

Isolation, by Affinity Chromatography, of Mutant *Escherichia coli* Cells with Novel Regulation of *lamB* Expression

THOMAS FERENCI* AND KIN-SANG LEE

Department of Microbiology, University of Sydney, Sydney, New South Wales, 2006, Australia

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Affinity chromatography was used as a positive genetic selection technique for the isolation of cells exhibiting high levels of surface receptor expression. Starting from a large population of *Escherichia coli* with no maltodextrin receptor due to a deletion of *malT*, the positive regulator gene required for receptor synthesis, cells were chromatographically enriched that could bind to starch-Sepharose, an immobilized ligand of the receptor. One such isolate showed over 25% of wild-type-induced levels of receptor in the absence of *malT* and levels higher than that of the wild type in a *malT*⁺ background. In contrast to wild-type cells, receptor expression in the isolate was insensitive to control by cAMP. The maltodextrin receptor synthesized by the mutant was identical to wild-type protein in terms of ligand affinity and electrophoretic mobility and was dependent on *lamB*, the structural gene for the receptor. The directed evolution of this novel form of *lamB* expression was dependent on at least two mutations in the isolate.

Affinity chromatography may be used to separate bacteria in a population altered in the structure or expression of a specific surface protein. For example, the chromatographic counterselection of wild-type cells that bind immobilized starch has recently been used to enrich mutants altered in the maltodextrin binding site of the lambda receptor (LamB protein [5]). In this study, the initial use of affinity chromatography as a positive selection technique is described in the isolation of regulatory suppressor mutants that exhibit high levels of LamB protein under conditions that do not permit normal induction of receptor expression.

The LamB protein in the outer membrane is part of a multicomponent transport system for the uptake of maltodextrins (4), and its expression is coregulated with other gene products of the *mal* operons (12). Regulation of the *malK-lamB* operon entails positive regulation through both the *malT* product (3) and the cAMP receptor protein (1); no mutants that escape MalT-cAMP receptor protein control of *lamB* have yet been described. Affinity chromatography offers a means of isolating such regulatory mutants, since the technique permits a positive selection of cells exhibiting *lamB* expression. Given that *malT* mutants show undetectable levels of LamB protein (2), we tested in this study whether chromatographic selection could be used to isolate regulatory suppressor mutants that escape *malT* control.

Indeed, a population of glucose-grown *Escherichia coli* cells with a *malT* deletion was found

to be even less well retained by starch-Sepharose than was a glucose-grown wild-type population, which in turn was considerably less well retained than fully induced, maltose-grown wild-type cells (Fig. 1a to c). In particular, no peak eluted by competing soluble ligand was found in the *malT* stain, confirming lack of expression of receptor. To isolate mutants that expressed *lamB* in the absence of normal *malT* activation, 10⁹ cells of a *malT* deletion strain (HFrG6Δ132, genotype: HfrG6 *his* Δ*malA132*, kindly provided by M. Schwartz [9]) mutagenized with ethylmethane sulfonate was applied to a starch-Sepharose column under the conditions of chromatography previously described (5). Over 80% of the bacteria were unretained and were eluted by washing the column with 10 ml of minimal medium A (MMA) (11). Less than 1% of the applied population was subsequently eluted when the eluting buffer was changed to 3 ml of MMA supplemented with 0.1 M maltose, a ligand able to reverse the LamB-dependent binding of bacteria to starch (5). Mutants in which the effects of the *malT* deletion are suppressed should be retained and eluted by maltose; hence, they appear in the maltose-eluted fraction. Isolation of this fraction should result in a bacterial population enriched with mutants expressing a maltodextrin receptor. Repetition of the chromatographic experiment, with the bacteria recovered from the maltose fraction and again grown up on glucose, should further increase the enrichment until a population is obtained that is not eluted by MMA but is eluted by maltose.

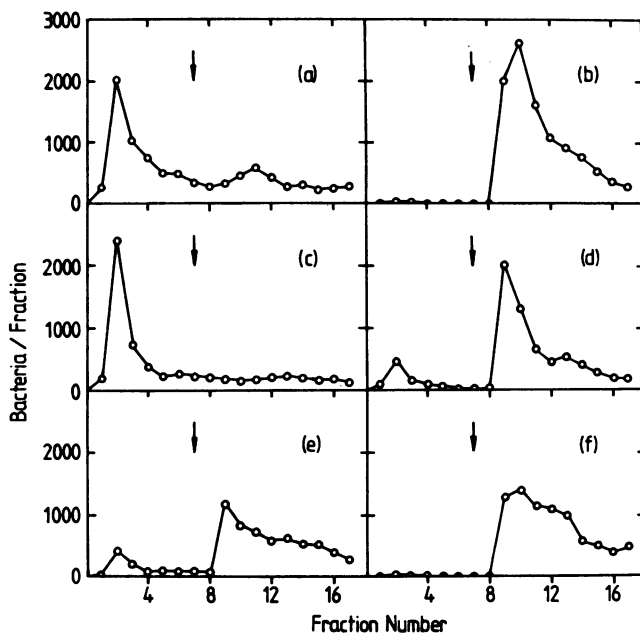


FIG. 1. Starch-Sepharose binding by *E. coli* strains altered in *lamB* expression. The starting strain of *E. coli* K-12 in these experiments was HFrG6 Δ 132 (genotype: HFrG6 *his* Δ *malA132*) that carried the Δ *malA132* deletion, removing all of *malT* (9). The strain BC26 was derived from HFrG6 Δ 132 as a member of a population able to bind starch-Sepharose and able to be eluted by maltose, under the conditions of chromatography previously described (5). BC261 is a Mal⁺ transductant of BC26 (using P1 lysate grown on wild-type HFrG6 [9]) in which the *malA* region was from wild-type cells. The wild-type strain in these studies, BW132, is near-isogenic to BC261 and was derived from HFrG6 Δ 132 through transduction to Mal⁺. The elution profiles of these strains on starch-Sepharose columns were determined as described previously (5), using 10⁴ applied cells. The first 7 fractions were eluted with MMA (11), and the last 10 were eluted with minimal medium containing 10 mg of soluble starch per ml. (a) Glucose-grown BW132, (b) maltose-grown BW132, (c) glucose-grown HFrG6 Δ 132, (d) glucose-grown BC26, (e) glucose-grown BC261, (f) maltose-grown BC261.

Using this strategy, it took 10 consecutive cycles of chromatographic enrichment, each starting with 10⁹ cells, to obtain a population of bacteria that were phenotypically binding positive. A single colony isolate from this population was effectively retained by starch-Sepharose and eluted by maltose (Fig. 1d), even after growth on glucose. The isolate (BC26) was Mal⁻, still retained the original deletion cotransducible with *aroB*, and was still unable to transport maltose, a character that requires expression of all of the genes in the *malB* region or "reversion" of *malT*.

When the *malT* deletion in BC26 was replaced by using P1 phage transduction with a lysate grown on wild-type *E. coli* and selecting Mal⁺ transductants, the derivatives still exhibited a constitutive expression of the starch-binding character (Stb^c). The Mal⁺ Stb^c transductants such as BC261 showed higher retention than in the wild type by starch-Sepharose after growth on glucose (Fig. 1e). However, maltose-grown BC261 exhibited higher retention than did glucose-grown cells (Fig. 1e and f), indicating that positive *malT* control of *lamB* expression was still operating in the isolates.

Glycerol-grown BC26 or BC261 exhibited similar retention to glucose-grown cells, and cAMP at 1 or 2 mM in the growth medium did not stimulate receptor synthesis (data not shown). Hence, the constitutive starch receptor synthesis was not subject to catabolite repression, in contrast to wild-type *lamB* expression, which is greatly increased in the presence of cAMP (15).

The starch-dependent retention by the Stb^c strains was due to constitutive expression of the *lamB* gene and not to the evolution of a novel starch receptor. First, analysis of outer membrane proteins in BC26 and BC261 showed that a protein migrating like wild-type LamB protein was present in the outer membrane of Stb^c strains (Fig. 2). However, unlike in wild-type cells, the protein was also present in glucose-grown cells, correlating with the starch binding properties shown in Fig. 1. From quantitative densitometric comparisons of the LamB peak to total outer membrane proteins (Fig. 2), the receptor made up 14% of total outer membrane protein in wild-type, maltose-grown bacteria but was undetectable in glucose-grown wild-type

cells or in the $\Delta 132$ strain. In glucose-grown BC26 and BC261, the receptor represented 4 and 6% of outer membrane proteins, respectively, or over a quarter of the fully induced wild-type level. The *Stb^c malT⁺* strain grown on maltose produced more receptor than did the wild-type strain, representing 19% of total outer membrane protein.

Comparison of the maltodextrin binding affinities of the isolate and the wild-type strain was made through assays of labeled amylopectin binding (6). It was found that the affinities of maltose-grown BC261 and wild-type cells were identical for both amylopectin and maltose (data not shown). This also suggested that the receptor responsible was the *lamB*-specified maltodextrin receptor.

Genetic inactivation of the *lamB* gene in BC261 resulted in both the loss of the *Stb^c* phenotype and the protein band corresponding to receptor. Phage λ *vir*-resistant mutants of BC261 were isolated since *Mal⁺ λ^r* mutants are known to be affected in *lamB* (16). *Mal⁺ λ^r* mutants of BC261 were checked for retention by starch-Sepharose. One such *Mal⁺ λ^r Stb⁻* isolate was analyzed by gel electrophoresis and was found to lack totally a *LamB* band in the outer membrane (data not shown). Hence, it was likely that the constitutive formation of receptor in BC261 was dependent on expression of the *lamB* gene which specifies the phage λ receptor (13) and not a novel starch-binding protein. In addition, it was found that the frequency of occurrence of spontaneous λ^r mutations in BC261 was equivalent to that found in wild-type cells. Therefore, it was unlikely that a duplication of the *lamB* gene had occurred in BC261 since λ^r mutations would have had to be rare double events.

The chromosomal location of *lamB* was still in the *malB* region since P1 lysates grown on BC26 could restore a *malK-lamB* deletion in strain *pop1730* (8), giving *Mal⁺ λ^s* transductants. None of these transductants which acquired the *malB* region from BC26 became *Stb^c*, suggesting that this phenotype was not due to a single mutation in the *malK-lamB* operon creating a *malT*-independent promoter for *lamB*. Indeed, preliminary mapping suggests that the *Stb^c* character is based on a complex regulatory circuit dependent on one *ilv*-linked mutation as well as on one or more unidentified mutations. In view of the recent reports implicating *rho* in control of *lamB* expression (2, 7), the nature of the *ilv*-linked mutation in BC26 will be of considerable interest.

In summary, it has been demonstrated that affinity chromatographic selection of bacterial cells can be used to isolate mutants with high levels of starch receptor expression. Indeed, the

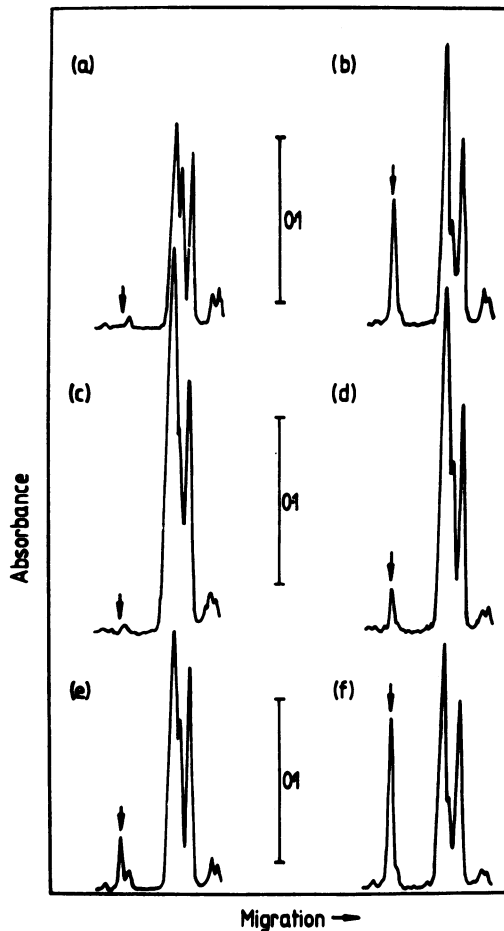


FIG. 2. Outer membrane proteins in *E. coli* mutants with altered regulation of *lamB* expression. The strains described in the legend to Fig. 1 were analyzed for outer membrane protein by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (10). The proteins were isolated after selective solubilization of cell extracts, as described by Schnaitman (14), and the outer membrane fractions were solubilized in the sample buffer of Lugtenberg et al. (10). The gels were stained with Coomassie brilliant blue, and the portion of the gel containing 30,000- to 60,000-molecular weight proteins was scanned with an LKB Ultrascan densitometer. The arrow points to the position on each track corresponding to *LamB* protein. The strains were (a) glucose-grown BW132, (b) maltose-grown BW132, (c) glucose-grown HFRG6 Δ 132, (d) glucose-grown BC26, (e) glucose-grown BC261, and (f) maltose-grown BC261.

selection pressure applied over 10 consecutive chromatographic enrichments was sufficient to result in the evolution of a novel regulatory mechanism of *lamB* expression independent of *malT* and cAMP control.

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