Investigation of the Regulation of the Escherichia coli btuB Gene Using Operon Fusions

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Operon fusions were isolated between Mu dX (lac CmR ApR) and btuB, the gene encoding the multivalent vitamin B_12 outer membrane receptor. Using these fusions, vitamin B_12-mediated repression of btuB in Escherichia coli was demonstrated. Mutations in metH, metE and ompR as well as exogenous methionine, membrane pertubants, high osmolar conditions and temperature had no major effect on the expression of the btuB gene.

INTRODUCTION

In Escherichia coli, vitamin B_12 is required as a cofactor by the metH-encoded homocysteine transmethylase (Taylor & Weissbach, 1967) and by ethanolamine ammonia lyase (Chang & Chang, 1975). Of the two enzymes capable of methylating homocysteine to yield methionine, one is vitamin B_12-dependent and the second, the metE product, functions without the vitamin. Synthesis of the outer membrane BtuB receptor is necessary for uptake of exogenous vitamin B_12 (Kadner & Liggins, 1973). Previous studies have suggested that vitamin B_12 is involved in the repression of the btuB gene (Kadner, 1978), and there is good evidence that vitamin B_12, in the presence of the metH and metE gene products, represses the gene encoding the MetE homocysteine transmethylase (Mulligan et al., 1982). In short, it would appear that under conditions where the level of vitamin B_12 within the bacterium is sufficient to satisfy the requirements for this cofactor (through uptake via the system involving the BtuB receptor), there is a reduction in the synthesis of both the BtuB receptor and the inefficient metE-encoded enzyme. In order to further our understanding of vitamin B_12-mediated gene regulation, we have isolated operon fusions between btuB and lacZ making use of Mu dX (lac CmR ApR) (Baker et al., 1983). We report the effect of a number of different parameters on the control of btuB expression.

METHODS

The btuB–lacZ operon fusions were initially isolated by Mu dX (lac CmR ApR) infection of PAP 488 [rpsL Δ(lac–pro)] with selection for Ap^8, Cm^8 and, following overnight incubation in L broth, resistance to colicin E2 on lactose–MacConkey agar plates. The Btu^- phenotype of Lac^+ clones was demonstrated by resistance to colicins E1 to E8 and phage BF23, and by the inability to detect the BtuB protein by SDS-PAGE of outer membrane preparations (Pugsley & Oudega, 1986). The presence of Mu dX in btuB was confirmed by showing that, first, P1 transduction of btuB::Tn10 (from J.-P. Bohin, Orsay, France) resulted in replacement of the Cm^R Ap^R phenotype by the Tc^R phenotype and, second, Btu^- and Cm^R Ap^R were cotransducible. Four independent fusions were transferred into KL320 [metE his-29(Am) proB trpA605(Am) lacI3 lacZ118(Oc) gyrA rpsL; Birge & Low, 1974] by P1 phage (Silhavy et al., 1984) with selection for Cm^8 Ap^8 and subsequent screening for the Btu^- phenotype. That a single copy of Mu dX, inserted in btuB, was present was confirmed by showing that transduction of btuB::Tn10 [source W3110 btuB460::Tn10 from RK4936 (CGSC 6405) Δ(argF–lac)205 metE70]
RESULTS AND DISCUSSION

butB-lac operon fusions were obtained in vivo using Mu dX (lac CmR ApR). Four independently isolated Lac+ fusions were transferred into KL320. CmR ApR transductants were selected and screened for inability to use exogenous vitamin B12, exploiting the fact that metE mutants grow in the absence of methionine by virtue of the vitamin B12-dependent homocysteine transmethylase (metH product). The presence of Mu dX in butB was confirmed by showing that acquisition of butB : Tn10 rendered the strains CmR ApS (see Methods).

Exogenous vitamin B12 reduced production of β-galactosidase in all butB-lac fusion strains (Fig. 1). In general, the maximum repression ratio – the ratio of β-galactosidase activity in the presence of the highest and lowest concentration of vitamin B12 – was similar for all the fusions tested. The concentration of vitamin B12 capable of reducing lac expression depended upon whether a functional butB gene was present. Thus, in fusions haploid for butB and, consequently, lacking a functional BtuB receptor due to Mu dX insertion, the inhibitory effect of exogenous vitamin B12 was only realized at concentrations in excess of 10−5 M. Introduction of a wild-type copy of butB in trans on an F-prime plasmid (F110) allowed lower concentrations of exogenous vitamin B12 (10−8 M or greater) to exert an effect (in agreement with P. D. Moir, M. G. Hunter, J. T. Armstrong and R. E. Glass, unpublished results). The maximum repression ratio was the same in both a Btu- and Btu+ background (Fig. 1).

These experiments led us to investigate the role of a number of gene products in the regulation of butB. First, because metH has been implicated in vitamin B12-dependent regulation of metE (Mulligan et al., 1982), we introduced a metH : Tn3 allele into two of our fusion strains (AJP361, AJP461). Comparison of the repression ratio in a MetH- background (data not shown) demonstrated that the metH gene did not play a major role, if any, in vitamin B12-mediated regulation of butB. Similar experiments were conducted for ompR and metE, and similar results were obtained (data not shown); ompR and metE did not affect butB expression. ompR was tested as it has been shown to be involved in the regulation of certain outer membrane proteins and because earlier results (Pugsley et al., 1983) suggested that ompR mutations affected colicin E2 and vitamin B12 receptor activity. The absence of any effect of ompR on butB expression in L broth or minimal medium cultures was confirmed by demonstrating the same amounts of BtuB protein in the outer membranes of ompR and ompR+ cells (data not shown). In the case of metE, we wanted to confirm that the presence of methionine in the growth medium (necessary for the growth of metE butB double mutants) did not affect butB expression.

Several other potential regulatory factors of butB-lac expression were examined. High osmolality, local anaesthetics and n-alcohols are known to affect the expression of a number of genes coding for exported proteins (Pugsley, 1983). However, 300 mM-NaCl, 15 mM-phenethyl alcohol, 20 mM-procaine, 350 mM-n-propanol and 70 mM-n-butanol were without effect on steady-state butB-lac expression in L broth cultures. Low temperature (27°C) has also been reported to stimulate production of the BtuB protein (Lundrigan & Earhart, 1984). However, we
Fig. 1. Effect of exogenous cobalamin on β-galactosidase activity in four independently isolated btuB-lacZ fusions. The data is for both Btu- [AJP150 (□), AJP350 (△), AJP450 (○) and AJP750 (●)] and Btu+ [AJP151 (■), AJP351 (●), AJP451 (●) and AJP751 (●)] strains. The maximum β-galactosidase activity (in units) shown by the strains (AJP150, 101.7; AJP350, 93.9; AJP450, 90.4; AJP750, 185.6; AJP151, 98.4; AJP351, 60.2; AJP451, 46.9; AJP751, 78.3) suggests that the majority of the fusions are different. No β-galactosidase activity was found in the red parent lacking a btuB-lac2 fusion. As a further control, we confirmed that β-galactosidase activity in a wild-type strain (W3110) was unaffected by the presence or absence of the concentration of vitamin B12 employed (10-10 to 10-12 M).

were unable to reproduce this low-temperature regulation of BtuB protein; incubation temperatures in the range 24–37 °C were without effect on btuB-lacZ expression (data not shown).

Our results confirm unambiguously that vitamin B12 reduces expression of the btuB gene. Assuming no effect of translational control on btuB mRNA synthesis or stability (see Silhavy & Beckwith, 1985), the observed vitamin B12-dependent alteration in β-galactosidase activity may be interpreted to reflect regulation at the transcriptional level. Approximately 75% repression was observed, a figure in close agreement with the 80–90% reduction in vitamin B12 uptake capacity achieved in the presence of cobalamin (Kadner, 1978). That the concentration of exogenous vitamin B12 giving this repression depended upon whether or not a functional BtuB receptor was present is in agreement with earlier work (Kadner & Liggins, 1973) which showed that E. coli btuB mutants are only capable of taking up vitamin B12 at concentrations of 10-6 M or higher. Our results indicate that vitamin B12-mediated regulation of btuB does not require the metH product. Finally, we have shown that temperature, osmolarity, membrane perturbants, methionine, and ompR and metE mutant alleles do not influence the regulation of btuB expression.

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REFERENCES


