶜ	77 ± 30ᵈ

ᵃ Values are mean ± standard error of the mean of three separate experiments.

ᵇ P = 0.047 by paired Student's t test; values calculated as indicated in Table 2.

ᶜ Values are mean ± standard error of the mean of four separate experiments.

ᵈ P = 0.035 by paired Student's t test; values calculated as indicated in Table 2.

After inhibition by nalidixic acid, Hfr transfer resumes with a timing suggesting reinitiation from the origin of transfer (oriT) rather than from the point of inhibition (6, 12). Nalidixic acid (12) or coumermycin added shortly after entry of a genetic marker inhibited Hfr transfer as measured by the number of recombinants attained at plateau 10 min later. With gyrA (Nalʳ) or gyrB (Couʳ) donors, respectively, the effects of nalidixic acid and coumermycin were abolished (data not shown), indicating the involvement of the respective gyrase A and B proteins in drug action. The gyrB (Couʳ) mutation in this experiment abolished the effect of coumermycin more than did another gyrB allele used in the experiments in Table 2. Whether this difference results from allelic differences or from the shorter periods of exposure of the Hfr matings to coumermycin is uncertain.

When nalidixic acid was added after formation of mating pairs containing a gyrA⁺ donor and diluted to a subinhibitory concentration before the entry of the argE⁺ marker, argE transfer resumed at a time (t = 60 min) approximating the sum of the time of drug dilution and the normal entry time (30 + 32 min) (Fig. 1A), suggesting reinitiation of transfer at oriT. In contrast, argE transfer resumed after coumermycin treatment (t = 40 min), for some recombinants at a time approximating the sum of the time of drug dilution and time of drug exposure (30 + 10 min) (Fig. 1B), suggesting resumption from the point of inhibition of transfer. These results also indicated that inhibition of transfer occurred after formation of mating pairs.

These experiments have shown that function of the gyrase B subunit in the donor bacterium is important for R64drd-11 transfer and likely for Hfr transfer. The action of coumermycin occurs after formation of mating pairs. The role of gyrase in conjugation suggests that DNA supertwisting might be important for DNA transfer. Alternatively, gyrase might function as an unwinding protein during strand transfer (20), although an F factor-encoded helicase might serve this function in Hfr transfer (1).

Coumermycin and nalidixic acid differed in their inhibition of conjugation. For Hfr Cavalli, the effect of coumermycin but not nalidixic acid was immediately reversible in some mating pairs. This finding is compatible with the hypothesis that formation of drug-enzyme-DNA complexes destructive to the growing fork occurs with nalidixic acid (15, 19), and thus transfer must be reinitiated from oriT after dilution of the drug. In contrast, coumermycin may function more simply as a reversible inhibitor of enzyme function, allowing

transfer to resume from the point of interruption. Alternatively, these differences might result from the different consequences of antagonizing the A and B subunits of DNA gyrase. Additional studies are needed to understand in greater detail the molecular events responsible for these differences and the exact role of DNA gyrase in conjugation.

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