POLYCATIONS WHICH DISORGANIZE THE OUTER MEMBRANE INHIBIT CONJUGATION IN *ESCHERICHIA COLI*

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Outer membrane disorganizing polycation, polymyxin B nonapeptide (PMBN), reduced drastically the production of recombinants when present at sub-MIC concentrations during F′-mediated *Escherichia coli* conjugation. The decrease of recombinants was accompanied by a less marked decrease of viability in the recipient population in a manner resembling lethal zygosis. No reduction was seen when either donor or recipient was grown in the same PMBN concentration, washed and resuspended to PMBN-free medium before mating. The same concentration of outer membrane disorganizing polycations of higher bactericidal activity (protamine and polylysine) caused only a moderate reduction in transconjugant frequency when present during mating. Spermine and tetralysine, which are not effective disorganizers of the outer membrane, did not reduce the recombinant frequency or the viability of the recipients.

Conjugation is a natural recombination process of enteric bacteria including *Escherichia coli*. The best characterized conjugation system is F-plasmid-mediated conjugation in *E. coli* (reviews[1,2]). Cells carrying the F-plasmid can initiate DNA transfer only when the *tra* (transfer) cistrons of the plasmid are transcribed and translated to Tra proteins. Most of the 19 known Tra proteins are associated with the cell envelope; seven of them have been localized in the outer membrane (OM), and two found both the inner and outer membrane fractions.[2]

Polycations, like polymyxin B and its derivative polymyxin B nonapeptide (PMBN), cause disorganization of OM seen *e.g.* as an increased hydrophobic permeability.[3,4] It seemed therefore of interest to investigate their possible effects on conjugation.

Experimental

The conjugations were carried out in broth between *E. coli* W3747 F13 *met*$^-$ [5] which carries the F′-plasmid F13 determining lactose fermentation (Lac$^+$), and the Lac$^-$ *E. coli* W3876 *sfa*3 *lac*$^-$ *rpsL*.[6]

If not otherways stated, matings were done as follows. The donor *E. coli* W3747 (Lac$^+$ Str^s) and the recipient *E. coli* W3876 (Lac$^-$ Str^r) were grown in Penassay broth[7] to logarithmic growth phase ($1\sim2\times10^8$ viable cells per ml). One ml of both cultures were then mixed and the mating was allowed to continue for 30 minutes at 37°C without shaking. The matings were stopped by vortexing with the highest speed for 10 seconds, and Lac$^+$ recombinants were then selected by plating appropriate dilutions on EM (eosine-methylene blue) minimal medium plates containing lactose and streptomycin.[8] The recombinant frequencies are given as the number of Lac$^+$ recombinants/number of donor cells in the mating mixture before conjugation.

PMBN was prepared from polymyxin B sulfate as described earlier.[9] Protamine chloride (from salmon sperm, grade V), spermine hydrochloride, poly-L-lysine hydrobromide (type II; degree of polymerization 20), and tetralysine hydrochloride were from Sigma (St. Louis, Mo., U.S.A.).

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Results

PMBN Inhibits Conjugation but only when Present in the Mating Medium

The presence of PMBN (10 μg/ml) in the conjugation mixture (Table 1) reduced the frequency of Lac$^+$ recombinants to 20 per cent of that obtained without PMBN (6.0×10^{-5}). This reduction was not due to changes in the synthesis of functional sex pili. When the donor was grown in the absence or presence of PMBN (10 μg/ml), washed and mated in its absence, the recombinant frequency was in both cases essentially the same (8.5×10^{-5} *versus* 8.7×10^{-5}). Growth of the recipient in the absence or presence of PMBN (10 μg/ml) did not affect the recombinant frequency either (5.0×10^{-5} *versus* 6.0×10^{-5}).

PMBN Decreases the Viability of the Mating Suspension

Accordingly, PMBN must be present in the mating mixture during conjugation to reduce the production of recombinants. Thus, the possible mechanisms of action could include the interference with DNA transfer, expression or establishment of the F13 plasmid, or the reduced viability of the conjugating recipient cells analogous to "lethal zygosis" earlier described in matings with a large excess of Hfr-donor cells.[10] The increased sensitivity of conjugating recipient cells seemed the most plausible explanation and experiments were designed to test this. In these experiments the conjugation mixtures were gently shaken (60 rpm), because aeration has been reported to stimulate lethal zygosis.[10]

PMBN (10 μg/ml) in the conjugation mixture reduced the frequency of Lac$^+$ transconjugants to 0.3 per cent of the control (Table 2). At the same time, the total number of cfu in the suspension decreased by half. A smaller concentration of PMBN (3 μg/ml) had a similar but smaller effect, reducing transconjugant frequency to 7 per cent and viable count to 74 per cent. Also other OM-disorganizing agents at 10 μg/ml reduced the transconjugant frequency but the effect was smaller, to 23 per cent by polylysine (lysine$_{20}$) and to 43 per cent by protamine. Lysine$_{20}$, like PMBN, reduced also the viability of the conjugating cells. Tetralysine and spermine which are not effective OM-disorganizers[4] did not inhibit conjugation or reduce the viability of the cells, even when used at 100 μg/ml (Table 2).

The antibacterial action of the polycations used has been studied earlier in some detail.[4,5] The

Table 1. The effect of PMBN (in the mating medium, or in the growth medium of either parent) on the frequency of Lac$^+$ recombinants in conjugation between *Escherichia coli* K-12 strains W3747 (F13) and W3876.[a,b]

	PMBN (10 μg/ml) in medium during			Recombinant frequency ($\times10^{-6}$)
	Growth of donor	Growth of recipient	Mating	
Expt 1 Control	−	−	−	60.1
PMBN-for-mating	−	−	+	11.8
Expt 2 Control	−	−	−	85.1
PMBN-for-donor	+	−	−	87.2
Expt 3 Control	−	−	−	50.1
PMBN-for-recipient	−	+	−	60.4

[a] The conjugations were done as described in the Experimental.

[b] When donor or the recipient was pregrown with PMBN, they (and their controls) were washed once and resuspended in the original cell density in Penassay broth.

Table 2. The effect of polycations on the frequency of Lac$^+$ recombinants and on the viability of cells in conjugation.[a]

Polycation present in the mating medium	Concentration of the polycation (μg/ml)	Recombinant frequency ($\times 10^{-6}$)	Viable count ($\times 10^8$/ml)
—	—	37.7	3.5
PMBN	3	2.6	2.6
	10	0.13	1.5
Protamine	3	41.7	2.9
	10	16.2	3.1
$Polylysine_{20}$	3	31.0	2.5
	10	8.6	1.8
Tetralysine	3	30.4	2.5
	10	31.8	2.5
	100	31.7	3.3
Spermine	3	41.5	3.0
	10	36.5	3.1
	100	52.2	3.6

[a] The conjugations were made as in Table 1, but the mating mixtures were shaken in a rotatory shaker (60 rpm) during conjugation. The total number of viable bacteria in the mating mixtures was assayed at the end of the conjugation by plating on L-agar[19] plates.

Table 3. The effect of PMBN on the viability of donors and recipients during conjugation.[a]

Concentration of PMBN in the mating medium	Recombinant frequency ($\times 10^{-6}$)	Total number of viable cells ($\times 10^8$/ml)	Number of viable recipient cells ($\times 10^8$/ml)	Calculated number of donor cells ($\times 10^8$/ml)	Conjugants per recipient cell ($\times 10^{-6}$)
—	29.30	4.07	1.41	2.66	46.80
1	12.70	3.14	0.79	2.35	36.20
3	1.16	3.19	0.31	2.88	8.39
10	0.36	2.07	0.26	1.81	3.08

[a] The conjugations were performed as in Table 2. The number of viable recipient cells in the mating mixtures at the end of the conjugation was assayed on L-agar plates containing streptomycin (100 μg/ml).

minimal inhibitory concentration (MIC) for protamine was 3 μg/ml, for $lysine_{20}$ 30 μg/ml and for PMBN, spermine and tetralysine over 100 μg/ml. Thus, the moderate reductions in recombinant frequencies in near to MIC concentrations of protamine or polylysine (Table 2) could be suggested to result from their bactericidal effects on the parents. However, as for the drastical reduction detected in sub-MIC concentrations of PMBN this explanation did not seem plausible. The effect of OM-disorganizing polycations on conjugation was thus investigated further with PMBN present in the mating medium.

PMBN Kills Mating Recipient Cells

The simultaneous reduction in transconjugant frequency and viability in conjugation mixtures with PMBN present was investigated by following the viability of the recipient populations (Table 3). The presence of PMBN (3 μg/ml) during mating reduced the cfu of the recipient population to 20 per cent (from 1.41 to 0.31×10^8), which accounted for the entire decrease in viability of the mating mixture. During these conditions the recombinant frequency was reduced to 4 per cent of the control. At high PMBN concentration (10 μg/ml), the corresponding decreases were to 18% (recipients), and to 1% (recombinants).

Discussion

Several earlier investigations have shown the effect of the extracellular milieu on the transfer frequency in F-type mating in *E. coli*. High solute concentrations (200~300 mM NaCl or 7.2~10.8% *myo*-inositol) reduce the transfer frequency possibly by affecting the cell envelope adhesion sites, from which the F-pili arise.[11] Zn^{2+} ions, which bind to the tips of F-pili, reduce the production of exconjugants in 1 mM concentrations by preventing the formation of mating pairs.[12] The temperature dependent formation of mating pairs is inhibited also by male specific phages, sex pili antisera or when the donor cells are treated with periodate.[13~16] The maturation of the mating pair capable of DNA transfer is inhibited by chemicals binding Zn^{2+}.[17] Furthermore, the presence of excess Hfr-donor activity reduces the recombinant frequency because of a lethal action on the recipient (lethal zygosis).[10]

The inhibitory action of PMBN — and possibly also of protamine and $lysine_{20}$ — on conjugation had the following characteristics: i) The inhibitory effect was detected in Penassay broth (55 mM NaCl) at low polycation concentrations (10 μg/ml). ii) It was accompanied by a decrease in viability of the recipient. iii) The function of the sex pili or their receptors were probably not effected.

Accordingly, the most likely mechanism of PMBN-mediated inhibition of conjugation is damage to the OM of the recipient cell. Co-operative action of PMBN and donor activity may result into disturbed physiology in a fashion resembling lethal zygosis. The OM-disorganizing properties of polycations were essential to their ability to inhibit conjugation. Polycations without the OM-disorganizing ability (tetralysine and spermine) did not reduce the recombinant frequency.

PMBN, which is a derivative of an antibiotic made by *Bacillus polymyxa* was recently shown to inhibit mating also in *Saccharomyces cerevisiae* by interfering with the sexual agglutination.[18] Systems which inhibit the exchange and spread of genetic information between micro-organisms might be considered to have evolutionary value in nature. Mating inhibiting effects are also reported for sex pili antisera and thus the existence of a defense system which inhibits the genetic adaptation of invading micro-organisms could be suggested.[15]

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