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Mutations Conferring Quantitative and Qualitative Increases in β-Galactosidase Activity in *Escherichia coli*

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Summary. Sodium lactobionate is not utilized as a carbon source by *Escherichia coli* because it is only poorly bound and hydrolyzed by β-galactosidase and it does not induce the formation of the enzyme. However, treatment with N-methyl-N′-nitro-N-nitrosoguanidine produced 32 independent mutants able to grow on lactobionate. Most of the mutants formed β-galactosidase constitutively, 29 of them having mutations in the regulatory gene and one possibly in the operator. In addition, the mutants possessed quantitatively — or qualitatively — altered β-galactosidase. In 28 mutants the β-galactosidase activity was 1.5 to 4.5 times that of the wild-type. The enzymes of these mutants were unaltered in thermostability and substrate binding. One enzyme that was titrated immunologically possessed a molecular activity indentical with the wild-type enzyme. These mutants appear to contain extra copies of the gene for β-galactosidase. The spontaneous mutation rate to constitutivity was 6.3×10^{-3} and to the formation of apparently extra genes, 9.2×10^{-3}.

The β-galactosidases of three mutants were qualitatively changed as judged from their increased thermosensitivity, altered substrate-binding constants and greatly increased ability to hydrolyze lactose and lactobionate. Affinity for 0-nitrophenyl-β-galactoside and galactose was increased by the mutations while that for lactose was decreased; maximum velocities for the hydrolysis of 0-nitrophenyl-β-galactoside were also decreased. Relative to their rates of hydrolysis of 0-nitrophenyl-β-galactoside, these altered enzymes hydrolyzed lactose at 6 to 8 times, and lactobionate up to 23 times, the rate given by the normal enzyme. The mutations appear to increase the hydrophobic nature of the enzyme near the aglycon binding site and facilitate the hydrolysis of more hydrophilic galactosides. The lactobionic acid positive character could be transferred to other bacteria by sexual conjugation when the enzyme changes were qualitative, but not when they were quantitative.

Introduction

The β-galactoside, lactobionic acid, is utilized for growth by *Corynebacterium simplex*, which possesses a β-galactosidase capable of hydrolyzing the carbohydrate (Bernaerts and de Ley, 1957). In contrast, the affinity and catalytic efficiency of the β-galactosidase of *Escherichia coli* is too poor with respect to lactobionic acid (Kuby and Lardy, 1953) to permit growth on the compound. Therefore, the mutational acquisition of lactobionic acid utilization appeared to be a suitable character to use in the study of the genetic and biochemical origin of new functions.

Materials and Methods

Strains. Mutations were induced in *Escherichia coli* 3000, an Hfr strain containing the wild-type genes of the lactose operon; i.e. $i^+o^+z^+y^+$, where i is the gene for regulator, o the gene for operator, z the gene for β-galactosidase and y the gene for galactoside permease.

Spontaneous mutation frequencies were also measured in strains 3300 (Hfr $i^-o^+z^+y^+$), 2000 ($F^-i^+o^+z^+y^+$), 2300 ($F^-i^-o^+z^+y^+$), RV/$Fi^+o^+z^+y^+$ (RV is a deletion of the whole lactose operon of the chromosome and the lactose genes are carried in an episome), RV/$Fi^-o^+z^+y^+$ and i^-lac^+/Fi^-lac^+. M-XIII, an F^- strain with a deletion of the lactose operon and the adjacent proline *a* and *b* genes, was used in crossing experiments. The genetic nature of constitutive mutants was tested by crosses with i^s/Fi^s, a homogenote containing the super-repressed allele (i^s) of the regulator gene (Willson *et al.*, 1964) and requiring arginine and histidine.

Methods. Mutations to lactobionic acid utilization were induced in strain 3000 by treatment with N-methyl-N′-nitro-N-nitrosoguanidine (MNN-guanidine) (Langridge and Campbell, 1968). The buffers, growth media, enzyme and protein assays and tests of enzyme sensitivity to heat have also been described earlier (Langridge, 1968 *a*, *b*, *c*).

For routine enzyme assays, bacteria were grown overnight in broth medium without inducer, centrifuged, suspended in phosphate buffer, sonicated and recentrifuged. Unless otherwise stated, β-galactosidase activity is expressed as μmoles 0-nitrophenyl-β-galactoside (ONPG) hydrolyzed per minute per μg of unfractionated bacterial protein in 0.1 M phosphate buffer at pH 7.0.

Lactobionic acid (galactose-β-D-gluconic acid) was obtained as calcium lactobionate and converted to the sodium salt by treatment with the sodium form of the cation exchange resin, Zeocarb 225. A solution of calcium lactobionate was shaken with the resin until the solution failed to give a precipitate with an ammoniacal solution of 4 percent ammonium oxalate.

Immunological titrations of β-galactosidase were performed by the method of Horiuchi *et al.* (1962).

The hydrolysis of the nonchromogenic galactosides, lactose and lactobionate, was measured with the aid of galactose dehydrogenase (EC 1.1.1.48; D-galactose : NAD oxidoreductase) purchased from Boehringer and Sons, Mannheim. The reaction mixture contained β-galactosidase, lactose (2×10^{-2} M) or Na lactobionate (10^{-2} M), galactose dehydrogenase (10 μg/ml) and NAD (2×10^{-3} M) in phosphate buffer at pH 7.0. Rates of β-galactoside hydrolysis were calculated from the increase in optical density at 340 mμ.

Measurements of catabolite repression in constitutive mutants were made as follows. Bacteria were inoculated into minimal liquid medium containing either 0.2 percent glucose or 0.2 percent glycerol as a carbon source and grown overnight with shaking at 37° C. The strains were then reinoculated into fresh medium of the same composition and grown under the same conditions. Samples were taken 60 and 150 minutes after inoculation, the bacterial density read at 600 mμ, made permeable to ONPG by treatment with toluene, and assayed for β-galactosidase activity.

Results

Thirty-two mutants, which had acquired the ability to grow on sodium lactobionate, were isolated after treatment with MNN-guanidine. An enzyme extract of each mutant was prepared by the sonication of bacteria suspended in phosphate buffer. Extracts were assayed for protein content and β-galactosidase activity to give the specific activity of each mutant, as described in the methods section. The temperature sensitivities of the β-galactosidases were determined and the mutants were tested for inducibility or constitutivity of the lactose operon. The resultant data are shown in Table 1.

In all but three of the mutants the uninduced specific β-galactosidase activity was higher than that of strain 3300 which is wild-type with respect to the quantity and quality of β-galactosidase, and which does not utilize lactobionic acid. Twenty-eight of the mutant enzymes were the same as the normal enzyme in thermostability, the remaining four enzymes (from mutants 3, 5, 13 and 25) being distinctly more sensitive to heat inactivation. As will be shown below, the mutants

Table 1. *Specific β-galactosidase activities, heat sensitivities and induction states of 32 mutants utilizing sodium lactobionate, as compared with the wild-type enzyme of strain 3300*

Strain	β-galactosidase activity (μmoles ONPG hydrolysed/ min/μg protein)	Heat sensitivity[a] (% activity remaining)	Induction state
3300 (*i-*)	0.96	65	Constitutive
3300-1	2.8	65	Constitutive
2	2.75	68	Constitutive
3	4.3	25	Constitutive
4	2.04	65	Constitutive
5	0.6	19	Constitutive
6	2.08	63	Constitutive
7	2.28	65	Constitutive
8	2.9	63	Constitutive
9	2.65	69	Constitutive
10	2.72	69	Constitutive
11	3.25	68	Inducible
12	2.3	64	Constitutive
13	0.48	17	Constitutive
14	1.68	58	Partially constitutive
15	1.96	66	Constitutive
16	1.86	69	Constitutive
17	2.04	66	Constitutive
18	2.38	69	Constitutive
19	2.9	66	Constitutive
20	3.26	63	Constitutive
21	2.88	63	Constitutive
22	1.74	61	Constitutive
23	1.5	64	Constitutive
24	1.42	64	Constitutive
25	0.91	26	Constitutive
26	1.6	65	Constitutive
27	2.4	58	Constitutive
28	2.32	65	Inducible
29	3.04	68	Constitutive
30	2.88	69	Constitutive
31	3.06	65	Constitutive
32	2.14	65	Constitutive

[a] Enzyme extracts heated for 10 minutes at 57° C.

with enzymes of unaltered stability have only quantitative changes in β-galactosidase in the sense that the molecules are identical with wild-type but increased in number, while most of the mutants with thermosensitive enzymes have qualitative alterations involving changes in protein structure.

1. Quantitative Enzyme Changes. As measured by the rate of hydrolysis of ONPG, this class of mutant synthesizes β-galactosidase constitutively with an activity 1.5 to 4.5 times that of the wild-type strain (Table 1).

The growth curves of one of these mutants (number 7) on sodium lactobionate and lactose are compared with those of the constitutive wild-type (3300) in Fig. 1. The generation times on lactose were: wild-type, 0.9 hours; mutant, 0.7 hours,

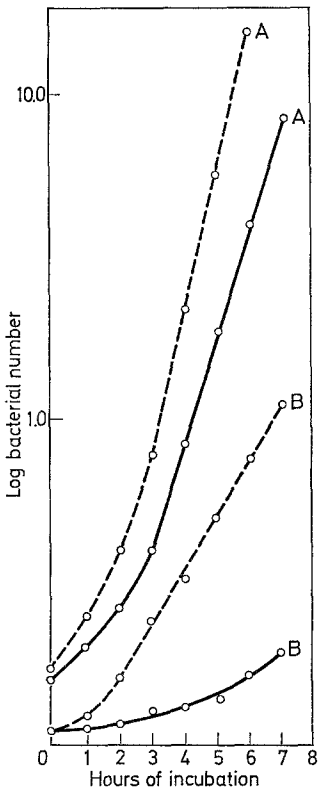

Fig. 1

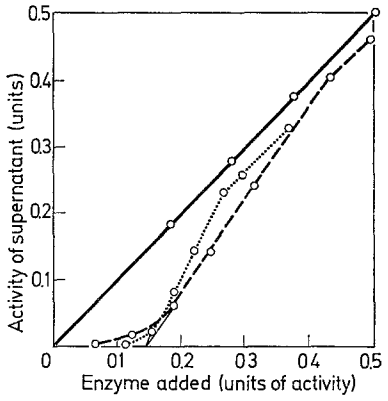

Fig. 2

Fig. 1 A and B. The growth of constitutive wild-type and mutant on lactose (A) and sodium lactobionate (B). Bacterial density in nephelometric units is plotted logarithmically (ordinate) against time of incubation at 37° C in hours (abscissa). Entire curves, growth of wild-type (3300); broken curves, growth of mutant 7 which utilizes lactobionate

Fig. 2. Immunological titration curves of β-galactosidase from constitutive wild-type (3300) and mutant 7. Increasing quantities of enzyme were added to a fixed quantity of β-galactosidase antibody. After removal of the antigen-antibody complex, the enzyme activity remaining in the supernatant (ordinate) was plotted against the units of enzyme activity initially added (abscissa). Dotted curve, enzyme of wild-type; broken curve, enzyme of mutant 7; entire line, control without antibody

and on lactobionate, wild-type about 13 hours; mutant, 1.8 hours. The 2.4-fold increase in β-galactosidase activity, although making little difference to lactose growth, causes more than a seven-fold increase in growth rate on lactobionate.

The high β-galactosidase activity of these mutants is readily lost during growth on substrates other than sodium lactobionate and during storage. After storage of the mutants for 10 months in tubes of complete agar, all but those with qualitative enzyme changes had lost the ability to grow on lactobionate. Since the quantitative mutants are clearly unstable, the variation in activity between different mutants could be due to the cultures in which enzyme activity was measured being contaminated with a varying fraction of non-mutant cells.

The molecular indentity of β-galactosidase from 3300 and mutant 7 is shown by the immunological titration curves of Fig. 2. The points of interception on the abscissa of the extrapolated slopes of the titration curves give the equivalence points of the enzymes. Since the two slopes extrapolate similarly, the enzymes have the same equivalence points and thus a given amount of antibody is neutralized by the same number of molecules in both enzymes. Therefore, the enzymes of wild-type and mutant are both immunologically and catalytically identical.

2. Qualitative Enzyme Changes. Although none of the enzymes of the "quantitative" class had altered affinity for ONPG, three of the four thermolabile enzymes showed changes in substrate affinity. Table 2 lists Michaelis constants (Km) for ONPG and inhibition constants (Ki) for lactose, lactobionate and galactose; maximum velocities (V) and Km's for ONPG from three mutants with only quantitative enzyme changes are included for comparison.

Table 2. *Maximum velocities (V), Michaelis constants (Km), inhibition constants (Ki) and initial velocities (v) for β-galactosidases from wild-type (3300) and two classes of mutants utilizing lactobionate*

Strain	V ONPG (% of wild-type)	Km ONPG ($\times 10^{-4}$M)	Ki Lactose ($\times 10^{-3}$M)	v Lactose[a] (moles $\times 10^{-9}$ in 30 min)	Ki Lacto-bionate ($\times 10^{-2}$M)	v Lacto-bionate[a] (moles $\times 10^{-9}$ in 30 min)	Ki Galactose ($\times 10^{-2}$M)
3300	100	3.1	3.1	5.4	6.5	1.0	4.15
Mutant 5	56.5	1.45	13.0	39.6	8.5	23.1	1.85
Mutant 13	45.5	2.3	9.3	32.5	8.5	15.9	2.65
Mutant 25	67	2.55	6.5	46.5	7.0	8.2	3.55
Mutant 3	482	3.1	3.1	3.1	6.5	0.5	4.25
Mutant 20	528	3.05	—	3.1	—	0.6	—
Mutant 21	452	3.15	—	2.4	—	0.4	—
Mutant 31	258	3.2	—	2.5	—	0.6	—

[a] Rates of lactose and lactobionate hydrolysis by enzyme extracts adjusted in concentration to give a rate of ONPG hydrolysis of 1.5×10^{-6} moles in 30 min.

In three of the qualitatively altered enzymes, the affinities for ONPG and galactose were increased, whereas those for lactose were decreased. If the different Km's for ONPG are plotted against the Ki's for lactose and galactose in the same mutants as in Fig. 3, a strict proportionality in substrate affinity is disclosed. The greater the increase in affinity for ONPG in a mutant enzyme, the greater is that for galactose and conversely, the less is the affinity for lactose.

These changes in the mutant enzymes are apparently not consequent upon ones increasing affinities for lactobionate which are in fact slightly lower than in the normal enzyme. Since this finding indicates that the alterations in substrate binding are not responsible for growth on lactobionate, the efficiency of catalysis in the mutant enzymes was examined. Enzyme solutions from mutants with qualitative and with quantitative changes were adjusted in concentration to give equivalent velocities of hydrolysis with ONPG. Extracts containing β-galactosidase of comparable activity with respect to ONPG hydrolysis were then used to determine initial velocities of hydrolysis of lactose and lactobionate. The galactose released by the action of β-galactosidase is oxidized by galactose dehydrogenase and the consequent reduction of NAD gives a measure of the rate of β-galactosidase action. The measured rates probably underestimate the actual rate of β-galactoside hydrolysis because of the occurrence of transgalactosidation (Wallenfels, 1960) and because the galactonolactone resulting from galactose dehydrogenase action is known to be inhibitory to β-galactosidase (Levy *et al.*, 1962). However, these rates permit a comparison between the catalytic efficiency

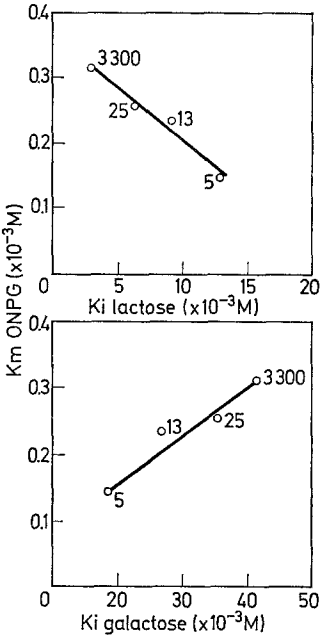

Fig. 3. Diagrams to show a correlated change in substrate binding as a result of mutation. Km ONPG (ordinate) for wild-type and mutant enzymes plotted against Ki lactose and Ki galactose (abscissa) for the same enzymes. Km and Ki are in millimolar units

Table 3. *Spontaneous mutation frequencies to the utilization of sodium lactobionate*

Strain	Number plated	Number growing	Mutants/ 10^6 bacteria	Remarks
3300 (Hfr i^-)	4.1×10^6	444	108	All grow slightly; mutants give large colonies
3000 (Hfr i^+)	8.1×10^6	129	16	No background growth; mutants give small colonies
RV/Fi^- (lac del. Fi^-)	2.3×10^4	all	—	Large colonies
RV/Fi^+ (lac del./Fi^+)	5.4×10^6	1,720	319	No background growth; mutants give large colonies
lac$^+$/F *lac*$^+$ (i^-/Fi^-)	4.0×10^4	all	—	Large colonies
2300 (F$^-i^-$)	3.3×10^6	223	68	All grow slightly; mutants give large colonies
2000 (F$^-i^+$)	3.9×10^6	35	9	No background growth; mutants give small colonies

of the mutant enzymes and that of the wild-type enzyme. The initial velocities in Table 2 show that the qualitatively altered enzymes from mutants 5, 13 and 25 hydrolyze lactobionate at rates respectively 23, 16 and 8 times faster than normal; lactose is hydrolyzed at from 6 to 8 times the normal rate. Mutants which have only quantitative changes in β-galactosidase are no different from wild-type in their rates of hydrolysis of lactose and lactobionate. Although the

heat sensitivity of the β-galactosidase from mutant 3 indicates a structural change in the enzyme, the maximum velocity for ONPG hydrolysis, the affinities for different substrates and the initial velocities for lactose and lactobionate hydrolysis are the same as in mutants with quantitative changes in enzyme activity.

3. Other Genetic Changes. As shown by Burstein (unpublished), lactobionic acid does not act as an inducer of the lactose operon nor is it transformed into an inducer by wild-type cells. When the β-galactosidase activities of the 32 lactobionate-positive mutants were measured with and without induction by 5×10^{-4} M isopropyl-thiogalactoside, the majority were found to form β-galactosidase constitutively (Table 1). Mutants 11 and 28 were, however, still inducible (uninduced activity less than 0.1% of induced activity) and mutant 14 was partially constitutive (uninduced activity about 5% of induced activity). Constitutive formation of β-galactosidase may result from a mutation in the regulator (*i* gene) or in the operator (*o* gene) (Jacob and Monod, 1961). Representative mutants of the completely constitutive class were shown to be regulator negative (i^-) and not operator constitutive (o^c) by the following tests. Mutants 3, 16, 21, 22 and 25 after transfer to F^- strains were mixed with an i^s/Fi^s strain, diluted and plated on lactose–tetrazolium agar. One hundred red colonies from each plating, which may be the i^s/Fi^s parent or heterogenotes containing both the allele conferring constitutivity and the super-repressed allele (i.e. i^-/Fi^s or o^c/Fi^s) were identified by streaking on minimal glucose medium on which the i^s/Fi^s parent fails to grow because of its requirement for arginine and histidine. About 35 percent of the red colonies were found to be heterogenotes which readily revert spontaneously to constitutive lactose-positive cells; i.e. i^-/Fi^s (lactose negative) to i^-/Fi^- (lactose positive). These tests showed that the five mutants examined were all i^-; if they had been o^c, no repressed forms of the lactobionate-positive parent would be isolated because the product of the super-repressed allele is not effective in an operator-constitutive genotype. The partially-constitutive mutant, 14, may have a mutation of the operator gene because it gives about three percent of the wild-type enzyme activity in the uninduced condition, which increases to 1.68 times the wild-type level upon induction with isopropyl-β-thiogalactoside.

In the living cell, certain products of galactoside hydrolysis may cause catabolite repression of the lactose operon (Magasanik, 1961). Since gluconic acid, which is released by the hydrolysis of lactobionic acid, leads to strong catabolite repression (Mandelstam, 1962), the lactobionate-positive mutants may have mutations reducing this repression. Therefore, six constitutive lactobionate-positive mutants were examined for repression as described in the methods section. The degree of catabolite repression was expressed as the activity of β-galactosidase formed during growth on glucose, which causes repression, as a percentage of that formed during growth on glycerol, which does not cause such repression. The results were: wild-type, 39.1%, mutant 8, 40.2%; 10, 32.6%; 12, 34.0%; 21, 41.3%; 22, 47.6%; 31, 35.2%. Thus the mutants were as sensitive as wild-type to this form of repression.

4. Origin of the Lactobionate-Positive Character. The frequency of mutation to utilization of sodium lactobionate was measured in strains of various genotype with respect to the lactose operon. Strains constitutive and inducible in the Hfr, F^- and F-prime states were examined, as well as a regulator-negative homogenote.

The results of Table 3 lead to the following conclusions. 1. Constitutive bacteria (3300, 2300) grow slightly on lactobionate, whereas inducible bacteria (3000, 2000) do not. 2. The presence of a constitutive lactose operon on an episome (RV/F*i*⁻, *i*⁻ *lac*⁺/F*i*⁻ *lac*⁺) allows better growth than the same operon in a chromosome (3300) and lactobionate-positive mutants cannot be detected. This is presumably because in a *lac*⁺/F *lac*⁺ strain there are on average three times the number of copies of the *z* gene as there are in a haploid *lac*⁺ strain (Revel, 1965). 3. F⁻ bacteria also give mutations to lactobionate utilization, suggesting that sexual fusion or recombination is not necessary in the generation of this class of mutation. 4. Good growth on lactobionate usually requires two mutations, one being inactivation of the repressor and the second a mutation leading to an increase in the specific activity of the enzyme. The spontaneous frequency of the mutation *i*⁺ to *i*⁻ as obtained in the strain RV/F*i*⁺, was 6.3×10^{-3} (rate corrected for the two episomes expected per chromosome). The frequency of the second type of mutation, as given by strain 3300, was 9.2×10^{-3}. Therefore the frequency of the double mutant, necessary to give good growth in strain 3000, would be 5.8×10^{-7}. However, the populations plated were not large enough to check the observed frequency with that expected. The colonies of strains 3000 and 2000 growing slowly on lactobionate were found on testing to be merely *i*⁻; they did not have a β-galactosidase activity greater than wild-type.

Tests were made of the ability of the lactobionate-positive character to be transferred by sexual conjugation. Three mutants with quantitative changes in β-galactosidase (mutants 14, 23 and 30) and two with qualitative changes (mutants 5 and 25) were crossed with M-XIII, an F⁻ streptomycin-resistant strain with a deletion of the lactose operon. Lactose-positive, streptomycin-resistant colonies were selected, purified and streaked on minimal plates containing sodium lactobionate. Where the lactobionate-utilizing character depended upon qualitative changes in the β-galactosidase molecule, it was transferred to F⁻ bacteria, but F⁻ lactose-positive recombinants did not grow on sodium lactobionate when the Hfr parent had only quantitative changes in β-galactosidase. The latter recombinants, however, spontaneously gave rise to lactobionate-utilizing colonies more readily than did normal constitutive bacteria.

Crosses of short duration (10 min) between Hfr mutants with quantitative enzyme changes and lactose-negative F⁻ strains failed to give colonies growing on lactose. Since strains carrying the lactose genes on an episome readily transfer their lactose-positive character under these conditions, the quantitative mutants do not possess an episome of the normal type.

Discussion

Wild-type *Escherichia coli* cannot utilize sodium lactobionate as a carbon and energy source. According to Kuby and Lardy (1953), lactobionate (presumably the calcium salt) has a low affinity for *E. coli* β-galactosidase, about 4×10^{-2} M, and the maximum velocity of enzymatic hydrolysis is abnormally low. In addition, sodium lactobionate does not induce the lactose operon (Burstein, unpublished). Sodium lactobionate is therefore not utilized because it fails to induce β-galatosidase, is poorly bound to the enzyme and hydrolyzed only with difficulty.

Mutants which grow on lactobionate are usually constitutive to overcome the lack of induction by the substrate. All the completely constitutive mutants examined contained a mutation of the regulator gene; a partially-constitutive mutant possibly had a mutation of the operator gene because it possessed the inducibility characteristics of known operator mutants (Jacob and Monod, 1961). Jacob *et al.* (1964) have demonstrated that most, if not all, inactivations of the operator require deletions of DNA, whereas the regulator gene may be inactivated by replacements of nucleic acid bases (Bourgeois *et al.*, 1965). Therefore, MNN-guanidine, which appears not to cause deletions (Langridge and Campbell, 1968), is expected to give constitutive mutants mainly by inducing changes in the regulator.

In addition to the requirement for constitutive enzyme synthesis, mutants must increase the quantity or quality of their β-galactosidase in order to grow on sodium lactobionate. If the mutant synthesizes constitutively two or three times the normal amount of enzyme, it apparently produces sufficient monosaccharide from lactobionic acid to allow slow growth. Revel (1965) has observed that bacteria with a lactose operon only on the episome possess β-galactosidase of twice the specific activity of strains with only a chromosomal operon; the homogenote (*lac*$^+$/F *lac*$^+$) gives three times the normal β-galactosidase activity. Such episomal strains grow slowly on sodium lactobionate (Table 3) and probably possess an average of two episomes per chromosome. However, the mutants growing on lactobionate which have high levels of β-galactosidase do not have episomes, at least of the normal type, because they do not show episomal transfer to F$^-$ strains. These mutants are very similar to the "hyper" strains obtained by selection for growth on low lactose concentrations for several hundred generations in a chemostat (Horiuchi *et al.*, 1962, 1963). Like the "quantitative" mutants, the "hyper" forms produced increased amounts of apparently normal β-galactosidase. The "hyper" mutants appeared to contain extra copies of the genes of the lactose operon because appropriate crosses showed segregation for the regulator and permease genes. Also, the presumed extra segments did not extend to the flanking phage-resistance and proline genes and were not associated with the sex factor. The similarities between the two types of mutant, "hyper" and "quantitative", suggest that the latter also may contain multiple lactose operons.

Mutants with the normal number of enzyme molecules, but with a hydrolytic efficiency two to three times greater than wild-type, would also be expected to grow on lactobionate. Mutants 5, 13 and 25 possess β-galactosidase which hydrolyzes lactose and lactobionate at greatly increased rates relative to ONPG hydrolysis and appear to have changes both in substrate binding and catalysis but not proportionately as was the case with mutant enzymes previously examined (Langridge, 1968a).

A comparison of rates of hydrolysis (Table 2) suggests that the greater the hydrogen-bonding property of the galactoside (lactobionate > lactose > ONPG), the greater the increase in hydrolysis by the mutant enzymes. In addition, the Michaelis and inhibition constants (Table 2) indicate that the mutations increase the binding of substrates with relatively hydrophilic aglycons (lactose, lactobionate). Apparently the amino-acid changes caused by the mutations, as well

as lowering the activation energy of hydrolysis for more hydrophilic substrates, also increase the hydrophobic nature of the enzyme in the vicinity of the aglycon binding site.

The position within the gene of mutations causing qualitative alterations in β-galactosidase could not readily be determined. With these mutations, unlike the usual ones which inactive β-galactosidase, recombinants cannot be detected by exerting selection against the parents. For this reason, genetic techniques are unsuitable for the location of this type of mutational site.

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