INDUCTION OF THE PLASMID TYPE IV DIHYDROFOLATE REDUCTASE

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An unusual group of trimethoprim [Tp] resistance plasmids, isolated from urinary pathogens from South India, confer only moderate levels of Tp resistance on their host and the minimum inhibitory concentration of Tp for strains harbouring them varies considerably (5-160mg/l) depending on the composition of the medium (Young & Amyes 1985). On the other hand, these plasmid bearing strains are able to grow in the presence of 160mg/l of Tp in liquid media, irrespective of its composition. The resistance mechanism of these unusual plasmids is the production of the new type IV plasmid-mediated dihydrofolate reductase [DHFR] (Young & Amyes 1986). This enzyme is ten times less sensitive to the action of Tp than the chromosomal enzyme and its synthesis is induced to high levels in the presence of Tp (Young & Amyes 1986).

The specific nature of induction was determined by challenging Escherichia coli J62-2(pUK1123) with other antifolate drugs. DHFR was prepared from litre cultures grown overnight in Oxoid Isosensitest broth [Iso] in the presence and absence of Tp (10mg/l), pyrimethamine [Py](10mg/l) and sulphamethoxazole [Sx] (100mg/l) by the method of Young and Amyes (1986). The DHFR specific activity (enzyme units/mg protein) was then calculated for each cleared lysate. The results (table) show that neither Sx nor Py was able to induce the synthesis of DHFR to a level similar to that obtained in the presence of Tp.

Media	Specific Activity
Iso	3.65
Iso + Tp	105.34
Iso + Sx	5.20
Iso + Py	4.10
DM	1.76
DM + Tp	4.95
DM + Met,Gly,Ad	1.27
DM + Met,Gly,Ad + Tp	26.86
DM + FUdR	2.01
DM + FUdR + Tp	12.98

The effect of Tp on DHFR synthesis was also investigated in Davis Mingioli minimal medium [DM]. The DHFR specific activity of cleared lysates, prepared from one litre overnight cultures grown in suitably supplemented DM medium containing Tp (10 mg/l) was found to be only three fold higher than similar cultures grown in the absence of Tp. DHFR synthesis is therefore not induced significantly in DM medium. However, when the additional supplements methionine [Met], glycine [Gly] and adenine [Ad] were present in DM medium containing Tp, DHFR synthesis was induced significantly. The effect of Met Gly and Ad is to preserve the tetrahydrofolate pool and thus the bacterium's ability to sythesise proteins is retained (Amyes & Smith 1974). 5-fluoro-2'-deoxyuridine [FUdR] also prevents the drain of the tetrahydrofolate pool. When FUdR was substituted for Met, Gly and Ad comparable induction of DHFR was observed. These results suggest that conservation of the tetrahydrofolate pool is a requirement for induction allowing the necessary protein synthesis needed for the increased production of the type IV DHFR.

Increased production of DHFR could be accounted for by multiple duplication of the DHFR gene, increased multiplication of the whole plasmid resulting in an increase in plasmid copy number or direct induction of the enzyme by a protein inducer. DNA analysis of strains grown in increasing concentrations of Tp show neither an increase in plasmid size nor an increase in plasmid copy number (estimated by increased fluoresence). Thus, it appears that increased DHFR synthesis results from direct action of an inducer protein on the DHFR gene.

We thank the Wellcome Trust for grant no. 13669/1.5 which supported this work.

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