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Effect of 1,10-Phenanthroline on Bacterial Conjugation in *Escherichia coli* K-12: Inhibition of Maturation from Preliminary Mates into Effective Mates

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The addition of 1 mM orthophenanthroline (OP) into a mating mixture drastically reduced the production of recombinants. Examination of the effect of OP on each step of conjugation showed that the effect on the following steps could not account for the up to 500-fold reduction of recombinant formation: (i) preliminary mate formation and (ii) deoxyribonucleic acid transfer and integration. Taking these results and additional experiments together, we conclude that OP inhibits the maturation of preliminary mates into effective mates. Kinetic experiments showed that there were two phases in the maturation of preliminary (OP-sensitive) mates into effective (OP-resistant) mates. The half-time (the time required to reach 50% OP-resistant mates) was 2.5 min for the first phase and 4 min for the second phase, with an overall half-time of 7.5 min. In contrast, only 3 min was required to reach 50% Zn^{2+}-resistant mates. The difference in half-times suggests that there is an intermediate step involved to form an effective mate from a preliminary mate.

The first step in bacterial conjugation is the collision of a female cell and an F pilus, the threadlike sex organelle of a male cell (4), to form a mating pair (3). This step can be inhibited by 1 mM Zn^{2+}, which is shown to act on the tip of the F pilus (19, 20). On the other hand, when F^- cells are grown in the presence of 1 mM Zn^{2+}, their ability to form mates (this term is used instead of the traditional term "mating pair" in view of the finding that many aggregates are formed in a mating mixture; see reference 1) increases (15). These two seemingly different effects of Zn^{2+} on two different cell surfaces can be explained as follows. Zinc ion is involved in the formation of receptor sites on the surface of the female, and the initial contact by F pili is between their tips and the Zn^{2+} of receptor sites. Thus, when there is an excess of Zn^{2+} in the mating medium, it competes for the tips of F pili and blocks the tips of F pili to reach receptor sites on the female. When F^- cells are grown in the presence of added Zn^{2+}, they increase receptor sites by incorporating Zn^{2+} and thus effectively compete for the tips of F pili to form mates. One way to test this hypothesis is to use chemicals that bind Zn^{2+} and thus block the availability of Zn^{2+} in the receptor site to F pili. One such chemical, a chelating agent, orthophenanthroline (OP) (1,10-phenanthroline), was chosen for its high affinity to Zn^{2+} to check the above hypothesis. The investigation presented here reveals that OP drastically reduces the recombinant production by primarily acting at the first step of conjugation, the formation of mates.

It is thought that there are two classes of mates in a mating mixture (5, 7): (i) a preliminary (specific) mate, which is a simple union between a female and a male cell and is thought to be the very first stage in conjugation; and (ii) an effective mate, which is defined as the mating pair that is ready to transfer or is transferring donor deoxyribonucleic acid (DNA) and is thought to be matured from the preliminary mate. Our examination suggests that 1 mM OP principally inhibits the maturation process of a preliminary mate into an effective mate, although at 1 mM it also inhibits the formation of preliminary mates, but with less efficiency. Furthermore, kinetic measurements indicate that the maturation process may involve several steps; we found at least two steps in this investigation: (i) a mate sensitive to 1 mM Zn^{2+} became Zn^{2+} resistant and (ii) then matured into an OP-resistant mate that allowed DNA transfer to occur.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains, which are all derivatives of *Escherichia coli* K-12, are listed in

Table 1. All bacterial strains were routinely grown in nutrient broth (20).

Media. All media have been described (20). OP (Fig. 1) and metaphenanthroline (MP) (1,7-phenanthroline; Fig. 2) were obtained from the G. Frederick Smith Chemical Co., Columbus, Ohio. Ten millimolar solutions of these drugs were made as the stock solutions.

Mating procedures. A 30-min mating technique was generally used as follows: male and female cells were grown to approximately 4×10^8 cells/ml, and equal volumes of the cultures of opposite sex were mixed. At 30 min after mating, samples were vigorously blended with the device invented by Low and Wood (14) to interrupt the mating and were plated onto appropriate selective plates for scoring recombinants. For interrupted matings and the measurement of mates that eventually produced recombinants, the DeHaan and Gross 5-min pulse-mixing technique (9) was used. For the determination of the effect of the drug on integration, the technique devised by Tomizawa (23) was used. All experiments were carried out at 37°C. The Coulter counter measurement of mate formation has been described (16).

RESULTS

Reduction in the frequency of recombinant formation by OP. The final step in an Hfr cross usually is the formation of recombinants. To determine the effect of OP, which binds Zn^{2+} with a binding constant of $K_1 = 6.4$ mol^{-1} (22), several crosses using a number of Hfr and F^- strains with and without OP were made and the frequencies of recombinant formation were measured. The results (Table 2) show that: (i) OP indeed affected conjugation by reducing up to 500-fold the frequency of recombinant formation; (ii) the effect was proportional to the concentration of OP added; and (iii) the effect of OP was not confined to a particular strain, but rather OP affected equally at least the four strains of Hfr and F^- used here.

Among the three concentrations of OP used, the highest effect was obtained at 1 mM. At this concentration, the growth of bacteria was halted (see below), but the bacteria were not killed. Therefore, 1 mM OP was used throughout the present investigation.

Effect of OP on the formation of mates as measured by a Coulter counter. Since OP strongly chelates Zn^{2+} and Zn^{2+} was suspected to be involved in the mate formation, we measured the kinetics of mate formation with and without 1 mM OP by a Coulter counter. The frequency of mate formation measured by a Coulter counter is obtained by taking the difference in the number of electrical pulses, monitored at times 0 and t, divided by the input number of the minority parent (14): $(P_t/M_0) = (I_0 - I_t)/M_0$, where P_t is the number of cells involved in mate formation, M_0 is the number of input minority parent (usually male cells), I_0 is the number of total cells in a mating mixture measured at time zero, and I_t is the number of cells in the mating mixture measured at time t. Therefore, this method does not measure the number of mating aggregates, but rather the mate formation as mating pairs. We shall therefore treat them as if all are mating pairs. This is not entirely inappropriate, for each reduction of a pulse is due to the attachment of a male or a female cell to a cell of opposite sex, presumably via an F pilus. Thus, the frequency

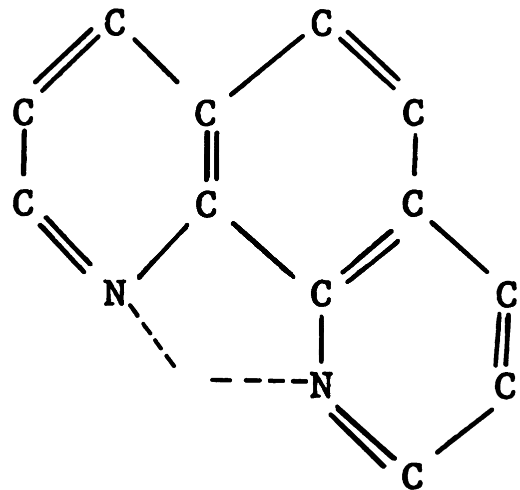

FIG. 1. *Structure of orthophenanthroline (1,10-phenanthroline).*

TABLE 1. *Escherichia coli K-12 strains*

Strain	Sex	Genotype[a]	Source[b]
OU11	Hayes type Hfr	*thi*$^-$	H. Marcovich
W3011	Cavalli type Hfr	*ton*$^-$	E. Lederberg
W1-3	F^-	*thi*$^-$ *leu*$^-$ *lac*$^-$ *tsx*$^-$ *str*$^-$	J. Tomizawa[c]
W1-4	F^-	*thi*$^-$ *proA*$^-$ *lac*$^-$ *tsx*$^-$ *str*$^-$	J. Tomizawa[c]
AB2102	F^-	*thi*$^-$ *mtl*$^-$ *mal*$^-$ *his*$^-$ *trp*$^-$ *gal*$^-$ *lac*$^-$ *proA*$^-$ *thr*$^-$ *leu*$^-$ *tsx*$^-$ *str*$^-$	E. A. Adelberg

[a] For the genetic symbols, see reference 22.
[b] See reference 2 for additional information on the pedigrees of strains.
[c] See reference 23.

of mate formation measured by a Coulter counter is essentially the frequency of F pili involved in the formation of mates.

A typical result is presented in Fig. 3. The formation of mates was inhibited to about 70% of control. In general, the inhibition of mate formation by OP measured at the plateau was 15 to 40%, which was not large enough to account for the drastic reduction in the recombinant frequencies obtained above. Therefore, in addition to affecting the formation of mates, OP appeared to affect some other conjugation steps.

Nature of mates formed in the presence of OP and the effect of OP on preformed mates. To find which conjugation step other than the formation of preliminary mates is affected by OP, we next examined the ability of the mates formed under various conditions of OP treatment to form recombinants. Male and female cells were mated with and without 1 mM OP for 5 min; the mating mixtures were then diluted 10^3-fold into broth with and without 1 mM OP to prevent further mate formation (9). At 30 min after mixing, the mating mixtures were blended to interrupt the mating, and samples were plated on appropriate plates for scoring the recombinants (Table 3). When the mating mixture was exposed to OP after mates were formed without OP (column B), the recombinant frequencies were reduced to 10 to 25% (column B/A) of control (column A). This fre-

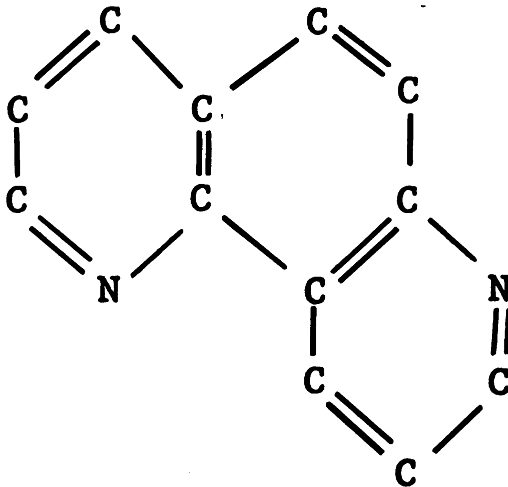

FIG. 2. *Structure of metaphenanthroline (1,7-phenanthroline).*

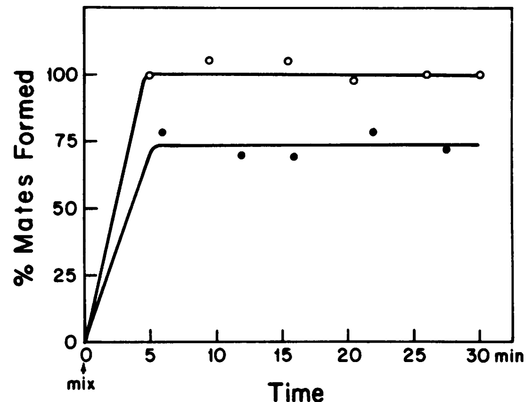

FIG. 3. *Effect of OP on the formation of mates as measured by a Coulter counter. Exponentially growing cells (~4 × 10^8 cells/ml) of opposite sex type (OU11 and W1-3) were mixed with and without 1 mM OP and incubated further with gentle shaking. At intervals, samples were taken and diluted gently, and the numbers of cells were monitored as electrical impulses in a Coulter counter. The number of mates was calculated from the formula previously described (16). Symbols: (○) Control (no OP); (●) with 1 mM OP.*

TABLE 2. *Reduction in the efficiency of Lac^+ Str^R recombinant formation by addition of OP into the mating mixture*

Expt no.	Donor Hfr	Recipient F⁻	Recombinant efficiency control (no OP) (recombinants/100 donors)	Recombinant efficiency (fraction of control)		
				0.1 mM OP	0.5 mM OP	1 mM OP
1	W3011	W1-3	11	0.60	0.16	0.02
2	W3011	W1-3	19	0.53	0.02	0.007
3	W3011	W1-3	10			0.03
4	W3011	W1-3	6			0.002
5	W3011	W1-3	25			0.005
6	W3011	W1-3	23			0.005
7	W3011	W1-4	1.6	0.31	0.016	0.0018
8	OU11	W1-3	14	0.68	0.08	0.003
9	OU11	W1-3	10	0.68	0.08	0.04
10	OU11	W1-3	11	0.68	0.08	0.03
11	OU11	W1-4	16	0.61	0.09	0.008
12	OU11	W1-4	18	0.61	0.11	0.007

TABLE 3. *Inhibition of the formation of effective mates in the cross HfrW3011 × F⁻W1-3 by 1 mM OP*

Expt no.	No. of Lac+ recombinants/100 male cells[a]				Ratio			% Mate formation (II/I × 100) at 5 min[b]
	I		II					
	A (No OP)[c]	B (+OP)	C (No OP)	D (+OP)	B/A	C/A	D/A	
1	18.9	4.5	14.4	0.15	0.23	0.76	0.007	
2	6.6	0.8	5.2	0.04	0.12	0.79	0.006	
3	20.5	9.1	7.2	0.02	0.44	0.35	0.001	
4	17.9	3.8	7.9	0.01	0.21	0.44	0.001	
5	2.8	0.4	2.1	0.05	0.14	0.75	0.018	
6	9.6	3.0	6.3	0.11	0.31	0.66	0.011	
7	19.3	2.3	9.7	0.03	0.12	0.50	0.002	83.0
8	6.4	0.3	5.1	0.01	0.05	0.79	0.001	60.3
9	10.2	3.5	7.4	0.30	0.34	0.73	0.03	58.0

[a] I: 5-min mating in broth and then 10^{-3} dilution in broth with and without OP. II: 5-min mating in broth and OP and then 10^{-3} dilution in broth with and without OP.

[b] Measured by a Coulter counter for the samples taken from I and II mating mixtures just before dilution.

[c] Mating efficiency varied from day to day; the reasons are unknown.

quency compared with column D (also see column D/A), which is equivalent to the experiment shown in Table 2, was rather high. The recombinant frequencies of column C were obtained from those mates that were formed in the presence of 1 mM OP, but their DNA transfer and integration were allowed to proceed in the absence of OP. The recombinant frequencies were higher than those of column B. This, together with the Coulter counter measurement (experiments 7 through 9) (also see Fig. 3), suggests that those mates formed in the presence of OP went on to produce recombinants after OP had been removed. Therefore, the effect of OP seems to be reversible. This result also suggests that the reduction in recombinant frequencies shown in column B is probably not due to disruption of the mates by OP.

Effect of OP on DNA transfer. Inhibition of DNA transfer can also reduce the production of recombinants in conjugation. To check on the possibility that OP might be affecting this step, we carried out the DeHaan and Gross type (9) of interrupted matings. After 5 min of mating, the mating mixture was gently diluted 10^3-fold to prevent further formation of mates. The diluted sample was then divided into three portions, two of which had 1 mM OP added, one at 5 min and the other at 20 min after mixing. Periodically samples were taken, blended to interrupt mating, and plated on appropriate plates for scoring of recombinants. The presence of 1 mM OP did not affect (i) the marker entry time or (ii) the slope of marker entry (Fig. 4). These data suggest that neither the rate of DNA transfer nor the breakage of transferring DNA was affected. Two differences, however, can be seen: (i) the plateau value for the mating mixture with OP added at 5 min was substantially lower, and (ii) the rising time (the time required to reach the plateau from the time of the first appearance of recombinant) was somewhat shorter for the mating mixture containing OP than for the control. The plateau value and the rising time were interrelated and were affected by at least two factors: (i) the probability of breakage of transferring DNA, and (ii) the number and the distribution of mating pairs that can transfer DNA. Because neither the entry time nor the slope of marker entry was affected, it is very likely, as will be shown below, that the reduction of the plateau value and the rising time was caused by the blockage of the processess for DNA transfer in those pairs that transferred DNA late in the transfer period. Similar results were obtained when the entry curves of *pro* and *leu* (entry times obtained were 12 and 20 min, respectively) were examined using HfrW3011 as the donor.

Kinetics of forming OP-resistant mates. The above interrupted mating experiments seem to indicate that younger mates are more susceptible to the inhibitory effect of OP than more aged mates. We therefore determined the kinetics of the mates becoming resistant to OP for the production of recombinants. Male and female cells were pulse mated for 2 min and gently diluted 10^3-fold into prewarmed broth. The diluted mating mixture was immediately divided into several portions, and 1 mM OP was added at predetermined times. At 40 min after mixing, mating mixtures were blended, diluted further if necessary, and plated onto appropriate selective plates for the measurement of recombinants. The kinetic curve (Fig. 5) obtained

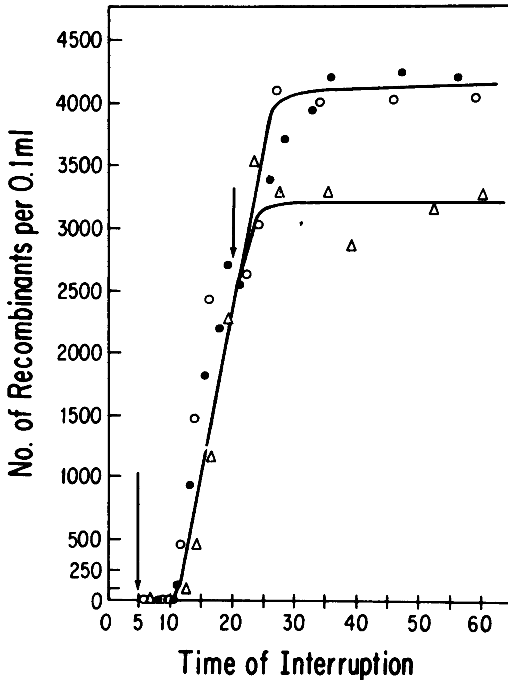

FIG. 4. *Effect of OP on DNA transfer. The donor and the recipient cells (OU11 and W1-3), exponentially growing at about 4 × 10^8 cells/ml, were mixed and allowed to form mates for 5 min. The mating mixture was then gently diluted 10^3-fold into nutrient broth and divided into three aliquots. OP was added to 1 mM to each of the diluted mating mixtures at 5 and 10 min after mixing. At intervals, 0.1-ml samples were taken, blended, and plated on the selective medium for leu$^+$ marker. Symbols:* (○) *Control (no OP);* (△) *1 mM OP added at 5 min;* (●) *1 mM OP added at 20 min.*

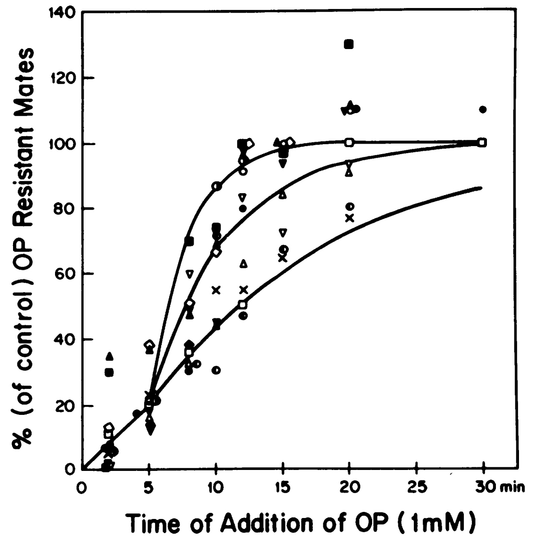

FIG. 5. *Kinetics of the formation of OP-resistant mates from OP-sensitive mates. Exponentially growing donor and recipient cells (~4 × 10^8 cells/ml) were mated for 2.0 min and then gently diluted for 10^3-fold to prevent further formation of mates. At designated times after mixing, 1 mM OP (final concentration) was added into each diluted mating mixture and incubated further. At 40 min after mixing, the mating mixtures were blended to interrupt mating and plated for measurement of recombinants. The ordinate is expressed as percentage of the recombinant efficiency of control, which was run parallel in each experiment; the abscissa is the time of 1 mM OP addition. This figure is composed of 12 independent experiments, using two Hfr (W3011 and OU11) and three F$^-$ (W1-3, W1-4, and AB2102) strains. The three theoretical curves were plotted with k = 0.346 min^{-1} (half-time = 2 min) for the upper curve; k = 0.173 min^{-1} (half-time = 4 min) for the middle curve; and k = 0.693 min^{-1} (half-time = 10 min) for the lower curve. Each symbol represents one independent experiment.*

showed two phases: (i) the number of OP-resistant mates increased linearly for 5 min to about 20% of total mates (that produced recombinants) formed; and (ii) after the first phase, the rate increased with a widely scattered range, but more steeply for the remaining 80% mates than for the mates in the first phase. Three curves were generated by a computer for the second-phase points from the formula $-dFs/dt = kFs$, where Fs is the number of F$^-$ cells in mates (in terms of mating pairs) that are sensitive to the presence of OP, t is time allowed to mate, and k is constant. Most points in the second phase fell between the upper ($t_{1/2}$ = 2 min, k = 0.346 min^{-1}) and the lower ($t_{1/2}$ = 10 min, k = 0.693 min^{-1}) curves and fit well with the middle curve ($t_{1/2}$ = 4 min, k = 0.173 min^{-1}). The half-time ($t_{1/2}$, the time required to reach 10%) was about 2.5 min for the first phase and 4.0 min for the second phase (the time required to reach 60% from 20%) when the middle curve is used. The overall half-time (the time required to reach 50%) was 7.5 min.

Kinetics of forming Zn^{2+}-resistant mates. The maturation of OP-sensitive mates into OP-resistant mates resembled the formation of Zn^{2+}-resistant mates from Zn^{2+}-sensitive mates as previously reported (20). Therefore, to determine whether the OP-sensitive mates were also sensitive to Zn^{2+}, we carried out similar kinetic experiments in which 1 mM Zn^{2+} was added instead of OP. As shown in Fig. 6, most points fell within the theoretical curves, which were, as in Fig. 5, generated by a computer from the formula shown above. If we use the center curve as the curve for these points, the time (3 min) required for 50% of the mates to become

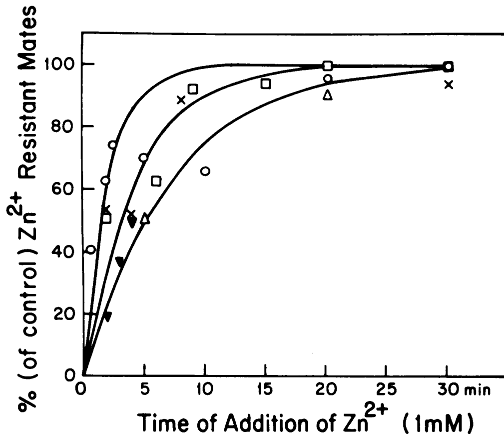

FIG. 6. *Kinetics of formation of Zn^{2+}-resistant mates from Zn^{2+}-sensitive mates in OU11 × W1-3. See Fig. 5 for the experimental procedures, except that (i) 1 mM Zn^{2+} was added instead of OP and (ii) the time allowed for mate formation was only 45 s for the experiment represented by open circles. This figure contains five independent measurements and is expressed in percentage of the recombinant efficiency of control as a function of the time of 1 mM Zn^{2+} (final concentration) addition. The three theoretical curves were plotted with $k = 0.462$ min^{-1} (half-time = 1.5 min) for the upper curve; $k = 0.231$ min^{-1} (half-time = 3 min) for the middle curve; and $k = 0.139$ min^{-1} (half-time = 5 min) for the lower curve. Each symbol represents one independent experiment.*

Zn^{2+} resistant was shorter than the 7.5 min (overall) that was required to form 50% OP-resistant mates (Fig. 5). Furthermore, the modes of maturation seem to be quite different, as indicated by the kinetic curves obtained (Fig. 5 and 6). Coulter counter examinations revealed that Zn^{2+}-sensitive mates were disrupted by the addition of 1 mM Zn^{2+}, confirming our previous observation (20). On the other hand, the addition of 1 mM OP into a mating mixture did not disrupt the preformed mates. This was expected, since mates can be formed in the presence of 1 mM OP (Fig. 3 and Table 3).

Effect of OP on integration. Inhibition at the integration step can also reduce the number of recombinants, as in the case of mating with recombination-deficient mutants (6). Therefore, the following experiments devised by Tomizawa (23) were carried out in order to determine the effect of 1 mM OP on integration of donor DNA. Thirty minutes after mixing of male and female cells, the mating mixture was blended to stop the mating and a sample was diluted 10^5-fold into broth containing 100 μg of streptomycin per ml. The diluted sample was divided into two aliquots, and 1 mM OP was added to one of them. Incubation was continued, and periodically samples were taken and plated on selective plates. Initially the number of recombinants decreased slightly when OP was added, but thereafter the number of recombinants no longer decreased (Fig. 7). The number of recombinants began to increase at about 100 min after mixing as in the control mixture. The much lower rate of increase was probably due to the effect of OP on growth, as can be seen from the growth curve of total cells. Thus, OP seems to affect integration slightly, but the extent of the inhibtion on integration cannot account for the drastic reduction shown in Table 2.

Does OP exert its effect by binding Zn^{2+}? OP is an effective Zn^{2+}-chelating agent. However the hydrophobic affinity of OP cannot be ignored. Therefore, an important question remains: does 1 mM OP affect conjugation by chelating Zn^{2+} or by its hydrophobicity? In an

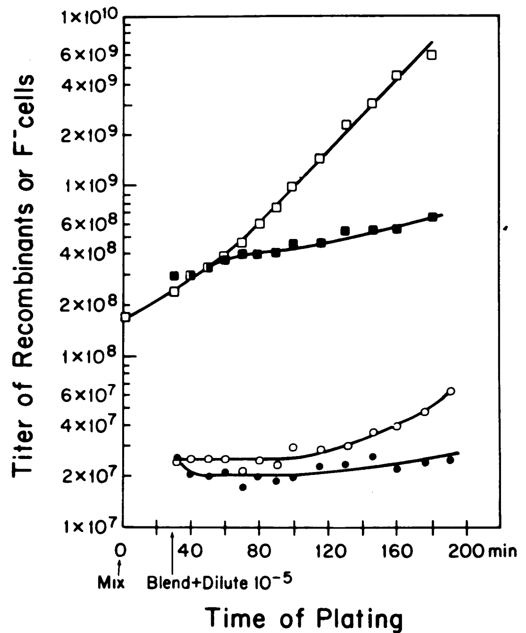

FIG. 7. *Effect of OP on integration. Exponentially growing (~4 × 10^8 cells/ml) W3011 and AB2102 were mated for 30 min without OP, and the mixture was then blended and diluted 10^5-fold into prewarmed and fresh nutrient medium containing 100 μg of streptomycin per ml to kill the donor cells. The diluted sample was divided into two aliquots, and to one of them 1 mM OP (final concentration) was added. At intervals, samples were plated for scoring Pro^+ recombinants. Upper curves were total viable cell counts: (□) without OP; and (■) with OP. Lower curves were Pro^+ recombinant counts: (○) without OP; and (●) with OP. The frequency of recombination was 13% at 30 min after mixing.*

attempt to answer this question, the following three approaches were used.

(i) Reversibility of the effect of OP by divalent ions. For this approach, direct measurements of recombinant production after 30 min of mating in various concentrations of Zn^{2+} with a fixed concentration of OP (1 mM) were carried out. As shown in Fig. 8, which is drawn by plotting the frequency of recombinant formation as a function of Zn^{2+} concentration, Zn^{2+} reversed the effect of OP, with the maximum reversal at 0.25 mM. If we used 0.33 mM Zn^{2+}, we could have presumably obtained a higher reversal than at 0.25 mM, since three OP molecules can chelate one Zn^{2+} ion. In this experiment (Fig. 8), a complete (100%) reversal was not obtained. This was expected because chelation is a reversible process and therefore, after reaching an equilibirum, there will be free Zn^{2+} and OP, both of which inhibit conjugation. Preliminary experiments showed that reversal of the OP effect could also be achieved by Co^{2+} (K_1 = 7.25 mol^{-1}) (21) and Cd^{2+} (K_1 = 6.4 mol^{-1}) (21), both of which did not inhibit mate formation as measured by a Coulter counter (unpublished data). Among the three cations (Zn^{2+}, Co^{2+}, and Cd^{2+}) tested so far, Zn^{2+} had the highest reversal effect and Cd^{2+} had the lowest. Mg^{2+} (K_1 = 1.2 mol^{-1}) was also used in a similar test but showed no effect, which was presumably due to little chelation of Mg^{2+} by OP.

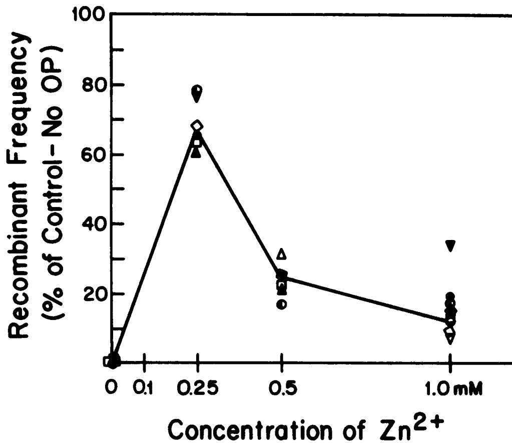

FIG. 8. *Reversal of the effect of 1 mM OP by Zn^{2+} on recombinant formation. The standard 30-min matings with and without 1 mM OP were performed, and to some of the mating mixtures containing 1 mM OP, various amounts of Zn^{2+}, ranging from 0.25 to 1.0 mM, were added. Thirty minutes after mixing, the mating mixtures were blended, diluted, and plated on selective plates for scoring appropriate recombinants. Lac⁺ recombinants were measured in W3011 × W1-3 (○, △, ●, ▲, and □) and in W3011 × W1-4 (▼ and ◇); Leu⁺ recombinants were selected in OU11 × W1-3 (■ and ▽); and Pro⁺ recombinants were scored in OU11 × W1-4 (◆ and ◐). Each symbol represents one independent experiment.*

(ii) Effect of OP in mating with Zn^{2+}-grown F^- cells. We have shown that Zn^{2+}-grown F^- cells increase their ability to form mates (15), and one explanation for this, as described earlier, may be that F^- cells incorporate Zn^{2+} to increase the receptor sites on the surface. If so, 1 mM OP should show little effect on mate formation involving Zn^{2+}-grown F^- cells. This was indeed the case when mate formation was measured by a Coulter counter. The frequency of mate formation involving Zn^{2+}-grown cells was consistently higher, even in the presence of 1 mM OP, than that of control mating using normally grown cells.

(iii) Effect of MP on mate formation and recombinant formation. Since OP is a chelating agent, the reversal by cations was expected. We therefore tested the effect of MP, an analogue of OP that has no chelating ability, on conjugation. The 5-min pulse-mating procedures of Table 3 were used for this test. The results (Table 4) show that: (i) MP had little effect on preformed mates (columns B and B/A); (ii) MP did not inhibit mate formation during the 5-min mate-formation period (columns C and C/A); and (iii) MP reduced recombinant formation to approximately 50% of the control

TABLE 4. *Effect of 1 mM MP on the formation of Lac⁺ recombinants in HfrW3011 × F⁻W1-3*

Expt. no.	No. of Lac⁺ recombinants/100 male cells				Ratio		
	5-min mating in broth		5-min mating in broth and MP				
	A (Diluted 10^{-3} in broth)	B (Diluted 10^{-3} in broth and MP)	C (Diluted 10^{-3} in broth)	D (Diluted 10^{-3} in broth and MP)	B/A	C/A	D/A
1	10.4	8.4	10.4	5.8	0.80	1.00	0.56
2	4.4	4.0	5.0	2.5	0.01	1.13	0.56
3	4.8	2.7	3.6	2.0	0.56	0.75	0.41
4	6.8	9.3	6.8	3.4	1.00	1.00	0.40
5	7.3	5.5	7.5	3.7	0.75	1.00	0.50

when MP was present for the entire 30-min mating period (columns D and D/A). These results suggest that OP affects conjugation primarily by chelating Zn^{2+}.

DISCUSSION

We have shown that OP inhibits the production of recombinants. Blockage at any known step in conjugation can reduce the production of recombinants. Therefore, we have examined the effect of OP on each conjugation step and reached the conclusion that 1 mM OP inhibits mainly the maturation of a preliminary mate into an effective mate. This conclusion is drawn from the following observations. (i) One millimolar OP reduces the formation of mates on the average only about 25% (Fig. 3). Therefore, this cannot account for a 500-fold reduction in recombinant formation. (ii) The mates formed in the presence of 1 mM OP produce recombinants when OP is removed. The removal of OP, therefore, seems to allow the resumption of the conjugation procedure. (iii) The addition of 1 mM OP does not detectably alter the transfer of DNA in any way (the rate of transfer and breakage of transferring DNA). It does, however, affect the plateau value, the final number of recombinants, and the rising time (Fig. 4). (iv) Furthermore, 1 mM OP has little effect on the integration process and reduces the production of recombinants only to about 20%, again not enough to account for the poor production of recombinants when mated in the presence of 1 mM OP.

Effective mates are derived from preliminary mates. That is to say, the formation of effective mates requires time. Thus, the fraction of effective mates (those resistant to OP) should increase as a function of time, and indeed it does (Fig. 5), with a half-time of 7.5 min (overall). However, the curve (Fig. 5) shows two phases: (i) the first 20% of the mates seem to mature linearly with a half-time of 2.5 min, and then (ii) there is a steep increase in maturation rate (half-time, 4 min) for the remaining mates (80%) to reach near completion. This suggests that most mates require some preparation to reach a critical stage from which the effective mates are quickly formed. The kinetics of maturation from OP-sensitive to OP-resistant mates differ considerably from the Zn^{2+} maturation kinetics (Fig. 6). The latter case seems to follow a simple exponential curve, with a half-time of about 3 min and a k value of 0.231 min^{-1}. These observations suggest that there may be an intermediate state between the Zn^{2+}- and the OP-resistant states that a mating pair goes through during the maturation process. Furthermore, it has been shown (16) previously that mates at an early stage can be disrupted by the addition of a high titer of the male-specific ribonucleic acid phage MS2 (multiplicity of infection, ~400) and that these MS2-sensitive mates mature into mates that are resistant to a high titer of MS2. Presumably the bacteria in MS2-sensitive mates are still connected to each other by F pili, since MS2 phage adsorb to F pili for infection. The maturation of MS2-sensitive mates into MS2-resistant mates follows a simple exponential kinetics, with a half-time of 12 min. However, the MS2-sensitive mates may already be effective mates, for a separate mating pair (a visible gap between them) connected by an invisible thread—presumably an F pilus—can transfer donor DNA (18). Considering these facts together, it seems that the process between the formation of a preliminary mating pair and the formation of an effective mating pair is rather complex and may involve Zn^{2+}. This process may include: (i) generation and transmission of a mating signal (17); (ii) after receipt of the mating signal, the start of protein synthesis for DNA transfer by the donor (8, 10, 13); (iii) the nicking at the transfer origin for DNA transfer (11, 12); (iv) the formation of a passageway between the donor and the recipient cells for DNA to go through (17); (v) formation of close mates that do not expose F pili among them (16, 18); and (vi) the actual transfer of DNA. At this moment we do not know which event OP inhibits, but we do know that OP inhibits the maturation, probably by binding Zn^{2+} on the recipient, since we have shown previously in a set of experiments (the reversal by cations that bind to OP, the ineffectiveness of MP and the Zn^{2+}-grown females) that OP affects conjugation by chelation. We now have additional data to show that OP also effectively inhibits mate formation when a higher concentration is used (manuscript in preparation). Thus, Zn^{2+} seems to play an important role in the early phase of conjugation.

The presence of the maturation step from the preliminary mating-pair stage into the effective mating-pair stage has long been postulated (5, 7). DeHaan and Gross (9) have shown its existence indirectly. We have now shown that, using either 1 mM Zn^{2+}, 1 mM OP, or a high titer of MS2 (16), one can demonstrate directly the maturation step. The order of maturation seems to be: preliminary mates, Zn^{2+}-resistant mates, OP-resistant mates, and then MS2-resistant mates.

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