RNA polymerase, PurR and MetR interactions at the *glyA* promoter of *Escherichia coli*

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In *Escherichia coli*, the MetR and PurR proteins positively and negatively regulate *glyA* gene expression, respectively. A DNase I footprint analysis showed that both proteins bind independently to the *glyA* control region. The PurR protein blocks RNA polymerase (RNAP) from binding to the *glyA* promoter. The presence of hypoxanthine, the co-repressor of PurR, increases the ability of PurR to prevent RNAP binding, providing a model for repression of the *glyA* gene by PurR. In contrast, MetR alters the RNAP footprint pattern of the *glyA* control region. In addition, the MetR footprint is increased in the presence of RNAP, suggesting that the two proteins might interact.

Keywords: gene regulation, transcription, activation, one-carbon metabolism, protein–protein interaction

INTRODUCTION

The *glyA* gene product of *Escherichia coli*, serine hydroxymethyltransferase, catalyses the interconversion of serine, glycine and 5,10-methylenetetrahydrofolate and is the cell's major source of one-carbon units (Mudd & Cantoni, 1964). One-carbon units are used in the synthesis of purines, thymine and methionine and in the formylation of aminoacylated initiator tRNA (Blakley, 1969). Thus, it is not surprising that the serine hydroxymethyltransferase levels, which play a major role in cell physiology, are carefully controlled within a narrow range and are regulated by a number of compounds involved in one-carbon metabolism (purines, methionine and folates) (Dev & Harvey, 1984; Greene & Radovich, 1975; Mansouri *et al.*, 1972; Taylor *et al.*, 1966).

PurR, a repressor protein for genes encoding enzymes used in purine and pyrimidine nucleotide biosynthesis (Rolfes & Zalkin, 1988a, b; Kilstrup *et al.*, 1989; Meng & Nygaard, 1990), negatively regulates the *glyA* gene (Steiert *et al.*, 1990, 1992). PurR was shown to bind upstream of the *glyA* promoter and protect from DNase I attack a 24 bp region that overlaps the *glyA* promoter (Steiert *et al.*, 1992). Hypoxanthine and guanine, co-repressors of PurR for genes in the *pur* regulon (Houlberg & Jensen, 1983; Rolfes & Zalkin, 1988b; Kilstrup *et al.*, 1989; Meng & Nygaard, 1990), increased the affinity of PurR for the *glyA* operator in an *in vitro* gel mobility-shift

assay (Steiert *et al.*, 1992). However, the mechanism by which PurR, hypoxanthine and guanine repress *glyA* expression was not elucidated.

MetR, a DNA-binding protein of the LysR-family (Schell, 1993), positively regulates several *met* regulon genes (Mares *et al.*, 1992; Cowan *et al.*, 1993; Urbanowski *et al.*, 1987; Urbanowski & Stauffer, 1989). Homocysteine, a methionine pathway intermediate, functions as a co-regulator for MetR-mediated regulation of these genes, but the mechanism of homocysteine involvement in the regulation of any of these genes is unknown. MetR, with homocysteine functioning as a co-activator, also positively regulates *glyA* gene expression (Plamann & Stauffer, 1989). For *glyA*, homocysteine was shown to increase MetR binding to its target sites in the *glyA* regulatory region (Lorenz & Stauffer, 1995). However, it is not known whether the only role of homocysteine is to increase MetR binding or whether it is also required to convert MetR to an activator form.

In a *purR* mutant, MetR protein is not required for *glyA* expression (Steiert *et al.*, 1992), suggesting that the mechanism of MetR-mediated activation may involve antagonism of PurR-mediated repression. However, since the MetR-binding sites (Lorenz & Stauffer, 1995) and the PurR-binding site (Steiert *et al.*, 1992) do not overlap (Fig. 1), the mechanism by which MetR might overcome PurR-mediated repression is unknown. In this study we tested the ability of PurR, MetR and RNA polymerase (RNAP) to bind to the *glyA* control region, individually and in combinations.

Abbreviation: RNAP, RNA polymerase.

PCR amplification. PCRs were carried out under standard reaction conditions using *Taq* DNA polymerase (Promega).

PurR was then added at a constant concentration of 88 nM, followed by a 5 min pre-incubation. This is greater

Fig. 2. DNase I footprint analysis of the *glyA* control region by MetR and PurR. A 397 bp 32 P-labelled fragment (less than 12 ng) containing the MetR- and PurR-binding sites was incubated with dilutions of MetR, PurR, or MetR and PurR, and digested with DNase I. The partial digestion products were electrophoresed on a denaturing 5% polyacrylamide/7 M urea gel adjacent to the Maxam & Gilbert (1980) sequencing reactions of the labelled DNA probe. Lanes 1 and 17 contain no protein. MetR was added at the following concentrations: lanes 2 and 12, 24 nM; lanes 3 and 13, 12 nM; lanes 4 and 14, 6 nM; lanes 5 and 15, 3 nM; lanes 6 and 16, 1.5 nM. PurR was added at the following concentrations: lane 7, 88 nM; lane 8, 44 nM; lane 9, 22 nM; lane 10, 11 nM; lane 11, 6 nM; lanes 12–16, 88 nM. In lanes 12–16, PurR was pre-bound for 5 min, followed by the addition of MetR for an additional 15 min. In addition, lanes 2–6 and lanes 12–16 had homocysteine added to a final concentration of 10 mM. The location of the MetR- and PurR-binding sites are indicated by the bars.

than a 10-fold excess of the minimum concentration of PurR used in this experiment that allows 100% protection of the PurR target site (6 nM; Fig. 2, lane 11). MetR was then added at concentrations ranging from 1.5 to 24 nM, followed by an additional 15 min incubation. This range of MetR concentrations should allow detection of increased or decreased binding of MetR in the presence of excess PurR protein. Homocysteine was added at 10 mM in lanes 2–6 and 12–16 since homocysteine was shown previously to increase binding of MetR to its target region (Lorenz & Stauffer, 1995). No decrease in the ability of MetR to bind and protect its two target sites from DNase I digestion was observed at any MetR concentration used,

even though PurR was bound to and fully protected its respective target site. At the higher concentrations of MetR used (> 12 nM), PurR appeared to increase MetR binding (compare lanes 2 and 3 with lanes 12 and 13). However, the degree of protection outside the MetR-binding sites varied between experiments at the higher MetR concentrations, and whether this is a true enhancement of MetR-binding by PurR is unknown. In a reciprocal experiment, MetR was pre-bound at 22 nM for 5 min in the presence of homocysteine. This concentration allows 100% protection of both MetR-binding sites. PurR was then added at various concentrations that allow 50–100% protection and incubation was continued for 15 min at 37 °C. No significant effect of the MetR footprint was observed at any PurR concentration used (not shown). Thus, MetR and PurR appear to bind to the *glyA* control region independently. These results, along with the *in vivo* data mentioned above, suggest that although the MetR-mediated activation and PurR-mediated repression might be part of a single mechanism for *glyA* gene control, the mechanism does not appear to involve competition of the two regulatory proteins for their target sites.

Effects of hypoxanthine and PurR on RNAP binding to the *glyA* promoter region

The binding site for the PurR protein overlaps the RNAP-binding site (Fig. 1), and hypoxanthine, one of the co-repressors for PurR, was shown to increase PurR binding to the *glyA* operator region *in vitro* (Steiert *et al.*, 1992). A simple model of PurR-mediated repression of *glyA* is that PurR binding to the *glyA* operator, facilitated by the co-repressor hypoxanthine, sterically inhibits RNAP binding to the *glyA* promoter. We used the DNase I footprint assay to test this hypothesis. RNAP alone at a concentration of 22 nM binds to and protects from DNase I digestion the *glyA* promoter from about bp –60 to bp +20 (Fig. 1). To test if PurR binding interferes with RNAP binding, PurR protein was pre-bound to the *glyA* control region at concentrations of 11 or 22 nM, in the presence or the absence of 2 μ M hypoxanthine, and RNAP was then added at a final concentration of 22 nM. PurR, at a concentration of either 11 nM or 22 nM, had no effect on the ability of RNAP to bind to the *glyA* promoter, displacing the pre-bound PurR protein and protecting the *glyA* promoter from DNase I digestion (not shown). However, the addition of hypoxanthine along with PurR resulted in protection of the PurR-binding site, and loss of protection of the RNAP-binding site (not shown).

We also tested whether a high concentration of PurR, without the co-repressor, prevents RNAP binding. RNAP at a concentration of 22 or 45 nM bound to and protected from DNase I digestion the *glyA* promoter from about bp –60 to bp +20 (Fig. 3, lanes 8 and 9) and PurR at a concentration of 44 nM bound to and protected from DNase I digestion a 30 bp region centred at bp –60 (Fig. 3, lanes 1 and 2). PurR was then pre-bound at concentrations ranging from 24 to 378 nM, followed by the addition of RNAP at a concentration of 45 nM. At a

Fig. 3. Effect of PurR on RNAP binding to the *glyA* control region. A 397 bp 32 P-labelled *Bam*HI–*Hind*III *glyA* promoter fragment was used as template (see Methods). RNAP was added at the following concentrations: lanes 1 and 2, 2 nM; lanes 3–7, 45 nM; lane 8, 22 nM; lane 9, 45 nM. PurR was added at the following concentrations: lanes 1 and 2, 44 nM; lane 3, 24 nM; lane 4, 47 nM; lane 5, 95 nM; lane 6, 189 nM; lane 7, 378 nM. In lane 1, RNAP was pre-bound for 5 min, and in lanes 2–7 PurR was pre-bound for 5 min. Additional proteins were then added and incubation was continued for an additional 15 min. The locations of the PurR and RNAP-binding sites are indicated by the bars.

PurR concentration of 47 nM or less, RNAP was able to bind to and protect from DNase I digestion the *glyA* promoter (Fig. 3, lanes 3 and 4). However, PurR, at a concentration of 95 nM and higher, bound to and protected the PurR-binding site from DNase I digestion, and prevented RNAP binding and protecting the *glyA* promoter from DNase I digestion (Fig. 3, lanes 5–7). These results suggest that the mechanism of PurR-mediated repression of the *glyA* gene is to block RNAP from binding to the *glyA* promoter, and that the role of

Fig. 4. The effects of MetR on RNAP binding to the *glyA* promoter. The 397 bp 32 P-labelled *Bam*HI–*Hind*III fragment carrying the *glyA* promoter region was used as template. Lane 1 contains no protein. MetR was added at the following concentrations: lanes 2, 7 and 8, 47 nM; lanes 3, 9 and 10, 23 nM; lanes 4, 11 and 12, 6 nM. Homocysteine was added at a concentration of 10 mM in lanes 2–4 and 7–12. RNAP was added at the following concentrations: lanes 6, 8, 10 and 12, 22 nM; lanes 5, 7, 9 and 11, 45 nM. In lanes 7–12, MetR was pre-bound in the presence of homocysteine for 5 min, followed by addition of RNAP, and incubation was continued for an additional 15 min at 37 °C. The mixtures were then subjected to partial DNase I digestion (see Methods) and the samples were run on a 5% polyacrylamide/7 M urea DNA sequencing gel alongside the Maxam & Gilbert (1980) C+T and A+G sequencing reactions.

the co-repressor hypoxanthine is to stabilize the PurR/DNA complex.

MetR effects on RNAP binding to DNA

MetR positively regulates *glyA* gene expression (Plamann & Stauffer, 1989). Homocysteine, the co-activator of

MetR for activation (Plamann & Stauffer, 1989), was shown to increase the affinity of MetR for the *glyA* promoter region about twofold in a DNase I footprint assay (Lorenz & Stauffer, 1995). Since MetR does not appear to increase *glyA* expression by interfering with PurR binding to the *glyA* operator, a possible mechanism for MetR-mediated activation of a *glyA-lacZ* fusion is by increasing the affinity of RNAP for the *glyA* promoter. We used a DNase I footprint assay to test whether MetR affects RNAP binding to the *glyA* promoter. MetR, at concentrations of 6, 23 and 47 nM protected MetR-binding sites 1 and 2 from DNase I digestion (Fig. 4, lanes 2, 3 and 4). RNAP, at concentrations of 22 and 45 nM, protected the *glyA* promoter from DNase I digestion (Fig. 4, lanes 5 and 6). We then pre-bound MetR at concentrations of 6, 23 and 47 nM, in the presence of 10 mM homocysteine, followed by the addition of RNAP at either 22 or 45 nM. At the lower concentrations of MetR (6 or 23 nM), both MetR and RNAP bound and protected their respective target sites (Fig. 4, lanes 9–12). However, at a higher concentration of MetR (47 nM), there was a significant change in the MetR and RNAP footprint patterns (lanes 7 and 8). Several nucleotides in the +1 to +20 RNAP-binding region were more sensitive to DNase I attack, and nucleotides from about –60 to –75 were protected from DNase I digestion. In addition, the MetR footprint was expanded from about –77 to –177 bp. Although the significance of the altered RNAP and MetR footprints are at present unknown, they are likely to be important in transcription activation of the *glyA* promoter.

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