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Subgenes expressing single lipoyl domains of the pyruvate dehydrogenase complex of *Escherichia coli*

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Subgenes encoding the lipoyl domains from the acetyltransferase components of two types of pyruvate dehydrogenase complex of *Escherichia coli* were made by site-specific oligonucleotide-directed nonsense mutagenesis of the corresponding *aceF* genes. One of the domains is capable of binding lipoic acid whereas the other is not. The subgenes were cloned into an expression vector under the transcriptional control of the λP_L and λP_R promoters and a temperature-sensitive λ repressor. Under non-permissive conditions expression of the lipoyl domains was not detected, but 6 h after thermo-induction the domains were amplified by at least 35–50-fold relative to the normal amounts of each type of covalently bound domain.

INTRODUCTION

The pyruvate dehydrogenase complex of *Escherichia coli* catalyses the overall conversion of pyruvate into acetyl-CoA and CO_2. It comprises a structural core of 24 dihydrolipoamide acetyltransferase (E2p) components, about which are assembled multiple copies of the pyruvate dehydrogenase (E1p) and lipoamide dehydrogenase (E3) components (Reed, 1974; Danson *et al.*, 1979). The substrate is transferred between three types of active sites via linkage to the lipoyl-lysine 'swinging arms' that are located in protruding regions of the E2p chains (Bleile *et al.*, 1979).

The multienzyme complex is encoded by the *aceE* (E1p), *aceF* (E2p) and *lpd* (E3) genes comprising the *aceEF–lpd* operon (Guest, 1978; Spencer & Guest, 1985), which has been fully sequenced (Stephens *et al.*, 1983*a*,*b*,*c*). The primary structure of the E2p component, deduced from the nucleotide sequence of the *aceF* gene (Stephens *et al.*, 1983*b*), reveals the presence of three tandemly repeated highly homologous segments of about 100 amino acid residues in the *N*-terminal half of the polypeptide chain. Each repeat contains a potential lipoylation site and is at least partially acetylated in the presence of pyruvate (Packman *et al.*, 1984). The repeating units also contain a region of 20–30 amino acid residues that is unusually rich in alanine, proline and charged amino acids, and is conformationally mobile, as judged by ^{1}H-n.m.r. spectroscopy (Perham *et al.*, 1981; Radford *et al.*, 1986).

The highly segmented structure of the E2p component inferred from the sequence (Stephens *et al.*, 1983*b*; Spencer *et al.*, 1984) has been confirmed by the isolation of the three lipoyl segments as distinct functional domains following *Staphylococcus aureus*-V8-proteinase cleavage in the three (Ala+Pro)-rich sequences (Packman *et al.*, 1984). These sequences link the lipoyl domains together and join them to another structurally distinct region of about 50 residues, which is involved in binding the E3 subunit (Packman & Perham, 1986). This in turn is connected by a shorter (Ala+Pro)-rich segment to the residual inner-core polypeptide containing the E1p-binding and E2p-binding sites and the catalytic site of the E2p component (Spencer *et al.*, 1984; Packman & Perham, 1986). One and even two of the three lipoyl domains can be removed by deletion *in vitro* of the corresponding portions of the *aceF* gene with no apparent loss of overall complex activity (Guest *et al.*, 1985). However, a lipoyl-Lys-244→Gln mutation in the 'one-lipoyl domain' complex abolishes the activity of the complex, presumably by preventing its lipoylation (Graham *et al.*, 1986).

Much is known of the quaternary structure of the pyruvate dehydrogenase complex from electron microscopy (Reed & Oliver, 1968), scanning-transmission-electron-microscopic analysis (Yang *et al.*, 1985), X-ray diffraction (Fuller *et al.*, 1979) and assembly experiments *in vitro* (Bates *et al.*, 1977), and the primary structure of all of the components is known (Stephens *et al.*, 1983*a*,*b*,*c*). However, little is known of the detailed tertiary structure of any of the components. This is mainly due to the large size of the complex (M_r approx. 5×10^6) and its components (E1p, M_r 99474; E2p, M_r 65959; E3, M_r 51274), and to the difficulty of obtaining sufficient homogeneous material for structural studies. A method for circumventing these problems is to use mutagenesis *in vitro* to create subgenes expressing functional subfragments of the polypeptide components, which are more amenable to further analysis. The segmented domain structure of the E2p component is ideally suited for such an approach. The present paper reports the construction and high-level expression of

Abbreviations used: E1p, pyruvate dehydrogenase (EC 1.2.4.1); E2p, dihydrolipoamide acetyltransferase (EC 2.3.1.12); E3, dihydrolipoamide dehydrogenase (EC 1.6.4.3); PDH complex, pyruvate dehydrogenase multienzyme complex; T, % (w/v) total acrylamide; C, % (w/w) bisacrylamide/acrylamide.

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aceF subgenes encoding lipoyl domains derived from the 'one-lipoyl domain' complex and the mutant derivative lacking the ability to bind lipoic acid.

EXPERIMENTAL

E. coli strains, plasmids and bacteriophages

The following *E. coli* strains were used as hosts for derivatives of bacteriophage M13mp18 (Yanisch-Perron *et al.*, 1985): BW313 (*dut ung thi-1 relA spoT1*/F'*lysA*) for producing uracil-containing DNA templates (Kunkel, 1985); BMH71-18mutL (*lac-pro*$_{\Delta}$ *supE thi mutL*::Tn*10*/F'*proA*$^{+}$*B*$^{+}$ *lacI*^{q} *ZΔM15*) for improving mutagenic frequency (Kramer *et al.*, 1984); JM101 (*lac-pro*$_{\Delta}$ *supE thi*/F'*traD36 proA*$^{+}$*B*$^{+}$ *lacI*^{q} *ZΔM15*) for routine preparation of DNA templates for sequence analysis (Messing, 1979). The PDH-complex-deletion strain JRG1342 (*aroP-lpd*$_{\Delta}$ *metB1 met-105 azi pox pps-1 tsx-87*? *ton*? *relA1 rpsL recA1*; Guest *et al.*, 1985) and strain CAG629 (*lac*$_{am}$ *trp*$_{am}$ *pho*$_{am}$ *htpR*$_{am}$ *mal rpsL lon supC*$_{ts}$; kindly provided by Dr. C. A. Gross) were used as hosts for expression of plasmid-encoded lipoyl domains. The plasmids pGS110 and pGS155, expressing the 'one-lipoyl domain' PDH complexes, have been described previously (Guest *et al.*, 1985; Graham *et al.*, 1986), as has the $\lambda P_L \lambda P_R$-expression vector pJLA502 (Schauder *et al.*, 1987), kindly provided by Dr. J. E. G. McCarthy.

Oligonucleotide-directed mutagenesis

The 'two-primer-extension plus ligation' method for mutagenesis was performed in accordance with Zoller & Smith (1984) except that uracil-containing M13 templates were used (Kunkel, 1985). Mutant-strand selection was by transfection of strain BMH71-18mutL (*ung*$^{+}$) cells followed by generation of M13 plaques in a lawn of strain JM101 cells to avoid unnecessary exposure of the bacteriophages to the mutator strain (Carter *et al.*, 1985). Mutant bacteriophages were identified by the dot-hybridization procedure, with ^{32}P-labelled mutagenic primer as a probe (Zoller & Smith, 1983).

Nucleotide sequence analysis

Single-stranded M13 templates were prepared and sequenced by the dideoxy chain termination method with the use of [α-[^{35}S]thio]dATP and salt-gradient gels (Sanger *et al.*, 1980; Biggin *et al.*, 1983). A series of synthetic oligonucleotides were used to confirm the sequence of the M13 constructs: S1, GTAAAACG-ACGGCCAGT ('universal' primer); S9, GTGAACAT-AAGCGTCGT (4767–4751); S11, TGCGCCTTAAAC-TTCGAA (4632–4615); S24, AGGATCTCGGTGATT-TC (3845–3829). Co-ordinates in parentheses correspond to the *aceF* sequence (Stephens *et al.*, 1983*b*).

Molecular cloning and cassette-replacement

Preparation of plasmids, restriction-endonuclease digestion, isolation of DNA fragments, ligation and transformation were done by standard methods (Maniatis *et al.*, 1982). Primer-extension cloning from M13 (Hong, 1981) was used to transfer the *aceF*' subgenes from M13 to the expression vector pJLA502. M13 ssDNA (1 pmol) was annealed to 'universal' primer (30 pmol) in 20 μl of annealing buffer (10 mM-Tris/HCl buffer, pH 8.5, containing 10 mM-$MgCl_2$). Samples (4 μl) of each of the four dNTPs (0.5 mM) were added and the extension reaction was started with 5 units of DNA polymerase (Klenow fragment). After 15 min a further 5 units of DNA polymerase were added. After a total of 30 min the reaction was stopped by heating at 70 °C for 10 min. The DNA was precipitated by adding 4 μl of 3 M-sodium acetate buffer, pH 4.8, plus 60 μl of cold ethanol and storing at −70 °C for 1 h. The DNA was then restricted with *Nco*I plus *Sph*I, and the 550 bp fragments containing the subgenes were cloned into the gel-purified receptor fragment of pJLA502.

Pulse–chase-labelling of proteins

Expression-plasmid transformants of strain JRG1342 were grown at 30 °C in minimal medium E (Vogel & Bonner, 1956) containing D-glucose (10 mM), potassium acetate (2 mM), potassium succinate (2 mM), L-methionine (30 μg/ml), thiamin hydrochloride (5 μg/ml) and sodium ampicillin (25 μg/ml) following inoculation (1:20) from an overnight culture grown in the same medium. The cultures were shifted to 42 °C at $A_{650} = 0.3$ and incubated for 20 min at the higher temperature. Samples (1 ml) were added to 5 μCi (100 μl) of [U-^{14}C]protein hydrolysate, the mixture was incubated for 90 s at 42 °C and then 200 μl of casein hydrolysate (50 g/l) containing 10 mM each of methionine and tryptophan was added as the chase solution. After 90 s the cells were spun down at 4 °C and either resuspended in SDS dissociation buffer for denaturing gel electrophoresis or disrupted by sonication for native gel electrophoresis. After electrophoresis the gels were stained with Coomassie Brilliant Blue (1 g/l), treated with the autoradiographic enhancer ENLIGHTNING according to the manufacturer's instructions, and exposed to Fuji RX X-ray film at −70 °C.

Over-expression of lipoyl domains

Expression-plasmid transformants of strains CAG629 and JRG1342 were grown at 30 °C in L-broth (Lennox, 1955) containing ampicillin (100 μg/ml) with glucose (1 g/l) as required following inoculation (1:100) from an overnight culture. The cultures were shifted to 42 °C at $A_{650} = 0.4$–0.6, and samples were taken at various intervals after induction of expression. The cells were washed in 40 mM-potassium phosphate buffer, pH 7.8, suspended to 1/25 of their original volume and disrupted by sonication. Cell debris was removed by centrifugation and supernatant extracts were subjected to polyacrylamide-gel electrophoresis.

Polyacrylamide-gel electrophoresis of protein samples

Polyacrylamide-gel electrophoresis was performed in accordance with Packman *et al.* (1984) in the presence or in the absence of SDS with resolving gel 27% T, 0.3% C, and stacking gel 5% T, 2.5% C. Staining was with Coomassie Brilliant Blue, sometimes followed by silver staining (Morrissey, 1981).

Materials

The [U-^{14}C]protein hydrolysate (57 mCi/mg-atom of C), [γ-^{32}P]ATP (3000 Ci/mmol) and [α-[^{35}S]thio]dATP (410 Ci/mmol) were supplied by Amersham International, and ENLIGHTNING was purchased from New England Nuclear. Restriction endonucleases, DNA polymerase (Klenow fragment), T_4 DNA ligase and T_4 polynucleotide kinase were from either Bethesda Research Laboratories or Boehringer Corp. The 'universal' sequencing primer

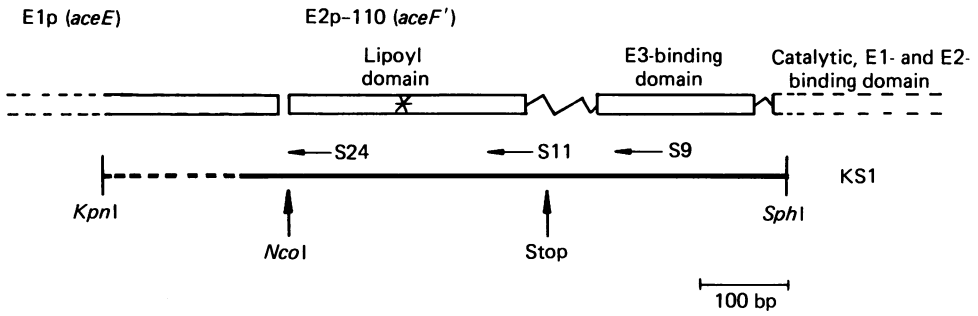

Fig. 1. Strategy adopted for constructing subgenes expressing single lipoyl domains

The 1.68 kb *Kpn*I–*Sph*I fragment cloned in the M13mp18 derivative KS1 is shown as a bold line. It encodes a *C*-terminal fragment of E1p (*aceE*) and an *N*-terminal fragment of the 'one-lipoyl domain' E2p-110 chain (*aceF'*) comprising the lipoyl and E3-binding domains (indicated as open bars). These domains are linked to each other and to the E1- and E2-binding and catalytic domain by (Ala+Pro)-rich segments of polypeptide chain indicated by zigzag lines. The asterisk shows the position of the lipoylatable lysine residue (Lys-244), and the horizontal arrows indicate the hybridization positions of three sequencing primers. The vertical arrows denote the mutagenesis targets for introducing *Nco*I sites and stop codons in the construction of subgenes expressing the lipoyl domain.

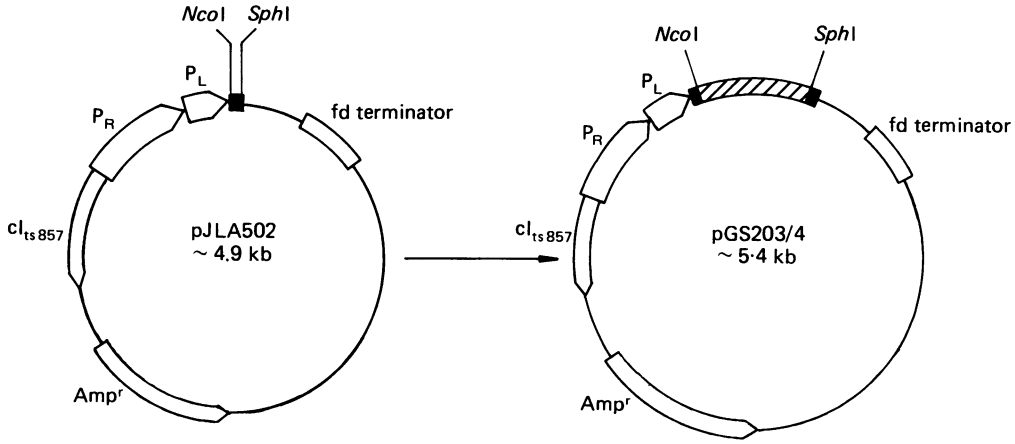

Fig. 2. Construction of plasmids expressing the lipoyl domains

The relevant features of the expression vector pJLA502 are shown. The λP_L and λP_R promoters drive transcription of genes cloned into the multi-cloning site (black box) but not beyond the fd terminator. The unique *Nco*I site (CCATGG) contains an initiation codon, which is placed optimally downstream of the *atpE* ribosome-binding site. At 30 °C the λcI_{ts} product represses transcription, whereas at 42 °C it is inactive and transcription occurs. The 0.55 kb *Nco*I–*Sph*I fragment from NS203 (hatched box) was cloned into plasmid pJLA502 to produce plasmid pGS203. This plasmid expresses a lipoylatable lipoyl domain. An analogous plasmid, pGS204, in which the lipoyl-lysine codon is replaced by glutamine codon, was constructed by the same procedure.

was from Celltech, and other oligonucleotides were either gifts from G. D. Searle Ltd. or made on an Applied Biosystems 380A DNA synthesizer.

RESULTS

Construction of subgenes for the expression of lipoylated and unlipoylated lipoyl domains

The strategy adopted for the construction and expression of subgenes encoding lipoyl domains with and without the potential for binding lipoic acid is summarized in Figs. 1 and 2. The M13mp18 derivative KS1 contains a 1.68 kb *Kpn*I–*Sph*I fragment from plasmid pGS110 (Guest *et al.*, 1985). It encodes a *C*-terminal fragment of E1p (*aceE*) and an *N*-terminal fragment of the 'one-lipoyl domain' E2p chain, E2p-110 (*aceF'*). The M13 clone KS155, derived from plasmid pGS155, is identical with KS1 except for a single point mutation in *aceF'* that converts the lipoyl-Lys-244 codon (AAA) into a glutamine codon (CAA), and abolishes lipoylation of the E2p-155 polypeptide chain (Graham *et al.*, 1986). Both bacteriophages thus contain DNA that is suitable for generating lipoyl-domain-encoding subgenes by oligonucleotide-directed mutagenesis.

The $\lambda P_L P_R$-expression vector pJLA502 has a unique *Nco*I site (CCATGG) containing a well-placed initiation codon just downstream of the efficient *atpE* ribosome-binding site (Fig. 2; Schauder *et al.*, 1987). In order to use this vector for expressing the lipoyl domains, *Nco*I sites were created at the beginning of the *aceF'* genes (Figs. 1 and 3). Uracil-containing single-stranded DNA templates of KS1 and KS155, prepared by the method of Kunkel (1985), were used separately in the 'two-primer-extension plus ligation' mutagenesis method of Zoller & Smith (1984). The mutagenic primer S30,

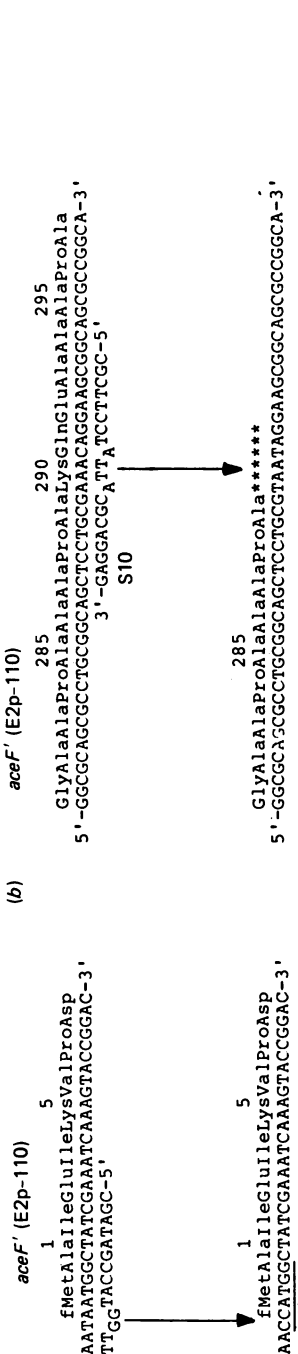

Fig. 3. Oligonucleotide-directed mutagenesis used to introduce *Nco*I sites and tandem stop codons in *aceF* genes

(*a*) The mutagenic 22-mer (S30) was used to introduce an *Nco*I site at the start of the *aceF* structural genes cloned in KS1 and KS155 without affecting the coding region. (*b*) Tandem stop codons were introduced into *aceF* genes by using the mutagenic 20-mer (S10). The Lys-290 and Gln-291 codons (AAA and CAG) were changed into ochre (TAA) and amber (TAG) respectively. The amino acid sequences are numbered in accordance with Stephens *et al.* (1983*b*), and the DNA sequence is that found in KS1 and KS155.

CGATAGCCATGGTTCTTTTACC, hybridizes to positions 3793–3772 in the *aceF* sequence (Fig. 3*a*; Stephens *et al.* 1983*b*) except for two mismatches (*), and was used in conjunction with S9, a perfect-matching 'helper' primer, to generate an *Nco*I site. The mutagenic reaction mixes were used to transfect BMH71-18mutL, and 12 derivatives from each experiment were tested by the dot-hybridization procedure with S30 as probe. All gave signals at room temperature, whereas six out of 12 (KS1) and eight out of 12 (KS155) were positive at 64 °C. The mutant M13s were plaque-purified, and the presence of *Nco*I sites was verified by nucleotide sequence analysis with S24 as primer (Fig. 1).

One of each type of *Nco*I mutant M13 was passaged through strain BW313 to provide templates for a further round of mutagenesis with a primer (S10, CGCTTCCTATTACGCAGGAG) designed to introduce tandem stop codons downstream of the lipoyl-domain-coding regions. The primer hybridizes at positions 4665–4646 with mismatches at 4654 and 4657 generating the codon changes AAA→TAA (Lys-29→ochre) and CAG→TAG (Gln-291→amber). The 'universal' primer was used as 'helper' in the mutagenesis reactions, and after transfection and dot-hybridization two out of 12 (KS1) and five out of 12 (KS155) were positive at 62 °C. One mutant of each type was plaque-purified, and the presence of the stop codon mutations was confirmed by sequencing with S9 as primer (Fig. 1). Finally, both M13 derivatives containing *Nco*I sites and tandem stop codons at the extremities of the lipoyl-domain subgenes were sequenced over the region of interest with S9, S11 and S24 as primers (Fig. 1). No changes other than those required were found (results not shown). The M13 derivatives having the wild-type lipoyl-Lys codon and the lipoyl-Lys→Gln mutation were designated NS203 and NS204 respectively.

In order to express the lipoyl domains, the relevant 0.55 kb *Nco*I–*Sph*I fragments from partially double-stranded primer extensions of NS203 and NS204 templates were subcloned into plasmid pJLA502 to produce plasmids pGS203 and pGS204 respectively (Fig. 2). The gross structures of these plamids were confirmed by using a variety of endonucleases giving diagnostic restriction patterns.

Expression of the lipoyl domains

Two *E. coli* strains were chosen as hosts for expression studies: JRG1342 (*ace-lpd*$_\Delta$ *recA*), which provides a background deficient in PDH complex including the acetyltransferase lipoyl domains, and CAG629 (*htpR lon*), which lacks the 'heat-shock' proteins and Lon proteinase. Amp^R transformants of JRG1342 carrying plasmids pGS203, pGS204 and pJLA502 were subjected to pulse–chase-labelling of proteins and denaturing polyacrylamide-gel electrophoretic analysis as described in the Experimental section. The strains harbouring plasmids pGS203 and pGS204 express polypeptides of M_r approx. 9000 and 9400 respectively, which are not present in the vector control (Fig. 4*a*). The M_r values of these plasmid-encoded polypeptides compare favourably with those predicted from the DNA sequence for the corresponding lipoyl domains: 9106 when lipoylated and 8918 for the non-lipoylated and lipoyl-Lys-244→Gln derivatives.

The lipoyl domain generated by *S. aureus*-V8-

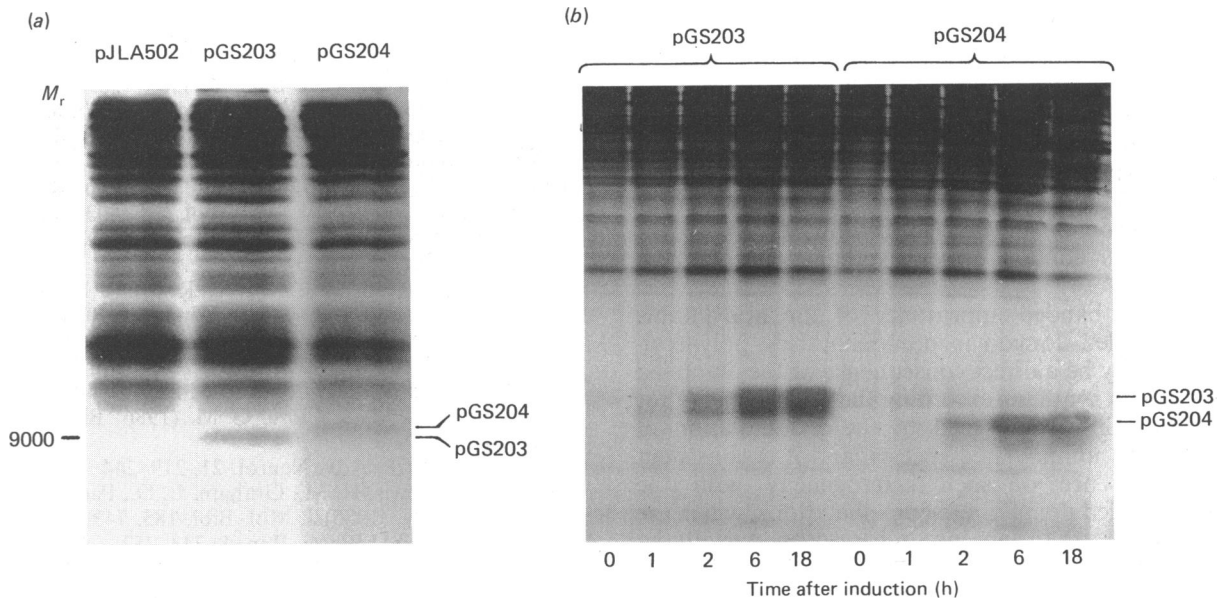

Fig. 4. Lipoyl domain expression

(*a*) Expression of lipoyl domains in *E. coli* strain JRG1342 from plasmids pGS203 and pGS204 following induction for 20 min and pulse–chase labelling with ^{14}C-containing protein hydrolysate. Whole cells were analysed by SDS/polyacrylamide-gel electrophoresis (27% T, 0.3 % C) and fluorography. Note the faster migration of the pGS203-encoded domain relative to that of pGS204, and the absence of these polypeptides (M_r approx. 9000) with plasmid pJLA502. (*b*) Expression of lipoyl domains in plasmid-containing *E. coli* strain CAG629. Cell-free extracts of cells harvested at different times after induction were analysed by native gel electrophoresis (27% T, 0.3% C) followed by Coomassie Brilliant Blue and silver staining. Note the slower migration of the pGS203-encoded domain relative to that of pGS204 in the absence of SDS.

proteinase cleavage of the E2p-110 (lipoyl-Lys-244) chain at Glu-292 (Fig. 3*b*) is known to migrate faster in denaturing polyacrylamide gels than the equivalent domain of E2p-155 (lipoyl-Lys-244→Gln), a feature that has been attributed to differences in the SDS-binding capabilities of the two domains (Graham *et al.*, 1986). However, the relative mobilities of the domains are reversed in native gels, where the resolution of different lipoyl domains is also greatly enhanced (Graham *et al.*, 1986). These reversals of electrophoretic mobilities provide a ready confirmation of the identities of the polypeptides expressed by plasmids pGS203 and pGS204. Native electrophoretograms of cell-free extracts of pGS203 and pGS204 transformants of strain CAG629 (*htpR lon*) prepared at different times after induction at 42 °C are shown in Fig. 4(*b*). The pGS203-specific product migrates as a doublet under these conditions (see the Discussion section), but both components are now retarded relative to the pGS204-specific product. Similar results were obtained with JRG1342 as the host strain (results not shown). Clearly, the plasmid-encoded polypeptides have the electrophoretic properties predicted for the two types of lipoyl domain.

The amounts of lipoyl domain relative to total protein in the cell-free extracts of strains CAG629(pGS203) and CAG629(pGS204) 6 h after induction were measured by densitometric analyses of Coomassie-Brilliant-Blue-stained native polyacrylamide gels. The values, 1.7% (w/w) for the pGS203-encoded domain and 1.3% (w/w) for the pGS204-encoded domain, are underestimates owing to the very poor staining properties of the lipoyl domains (Bleile *et al.*, 1979; Guest *et al.*, 1985). They are also further underestimated relative to total cell protein because phase-contrast-microscopic examination revealed the presence of inclusion bodies in the over-expressing strains at 6 h and subsequent times after induction, and the amount remaining in the debris after ultrasonic disruption was not determined.

DISCUSSION

The acetyltransferase component (E2p) of the pyruvate dehydrogenase complex comprises several distinct structural and functional domains concerned with lipoic acid-binding (Stephens *et al.*, 1983*b*; Packman *et al.*, 1984), E3-binding and acetyltransferase activity (Packman & Perham, 1986). To facilitate further analyses of these domains, it is desirable to have enriched sources from which to purify them. Previous studies with tyrosyl-tRNA synthetase (Waye *et al.*, 1983), DNA polymerase I (Freemont *et al.*, 1986) and the cyclic AMP receptor protein (Gronenborn & Clore, 1986) have shown that subgenes, synthesized by deletion *in vitro*, can be used to express protein domains possessing partial activities such as tyrosyladenylate formation, DNA polymerization and DNA-binding respectively. Comparable subgenes of *aceF* expressing the hybrid lipoyl domains of the 'one-lipoyl domain' complexes (Guest *et al.*, 1985; Graham *et al.*, 1986) have now been constructed. However, in this case oligonucleotide-directed mutagenesis was used to introduce tandem stop codons directing premature termination of polypeptide chain synthesis at a predetermined position. This method should be generally applicable to any gene–protein system for generating *N*-terminal segments of polypeptide chains.

The $\lambda P_L P_R$ promoter vector pJLA502 (Schauder *et al.*, 1987) enables the controllable expression of lipoyl domains to a high level. Before induction of expression

there is little or no synthesis of lipoyl domains (Fig. 4*b*), owing to the plasmid-encoded λ repressor, but 6 h after induction at 42 °C the domains account for at least 1.3–1.7% of the protein in the extracts without allowing for their poor stainability or for the domain content of the inclusions. This is somewhat greater than the normal cellular content of pyruvate dehydrogenase complex (about 1%; Smith & Neidhardt, 1983), and it represents an amplification of at least 35–50-fold relative to the normal cellular content of each specific type of lipoyl domain.

The double-banded appearance of the lipoylatable pGS203-encoded domain in non-denaturing polyacrylamide gels may be a direct consequence of the amplified rate of domain synthesis exceeding the cell's capacity for lipoylation. This would lead to the accumulation of lipoylated and unlipoylated species, and it is consistent with the absence of such heterogeneity with the pGS204-encoded domain. Other explanations, which are also consistent with the absence of heterogeneity with the pGS204-encoded domain, likewise depend on post-translational changes such as acetylation, oxidation or mixed-dithiol formation of the lipoylated species.

The individual types of lipoyl domain have previously been isolated from the purified complex in very small amounts after limited proteolysis, gel filtration and ion-exchange chromatography (Packman *et al.*, 1984). The availability of an enriched source of free lipoyl domain should facilitate the isolation of relatively large quantities, especially if native domain can be recovered from the inclusions. This in turn should advance the structural determination by X-ray diffraction or two-dimensional n.m.r. analysis to shed light on the role of the small internal repeat within each domain and the possible relationship to the small dithiol proteins glutaredoxin and thioredoxin (Spencer *et al.*, 1984).

The lipoyl-lysine residue of the lipoyl domain is a good substrate for reductive acetylation by free E1p, whereas free lipoic acid and lipoyl-lysine are not (Packman *et al.*, 1984). Presentation of lipoamide to the E1p active site by the lipoyl domain is thus important for catalysis; the engineered lipoyl domains with and without the ability to bind lipoic acid should aid the investigation of the protein–protein interactions involved. Similarly there is now the possibility of supplying lipoyl domains (*in trans*) for studying complementation *in vivo* and *in vitro* of an engineered pyruvate dehydrogenase complex that lacks its own lipoyl domains (Angier *et al.*, 1987).

It is hoped that protein engineering, expression and purification of further domains of the acetyltransferase component will lead to a better understanding of the structure and function of the pyruvate dehydrogenase multienzyme complex.

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