Study of Vitreoscilla globin (vgb) gene expression and promoter activity in E. coli through transcriptional fusion

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ABSTRACT

Bacterial hemoglobin (VtHb) is produced by the gram-negative bacterium, Vitreoscilla, in large quantity in response to hypoxic environmental conditions. The vgb gene coding for VtHb has been cloned in E. coli where it is expressed strongly by its natural promoter. The expression of the vgb gene in Vitreoscilla is transcriptionally regulated by oxygen. When E. coli cells were shifted from 20% to 5% oxygen, vgb specific transcript increased. In E. coli cells with plasmids carrying transcriptional fusions of the vgb gene promoter to either CAT (chloramphenicol acetyl transferase) or xylE (catechol-2,3-dioxygenase) genes, the promoter activity depended on the oxygen level. The concentration of CAT and xylE gene products in cells grown under 5% oxygen was 5–7 times that of aerobically (20% oxygen) grown cells. When the vgb gene promoter was deleted, VtHb was not produced under any conditions. When the promoter was replaced by the E. coli tac promoter, hypoxic oxygen did not affect the level of expression of vgb, but adding IPTG did increase the expression of this gene. These results indicate that the vgb gene promoter is transcriptionally regulated by oxygen even in E. coli, and that microaerobiosis is sufficient to induce vgb expression. The size of S1 nuclease-resistant hybrids, prepared using RNA transcripts protected with restriction enzyme fragments containing the promoter proximal region of vgb, was the same for both Vitreoscilla and E. coli, further evidence that the same promoter is used in both organisms. Transcriptional fusion of the vgb gene promoter to the xylE reporter gene on the broad host range plasmid, pKD-49, was used to demonstrate that the vgb promoter can be expressed in other gram-negative organisms, including Pseudomonas, Azotobacter, and Rhizobium.

INTRODUCTION

Vitreoscilla hemoglobin (VtHb), is the only hemoglobin of prokaryotic origin that is well characterized with regards to its structural and functional properties (1,2,3). It has been speculated, based on its oxygen binding kinetics (1), that the function of VtHb is to facilitate oxygen flux to vigorously respiring membranes of Vitreoscilla, which is an obligate aerobe usually found in hypoxic habitats. This assumption is also based on the fact that the cellular level of heme in Vitreoscilla increases many fold when cells are exposed to hypoxic conditions (4,5).

The gene (vgb) responsible for VtHb production has been cloned in E. coli along with its natural promoter (3,6). A single copy of the vgb gene on the Vitreoscilla genome was identified after Southern blot analysis, and an approximately 500 base long RNA transcript specific for vgb was detected in Vitreoscilla after Northern hybridization (3). The nucleotide sequence of the coding region (6) of vgb matches the previously reported primary protein sequence of VtHb (2) and exhibits some sequence similarity with leghemoglobin genes (6). However, the non-coding upstream regulatory region is remarkably similar to E. coli promoters (7). The regulation of expression of vgb has been shown to be at the level of transcription (8,9). The cellular level of the enzymes, delta-aminolevulinic acid synthetase (ALAS) and methemoglobin (metHb) reductase, necessary for structural and functional integrity of VtHb, also increased in hypoxic conditions (8). Since the vgb gene is expressed at a high level in E. coli under its own promoter, we further characterized regulation of this gene in response to oxygen by monitoring its activity through CAT and xylE reporter genes after transcriptional fusion.

MATERIALS AND METHODS

Strains and Growth conditions

E. coli strains HB101 and JM83 and plasmid pUC8:16 (3) were used in this study. Growth conditions and maintenance of different oxygen levels have been described previously (3,8). When minimal medium was needed, M9CA (10) was used. Glycerol (0.2%) and potassium nitrate (0.5%) were added for anaerobic growth of E. coli. All restriction and DNA modifying enzymes were obtained from Bethesda Research Laboratories (Gaithersburg, MD) or New England Biolabs Inc. (Beverly, MA). Plasmid pDR 540 and promoter probe vector pKK232-8 (11) were obtained from Pharmacia, Piscataway, NJ, and pVDX18 (12) was a generous gift from Dr. Vojo Deretic.

Plasmid construction

The strategy for constructing reporter plasmids with CAT (chloramphenicol acetyl transferase) and xylE (catechol 2,3-dioxygenase) transcriptional fusion is shown in Fig. 2. Fusion with CAT gene was created by ligating the HindIII-MluI/Klenow fill-in fragment of plasmid pUC8:16 (Fig. 2B, this fragment is approximately 500 bp including about 180 bp upstream of the
structural gene) at the Smal site of promoter probe vector pKK232-8 which contains the promoterless CAT gene preceding the multiple cloning site. The resulting plasmid pKD-10 (Fig. 2) contains the vgb promoter and two-thirds of the vgb coding region. Orientation of the insert with respect to the CAT gene was checked by AffII digestion which has a unique site within the non-coding region of the vgb. Plasmid pKD-14, where only the vgb promoter region was directly coupled with CAT gene, was derived from pKD-10 by removing an AffII-MluI restriction fragment and self ligation of the plasmid after filling the ends with the Klenow fragment of DNA polymerase. Both plasmid constructs provide chloramphenicol resistance to the host cells.

Plasmid pUC8:17 carrying the promoterless vgb gene was constructed by removing the HindIII-AffII fragment from plasmid pUC8:16 (Fig. 2B) after treatment with these enzymes. The restriction fragments were separated on low melting agarose gel (1.1%) and the larger HindIII-AffII fragment was isolated, purified through a Nick Pack column (BRL), and self-ligated with T4 DNA ligase after filling the ends with Klenow DNA polymerase.

Plasmids pDR 540:16 and pDR 540:17 which carry the vgb gene with its native promoter and the E. coli tac promoter, respectively, were constructed according to the scheme shown in Fig. 4, I. The HindIII-BamHI fragment of plasmid pUC8:16, which contains the vgb gene along with is promoter, was ligated at the HindIII-BamHI site of plasmid pDR 540 to produce pDR 540:16. In the control plasmid, pDR 540:17, the vgb promoter was replaced with the E. coli tac promoter by cloning the AffII-SalI fragment of pUC8:16 at the BamHI site of pDR 540 after filling in the ends with Klenow DNA polymerase followed by blunt end ligation. Colonies expressing the vgb gene under control of the tac promoter were identified on plates supplemented with 5 mM IPTG on the basis of their color difference due to high expression of VtHb. The presence and orientation of the insert was checked by restriction enzyme analysis. E. coli JM103 carrying the above plasmid constructs, i.e., pDR 540 (control), pDR 540:16, and pDR 540:17 were used to prepare RNA for comparative vgb transcript analysis after Northern blotting as described previously (8).

Another transcriptional fusion was created on the broad host range plasmid pVDX18 to assess vgb promoter activity in organisms other than E. coli. For that, the HindIII-AffII fragment of pUC8:16 (AffII end made blunt with the Klenow fragment of DNA polymerase) was ligated at the HindIII-EcoRI (EcoRI end made blunt) site of pVDX18, which keeps the vgb promoter in the right orientation with respect to the promoterless xylE gene. Clones that contained an insert which promoted transcription in the direction of the xylE gene were detected by the yellow color of the colonies which appeared after spraying with a solution of 100 mM catechol in 50 mM potassium phosphate buffer (pH 7.5).

S1 protection analysis of vgb transcripts
This was performed using a radioactive DNA probe containing the 5′ promoter proximal region and part of the vgb coding region. To prepare the labelled probe, plasmid pUC8:16 was treated with MluI and the 5′ end was labelled with polynucleotide kinase and [γ-32P]ATP, after removing the 5′ terminal phosphate by treatment with calf intestine phosphatase, essentially as described by Maniatis et. al (10). The labelled fragments were separated on a 2% low melting agarose gel, and the HindIII-MluI fragment was isolated and purified through a Nick Pack column (BRL). Approximately 200 μg of RNA from each strain (i.e.,
Fig. 2. A. Detailed map of vgb-CAT and vgb-xylE fusion plasmids. Recombinant plasmids, pKD-14 and pKD-49 were created by fusing the HindIII-AflII fragment of pUC8:16 (shown in Fig. 2. B) at the Smal site of pKK232-8 and the HindIII-EcoRI site of pVDX18, respectively. Details are given in Materials and Methods. Plasmid pKD-49 is a non-self transmissible RSF 1010 derivative and can be mobilized using helper plasmid pRK 2013 (32). B. Map of plasmid pUC8:16 showing cloned portion of the Vitreoscilla genome containing the vgb gene. Sites for restriction enzymes are denoted by A, AflII; B, BamHI; E, EcoRI; H, HindIII; K, KpnI; M, MluI; P, PstI; and S, Sall. Designations for genetic markers are AmpR, ampicillin resistance; xylE, gene encoding catechol 2,3 dioxygenase; CAT, gene encoding chloramphenicol acetyl transferase; vgb, gene coding for globin part of Vitreoscilla hemoglobin; oriV, origin of replication; nic, relaxation nick site; mob, determinant essential for plasmid mobilization. The double arrows, ——, indicate the portions of vgb that were cloned on the respective promoter probe vectors.

The cells were collected by centrifugation, washed in Tris-Cl buffer (pH 7.8) containing 30 μm DTT, and sonicated for approximately two min (10 sec. pulses, with at least 15 sec incubation on ice between the pulses). Cell debris was removed after centrifugation and the supernatant was used for the enzyme assays. CAT activity was measured spectroscopically by the method of Shaw (14). Briefly, 40 μl of crude lysate was added to 1.18 ml of CAT substrate (14750008T) and equilibrated at 37°C for 30 min. The background rate of acetylation was determined by monitoring the change at 412 nm for 30 min. A 24 μl aliquot of chloramphenicol (5 mM) was added and the reaction monitored for 3 min. Specific activity of enzyme was calculated as A412/0.0136/mg protein. The activity of CDO (catechol 2,3-dioxygenase), the xylE gene product, was measured essentially as described by Konyecsni and Deretic (12). Protein concentrations were determined by the method of Bradford (15). Detailed methods for determination of heme and hemoglobin have been described previously (3).

RESULTS

Effect of oxygen on vgb gene expression and hemoglobin production in E. coli

Oxygen regulates the expression of the vgb gene and hemoglobin production in Vitreoscilla. To evaluate the role of oxygen and the mechanism by which it regulates VtHb production, we studied expression of the vgb gene in E. coli in response to different oxygen levels. E. coli JM83 with recombinant plasmid pUC8:16 was grown at 20% and 5% oxygen and the amount of vgb specific RNA transcript was measured. Both VtHb mRNA and the protein itself were significantly more abundant in cells grown at 5% oxygen. Only a small amount of vgb specific transcript was detectable in cells grown at atmospheric oxygen (Fig. 1 II, III). The growth responses of cells containing plasmid pUC8 with and without the vgb gene under the above conditions were compared: the growth rate of cells with plasmid pUC8 alone was lower than with cells containing pUC8:16 with vgb present (Fig 1, I). These results confirm that the synthesis of hemoglobin in E. coli is regulated in response to oxygen as it is in Vitreoscilla (8) and that its presence is beneficial for these cells when oxygen is limited.

Coupling of vgb promoter with CAT and xylE reporter genes

Fig. 2 shows the construction of different transcriptional fusions which were created by putting promoterless CAT and xylE genes, which are present on promoter probe vectors pKK232-8 and pVDX18, respectively, under the control of the vgb promoter. Two constructs, pKD-10 and pKD-14 (Fig. 2 A, B) show different response in terms of chloramphenicol resistance, pKD-14 being more resistant (up to 300 µg/ml chloramphenicol) than pKD-10 (100 µg/ml); the latter contains two-thirds of the vgb gene in addition to the vgb promoter and the RBS (ribosome binding site). This may interfere because of transcriptional
polarity with efficient translation of the CAT gene from the second RBS site present on plasmid pKK232-8 (11) since pKD-14, which has a different attachment of the vgb promoter region, strongly expressed CAT. This plasmid is constructed from the HindIII-AflIII fragment of pUC8:16, in which the AflIII site is within the RBS site of vgb. When this fragment was cloned on pVDX18 in the proper orientation, the xylE gene was strongly expressed. The xylE fusion was specially created to assess the vgb promoter activity in organisms other than E. coli because the presence of this reporter gene is easily detectable by the yellow color of colonies on spraying plates with a solution of catechol.

Expression of CAT and xylE genes in response to growth phase and oxygen level

The observed increase in vgb specific transcript in both Vitreoscilla (8) and E. coli (Fig. 1, III) harboring pUC8:16 when oxygen was limited suggested that the transcriptional activity of the vgb promoter may be regulated by oxygen. To test this hypothesis, plasmids pKD-14 and pKD-49 having transcriptional fusion with CAT and xylE genes, respectively, were used to monitor and compare vgb promoter activity under various conditions. The specific activities of the protein products of these two genes increased sharply after mid-exponential phase in aerobically growing cells, reaching maximum values at a cell density of A500 = 1.2, and remaining fairly stable thereafter (Fig. 3). The specific activities were 5–8 times higher in cells grown under limited oxygen (5%). Our results also indicate that vgb promoter activity was induced earlier in the exponential phase of cells grown under low oxygen compared to cells grown at atmospheric oxygen (Fig. 3).

In cells grown under atmospheric oxygen the vgb promoter activity was not induced until stationary phase. To test whether microaerobiosis alone is sufficient to induce the vgb promoter, aerobically growing E. coli cells containing fusion plasmids were shifted to a different level of oxygen during early exponential phase (Table 1). The results indicated that vgb promoter activity is not dependent upon the growth phase and can be induced by oxygen limitation. Promoter activity was maximal at an oxygen level of 2%, 10–15 times higher than at an atmospheric level (Table 1).

Cells grown in limited oxygen have an increased level of vgb specific message (Fig. 1, III), higher hemoglobin content, and higher activity of reporter gene products (Table 1). These results indicate that oxygen has a repressive effect on the transcriptional activity of the vgb promoter which is relieved when oxygen falls below a certain level. To determine whether lowering oxygen below 2% would further increase expression of the vgb promoter, we grew E. coli JM83 harboring plasmids pUC8:16 and pKD-14 in the presence of different carbon sources in growth flasks flushed with nitrogen for 30 min and sealed (Table 2). Promoter activity under these conditions was higher when cells were grown in minimal media (containing 0.04% glucose and 0.2% casamino acids) supplemented with a carbon source, except additional glucose, than in the minimal medium alone and was maximum in the presence of glycerol. Glucose increased the growth rate but reduced the level of hemoglobin as well as reporter gene products. The vgb promoter activity was maximum in the presence of glycerol which does support the anaerobic growth of E. coli (Table 2). The repression of vgb expression by glucose in E. coli suggests the involvement of cyclic AMP (16,17).

Deletion and replacement of the vgb promoter and effects on vgb specific transcript

To ascertain whether the HindIII-AflIII fragment of pUC8:16 contains the vgb promoter and whether the CAT and xylE fusions were utilizing this promoter and not some upstream vector sequences, we constructed plasmid pUC8:17 with the HindIII-AflIII portion deleted. Cells transformed with pUC8:17 did not produce hemoglobin under any conditions tested, including oxygen limitation, indicating that this HindIII-AflIII region is required for vgb expression. Additionally, when this fragment was inserted at the Smal site of plasmid pKK232-8, CAT was expressed only when it was in the proper orientation and not in the reverse orientation, evidence that the CAT gene utilizes the vgb promoter for its expression.

Two plasmid constructs (Fig. 4, 1) carrying the vgb gene with

<table>
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<tr>
<th>Oxygen level</th>
<th>CAT activity U/mg protein</th>
<th>CDO activity U/mg protein</th>
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<tbody>
<tr>
<td>20% (a)</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>20% (b)</td>
<td>44</td>
<td>68</td>
</tr>
<tr>
<td>5%</td>
<td>99</td>
<td>157</td>
</tr>
<tr>
<td>2%</td>
<td>198</td>
<td>240</td>
</tr>
<tr>
<td>Nitrogen (c)</td>
<td>191</td>
<td>241</td>
</tr>
</tbody>
</table>

E. coli JM83 harboring fusion plasmids were grown in LB medium at 37°C with vigorous shaking (250 rpm) for 3 h and shifted immediately to the specified oxygen level and maintained at that level as previously described (8). After 1 h incubation the amounts of reporter proteins were measured. a. 20% oxygen level was maintained by continuously bubbling air. b. Oxygen level was not maintained (standing culture). c. Culture was bubbled with nitrogen and sealed.
Table 2. Effect of carbon sources on expression of hemoglobin and CAT.

<table>
<thead>
<tr>
<th>Carbon sources</th>
<th>Growth atmosphere</th>
<th>Total Hb (nmol/g wet wt)</th>
<th>CAT activity (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>air</td>
<td>183</td>
<td>81</td>
</tr>
<tr>
<td>Succinate</td>
<td>N2</td>
<td>215</td>
<td>101</td>
</tr>
<tr>
<td>Lactate</td>
<td>N2</td>
<td>201</td>
<td>122</td>
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<tr>
<td>Glucose</td>
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<td>63</td>
<td>38</td>
</tr>
<tr>
<td>Glycerol</td>
<td>N2</td>
<td>297</td>
<td>202</td>
</tr>
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E. coli JM83 cultures with plasmids pUC8:16 or pKD-14 were grown overnight in 100 ml minimal media (9) supplemented with nitrate and different carbon sources (0.2%) under the indicated growth atmosphere at 37°C. Oxygen was flushed by bubbling nitrogen into each 250 ml flask for 30 min through a sterilized 0.45 µ Millipore filter and the flasks sealed with a rubber stopper plus parafilm.

E. coli JM103 carrying plasmid pDR 540:16 exhibited a relatively low level of vgb specific RNA in comparison to cells carrying plasmid pDR 540:17 (Fig. 4, II A and C). However, when exposed to hypoxia (2% oxygen) a large increase in vgb transcript was observed in cells carrying the former but not the latter plasmid (Fig. 4, II B and D). This result shows that expression of vgb is induced in response to oxygen limitation only when it is under the control of its natural promoter. When the vgb gene...
was cloned under control of the tac promoter it did not respond to hypoxia, but addition of IPTG, an inducer of the tac promoter, increased the level of vgb transcript (Fig. 4, II E). No vgb specific mRNA was detected in cells containing control plasmid pDR 540 (Fig. 4, II F).

S1 protection analysis of vgb transcripts
To determine whether transcription of vgb is initiated in both Vitreoscilla and E. coli using the same promoter we performed S1 mapping with in vivo RNA transcripts which protected restriction enzyme fragments containing the promoter-proximal region of the vgb gene. RNA was prepared from Vitreoscilla and E. coli JM83 carrying recombinant plasmid pUC8:16 during the stationary phase of growth and hybridized with a radioactive probe labelled at the 5' end of the MluI site within the vgb gene (Fig. 2 B). The size of the S1 nuclease resistant hybrids, analyzed on a denaturing polyacrylamide gel (Fig. 5) was the same for both Vitreoscilla and E. coli which shows that the same promoter is utilized in both organisms. An additional faint band observed in the case of E. coli (Fig. 5 C) may be a partial product of S1 digestion. The size of the untreated control probe (Fig. 5 D) was about 500 bp, and the size of the S1 nuclease-resistant fragments were approximately 380 bp. No protected DNA fragments were observed with RNA prepared from E. coli carrying plasmid pUC8:17 which lacks the vgb promoter (Fig. 5 A).

Expression of the vgb promoter in organisms other than E. coli
It has been observed that VtHb provides a growth advantage to cells under certain situations (Fig. 1, I, and reference 18). The transfer of the globin gene to other organisms, especially those which are industrially or agriculturally important, may yield commercial benefits. To test whether the vgb promoter is expressed and regulated in other organisms besides Vitreoscilla and E. coli, we constructed promoter fusion vector pKD-49, which has the vgb promoter fused with xylE gene and contains a broad host range replicon. The promoter activity can be indirectly assessed by the rate and intensity of color development in colonies harboring this plasmid. Some gram-negative bacteria in which the level of vgb promoter expression has been measured are listed in Table 3 which shows that the vgb promoter is active in several other microorganisms.

DISCUSSION
In this study, we have analysed the regulation of the Vitreoscilla globin gene expression by characterizing its regulatory region. Experiments using transcriptional fusion and direct measurements have revealed the oxygen-responsive nature of the vgb promoter, although the mechanism involved in transducing the signals to the vgb promoter is not yet clear. We have assessed and compared the vgb promoter activity through two different transcriptional fusions in two different types of plasmids in E. coli and they showed similar responses in terms of the oxygen-dependent induction of the vgb promoter. It was shown previously that the biosynthesis of hemoglobin in Vitreoscilla is regulated at the level of transcription (8,9) and that the vgb promoter is efficiently recognised in E. coli (3,6). The present study indicates that it is regulated similarly in response to oxygen in both organisms. The vgb regulatory region when fused with reporter genes, encoding chloramphenicol acetyl transferase (CAT), or catechol 2-3 dioxygenase (CDO) in two different constructions, efficiently expressed these fusion gene products in E. coli, and production of both reporter gene products increased at oxygen levels below 5%. Other genetic loci which are regulated by oxygen in prokaryotes are positively regulated by fnr levels below 5%. Other genetic loci which are regulated by oxygen in prokaryotes are positively regulated by fnr levels below 5%. Other genetic loci which are regulated by oxygen in prokaryotes are positively regulated by fnr levels below 5%. Other genetic loci which are regulated by oxygen in prokaryotes are positively regulated by fnr levels below 5%

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<th>Strains</th>
<th>Visual Expression level</th>
<th>CDO activity U/mg protein</th>
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<tbody>
<tr>
<td>E. coli JM83</td>
<td>* * * *</td>
<td>124</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>* * *</td>
<td>147</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>* * *</td>
<td>144</td>
</tr>
<tr>
<td>Azotobacter vinellandi</td>
<td>* * *</td>
<td>- (b)</td>
</tr>
<tr>
<td>(ATCC 475)</td>
<td>*</td>
<td>28</td>
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Fusion plasmid pKD-49 was transferred into different hosts by triparental filter mating (32) using E. coli HB101 harboring the helper plasmid pRK 2013. a. The yellow color of the highest intensity that was observed in recombinant colonies after spraying with catechol solution is indicated by * * * * *. b. Not determined.
in gross changes in chromosomal superhelicity, which in turn activate expression of a large number of genes. Oxygen regulated transcription of several genes, for example nif genes in Rhodopseudomonas capsulata and Klebsiella pneumoniae (25) and photosynthetic genes in Rhodobacter capsulatus (26), may be mediated by changes in the DNA superhelix. Similarly, the tonB promoter is very sensitive to changes in DNA supercoiling which occur in response to oxygen availability (27). Further experimentation is required to determine if such a mechanism also governs vgb expression, or whether there is negative or positive regulation, governed by repressor or activator proteins, respectively.

One negative regulatory mechanism that affects the expression of vgb besides oxygen is suggested by the inhibition of the synthesis of globin and reporter gene proteins in the presence of glucose. In microorganisms, several genes are controlled through glucose repression (16) which operates by affecting the cellular level of cyclic AMP (17) and such a mechanism may also control vgb expression in E. coli. Independent evidence for the involvement of cyclic AMP or a cyclic AMP receptor protein, which are key elements in transcriptional control of the glucose sensitive operon (28), in vgb gene regulation has been reported recently (9). The transcriptional fusions described in this report will be helpful in extending the study in this direction.

In Vitreoscilla the biosynthesis of two enzymes, δ-aminolevulinic acid synthetase, the first enzyme in the heme biosynthetic pathway, and NADH-methemoglobin reductase, which keeps the protein in its physiologically functional ferrous form, are also regulated by oxygen (8) as is Vitreoscilla catalase which has the probable function of protecting the cell from hydrogen peroxide produced by the autooxidation of VtHb (29). Whether these proteins are regulated coordinately by oxygen using the same or independent mechanisms is not yet known.

The S1 mapping analysis (Fig. 5) furnished additional evidence that both Vitreoscilla and E. coli use the same promoter. Only one S1-protected DNA fragment was observed in Vitreoscilla and it was identical to the size of the major fragment from E. coli. An additional minor band, about 35 bp larger and representing 20% of the total intensity, observed in the polycarylamide gel for E. coli (Fig. 5 C) could be the result of incomplete S1 nuclease digestion. Alternatively, it could be interpreted as evidence for the existence of a second promoter which has been reported by others using primer extension analysis for publication).

Under hypoxic conditions, E. coli cells that can synthesize VtHb grow better than those cells that cannot. In Rhizobium, leghemoglobin has been shown to facilitate oxygen diffusion and respiratory activity under conditions of very low oxygen pressure (30). VtHb is closely associated with respiratory membranes in Vitreoscilla (1,31) and also in recombinant E. coli (31), which location may facilitate respiration by a similar mechanism.

The vgb promoter in the broad host range pKD-49 was also expressed in some other gram-negative bacteria with varying efficiency (Table 3). We have also transferred the entire vgb gene together with its own promoter into Pseudomonas where it is highly expressed and produces hemoglobin in relatively large amounts. Manipulation of vgb and other genes in organisms besides Vitreoscilla and E. coli using the regulatory properties of this promoter appears very feasible.

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