Resistance to ultraviolet-induced mutagenesis in *Proteus*

R. Schwarzhoff and U. Santhanam[1]

Department of Biology, Wichita State University, Wichita (Kansas 67208, USA), 31 December 1980

Summary. A survey of the mutagenic activity of UV-irradiation in 20 bacterial strains of the *Proteus* group indicated that resistance to UV-mutagenesis is characteristic of these organisms.

The use of UV-irradiation for induction of mutations has been an invaluable tool in virtually all areas of microbiology, and the molecular events involved in UV-mutagenesis in bacteria have been intensively investigated (for review see Witkin[2]). All organisms are not equally mutable by UV-light; *Haemophilus influenzae*[3], *Micrococcus radiodurans*[4], *Streptococcus pneumoniae*[5], and *Proteus mirabilis*[6,7] have all been reported to be particularly resistant to UV-mutagenesis. Preliminary attempts in our laboratory to induce antibiotic resistant mutants of *Proteus vulgaris* with UV-light were unsuccessful; in view of the similar resistance of *P. mirabilis* to UV-mutation, it was of interest to determine if resistance to UV-mutagenesis might be characteristic of the *Proteus* group generally.

Materials and methods. A total of 20 *Proteus* strains, 4 from each of the 5 species recognized in the 8th edition of Bergey's Manual of Determinative Bacteriology[8], were tested for UV-induced mutagenesis to antibiotic resistance. The strains were obtained from laboratory culture collections and from clinical specimens, and the identity of each strain was confirmed using conventional physiological tests and the API-20E rapid identification system (Analytab Products). *Escherichia coli* B (ATCC 11303) was included in the study as a positive control for UV-mutability.

Cells for exposure to UV-light were grown in a broth medium prepared with 1% tryptose (Difco) and 0.5% NaCl, harvested during the mid-log growth phase, and suspended at a density of about 10^8 viable cells per ml in phosphate buffer (0.02 M, pH 7.0). UV-irradiation was performed using a 4-W germicidal lamp (General Electric) positioned 36 cm from a glass petri dish containing the bacterial suspension. The dose rate at the surface of the suspension was 0.24 J m^{-2} s^{-1}. Following irradiation the cells were transferred to tryptose broth, incubated for phenotypic expression, and inoculated onto nutrient agar (Difco) containing either rifampicin (10 µg/ml) or nalidixic acid (10 µg/ml). All manipulations during and after UV-exposure were performed under low level red light to prevent photoreactivation. Resistant colonies were counted after 5 days of incubation at 37 °C. Mutation frequencies to antibiotic resistance were also determined following exposure to N-methyl-N'-nitro-N-nitrosoguanidine according to the procedure of Adelberg et al.[9].

Results and discussion. The effect of UV-irradiation on survival and mutation frequencies for the 21 strains are shown in the table. Whereas the mutation frequencies induced by 21.6 J m^{-2} in *E. coli* B were increased over the spontaneous frequencies by factors of 200 times for rifampicin and 125 times for nalidixic acid, the frequencies in the 20 *Proteus* strains remained low. The average increases for the 20 strains were 5.5 times for rifampicin and 5.2 times for nalidixic acid.

Single representative strains from each of the 5 *Proteus* species were compared with *E. coli* B for mutability at 7 UV-doses between 3.6 and 43.2 J m^{-2}. The results from these experiments are not shown but are consistent with those presented in the table. *E. coli* B demonstrated a continuous increase in mutation frequency over the range

UV-induced mutations to rifampicin (10 µg/ml, Rif) or nalidixic acid (10 µg/ml, Nal) resistance in *E. coli* B and *Proteus*

Strain		Unirradiated Mutants/10^7 survivors[a]		Irradiated 3.6 J m^{-2}			Irradiated 21.6 J m^{-2}		
					Mutants/10^7 survivors[a]			Mutants/10^7 survivors[a]	
		Rif	Nal	% Survival	Rif	Nal	% Survival	Rif	Nal
Escherichia coli	B	0.59	0.02	82	37	0.08	23	125	2.5
Proteus vulgaris	1	0.34	0.11	90	0.96	0.39	9.0	3.1	1.2
	2	0.32	0.05	88	0.73	0.06	8.4	3.4	0.01
	3	0.64	0.08	55	0.92	0.08	11	3.1	0.54
	4	0.20	0.17	83	0.41	0.29	5.1	4.5	3.4
Proteus mirabilis	1	0.48	0.85	89	1.1	1.0	5.0	1.7	4.0
	2	1.0	0.65	89	1.1	1.6	15	1.9	4.8
	3	0.32	1.8	73	0.68	3.5	0.76	0.48	5.0
	4	0.57	0.39	83	0.51	0.34	0.94	2.2	0.5
Proteus morganii	1	0.74	0.06	79	1.4	0.01	11	5.7	0.61
	2	1.3	0.55	89	1.9	0.44	4.6	3.0	0.38
	3	1.2	0.14	98	0.89	0.23	43	3.0	0.38
	4	1.1	0.12	94	0.58	0.33	61	1.4	0.68
Proteus rettgeri	1	1.1	1.3	96	2.2	1.7	18	9.7	7.4
	2	0.53	13	90	0.92	13	23	2.7	21
	3	0.23	0.64	89	0.31	0.59	3.9	1.4	1.1
	4	0.38	0.24	79	1.0	0.68	2.5	4.8	2.9
Proteus inconstans	1[b]	0.21	1.4	64	0.31	0.78	1.3	0.11	0.39
	2[b]	0.69	1.2	25	1.4	1.8	0.05	0.5	0.74
	3[b]	0.24	0.67	71	0.38	0.61	4.3	1.6	2.5
	4[c]	1.9	0.04	72	1.7	0.38	0.08	0.85	0.17

[a] Mutation frequencies represent means based on 5 determinations each from duplicate experiments; [b] *Providencia stuartii*; [c] *Providencia alcalifaciens.*

of UV-doses reaching maximum increases over spontaneous frequencies of 500 times for rifampicin and about 1000 times for nalidixic acid. The induced mutation frequencies in the *Proteus* strains remained low over the range of UV-doses; none of the frequencies was increased over the spontaneous frequencies by more than 15 times, and the average maximum increases for the 5 strains were 9.0 times for rifampicin and 7.7 times for nalidixic acid. It should be noted that lysogeny is reportedly common among *Proteus* strains[10]; however, no evidence for UV-induction of prophage was observed in any of these experiments. In contrast to UV-irradiation, nitrosoguanidine proved to be an effective mutagenic agent for *Proteus*. Exposure of these strains to nitrosoguanidine (25 or 50 µg/ml for 30 min at 37 °C) caused increases in mutation frequencies to rifampicin resistance that ranged from 300-to 3000-fold.

Recent investigations by Hofemeister et al.[11] indicate that resistance to UV-mutagenesis in *P. mirabilis* is caused by the absence of specific genetic components that, in other bacteria, are associated with error-prone repair of UV-induced lesions in DNA, and the results from our study suggest that this trait is a distinctive feature of all *Proteus* species. Because of significant physiological differences between the organisms in the *Proteus* group, their assignment to a single genus, as proposed in the latest edition of Bergey's Manual, is not uniformly accepted. The observation that these organisms all share the unusual characteristic of resistance to UV-mutability is, therefore, significant and could be a consideration in their classification.

1 Acknowledgment. We greatly appreciate the helpful discussion provided by A. Sarachek during the course of the study and the preparation of the manuscript.
2 E.M. Witkin, Bact. Rev. *40*, 869 (1976).
3 R.F. Kimball, M.E. Boling and S.W. Perdue, Mutat. Res. *44*, 183 (1977).
4 D.M. Sweet and B.E.S. Moseley, Mutat. Res. *23*, 311 (1974).
5 A.M. Gasc, J.P. Claverys and A.M. Sicard, Mutat. Res. *70*, 157 (1980).
6 H. Böhme, Microbiol. genet. Bull. *19*, 15 (1963).
7 M. Mates, Z. allg. Mikrobiol. *4*, 22 (1964).
8 H. Lautrop, in: Bergey's Manual of Determinative Bacteriology, 8th edn, p. 327. Ed. R. Buchanan and N. Gibbons. The Williams and Wilkins Co., Baltimore 1974.
9 E.A. Adelberg, M. Mandel and G.C.C. Chen, Biochem. biophys. Res. Commun. *18*, 788 (1965).
10 T. Bergen, Meth. Microbiol. *11*, 243 (1978).
11 J. Hofemeister, H. Kohler and V.D. Filippov, Molec. gen. Genet. *176*, 265 (1979).

Isozymes of cathepsin B1 in developing human placenta

Maria Warwas

Department of Biochemistry, Institute of Bioanalysis and Environmental Research, Medical Academy, Szewska 38/39, PL-50-139 Wrocław (Poland), 27 January 1981

Summary. Cathepsin B1 was purified from human placentas of different gestational ages and analyzed by isoelectric focusing on polyacrylamide gel. The enzyme was shown to consist of 3 or 4 isozymes with pI values between 5.1 and 5.7.

Cathepsin B1 (EC 3.4.22.1), a lysosomal thiol-dependent proteinase, has been found in various mammalian tissues including human placenta[1,2] and fetal membranes[3]. In view of changes in the cathepsin B1 activity during the development of the placenta[4] and the presence of isozymes in chorion[3] it seemed worthwhile to investigate changes in the isozyme pattern in placentas from different gestational ages. The results are reported in this paper.

Materials and methods. Human placentas were obtained from the Institute of Obstetrics and Gynecology immediately after delivery. The placenta was washed with cold water to remove adherent blood, separated from fetal membranes and stored at −20 °C until preparation. Cathepsin B1 activity was assayed according to Barrett[5] with α-N-benzoyl-DL-arginine-β-naphthylamide hydrochloride as a substrate. Protein concentration was determined by the procedure of Lowry et al.[6]. Isoelectric focusing in polyacrylamide gel was performed at 4 °C according to Drysdale et al.[7]. Ampholine of pH 4–6 was used at the concentration of 2%. The cathepsin B1 activity was located as described by Barrett[8]. For the pH determination the gels were cut into 30 or more sections and immersed in 1 ml of distilled water. Purification of cathepsin B1 was carried out at 4 °C in the presence of 1 mM EDTA. Frozen human placenta was thawed and homogenized in 0.2 M acetate buffer, pH 4.3 (2 ml/g). The homogenate was incubated for 16 h at 37 °C. Early placentas (5–13 weeks) were centrifuged immediately after homogenization. In this case autolysis was not necessary because in the preliminary experiments no increase of the activity had been observed. After centrifugation the fraction precipitated between 0.4 and 0.7 ammonium sulphate saturation was dialyzed

Isoelectric points of cathepsin B1 isozymes during the development of the human placenta

Weeks of gestation	Activity* of homogenate units/g wet tissue	Specific activity** after purification	Isoelectric points of isozymes			
			I	II	III	IV
5–13, formation of placenta, n = 1	0.083	0.86	5.1	5.2	*5.4*	
21, growth and differentation, n = 1	0.045	0.64	5.1	*5.2*	5.4	
40, fully developed placenta, n = 3	0.034	0.40	5.1	*5.2*	*5.4*	
42, involution of placenta, n = 3	0.027	0.32	5.1	5.2	*5.4*	5.7

Main fractions are in italics. In the case where n > 1, the data are mean values. *Activity unit is expressed in µmoles of substrate hydrolyzed per min. **Specific activity was calculated in units per mg of protein.