Mutant *Lac* Repressor

from our Molecular Genetics Correspondent

MUTATION of *Escherichia coli* seems to produce an almost infinite variety of forms of the repressor of the lactose operon, for yet another new state is reported by Jobe and Bourgeois (*J. Mol. Biol.*, **72**, 139; 1972) in the latest addition to their series of articles on the interaction of lactose repressor and operator. The bizarre i^r class of regular mutants has an inversion of the normal control process so that at low concentrations of a co-inducer synthesis of the lactose enzymes is repressed instead of induced. Of the two possible mechanisms which might produce this result, only one has previously been identified; an i^{rc} mutant requires β-galactosides for proper synthesis and assembly of the repressor, so that the control protein is only active in the cell when co-inducer has been added.

The i^{X86} mutant, the properties of which Jobe and Bourgeois now report, seems to have a different type of defect; the allosteric-like properties of the repressor protein are altered so that at low concentrations mutant cells behave as though the co-inducer increases its affinity for the DNA of the operator instead of decreasing it. At higher concentrations of co-inducer, a situation more akin to the normal state of the cell is restored and there is partial induction of the enzymes of the operon. The rate of β-galactosidase synthesis as a function of co-inducer concentration therefore follows an unusual curve. As co-inducer is added in increasing amounts there is first a dip in the curve as synthesis of the enzyme is almost completely repressed from the low constitutive level found in uninduced cells; and this is then succeeded by a rise in synthesis to a maximum level which is about seven per cent of that achieved by a wild type cell.

The affinity of the repressor for the co-inducer IPTG is the same as that of wild type repressor, but its affinity for the operator *in vitro* is different from that of normal repressor. In the presence of IPTG, the X86 repressor continues to bind to the operator when wild type repressor would have been released. But the affinity of repressor for operator is lower in the presence of IPTG. Although the rate of association of repressor and operator is very similar to that of the wild type system—both in the presence and absence of IPTG—the rate of dissociation is slower, with a half-life some twenty to forty times greater than that of wild type repressors. The half-life is reduced from some hours to about 3 minutes when IPTG is added.

These results are consistent with the behaviour of the i^{X86} mutant *in vivo* at higher concentrations of co-inducer. The affinity of the repressor for the operator is reduced when IPTG is present in sufficient amounts, although it remains greater than that of wild type repressor so that the repressor-inducer complex continues to repress the operator to some extent, restricting the expression of the operon when switched on to only a few per cent of its usual level. But the observation that cells lacking IPTG show a rather low constitutive level of expression which is reduced by addition of IPTG in low amounts cannot be explained. The idea that these cells are similar to the i^{rc} mutants and require co-inducer for synthesis of repressor or assembly of the tetramer is refuted by the isolation of active X86 repressor protein from cells grown in the absence of IPTG. One possible reconciliation is that the repressor is present in i^{X86} cells only in an inactive form, but that it is activated during its extraction.

Whatever the reason for the peculiar behaviour of this mutant, it offers the prospect that its unusual affinity for the operator may help to isolate this DNA binding site. Previous attempts to bind repressor to DNA, degrade unprotected DNA with nucleases, and thereby isolate the protected binding site, have come to grief because the repressor does not remain attached to its operator for long enough. A half-life of 30 hours is a sufficient improvement on that of one hour to raise the hope that future experiments with the X86 repressor may be more successful.

In the preceding article in this series of publications, Jobe and Bourgeois (*J. Mol. Biol.*, **69**, 397; 1972) produce a surprise about the interaction of wild type repressor with DNA. The principal product of the lactose operon is the enzyme β-galactosidase and it has often been taken for granted that the co-inducer of the operon is the substrate for the enzyme, lactose. Most of the lactose upon which the enzyme acts is hydrolysed to galactose and glucose, but some side reactions also take place, one of which is the rearrangement of lactose to its isomer allolactose. It seems that the allolactose, not galactose, is the co-inducer for the operon.

When repressor protein was isolated from cells grown on lactose, Jobe and Bourgeois found that the sugar bound to it was allolactose. Taking this hint, they tested different sugars for their ability to release repressor from DNA *in vitro* and found that allolactose is as effective as IPTG; *in vivo*, it is even more effective. This implies that *E. coli* cells must contain a small basal amount of β-galactosidase activity which acts on any galactose present in the incubation medium. Sufficient allolactose must be produced by the side reaction to induce the operon and ensure that β-galactosidase is synthesized so that further galactose molecules can be metabolized. One implication of this subtle control is that mutants in β-galactosidase itself which cannot metabolize galactose should not produce allolactose and should therefore fail to induce the enzymes of the operon.

Ion Pair Partition at Lidingö, Sweden

from a Correspondent

THE Swedish Academy of Pharmaceutical Sciences and A. B. Hässle jointly sponsored a symposium on ion pair partition and its role in analytical and preparative organic chemistry, and in membrane transport, on the island of Lidingö, near Stockholm, from October 24 to 26.

The symposium was opened by the president, Dr H. Freiser (University of Arizona), and the five sessions which followed covered extractive and chromatographic isolation of organic compounds based on ion pair partition; quantitative determinations of organic compounds as ion pairs; ion pairs as reactive intermediates in organic synthesis; ion selective electrodes; and ion pair movements across membranes.

In the first session Dr R. M. Diamond (Lawrence Radiation Laboratory, California) pointed out the need to consider activity corrections for ion pair complexes dissolved in "poor" organic solvents (of low dielectric constant and relatively inert). Although Drs E. Högfeldt (KTH, Sweden) and A. S. Kertes (Hebrew University of Jerusalem) argued in favour of ions as participants in the formation of higher aggregates and micelle formation in ion pair partition, Dr Y. Marcus (Hebrew University of Jerusalem) pointed out the possibility of explaining the same phenomenon by means of a simple dimer plus an activity correction which includes non-specific interactions. Dr M. Szwarc (State University of New York, Polymer Research Institute, Syracuse) emphasized the value of stressing the positive aspect of ion pair formation, for example, by utilizing their unique properties where appropriate—such as ESR, NMR and electronic spectroscopy—rather than the "negative aspects", that is, the disappearance of ions as characterized by many thermodynamic studies. In a similar vein Dr P. Mukherjee (University of Wisconsin) discussed spectro-