INDUCTION OF REVERSE MUTATIONS IN A THYMINE-DEPENDENT STRAIN OF Escherichia coli AND STUDY OF DONOR ABILITY OF THE REVERTANTS

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Treatment of cells of a thymine-dependent strain of Escherichia coli with 5-bromouracil has a mutagenic action. As a result, the frequency of recombinations is disturbed in isolated reverse mutants compared with the initial type.

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The thymine analog 5-bromouracil, when incorporated into the DNA of bacteria and bacteriophages, produces a mutagenic action. Investigations of its mutagenic action on bacteriophages have been particularly numerous [4, 8]. Work has also been done on the study of induction of both direct and reverse mutants in bacteria by the action of 5-bromouracil [1-3, 9].

In face of results indicating that the sex factor is DNA and can be regarded as an additional ring chromosome of the donor cell, it can be assumed that 5-bromouracil can also be incorporated into the sex factor of bacteria, resulting in its mutation. A number of recent investigations have confirmed the possibility of obtaining such mutations [5-7].

In the present investigation we attempted to induce reverse mutations in cells of the thymine dependent strain Escherichia coli HfrH by the action of 5-bromouracil and we studied the donor ability of the isolated revertants in order to detect possible mutations of the sex factor.

EXPERIMENTAL METHOD

Strains of E. coli differing in respect to sex were used in the investigation: E. coli HfrH Thy^- B_1^- S^S λ-T_6^S; E. coli J62 F^- Pro^- Try^- His^- lac^- S^r.

Reverse mutations were induced by treatment with 5-bromouracil in liquid M-9 minimal medium. A 12-h culture of the thymine-dependent strain of E. coli grown in M-9 medium with the addition of essential growth factors for the strain (50 μg/ml thymine and 1 μg/ml vitamin B_1) was centrifuged, and washed twice with buffer solution to remove excess thymine. The culture was then suspended in a minimal volume of M-9 medium to which 5-bromouracil was added in doses of 50, 100, and 200 μg/ml. A control suspension was prepared in the same way (but without 5-bromouracil) to determine the background of spontaneous mutations. The prepared specimens were incubated at 37° with aeration for 10-12 h, and then seeded on selective medium to detect revertants.

Donor ability of the revertants was determined by crossing in the usual way, and selection was carried out relative to proline, tryptophan, and histidine.

EXPERIMENTAL RESULTS

The results obtained by treatment of the thymine-dependent mutant of E. coli with 5-bromouracil are given in Table 1, showing that the incidence of reverse mutants from thymine-dependence to prototrophism under the influence of 5-bromouracil was significantly higher than the spontaneous incidence of reversions. The most marked mutagenic effect was given by a dose of 50 μg/ml, which increased the frequency of reversions by 18 times compared with the control value ($P < 0.01$). A dose of 100 μg/ml gave a threefold increase in frequency, while a dose of 200 μg/ml did not yield a statistically significant increase in the incidence of reversion, possibly because of considerable mortality among the cells of this strain after treatment

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TABLE 1. Induction of Reverse Mutations in Thymine Dependent Strain of E. coli with 5-Bromouracil in Doses of 50 μg/ml

Expt. No.	Substances administered	Survival rate	Incidence of mutants per $1 \cdot 10^7$ cells
1	5-bromouracil	$2.6 \cdot 10^7$	13.8
	thymine	$6.9 \cdot 10^7$	0.28
2	5-bromouracil	$1.2 \cdot 10^7$	5.0
	thymine	$1.3 \cdot 10^7$	1.3
3	5-bromouracil	$4.9 \cdot 10^7$	4.8
	thymine	$8.6 \cdot 10^7$	0.13
4	5-bromouracil	$2.5 \cdot 10^7$	11.0
	thymine	$3.0 \cdot 10^7$	0.26
5	5-bromoruacil	$1.1 \cdot 10^7$	5.0
	thymine	$1.7 \cdot 10^7$	0.1

Mean incidence of mutants: bromouracil 7.4 ± 1.45; $P < 0.01$, thymine 0.4.

with this particular dose. Doses of 50 and 100 μg/ml, judging from the survival rate in our experiments and data published by other authors, are the most physiological and, at the same time, ensure adequate incorporation of the analog into DNA to yield a significant mutagenic effect.

We isolated 68 revertants, indistinguishable from the original mutant type in colony morphology and biochemical and staining properties.

The results of crossing experiments showed that the incidence of recombinations in the seven isolated strains of revertants in the seven selected strains of revertants was much lower than in the original mutant strain. The mean figures obtained by statistical analysis of the results of five experiments for each strain showed a significant decrease in frequency with several strains of revertants. The frequency of recombinations was assessed relative to three principal characteristics: proline, tryptophan, and histidine. Whereas the incidence of recombinations of the initial mutant strain relative to proline was 1.72 ± 0.22, to tryptophan 0.11 ± 0.0121, and to histidine 0.11 ± 0.12 respectively, for the revertants the incidence varied relative to proline from 0.09 ± 0.053 to 0.574 ± 0.31, to tryptophan from 0.021 ±0.0075 to 0.064 ± 0.0169, and to histidine from 0.0024 ± 0.00037 to 0.0114 ± 00036. Revertants No. 9 gave a particularly low incidence. In every case $P < 0.01$. No disturbances were found in the order of transfer of the characteristics.

For two strains of revertants Nos. 6 and 16 no significant difference was found in the incidence of recombinations compared with the original strain. Bearing in mind that in the preliminary crossing on agar they behaved like the original mutant strain, yielding recombinants, and that their sensitivity to specific male phage was the same as that of the original strain, it can be postulated that with respect to donor abilities they were indistinguishable from the mutant strain from which they were isolated.

The results obtained show that 5-bromouracil, when incorporated into bacterial DNA, is capable of exerting a considerable mutagenic action, although in large doses it lowers the viability of the cells. Remembering that although 5-bromouracil is a thymine analog, it differs from it in chemical structure and is not a normal component of the cell, we may postulate that it can produce sufficiently gross changes in the bacterial cell and in its DNA. The changes in frequency of recombination can be explained by changes in the sex factor of the bacteria under the influence of the analog, although other possible causes cannot be ruled out, such as disturbance of cell contacts, delay in transfer of markers during conjugation, and a decrease in the frequency of integration of markers and the recipient's chromosome.

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