Transduction of Isoniazid Susceptibility-Resistance and Streptomycin Resistance in *Mycobacteria*

D. SAROJA AND K. P. GOPINATHAN

Microbiology and Pharmacology Laboratory, Indian Institute of Science, Bangalore-560012, India

Received for publication 10 July 1973

Transduction of resistance to isoniazid and streptomycin as well as susceptibility to isoniazid in *Mycobacterium smegmatis* SN2 has been demonstrated. A method has been described for the selection of isoniazid-susceptible variants after transduction of susceptibility.

The isolation of a transducing mycobacteriophage (6, 7) offers another means of examining the genetics of mycobacteria and particularly the action of drugs and the development of resistance to drugs to which only mycobacteria are susceptible (e.g., isoniazid, INH). So far, transduction has been demonstrated for certain auxotrophic markers such as histidine, arginine, alanine, glycine, and adenine in *Mycobacterium smegmatis*, but attempts to transduce drug resistance markers have not been successful (6, 7). More recently, the occurrence of an "R factor" type of drug resistance to streptomycin in mycobacteria and its transfer by using the mycobacteriophage D29 have been demonstrated; however, the chromosomal streptomycin resistance could not be transduced (2). Evidence for genetic recombination between several strains of mycobacteria, due to the presence of different mating types, has also been documented, although the possibility of cell fusion followed by exclusion and leading to the appearance of recombinant types could not be completely ruled out (8). The available information on the genetics of mycobacteria has been summarized in a recent review (5). In the present communication, we report the transduction of resistance to INH and streptomycin, as well as susceptibility to INH in *M. smegmatis* strain SN2 by employing the mycobacteriophage I3. A method for the selection of INH-susceptible variants after the transduction of susceptibility has also been described.

Growth of organisms. *M. smegmatis* strain SN2 was grown in Luria broth (3) containing 0.05% (vol/vol) Tween 80 to minimize clumping of the organism. The phage employed was the transducing mycobacteriophage I3. It was prop-

agated on its host, *M. smegmatis*, in Luria broth or on Luria agar (Luria broth solidified with 2% agar) at 37 C.

Isolation of drug-resistant mutants of *M. smegmatis*. *M. smegmatis* strains resistant to streptomycin and to INH were isolated by treating log-phase cells of the wild-type cultures with 500 μ g of *N*-methyl-*N*-nitro-*N*-nitrosoguanidine per ml by using the standard procedure (1, 7). After treatment with the mutagen, the cells were washed and plated on Luria agar plates containing 100 μ g of INH or streptomycin per ml. A few colonies that appeared on drug-containing plates after 4 to 5 days of incubation at 37 C were purified by three independent steps of purification. Spontaneous drug-resistant mutants at the 100 μ g/ml level could not be isolated (the minimal inhibitory concentration of streptomycin and INH for *M. smegmatis* SN2 was 1 μ g/ml).

Transduction of streptomycin and INH resistance. The recipient cells (wild-type *M. smegmatis*) grown in Luria broth containing 0.05% Tween 80 to a concentration of 2×10^8 cells/ml were incubated with phage I3 grown on a streptomycin-resistant strain at a multiplicity of infection of 2 or more. At the end of 45 min of incubation, the cells were centrifuged and washed three times to remove free phage. The cells were suspended in fresh medium and incubated with shaking at 37 C for 6 h (nearly four generations). Because phage I3 has a latent period of 300 min, followed by a slow rise period lasting for nearly 150 min, there is no lysis of the host cells in 6 h in the course of transduction. Samples of culture (0.1 ml) were plated on Luria agar plates containing streptomycin (100 μ g/ml) and incubated at 37 C. Suitable control

cultures, incubated without added phage, were always included.

For transduction of INH resistance, the procedure was similar to that employed for streptomycin resistance, except that the phage used was propagated on *M. smegmatis* resistant to INH (Table 1).

Transduction of INH sensitivity. Because the frequency of transduction of drug resistance markers is lower than that of the auxotrophic markers, an attempt was made to see whether "susceptibility" could be transduced to resistant cells.

The procedure was similar to that used for the transduction of resistance markers, except that the recipient cells were INH-resistant *M. smegmatis*, and the phage was propagated on wild-type (INH-sensitive) *M. smegmatis*. The method used for the selection of INH-sensitive transductants was based on the observation that upon development of resistance to INH, mycobacteria lose catalase-peroxidase activities, which makes the INH-resistant cells more susceptible to the action of hydrogen peroxide than INH-sensitive cells. The killing rate of *M. smegmatis* by hydrogen peroxide was determined (Table 2).

With 0.05% (wt/vol) hydrogen peroxide there was 12% survival in the case of INH-sensitive cells, whereas the survival was less than 0.2% in the case of INH-resistant cells. This concentration of hydrogen peroxide was, therefore, employed for isolating INH-sensitive transductants.

After transduction experiments, the bacteria were treated with 0.05% (wt/vol) hydrogen peroxide for 30 min at 37 C and plated on Luria agar plates (without INH). In each experiment, more than 1,000 colonies were picked, and

TABLE 1. Transduction of streptomycin and INH resistance in *Mycobacterium smegmatis*^a

Character transduced	Expt no.	Multiplicity of infection	No. of transductants
Streptomycin resistance	1	2.5	22
	2	3.0	26
	3	3.5	30
	4	3.5	25
	5	4.0	101
INH resistance . . .	1		25
	2		27

^a Frequency of transduction was calculated on the basis of phage input was 5×10^{-7} . The details of the transduction procedure are given in the text.

replica were plated onto Luria agar plates containing 100 μ g of INH per ml. The drug-sensitive colonies, which appeared only on plates not containing INH, were further purified and rechecked for their drug susceptibility. The frequency of transduction of INH-susceptibility was approximately 1×10^{-6} (Table 3).

Nearly 50% of all transductants (INH^r, INH^s, or streptomycin^r) showed lysogeny, as indicated by the release of free phages spontaneously or after ultraviolet irradiation for 2 min.

From our results it is clear that INH sensitivity can be transduced at a higher frequency than can INH resistance. Presently there is no information available on the dominance of susceptibility-resistance in the action of isoniazid. The development of INH resistance results in the loss of a biochemical character (catalase-peroxidase activity), and there is every likelihood that susceptibility may be dominant to resistance. The results reveal that the attainment of INH sensitivity (by transduction) is

TABLE 2. Effect of hydrogen peroxide on the viability of *M. smegmatis*^a

Hydrogen peroxide (wt/vol) used (%)	Bacterial population (per ml)	
	INH-resistant cells	INH-sensitive cells
None (control)	1.4×10^8	1.3×10^8
0.001	1.0×10^8	1.2×10^8
0.002	1.0×10^8	1.0×10^8
0.003	2.5×10^7	1.0×10^8
0.005	2.0×10^7	6.8×10^7
0.01	1.8×10^6	5.0×10^7
0.02	5.0×10^5	2.9×10^7
0.05	2.5×10^5	1.6×10^7
0.10		3.0×10^6

^a *M. smegmatis* (INH-sensitive and -resistant strains) were grown to log phase and treated with various concentrations of hydrogen peroxide. At the end of 30 min at 37 C (with shaking), the cells were centrifuged, washed free of hydrogen peroxide, and plated out for survivors after appropriate dilutions.

TABLE 3. Transduction of INH sensitivity in *M. smegmatis*

Multiplicity of infection	No. of colonies (INH sensitive) per 1,000	
	Experimental	Control ^a
2.5	18	None
3.0	20	None
3.0	15	None

^a In the control series, broth was added in place of phage.

concomitant with the attainment of catalase activity, giving additional proof for the concept that a single protein is responsible for both characters (9; B. Gayathri Devi and K. P. Gopinathan, Proc. Soc. Biol. Chem. [India], Proc. Abstr. 31:21, 1972). Furthermore, the action of drugs can now be analyzed genetically in *Mycobacterium* by transductional analysis, especially because one-way and two-way cross-resistance between drugs (e.g., streptomycin, kanamycin, capreomycin, and viomycin) is known to exist in this genus.

We thank T. Ramakrishnan for helpful discussions and Rekha Sujeer for excellent technical assistance.

LITERATURE CITED

1. Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1969. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. 18:788-800.
2. Jones, W. D., and H. L. David. 1972. Preliminary observations on the occurrence of a streptomycin R-factor in *Mycobacterium smegmatis* ATCC 607. Tubercle 53:35-42.
3. Luria, S. E., and J. W. Burrows. 1957. Hybridization between *Escherichia coli* and *Shigella*. J. Bacteriol. 74:461-476.
4. Mizuguchi, Y., and T. Tokunaga. 1971. Recombination between *Mycobacterium smegmatis* strain Jucho and *Lacticola*. Jap. J. Microbiol. 15:359-366.
5. Ramakrishnan, T., P. Suryanarayana Murthy, and K. P. Gopinathan. 1972. Intermediary metabolism of mycobacteria. Bacteriol. Rev. 36:65-108.
6. Sundar Raj, C. V., and T. Ramakrishnan. 1970. Transduction in *Mycobacterium smegmatis*. Nature (London) 228:280-281.
7. Sundar Raj, C. V., and T. Ramakrishnan. 1971. Genetic studies in mycobacteria: isolation of auxotrophs and mycobacteriophages for *Mycobacterium smegmatis* and their use in transduction. J. Indian Inst. Sci. 53:126-140.
8. Tokunaga, T., Y. Mizuguchi, and K. Suga. 1973. Genetic recombination in mycobacteria. J. Bacteriol. 113:1104-1111.
9. Winder, F. 1960. Catalase and peroxidase in mycobacteria: possible relationship to the mode of action of isoniazid. Amer. Rev. Resp. Dis. 81:68-78.