Relationship between Cytostatic Activity of Oxazolopyridocarbazoles and Accessibility of DNA Intercalation Sites in Living Bacteria†

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ABSTRACT: The ability of oxazolopyridocarbazole (OPC) derivatives to interact with DNA in living bacteria through reversible intercalation has been determined by using as probes (i) their selective mutagenic effect on *Salmonella typhimurium* TA 1977 and TA 1537 as detected by frame-shift −1 reversion, (ii) the absence of intervention of the error-prone repair system on the mutagenic efficiency, (iii) the absence of induction of the SOS functions, and (iv) the absence of effect of *recA* and *uvrB* mutations on their bacteriostatic properties. Involvement of simple reversible intercalation as the event responsible for the bacteriostatic effect of the drugs has been further investigated by the establishment of a significant correlation between the maximum number of accessible intercalating sites in living bacteria and the bacteriostatic effect expressed in terms of the ED_{50}. This correlation has been established by using bacteria spontaneously exhibiting different sensitivities toward the drugs as well as a resistant strain obtained by adaptation in the presence of increasing amounts of isopropyl-OPC. The number of intercalating sites in living bacteria was determined by using the change in the fluorescence properties of the drugs upon binding to intercalating sites. The results obtained clearly demonstrate that the number of intercalating sites is the parameter that controls the bacteriostatic effect of the drugs, indicating that DNA is the target for these drugs and that reversible intercalation is responsible for the cytostatic effect.

Oxazolopyridocarbazoles (7,10,12-trimethyl-6*H*-[1,3]oxazolo[5,4-*c*]pyrido[3,4-*g*]carbazole) (OPC derivatives) (Figure 1) form a homologous series of intercalating compounds of varying hydrophobicity prepared through the covalent addition of aliphatic amino acids to the antitumor drug N^2-methyl-9-hydroxyellipticinium (NMHE) (Auclair et al., 1984; Gouyette et al., 1985). OPC derivatives are cytotoxic in cultured L1210 leukemia cells and bacteriostatic in various strains including *Escherichia coli Ebs* and *Salmonella typhimurium rfa* (deep rough mutants) (Auclair et al., 1984; René et al., 1985). The change in fluorescence properties of OPC upon binding to DNA has allowed quantitative measurement of drug accessibility to the intercalating sites in living bacteria (Banoun et al., 1985). Data so obtained have indicated that the bacteriostatic activity of OPC was related to their ability to fill the intercalating sites in bacterial nucleic acids, suggesting (i) that double-stranded nucleic acids are the target for these drugs and (ii) that reversible intercalation could be the sole event responsible for the toxic effect. If these assumptions are true, a significant correlation should be observed between the state of accessibility of the intercalating sites in the living cells and their sensitivity toward the drugs. In attempting to establish such a correlation, we have undertaken further investigations using the following experimental approach: (i) we have first of all verified that OPC derivatives effectively interact with bacterial DNA according to a simple reversible intercalation mode only, and (ii) we have determined the maximum number of accessible intercalating sites on OPC and isopropyl-OPC (iPr-OPC) (the least and the most bacteriostatic OPC derivatives, respectively) in a sensitive and a resistant *S. typhimurium* strain obtained by adaptation to increasing concentrations of iPr-OPC as well as in various strains spontaneously exhibiting different sensitivities toward the drugs. The occurrence of reversible intercalation in bacterial DNA has been investigated by using as probes (i) the selective reversion of the *his C3076* mutation in *S. typhimurium*, (ii) the absence of effect of the *recA* mutation on the mutagenic properties of the drugs, (iii) the inhability of the drugs to induce the SOS functions, and (iv) the absence of effect of *uvrB* and *recA* mutations on the bacteriostatic activity of the drugs.

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Materials and Methods

Chemicals. The conjugation of N^2-methyl-9-hydroxyellipticinium and the aliphatic amino acids Gly and Val was performed as previously described (Auclair et al., 1984; Gouyette et al., 1985). The corresponding covalent adducts obtained (Figure 1) are referred to as OPC (7,10,12-trimethyl-6*H*-[1,3]oxazolo[5,4-*c*]pyrido[3,4-*g*]carbazole) and isopropyl-OPC, respectively.

Bacterial Strains and Media. E. coli K12 strain AB 1157 was kindly provided by Dr. N. Otsuji (Fukuoka, Japan).

E. coli BL 101 strain was provided by Dr. B. Lambert (Villejuif, France). This strain derives from AB 1157 after a single mutation and was isolated and selected for its sensitivity to ethidium bromide; it exhibits an Ebs phenotype similar that of the *acrA* mutation (Lambert & Le Pecq, 1982).

E. coli AB 1157 R/T_7 deep rough mutant was isolated from *E. coli* AB 1157 according to van Alphen et al. (1976) (through resistance to bacteriophage T_7): the resistant colonies appeared at a frequency of 10^{-7}. We checked the deoxycholate, rifampicin, methylene blue, crystal violet, and novobiocin sensitivity of the strain AB 1157 R/T_7 and found the same pattern of sensitivity as for the *rfa E. coli* strain isolated by Coleman and Leive (1979) (data not shown).

E. coli deep rough K12 strain GY 5057 (*thr*, *leu*, *uvrB*, *tonA*, C_{21} resistant) was provided by Dr. R. Devoret (Gif-sur-Yvette, France).

E. coli K12 PQ 37 was *sfiA*::mud(*Ap*, *lac*) *cts*, *lac* Δ*U169*, *rfa*, *uvrA*.

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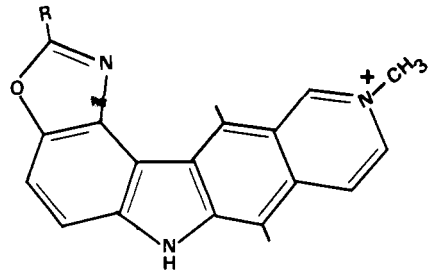

FIGURE 1: Structure of oxazolopyridocarbazole derivatives: OPC, R = –H; iPr-OPC (isopropyl-OPC), R = $-CH(CH_3)CH_3$.

E. coli K12 ES 548 [*Trp A 540, str A, lac Z (ICR 36)*] and ES 866 [*Trp A 540, str A, lac Z (ICR 36), recA*$^-_1$] (Vaccaro & Siegel, 1977) were kindly supplied by Dr. M. Radman (Paris). Permeable derivatives (to bulk and hydrophobic compounds) ES 548/7 and ES 866/7 were isolated (respectively from ES 548 and ES 866) by selecting for resistance to phage T_7 (Hancock & Reeves, 1976) and checked for crystal violet and sodium deoxycholate permeability (Ames et al., 1973).

S. typhimurium LT_2 histidine auxotrophic strains were kindly supplied by Dr. B. N. Ames (Berkeley, CA): TA 1977, *his C 3076* (+1 frame-shift mutation), *rfa*, *uvrB* wild; TA 1537, *his C 3076*, *rfa*, *ΔuvrB*; TA 1978, *his D 3052*, *rfa*, *uvrB* wild; TA 1538, *his D 3052*, *rfa*, *ΔuvrB*; TA 98, *his D 3052* (–1 frame-shift mutation), *rfa*, *ΔuvrB*, pKM 101; TA 100, *his G 46*, *rfa*, *ΔuvrB*, pKM 101; TA 102, *his D(G) 8476*, *rfa*, *uvrB* wild, pKM 101, pAQ1. These strains carry the *rfa* mutation, resulting in a deep rough phenotype (Ames et al., 1973).

Mg medium contains Na_2HPO_4 (6 g), KH_2PO_4 (3 g), NaCl (0.5 g), NH_4Cl (1 g), vitamin B_1 (5 mg), casamino acids (Difco, Detroit, MI) (3 g), glucose (2 g/L), $MgSO_4$ (1 mM), and $CaCl_2$ (0.1 mM). Oxoid nutrient broth no. 2 (for *S. typhimurium* strains) was obtained from Oxoid Ltd. (England). *E. coli* were cultured in LB medium (Miller, 1972). Lactose reversion plates were solid M9 containing 2 g/L lactose, 5 mg/L thiamine, 40 mg/L tryptophan, and 0.14 g/L D-glucose; top agar was supplemented with 40 mg/L tryptophan.

Antibacterial Tests. Inocula were grown overnight at 37 °C in oxoid medium and then diluted 100-fold in the same oxoid medium. Cultures were grown at 37 °C in Erlenmeyer flasks on a rotary shaker, up to an absorbance of 0.1 at 650 nm, and then divided into 10-mL fractions, and the drug was added. Growth was followed by monitoring the turbidity at 650 nm.

From the growth rate of each culture, the ED_{50} was determined, which is the dose of drug reducing by 50% the bacterial growth as compared to control [at ED_{50} concentrations, only bacteriostatic effects were observed as previously shown by Renē et al. (1985)].

For the viability experiments, aliquots (100 μL) of each suspension were diluted in oxoid medium, added to 2.5 mL of top agar, and plated into oxoid plates.

Mutagenic Tests. (1) Reversion of the Histidine Mutations. The Ames test (Maron & Ames, 1983) was modified as follows: bacteria were grown overnight at 37 °C in oxoid medium and then diluted (1‰) in oxoid medium and incubated at 37 °C to an absorbance of 0.1 at 650 nm (10^8 cells/mL). Then bacteria were subdivided in several samples and reincubated for 5 hours with or without drug. The samples were centrifuged and the cells resuspended in oxoid to obtain the absorbance of the control without drug. Aliquots (100 μL) of each suspension were added to 2.5 mL of top agar and plated into reversion plates as for the standard Ames test.

(2) Reversion of the Lactose Mutation. Bacteria were grown overnight in LB medium, then diluted (6‰) in M9 medium supplemented with 40 mg/L tryptophan, and incubated at 37 °C to an absorbance of 0.1 at 650 nm. Next the drug was added, and the bacteria were reincubated for 1 hour. The samples were centrifuged and the cells resuspended in M9 medium to an absorbance of 2 at 650 nm. Aliquots (100 μL) were added to 2.5 mL of top agar and plated on selective plates for lactose reversion (see above).

Determination of RecA Protein Content and of β-Galactosidase Induction. Determination of the recA protein content in *S. typhimurium* TA 1977 was performed by using an immunoradiometric assay essentially as described by Paoletti et al. (1982). Purification of recA protein from *S. typhimurium* and preparation of antibodies against recA protein were performed as described by Paoletti et al. (1982). Both recA protein and antibodies were a gift of Dr. A. Pierrē (SANOFI Co., Toulouse, France).

SOS chromotest was carried out according to published procedures (Quillardet et al., 1982). An exponential phase culture of PQ 37, grown in LB medium plus ampicillin at 37 °C, was diluted 1:10 into fresh medium. Fractions (0.6 mL) were distributed into glass test tubes containing the drug. After a 2-h incubation at 37 °C, β-galactosidase and alkaline phosphatase activities were assayed in each sample as described (Quillardet et al., 1982). Induction of the error-prone repair system was expressed as the SOS induction factor (SOSIP), which takes into account the protein synthesis inhibition checked by the activity of the constitutive alkaline phosphatase.

Preparation of S. typhimurium (TA 1977 OPCr) Resistant to iPr-OPC. Sensitive TA 1977 cells were subjected to three cycles of adaptation to iPr-OPC. One cycle consisted of the following: bacteria were grown in M9 medium up to an OD of 0.1 at 650 nm, and then iPR-OPC (2 μM) was added; after a 3-h incubation, bacteria were centrifuged, rediluted in M9 medium to an OD of 0.1 at 650 nm, and allowed again to grow for 3 h in the presence of 2 μM iPr-OPC; after this time, bacteria were centrifuged, resuspended in oxoid medium, and left to grow overnight with 20 μM iPr-OPC (in oxoid medium, OPC derivatives are about 10 times less toxic than in M9 medium). After these cycles, the TA 1977 cells acquire a high level of resistance to iPr-OPC as compared to untreated cells (the ratio of resistance was 42 as measured by cloning efficiency after 1 h of drug contact in M9 medium). One clone of highly resistant cells was isolated for the experiments described below. After several growth passages in drug-free medium, the TA 1977 resistant clone never regains sensitivity to iPr-OPC. The genetic characters, including the deep rough phenotype, in the TA 1977 resistant clone were checked and found to be the same as in the sensitive strain TA 1977.

Fluorescence Measurement of iPr-OPC in Vivo Accessibility to Bacterial Nucleic Acids. The amount of drug bound to nucleic acids is measured as previously described (Lambert & Le Pecq, 1984; Banoun et al., 1985). Bacteria were grown overnight in oxoid medium and then diluted (1%) in M9 medium and grown up to an OD of 0.1 at 650 nm; then the growth was stopped at 4 °C. At this absorbance, the number of cells is 10^8 cells/mL. Aliquots of this suspension were successively prewarmed in 1-mL quartz cuvettes. Various amounts of drug were then added, and the fluorescence increment was continuously recorded (excitation, 330 nm; emission, 550 nm) in a SFM 23/B Kontron spectrofluorometer (Zürich, Switzerland). When equilibrium was achieved, the amount of bound drug was calculated from this fluorescence increment according to Le Pecq and Paoletti (1967). The

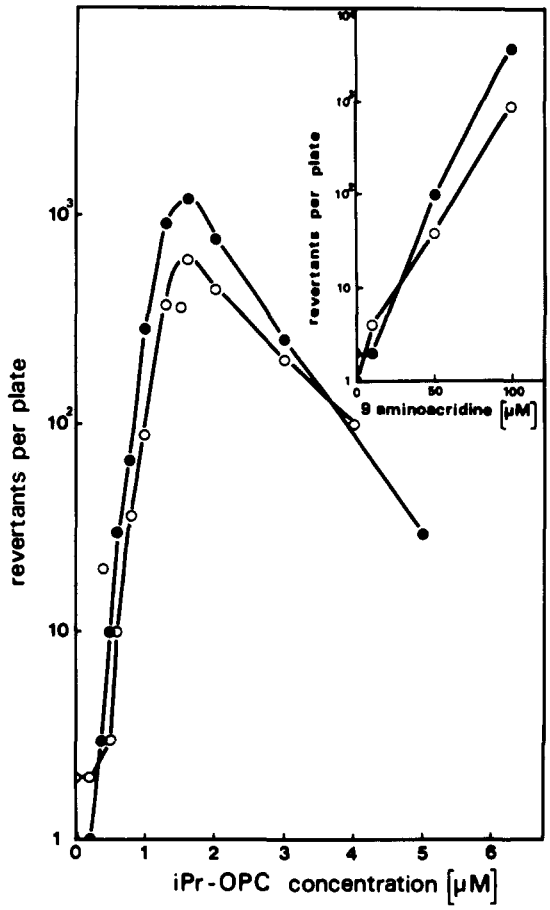

FIGURE 2: Mutagenic activity of iPr-OPC on the *lac Z* (*ICR 36*) mutation in *E. coli recA*$^+$ (●) and *recA*$^-$ (○). Drug was added to a bacterial suspension containing 10^8 bacteria/mL. After a 1-h incubation, bacteria were centrifuged, washed, and resuspended in M9 medium and plated on M9 Lac medium. Revertants were counted after 48 h of incubation at 37 °C. The inset show the mutagenic activity of 9-aminoacridine performed in similar experimental conditions.

maximum number of binding sites for each drug was obtained from the treatment of the data according to the Scatchard equation.

RESULTS

Mutagenic Properties of OPC Derivatives. Information on the nature of the interaction between OPC derivatives and bacterial DNA can be provided first of all by the determination of the mutagenic properties of the drugs (McCann et al., 1975a). This has been examined by using various *S. typhimurium* tester strains that may detect different types of mutations including frame shift −1 in TA 1977 and TA 1537, frame shift −2 in TA 98, base pair substitution in TA 100 and TA 102, and small deletions in TA 102 as well (Ames et al., 1973, 1975). The results summarized in Table I show that iPr-OPC exhibited a selective and strong mutagenic effect on both TA 1977 and TA 1537 strains exhibiting the frame shift −1 reversion. Similar behavior was observed with OPC (data not shown). As previously reported (Podger & Hall, 1985), and in agreement with the various models related to the mechanism of intercalator-induced frame shift mutations, the frequency of such a mutation should not be affected by the activity of the error-prone repair system. In order to verify this property in the case of OPC derivatives, we have investigated the recA dependence of the iPr-OPC-induced −1 frame shift mutation *lacZ* (ICR 36) in *E. coli* K12. Dose–response curves for iPr-OPC in *recA*$^-$ strains indicated in Figure 2 show

Table I: Mutagenic Activity of OPC and iPr-OPC on *S. typhimurium* Tester Strains[a]

strains	TA 100	TA 98	TA 1977	TA 1537	TA 102
OPC (μM)					
0.2	84	50	43	75	276
0.5	96	38	210	240	282
1.0	87	43	1800	640	267
5.0	76	40			210
10	64	38			208
50	52	30			196
iPr-OPC (μM)					
0.1	92	53	110	32	320
0.2	87	56	380	48	287
0.5	80	59	2800	250	234
1.0	76	50		1500	206
5.0	56	45			198
10	46	32			172
50	34	21			134
spontaneous revertants	88 ± 12	42 ± 8	8 ± 2	12 ± 2	278 ± 22

[a] Mutagenic activity was performed by using the standard plate incorporation assay essentially as described by Maron and Ames (1983). The numbers of revertants per plate are average values from at least four different experiments.

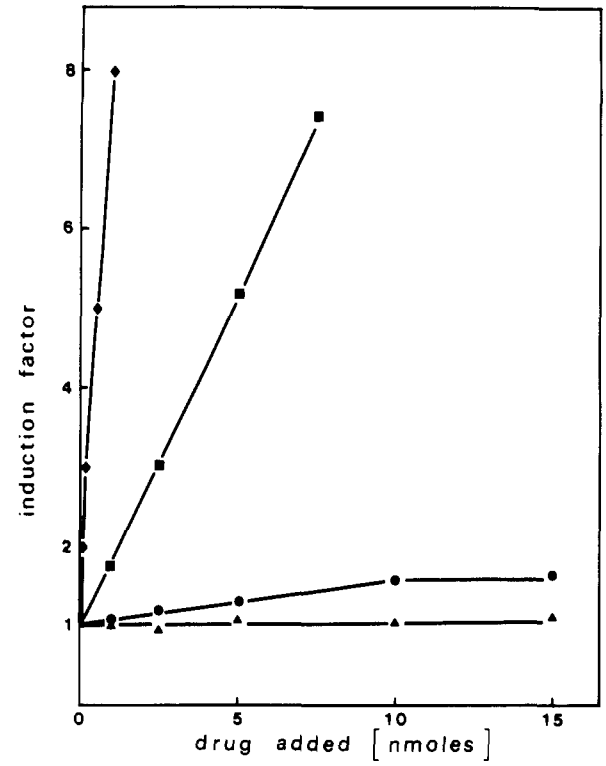

FIGURE 3: Induction of β-galactosidase activity in *E. coli* PQ 37. Determination of the β-galactosidase activity was performed as described by Quillardet et al. (1982) (see Materials and Methods). The induction factor was estimated from the increase of the β-galactosidase activity corrected for the inhibition of protein synthesis as measured by the alkaline phosphatase activity. Symbols: ▲, OPC; ●, iPr-OPC; ♦, mitomycin C; and ■, nalidixic acid.

that the mutation rates are similar in both strains. Figure 2 shows that, under identical experimental conditions, the reversible intercalator 9-aminoacridine exhibited similar behavior as described by Podger and Hall (1985).

Induction of β-Galactosidase Activity and RecA Protein. Despite the lack of effect of the *recA* mutation on both antibacterial and mutagenic activity of OPC derivatives, the drugs may induce structural and/or conformational modifications in DNA, resulting in the induction of SOS functions. The possible induction of the SOS response upon binding of OPC to bacterial DNA was first investigated by determining

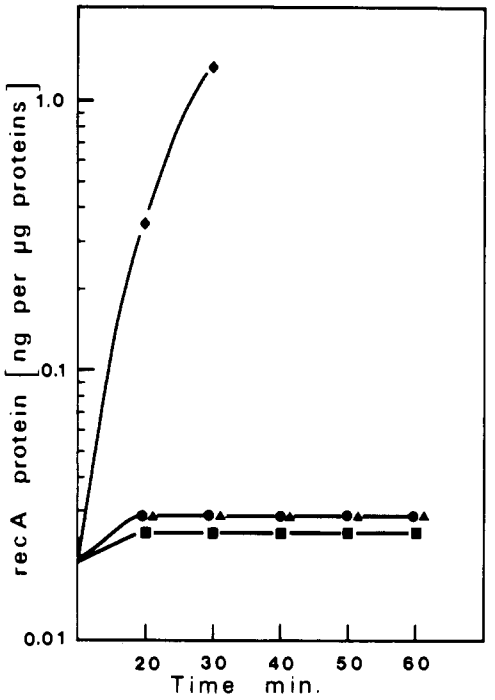

FIGURE 4: Kinetics of induction of recA protein in *Salmonella* TA 1977. RecA protein content was estimated by the radioimmunoassay technique as described under Materials and Methods. In each experiment the concentration of drug used was equal to its respective ED_{50}. Symbols: ▲, OPC; ●, iPr-OPC; ♦, mitomycin C; and ■, basal level of recA protein.

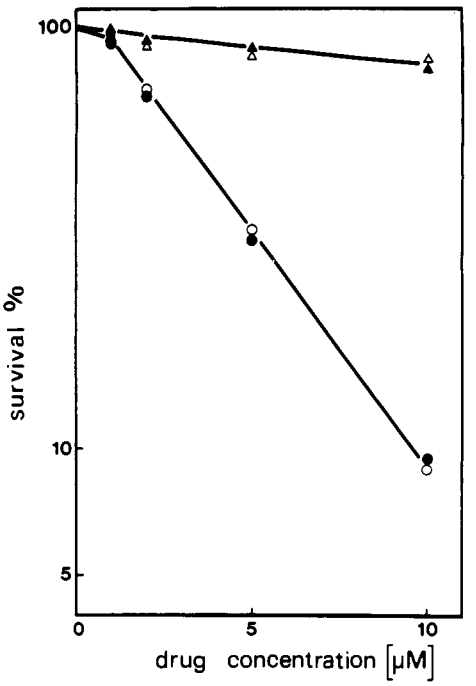

FIGURE 5: Effect of OPC (▲ and △) and iPr-OPC (● and ○) on the survival of *S. typhimurium* TA 1977 (filled symbols) and TA 1537 (open symbols). In all strains, survival was tested according to the standard plate incorporation assay.

the induction of the β-galactosidase activity in *E. coli* PQ 37 (*sfiA::lac* fusion) (Quillardet et al., 1982). Figure 3 shows that at all concentrations OPC fails to induce β-galactosidase activity whereas, in contrast, iPr-OPC produces a very slight but significant inducible effect. In similar experimental conditions mitomycin C and nalidixic acid used as positive controls markedly induce the β-galactosidase activity. In order to determine whether iPr-OPC effectively induces the error-prone repair system, we have determined the kinetics of the induction of the recA protein content in *S. typhimurium* strains TA 1977 and TA 1537 in the presence of concentrations of drugs corresponding to the ED_{50}. Results in Figure 4 show that, in these conditions, neither OPC nor iPr-OPC induces the recA protein in TA 1977 whereas, as expected, mitomycin C acts as a potent inducer. Similar results were obtained in TA 1537 (data not shown).

Effect of uvrB and recA Mutations on Antibacterial Activity of OPC Derivatives. Additional information on the nature of the interaction between OPC derivatives and bacterial DNA can be investigated by studying the effect of *uvrB* and *recA* mutations on the antibacterial activity of the drugs. For example, it is known that bifunctional agents such as mitomycin C and psoralen plus visible light as well as bulky monofunctional carcinogenic compounds such as 4-nitroquinoline 1-oxide and 7-(bromoethyl)benz[*a*]anthracene, which interact with DNA through covalent binding, are markedly more toxic in bacteria carrying mutations in genes coding for steps in the excision repair pathway (*uvr*$^-$) (Roberts, 1979). Conversely, various compounds suspected of behaving as reversible intercalating agents have been found to exhibit similar toxic effects on *S. typhimurium uvr*$^+$ and *uvr*$^-$ by using the repair test procedure (Ames et al., 1973; McCann et al., 1975b). We have previously demonstrated that OPC derivatives were strongly bacteriostatic in the *S. typhimurium* TA 98 strain, which exhibits a deletion in the *uvrB* gene (Auclair et al., 1984). In order to determine whether OPC derivatives may induce DNA lesions that can be repaired by the excision repair system, we have checked the sensitivity of two *S. typhimurium* strains TA 1977 (*uvr*$^+$) and TA 1537 (*uvrB*) to these drugs. These strains have been previously used for the determination of the mutagenic properties of the drugs. Figure 5 shows the dose effect of OPC and iPr-OPC on *S. typhimurium* TA 1977 and TA 1537. The survival curves clearly show that the drugs exhibited identical antibacterial effect on the two strains. Along the same line of evidence, and in order to ascertain that postreplication repair and/or error-prone repair cannot contribute to the recovery of the *S. typhimurium uvr*$^-$ strain from the damaging effects of OPC derivatives, we have determined the effect of iPr-OPC on the survival of *E. coli* K12 *rec*$^+$ and *recA*$^-_1$ (see Materials and Methods) since both repair systems are under the control of the *rec* genes. Both *E. coli* strains exhibited similar sensitivities to iPr-OPC. Similar behavior was observed with OPC (data not shown).

Relation between Sensitivity of Bacteria to OPC and Maximum Number of Intercalating Binding Sites. We have previously described that the addition of OPC derivatives to a suspension of living bacteria resulted in a fluorescence increment of the drugs following pseudo-first-order kinetics (Banoun et al., 1985). On the basis of the determination of fluorescence lifetimes and observation of energy transfer, this fluorescence increment has been demonstrated to result from the binding of the drug to intercalating sites in bacterial nucleic acids. At equilibrium, the extent of fluorescence enhancement allows one to determine the amount of drug bound to the intercalating sites. The variation of the amount of drug intercalated as a function of the concentration of free drug can be plotted according to Scatchard. The binding curves so obtained permit estimation of the maximum binding at saturating concentration of drug, which reflects the number of accessible intercalating sites. This parameter was first determined in *S. typhimurium* TA 1977 sensitive and resistant to OPC derivatives. The resistant strain was obtained by adaptation of the sensitive strain in the presence of increasing amounts of iPr-OPC (see Materials and Methods). The re-

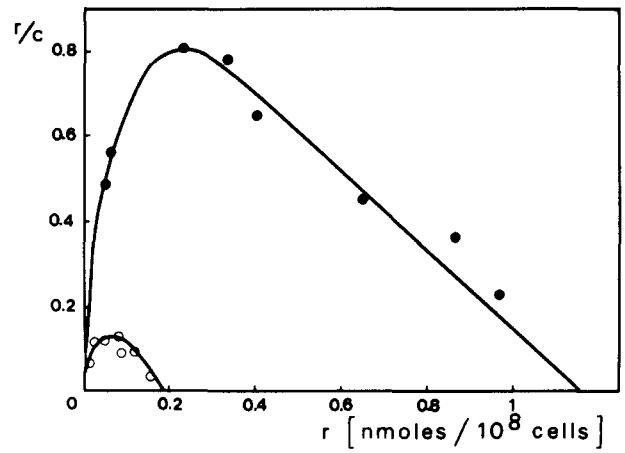

FIGURE 6: Scatchard representation of the binding curves of iPr-OPC to bacterial nucleic acids in *S. typhimurium* TA 1977 sensitive (●) and resistant (○) strains. The amount of drug intercalated in bacterial DNA was estimated by fluorescence as described under Materials and Methods.

sistance ratio obtained under the conditions used was 42. Figure 6 shows the Scatchard plots of the binding curves of iPr-OPC to bacterial DNA in the sensitive and resistant TA 1977 strains. The curves clearly show that the maximum number of accessible intercalating sites is markedly decreased (80%) in the resistant strain. The maximum number of accessible intercalating sites has been further determined in a similar way for both OPC and iPr-OPC in different strains including *S. typhimurium* TA 98 and *E. coli* BL 101, AB 1157 R/T_7, and GY 5057 that exhibit a large range of sensitivity to the drugs. Figure 7 shows the relation between the bacteriostatic activity of the drugs expressed as the ED_{50} and the maximum number of intercalating sites. The correlation coefficient estimated from the linear regression line ($r = -0.954$) is statistically significant at $p \leq 0.01$.

Discussion

The results described in this paper show, first of all, that in living bacteria the interaction of OPC with bacterial DNA results in a selective reversion of the frame-shift +1 mutation in TA 1537 and TA 1977 occurring independently of the intervention of the inducible error-prone repair system. This property can be considered as evidence of reversible intercalation (Ames et al., 1973). The absence of mutagenic effects on TA 98, TA 100, and TA 102 allows us to exclude various types of damage including covalent binding and DNA strand breaks leading to small deletions. The absence of significant induction of the error-prone repair system (as for 9-aminoacridine) (Podger & Hall, 1985) is in agreement with this assumption and suggests that no major structural modifications were introduced in the DNA by the drugs. Finally, the absence of effect of both *uvrB* and *recA* mutations on the survival of treated bacteria is also well in agreement with the absence of DNA damage. From these first series of observations, it is reasonable to assume that OPC derivatives interact with bacterial DNA through a reversible intercalation process only.

The question then arises as to whether intercalation is responsible for the bacteriostatic activity of the drugs. In such a case, intercalating sites should be considered as the target for OPC derivatives. We have previously shown that the toxic effect of OPC and iPr-OPC was related to their ability to reach the intercalating sites, which was in turn controlled by the hydrophobicity of the drugs and the phenotype of the bacteria (Banoun et al., 1985). In order to definitively demonstrate the direct linkage that may exist between intercalation and

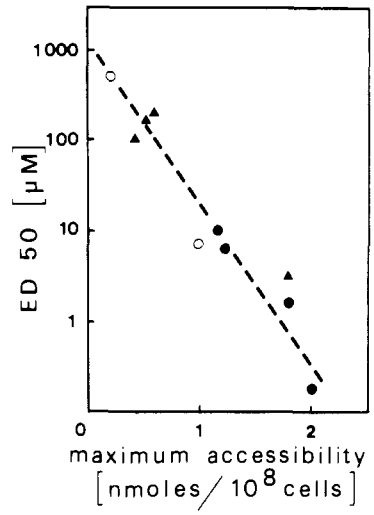

FIGURE 7: Regression line of the bacteriostatic activity as a function of the maximum number of binding sites: (▲) OPC and (● and ○) iPr-OPC; filled symbols refer to *E. coli Ebs* BL 101, *E. coli* deep rough AB 1157 R/T_7, and GY 5057, and *S. typhimurium* deep rough TA 98; open symbols correspond to sensitive and resistant deep rough *S. typhimurium* TA 1977 strains. The correlation coefficient was $r = 0.954$, significant at $p \leq 0.01$.

toxicity, a suitable strategy consists in the establishment of a significant correlation between the toxicity of the drugs and the extent of accessibility of the intercalation sites in living bacteria. Determination of the state of accessibility of intercalating sites can be achieved by Scatchard representation of the binding curves of the drugs to bacterial DNA, from which the number of binding sites can be extrapolated. This parameter is independent of the diffusion of the drugs through the bacterial membrane or influx/efflux equilibrium since the number of accessible binding sites is extrapolated at saturating concentration of drug.

Strains of variable sensitivity have first of all been obtained by the selection of resistant bacteria to iPr-OPC. Resistant bacteria exhibited no phenotypic modification in terms of repair capability and membrane permeability whereas the maximum number of accessible sites was strikingly decreased. This suggests that the resistance may come from the decrease in the accessibility of intercalating sites. In order to establish a statistical correlation between the extent of accessibility of intercalating sites and toxicity, we have screened various *E. coli* and *S. typhimurium* strains available in our laboratory for their sensitivity toward OPC and iPr-OPC and selected some of them. Among OPC derivatives, OPC and iPr-OPC have been used because of their marked difference in toxicity toward the same strains, leading to a large interval of variation in the cytotoxic activity values. The linear regression coefficient of the curve that relates the number of accessible intercalating sites to the toxicity is statistically significant. It is of interest to note that the experimental points corresponding to the sensitive and resistant strains are located fairly close to the regression line.

It should be pointed out that the number of accessible sites is a parameter that controls the amount of drug bound to the intercalating sites at any drug concentration. This appears clearly from the equation underlying the equilibrium:

$$D_b = N/(1/KD_f + 1)$$

where D_b is the amount of bound drug, N the number of binding sites, K the association constant, and D_f the amount of free drug. However, an additional problem arises from the positive cooperativity in the binding process occurring in living bacteria as indicated by the shape of the Scatchard plots. In

that case, at low drug concentrations, the virtual number of binding sites is a fraction of N which increases as the drug concentration increases. Nevertheless, the significant correlation that exists between the maximum number of binding sites N and the antibacterial effect expressed in term of ED_{50} indicates that this parameter controls the intrinsic sensitivity of the bacteria at any drug concentration. This indicates that the binding of the drugs to nucleic acids is the event responsible for the inhibition of cellular multiplication. The additional information is that the binding sites are the intercalating sites, which was obvious from the occurrence of in vivo energy transfer from nucleic acids to the drug (Banoun et al., 1985). This moreover indicates that intercalation per se is very likely responsible for the toxic effect. The striking feature is that the accessibility to intercalating sites appears to be subject to large variations. In the strains resistant to iPr-OPC, 80% of the intercalating sites remain inaccessible compared to the sensitive strains. This indicates that the extent of accessibility corresponds to a stable phenotypic character that controls the cytotoxic efficiency of intercalating agents.

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