CELL ENVELOPE ALTERATIONS ASSOCIATED WITH SENSITIVITY TO ANTIBACTERIAL AGENTS OF GAMMA-IRRADIATION RESISTANT MUTANTS OF *ESCHERICHIA COLI*

AKHAND P. SINGH*

*Department of Microbiology, Macdonald Campus of McGill University,
Montreal, P. Quebec, Canada*

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The principle underlying the sensitivity to antibacterial agents of wild type *Escherichia coli* P and its γ -irradiation resistant mutants was studied. These strains were classified as typical rough (R) strains, belonging to classes Ra (P and γ), and Rc (6γ and 12γ). The galactose-deficient (Rc) strains, 6γ and 12γ , leaked large amounts of alkaline phosphatase, acid phosphatase, 2,3-cyclic phosphodiesterase, and β -galactosidase into the culture media during active growth, indicating defects in both the outer and inner membranes. Unlike strains P and γ , strains 6γ and 12γ were hypersensitive to Actinomycin-D, Bacitracin, Erythromycin, Kanamycin, Novobiocin, penicillin G, Rifamycin, Vancomycin, sodium deoxycholate (DOC), sodium dodecyl sulfate (SDS), ethylenediaminetetraacetic acid (EDTA), lysozyme, and Methylene Blue. Strains P, 6γ , and 12γ showed similar sensitivity to phages T2, T3, T4, T5, T6, and T7, and differed from strain γ , which was sensitive only to phages T2 and T4. Strains P and 6γ were sensitive to colicins E₁, E₂, E₃, G, H, and K, whereas strain γ was resistant to colicin H, and 12γ was tolerant to colicins H and K.

On the basis of these observations it is suggested that the leakage of periplasmic enzymes from and the sensitivity of these mutant strains to antibacterial agents are associated with both defects in the cell wall and plasma membrane.

Studies on γ -irradiation resistant mutants of *Escherichia coli* have shown that, unlike the less radio-resistant mutant, γ , and the wild type parent, the more radio-resistant mutant strains, 6γ and 12γ , are highly sensitive to lysozyme and Actinomy-

* Present address: Department of Biochemistry, Faculty of Medicine, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

cin-D. In addition, they lack galactose in their lipopolysaccharides, and leak alkaline phosphatase and β -galactosidase into the culture medium during active growth (1-5). Other differences between the wild type and radio-resistant mutants of *E. coli* include sensitivity to penicillin G (6) and the ability to form 'minicells' (7). It has also been shown that the radio-resistant strains, 6 γ and 12 γ , are filamentous, and contain a large number of separate mesosomes and cell nuclei (7, 8).

This paper describes the results of a comparative study of the sensitivity of wild type and γ -irradiation resistant mutants of *E. coli* to antibiotics, detergents, dyes, lysozyme, EDTA, phages, and colicins. The chemical composition of the lipopolysaccharides of the wild type and mutant strains was also compared. A preliminary report on some of the characteristics of the γ -irradiation resistant mutants of *E. coli* has been published (9).

MATERIALS AND METHODS

Chemicals. Bacitracin, Kanamycin, Erythromycin, Mitomycin-C, and Penicillin G were purchased from Sigma Chemical Co., St. Louis. Rifamycin, Oxy-Tetracycline, and Tetracycline were obtained from Calbiochem, Los Angeles, Calif. Actinomycin-D, Chloramphenicol, Novobiocin, and Vancomycin were gifts of Merck, Sharpe and Dohme of Canada Ltd., Point-Claire, Quebec; Parke, Davis and Co., Ltd., Brockville, Ontario; Upjohn Co., of Canada Ltd., Don Mills, Ontario, and Eli, Lilly and Co., Indianapolis, respectively.

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49), lactate dehydrogenase (EC 1.1.1.27), containing pyruvic kinase (EC 2.7.1.40), phosphoglucomutase (EC 2.7.5.1), uridine 5-diphosphate glucose dehydrogenase (EC 1.1.1.22) type III, lysozyme (EC 3.2.1.17), adenosine 5-triphosphate (ATP), bis-p-nitrophenyl phosphate (bis-PNP), glucose 1-phosphate, glucose 1,6-diphosphate, β -glycerophosphate, nicotinamide adenine dinucleotide (NAD), and nicotinamide adenine dinucleotide phosphate (NADP), phosphoenol pyruvate, uridine-5-diphosphogalactose (UDP-Gal), and uridine 5-diphosphoglucose (UDPG) were purchased from Sigma Chemical Co., St. Louis. Glucose oxidase (EC 1.1.3.4) and galactose oxidase (EC 1.1.3.9) were obtained from Worthington Biochemical Corp., Freehold, N.J. Isopropyl- β -D-thiogalactoside (IPTG) and o-nitrophenyl- β -D-galactopyranoside (ONPG) were products of Schuchardt, München. L-Glycero-D-mannoheptose phosphate was a gift of Dr. M. J. Osborn.

Media. Luria broth and agar (10), λ broth (11), nutrient broth and agar (Difco), and penassay broth (Difco) were used as enriched media. Chemically defined medium (CDM) was prepared as described by ROBERN and THATCHER (12).

Strains. The bacterial and phage strains used in this study are listed in Table 1.

Sensitivity to antibiotics. The sensitivity of *E. coli* P and its mutant strains to various antibiotics was measured in penassay broth by the method of TAMAKI *et al.* (13).

Table 1. Bacterial and phage strains employed^a.

Strain	Relevant properties	Immediate source
<i>E. coli</i> :		
P	Wild type parent	E. I. Erdman
γ	γ -radiation resistant mutant	E. I. Erdman
6 γ	Leu ⁻ , Met ⁻ , Pro ⁻ , and resistant to γ -radiation	E. I. Erdman
12 γ	Arg ⁻ , Cys ⁻ , His ⁻ , Leu ⁻ , Met ⁻ , Pro ⁻ , Trp ⁻ , Val ⁻ , Lac ⁻ , Gal ⁻ , Uracil ⁻ , resistant to γ -radiation	E. I. Erdman
CA38 (col E3/I)	Produces colicin E3/I	J. Smarda
CA42 (col E2)	Produces colicin E2	J. Smarda
CA46 (col G)	Produces colicin G	J. Smarda
CA58 (col H)	Produces colicin H	J. Smarda
K235 (col K)	Produces colicin K	B. D. Sanwal
Y20 (col E)	Produces colicin E1	B. D. Sanwal
B2156	Host strain for phages C21, T3, T5 & T7	G. Schmidt
<i>S. typhimurium</i> :		
RC903 (col E1)	Produces colicin E1	S. Silver
RC906 (col E2)	Produces colicin E2	S. Silver
SL869	Host strain for phage P221	G. Schmidt
SL1546	Host strain for phage P22	G. Schmidt
<i>S. paratyphi</i> :		
B309	Host of phage FO	G. Schmidt
Kroger I/2	Host of phages Br2, Br10, Br60	G. Schmidt
BR	Host of phage Ffm	G. Schmidt
A 59/28	Host of phage 6SR	G. Schmidt
Bacteriophage:		
P22	LPS specific phage, attacks only smooth strain	G. Schmidt
FO	Lyses both smooth and rough strains	G. Schmidt
P221, Ffm, 6SR, Br2, Br10, C21	Lyses rough strains only	G. Schmidt
T2, T3, T4, T5, T7	Coli phage	G. Schmidt
BF23	Same receptor as E-group colicins	P. Fredericq
T6	Same receptor as colicin K	P. Fredericq

^a The definition of the genetic symbols follow TAYLOR and TROTTER (66). For full description of phages, see LINDBERG (28).

Sensitivity to detergents and dyes. The effect of sodium deoxycholate (DOC), sodium dodecyl sulfate (SDS), and EDTA on the growth of *E. coli* P, γ , 6 γ , and 12 γ was determined by the procedures described by NAGEL DE ZWAIG and LURIA (11). Sensitivity to dyes was estimated by determining the colony-forming ability of the bacteria on nutrient agar plates containing different concentrations of the various dyes.

Effect of detergents, lysozyme, and EDTA on cell lysis. The sensitivity of wild type P and mutant strains to DOC, SDS, lysozyme alone and in combination with EDTA was measured by determining the lysis of a washed cell suspension

in the presence of lytic agents. The reaction mixture in a total volume of 3 ml contained 50 mM Tris-HCl buffer, pH 8.0, and cells plus the different additives at the concentrations indicated. The absorbance change was recorded at 600 nm with a Coleman 124 recording spectrophotometer. The lysis of cells in the presence of Tris buffer was used as the control.

Phage and colicins. Sensitivity to phages and colicins was determined by the drop on lawn method (14, 15). About 10^8 cells of the test strains were spread on nutrient agar (NA) plates and the plates were dried for 10 to 20 min at 37° . One drop of phage suspension (10^8 pfu/ml) or colicin solutions (10^{11} to 10^{12} killing particles/ml) was spotted on dried plates which were then incubated at 37° . Results were recorded after 5 and 12 hr.

Binding of colicin to intact cells. The binding of colicin K and H to intact cells of P, γ , and 12γ strains was estimated by the method of SABET and SCHNAITMAN (16).

Isolation and chemical analysis of lipopolysaccharide. Lipopolysaccharide (LPS) was extracted from cells grown on nutrient broth containing 0.3% yeast extract (NBYE) by the phenol-water method described by WESTPHAL and JANN (17). Sugars were estimated by colorimetric methods or by paper chromatography as described previously (1, 4). Glucose and galactose were determined by glucose and galactose oxidase, respectively; 1-glycero-D-mannoheptose and 2-keto-3-deoxyoctonate (KDO) were measured by the method of OSBORN (18). Phosphorus was estimated by the procedure of CHEN *et al.* (19).

Enzyme assays. Galactokinase (EC 2.7.1.6), galactose-1-phosphate uridyl transferase, uridine diphosphogalactose-4-epimerase, 2,3-cyclic phosphodiesterase, acid phosphatase, alkaline phosphatase, and β -galactosidase were measured according to published procedures (20-26).

RESULTS

Cultural characteristics

The colony morphology and agglutinability of strains P, γ , 6γ , and 12γ of *E. coli* in 0.3% auramine, 0.2% acriflavine, and 0.85% NaCl are shown in Table 2.

Table 2. Colony morphology and agglutination characteristics of wild type *Escherichia coli* P and its γ -irradiation resistant mutant strains.

<i>E. coli</i> strains	Colony morphology ^a on NAYE plate	Agglutination in ^{b, c}		
		Auramine	Acriflavine	NaCl
P	R	+	+	—
γ	SR	—	+	+
6γ	R	+	+	+
12γ	R	+	+	+

^a R=rough; SR=smooth and rough.

^b +=agglutinated; —=not agglutinated.

^c Microscopic slide agglutination test was used to determine the auto-agglutination ability of *E. coli* P and its mutant strains in auramine, acriflavine and NaCl.

The colony morphology of strains P, 6 γ , and 12 γ on nutrient agar plate indicated that these strains were rough (R) whereas the γ strain produced smooth (S) colonies. Strains 6 γ and 12 γ gave positive agglutination tests in auramine, acriflavine, and NaCl, whereas strains P and γ did not agglutinate in NaCl and auramine, respectively (Table 2 and Ref. 1). Auto-agglutination ability of *E. coli* P and its mutant strains in auramine, acriflavine and NaCl was determined by microscopic slide agglutination test (see legend to Table 2). The results reported on auramine agglutination were also confirmed in Dr. Luderitz's laboratory (Dr. G. Schmidt, personal communication, 1972).

Pathogenicity

The roughness of the P, 6 γ , and 12 γ strains was further confirmed by pathogenicity trials in mice. About 10^9 viable cells/ml of *E. coli* P, γ , 6 γ , and 12 γ were injected intraperitoneally into mice. Under these conditions no deaths occurred during the 15-day observation period.

Sensitivity to LPS-specific phages

The smooth (S) and rough (R) strains of *E. coli* and *Salmonella typhimurium* have also been characterized by their sensitivity to a series of S and R specific phages, and a correlation has been obtained between phage sensitivity pattern and the polysaccharide composition of their LPS (27-29). The sensitivity to different LPS specific phages of *E. coli* P and its γ -irradiation resistant mutants was therefore tested. Strains 6 γ and 12 γ were sensitive to R-specific phages P221, Ffm, Br2, Br10, Br60, and C21 (Table 3). The phage sensitivity pattern of these strains resembled that of *S. typhimurium* mutants with no functional UDPGal-4-epimerase (28), but differed from that of the γ -irradiation resistant mutants of *Salmonella* spp. (30-33). Strain P gave a phage sensitivity pattern similar to those of 6 γ and 12 γ , with the exception that it was resistant to phages Ffm and Br60. Strain γ was resistant to all phages except the R-specific phage, Ffm. These results were also corroborated by Dr. G. Schmidt (personal communication, 1972).

Table 3. Phage sensitivity pattern of wild type and radiation resistant mutants of *E. coli*.

<i>E. coli</i> strains	Sensitivity to phage types ^a								
	S P22	SR FO	P221	6SR	R Ffm	Br2	Br10	Br60	C21
P	—	—	+	—	—	+	+	—	+
γ	—	—	—	—	+	—	—	—	—
6 γ	—	—	+	—	+	+	+	+	+
12 γ	—	—	+	—	+	+	+	+	+

^a + = lysis; — = no lysis; S = smooth specific; SR = semirough; R = rough specific.

Table 4. Sensitivity of wild type and radiation-resistant mutants of *E. coli* to various T phages.

<i>E. coli</i> strains	Sensitivity to phage types ^a						
	T1	T2	T3	T4	T5	T6	T7
P	N.T.	+	+	+	+	+	+
γ	N.T.	$\pm$	—	+	—	—	—
6 γ	N.T.	+	+	+	+	+	+
12 γ	N.T.	+	+	+	+	+	+

^a + = clear lysis; $\pm$ = turbid lysis or sometime single plaque; — = no lysis; N.T. = not tested.

Sensitivity to T phages

It has been reported (28, 34, 35) that the phages, T3, T4, and T7, are rough specific and have their receptors in the LPS. Strains P, 6 γ , and 12 γ were sensitive to all T phages tested (Table 4), whereas strain γ was resistant to phages T3, T5, T6, and T7 but was sensitive to phages T2 and T4 at very high multiplicity (10^{10} pfu/ml).

Table 5. Sensitivity of wild type and radiation-resistant mutants of *E. coli* to various colicins and phages BF23 and T6.

<i>E. coli</i> strains	Colicin sensitivity ^a						Phage sensitivity ^a	
	E ₁	E ₂	E ₃	G	H	K	BF23	T6
P	+	+	+	+	+	+	—	+
γ	+	+	+	+	—	+	—	—
6 γ	+	+	+	+	+	+	+	+
12 γ	+	+	+	+	—	—	—	+

^a + = lysis; — = no lysis.

Sensitivity to colicin

The colicins of the E-group share a common receptor with phage BF23, and colicin K with that of phage T6 (11, 36). The sensitivity of *E. coli* strains P, γ , 6 γ and 12 γ to various colicins and phages, T6 and BF23, is shown in Table 5. Strains P, γ , 6 γ , and 12 γ were sensitive to colicins E₁ to E₃ and G, but they differed in their response to colicins H and K, and phages BF23 and T6. The binding of colicin H and K to whole cells of strains γ and 12 γ , respectively, indicated that the strain γ was resistant (receptor *minus*) to colicin H while 12 γ was tolerant (contains receptor but lacks specific transmission mechanism) to colicin K. These results also suggest that phage BF23 do not share a common receptor with colicin E and phage T6 with that of colicin K (11, 36).

Chemical composition of LPS

The roughness of strains P, γ , 6 γ , and 12 γ was also confirmed by chemical analysis of their LPS (Table 6). The LPS of all the strains lacked mannose and

Table 6. Polysaccharide and phosphate composition of the lipopolysaccharides of *E. coli* P and its γ -irradiation resistant mutant strains.

<i>E. coli</i> strains	Composition of lipopolysaccharide (nmol/mg of LPS)					
	Rhamnose	Glucose	Galactose	Heptose	KDO	PO ₄
P	0	493	205	493	205	78
γ	0	513	206	493	200	40
6 γ	0	376	0	633	195	64
12 γ	0	707	0	598	184	39

Abbreviations: KDO=2 keto-3-deoxyoctonate; PO₄=phosphate.

Table 7. Specific activities of various galactose operon enzymes in cell-free extracts prepared from wild type *E. coli* P and its γ -irradiation resistant mutants.

<i>E. coli</i> strains	Galactose operon enzymes specific activity (μ mol/mg protein/hr) ^a		
	Kinase	Transferase	Epimerase
P	60.7	40	34
γ	58.2	41	32.5
6 γ	44.5	36.2	1.1
12 γ	0.6	39.8	0.4

^a Specific activities were corrected for the uninduced basal level of the various enzymes. Enzymes were induced by growing the bacteria at 37°C in peptone broth containing 10⁻² M D-galactose.

rhamnose, indicating that these strains lack the *o*-antigenic side chain (29). This conclusion is consistent with the phage-sensitivity pattern shown in Table 3. The amount of heptose and KDO was approximately the same in all the strains, but the content of phosphate was 40 to 50% less in the LPS of γ and 12 γ strains (Table 6). As reported previously (4), no galactose was found in the LPS of the 6 γ and 12 γ strains, and the content of glucose was slightly higher in 12 γ LPS (Table 6).

The absence of galactose in the LPS of strains 6 γ and 12 γ was due to the lack of UDPGal-4-epimerase (Table 7). Thus these strains are unable to synthesize LPS-galactose in the absence of exogenous galactose. In addition, strain 12 γ also lacked galactokinase (Table 7).

Effect of galactose on growth

The addition of galactose to either a complex or a chemically defined medium containing glycerol as the carbon source caused severe inhibition of the growth of the 12 γ strain (Fig. 1 and Ref. 37-40). In contrast, the growth of the 6 γ strain was not affected by galactose (Fig. 1). In the presence of an osmotic stabilizer, galactose inhibited the growth of strains 6 γ and 12 γ , and also caused the formation of spheroplasts from the growing cells of these strains.

Fig. 1. Effect of galactose (0.1%) on the growth of *E. coli* 6 γ and 12 γ in chemically defined medium (12) containing 0.5% glycerol as a carbon source.

Symbols: t =time of addition of galactose, —●— C (control, without galactose), ---○--- + galactose. Under these conditions, galactose had no effect either on the growth of wild type *E. coli* P or less radiation resistant mutant strain.

Table 8. Concentration of various antibacterial agents required to inhibit 50% growth (IG₅₀) of *E. coli* P and its γ -irradiation resistant mutants at 37°C under shaking conditions.

Antibacterial agents	<i>E. coli</i> strains			
	P	γ	6 γ	12 γ
		IG ₅₀ (μ g/ml)		
Bacitracin	680	550	410	80
Penicillin G	41.5	8	4	1.5
Novobiocin	46	12.5	10	10
Vancomycin	71	33	25.5	6
EDTA	> 2000	750	370	120
Actinomycin D	70	47	36	3
Rifamycin	97.5	76.5	41.5	5
Erythromycin	14.5	15.6	3	0.5
Kanamycin	2.7	2.8	1.2	0.4
Tetracycline	0.1	0.1	0.05	0.4
Oxytetracycline	0.14	0.18	0.09	0.3
Methylene Blue	> 100	> 100	25	5
Deoxycholate	> 5000	> 5000	> 5000	1500
Sodium dodecyl sulfate	> 4000	> 4000	1500	150

^a Antibacterial agents are grouped according to their site of action. The first 5 agents act either at the cell wall or outside the plasma membrane, and the remaining agents act either on the plasma membrane or in the cytoplasm (65).

Fig. 2. Effect of different concentrations of Actinomycin D, Bacitracin, Erythromycin, Kanamycin, Novobiocin, penicillin G, Rifamycin, Vancomycin, tetracycline, and oxytetracycline on the growth of wild type *E. coli* P and its γ -irradiation resistant mutant strains, γ , 6γ , and 12γ .

Cells were grown at 37° in Penassay broth (Difco) under shaking conditions, for 4 hr (P, γ), 6 hr (6γ), and 10 hr (12γ). Cell growth was estimated from the absorption at 660 nm. Under these growth conditions, the mean generation time was 30 min (P, γ), 45 min (6γ), and 96 min (12γ).

Effect of antibacterial agents on wild type and mutant strains

(a) *Antibiotics.* It has been observed that smooth (S) strains of both *E. coli* and *Salmonella* species are more resistant to the lethal action of certain antibiotics than the rough (R) strains; and a correlation between sensitivity to antibiotics and LPS structure has been reported (41–48). The wild type *E. coli* P and its γ -irradiation resistant mutants were therefore tested for sensitivity to various antibiotics, and the results are summarized in Table 8 and Fig. 2. In contrast to the γ -irradiation resistant mutants of *Salmonella* species (30–33), the γ -irradiation resistant mutant strain 12γ was most sensitive to Actinomycin-D, Bacitracin, penicillin G,

Fig. 2 (continued).

Rifamycin, and Vancomycin. Strains 6 γ , γ , and P showed progressively less sensitivity to these antibiotics (Fig. 2 and Table 8). In general, the reduction in growth of 12 γ was directly proportional to the concentration of the antibiotics (Fig. 2).

Strain 12γ was also found to be most sensitive to Kanamycin and Erythromycin, whereas strains 6γ , P, and γ displayed successively decreasing sensitivity to these antibiotics (Fig. 2). Strain P was less sensitive to Novobiocin than strains γ , 6γ , and 12γ which exhibited a similar degree of sensitivity (Fig. 2). Strain 12γ was found to be most resistant to tetracycline and oxytetracycline, strain 6γ was most sensitive, and intermediate values were obtained for strains P and γ (Fig. 2).

(b) *Dyes*. In contrast to the LPS-defective mutants of *Salmonella* spp. (41–43, 45, 46), the wild type *E. coli* P and its γ -irradiation resistant mutants were all insensitive to acriflavine, Acridine Orange, Crystal Violet, and Melachite Green but differed in their reaction to Methylene Blue (Fig. 3). The increased sensitivity of 12γ to Methylene Blue was due to the greater uptake and binding of the dye

Fig. 3. Effect of different concentrations of Methylene Blue on the colony-forming ability of *E. coli* P and its mutant strains, γ , 6γ , and 12γ .

Serially diluted mid log phase cells (0.2 ml) of various test strains were plated in triplicate on the surface of various nutrient agar plates containing different concentrations of the dye. Plates without dye were used as controls. The colonies were counted after 24 or 48 hr (12γ) of incubation at 37° .

Table 9. Uptake and/or binding of Methylene Blue by cells of wild type *E. coli* P and its mutant strains.

<i>E. coli</i> strains	Methylene Blue bound ^a (% of control)
P	1.8
γ	1.8
6γ	5.0
12γ	20.0

^a Bacteria (0.5 mg protein/ml) were incubated for 15 min at 37° in chemically defined medium (12) containing 20 μ g/ml Methylene Blue (100% control). Samples were removed at time zero (zero control) and, after 15 min, cells were removed by centrifugation and the amount of dye remaining in the supernatant was measured spectrophotometrically at 665 nm.

to the cell surface of this strain (Table 9).

(c) *Effect of detergents.* It has been shown that colicin-tolerant and antibiotic-sensitive mutants of *E. coli* are hypersensitive to the detergents, DOC and SDS (11, 49-52). Strain 12 γ was most sensitive to DOC and SDS, and strain γ was least sensitive, while strain 6 γ showed an intermediate level of sensitivity to these detergents (Table 8 and Fig. 4). The wild type strain P was not affected by either DOC or SDS.

Fig. 4. Time-dependent changes in the turbidity of cell suspensions of wild type *E. coli* P and its γ -irradiation resistant mutant strains γ , 6 γ and 12 γ , upon treatment with (A) 6 mg/ml sodium deoxycholate (DOC), (B) 1 mg/ml sodium dodecyl sulfate (SDS), (C) 5 μ g/ml lysozyme, or (D) 10 μ g/ml lysozyme plus 5×10^{-5} M EDTA at 25°.

(d) *Effect of EDTA and lysozyme.* It has been suggested (50, 53, 54) that lysozyme sensitivity of Gram-negative bacteria may be an effective means of differentiating cell envelope-defective mutants from the wild-type parent. In order to obtain more information on the nature of the cell surface of *E. coli* P and its mutant strains, their sensitivity to lysozyme, either alone or in combination with Tris and EDTA, was investigated. Lysozyme treatment alone caused the greatest turbidity decrease in cell suspensions of strain 12 γ . Suspensions of strain 6 γ showed a slight decrease in turbidity while the strain γ cell suspension was unaffected, and suspensions of strain P increased in optical density (Fig. 4C). The results obtained with strains 6 γ and 12 γ are compatible with those reported for Actinomycin-D sensitive mutants of *E. coli* (50, 54).

Lysozyme in combination with EDTA (5×10^{-5} M) caused a 50% decrease in optical density in cell suspensions of strains 6 γ and 12 γ within 2 min (Fig. 4D). No effect was detected with cell suspensions of strains P and γ . The optical density decrease after a 12-min incubation was 84%, 75%, 18%, and 8% in strains 6 γ , 12 γ , γ , and P, respectively (Fig. 4D). Increasing the concentration of EDTA from 5×10^{-5} M to 5×10^{-4} M caused very little or no lysis of strain P within a 2-min period (results not shown). Incubation for 12 min increased lysis but not to the extent observed with strains 6 γ and 12 γ in the presence of 5×10^{-4} M EDTA for 4 min.

Leakage of enzymes

In previous studies (3–5) we showed that γ -irradiation resistant mutants of *E. coli*, 6 γ and 12 γ , leak a periplasmic enzyme, alkaline phosphatase, and a cytoplasmic enzyme, β -galactosidase. In addition to these enzymes, strains 6 γ and 12 γ were also found to leak two other periplasmic enzymes, acid phosphatase and 2,3-cyclic phosphodiesterase. Neither the γ nor P strains leak any enzyme into the growth medium (Table 10).

Table 10. Specific activities of various enzymes and percentage of total activity released into the growth medium during normal growth of wild type *E. coli* P and its γ -irradiation resistant mutant strains.

<i>E. coli</i> strains	Amount of enzyme present in cells and culture filtrates (units/ml of culture or culture filtrate)				
	APase	Acid phosphatase	2,3-Cyclic phosphodiesterase	β -Galactosidase	NADH oxidase
P	154 (1.5)	34 (0)	16 (0)	4477 (0)	350 (0)
γ	321 (1.0)	23 (0)	56 (0)	4552 (0)	352 (0)
6 γ	442 (13)	20 (2)	42 (15)	4697 (3)	360 (0)
12 γ	620 (29)	20 (12)	44 (17)	2841 (5.5)	358 (0)

Numbers in parentheses represent the % of total cellular activity released into the culture fluid. APase=alkaline phosphatase. The culture had an optical density of 1.0 at 660 nm, which corresponds to 250 μ g of protein/ml.

DISCUSSION

The observation that γ -irradiation resistant mutants (6γ and 12γ) of *E. coli* are sensitive to various antibiotics, dyes and detergents suggests that a correlation may exist between sensitivity to antibacterial agents and resistance to γ -irradiation. Alternatively sensitivity to antibacterial agents may be due to cell envelope alterations rather than radiation resistance. The latter interpretation is consistent with the finding that cell surface-defective mutants obtained by chemical mutagenic agents are also sensitive to various antibiotics, dyes, and detergents (13, 51, 54-57).

The high sensitivity of the 6γ and 12γ strains to various antibiotics, detergents, and Methylene Blue appears to be associated with the absence of galactose in their LPS. However, these studies do not eliminate the possibility that other components of the cell envelope may also be involved in the sensitivity of these strains to antibacterial agents, and in the leakage of periplasmic and cytoplasmic enzymes. The tolerance of strain 12γ to colicins H and K, and leakage of β -galactosidase from cells of 6γ and 12γ , and their sensitivity to DOC and Methylene Blue, all suggest that, in addition to defects in the outer membrane, these strains may have an altered plasma membrane structure. A direct effect of γ -irradiation on the plasma membrane has been reported for other strains of *E. coli* (58-61). Evidence for altered membrane permeability in these strains has been obtained from experiments in which formaldehyde was used as a blocking agent. Formaldehyde blocks the entry of substrates such as *o*-nitrophenyl- β -D-galactoside (ONPG) into cells which have an intact permeability barrier, but it has either very little or no effect on broken or leaky cells (62, 63). In contrast to the P and γ strains, the *in vivo* hydrolysis of ONPG in 6γ and 12γ was unaffected by formaldehyde (2, 64).

From these studies it is concluded that alterations in the cell envelope (cell wall and plasma membrane) are responsible for leakage of enzymes from γ -irradiation resistant mutants of *E. coli* and for the high sensitivity of these mutant strains to antibacterial compounds.

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