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A Carboxy-Terminal Deletion Impairs the Assembly of GroEL and Confers a Pleiotropic Phenotype in *Escherichia coli* K-12

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A series of COOH-terminal deletions of the chaperonin GroEL have been examined for effects in vivo at haploid copy number on the essential requirement of GroEL for cell growth. Strains with a deletion of up to 27 COOH-terminal amino acids were viable, but no viable strain could be isolated with a deletion of 28 or more codons. When substitutions were placed in the COOH-terminal amino acid Val-521 of the 27-amino-acid-deleted (Δ27) mutant, we found variable effects—Trp and Glu led to inviability, whereas Arg and Gly were viable but slow growing. The effects of the Arg substitution plus deletion (V521RΔ) were examined in more detail. Whereas the Δ27 mutant with the wild-type residue Val-521 grew as well as a strain with wild-type GroEL, the V521RΔ mutant strain (*groEL202*) exhibited a broad range of phenotypic defects. These include slow growth; filamentous morphology; a defect in plating λ; absence of activity of expressed human ornithine transcarbamylase, as seen in other GroEL mutants; and several newly observed defects, such as absence of motility, sensitivity to UV light and mitomycin, a defect in one mode of specialized transduction, and inability to grow on rhamnose. Sucrose gradient analysis of extracts from the V521RΔ cells showed a substantially reduced level of GroEL sedimenting at the normal 20S position of the assembled tetradecamer and a relatively large amount of more lightly sedimenting subunits. This indicates that the substitution-deletion mutation interferes with oligomeric assembly of GroEL into its functional form. This is discussed in light of the recently determined crystal structure of GroEL.

The history of analysis of GroE function in *Escherichia coli* K-12 has been remarkable in terms of the number of puzzling, apparently disparate observations on the properties of mutants for this function. Originally discovered as a gene whose mutations, *groE*, can lead to the failure of morphogenesis of bacteriophage λ and other phages (6, 13, 44, 46, 52), subsequent genetic analysis produced mutants in one of two components, *groEL* (*mopA*) or *groES* (*mopB*) (47), which are defective in either DNA and RNA synthesis (50), SOS-induced mutagenesis and Weigle (UV) reactivation of UV-irradiated phage (7, 26, 33, 43), protein export (31), proteolysis (45), septation (12), coupling of replication between F factor and the chromosome (36), or ability to grow at some or all temperatures (11, 20). Further experiments implicated alterations in GroE levels or configuration in indirect suppression of a variety of mutations in other genes such as *ssb* (32, 41), *rpoH* (30), *dna* (10, 23), and various biosynthetic genes (48).

A more unified picture of GroE function has evolved from studies in vitro, which offer an explanation for all of the preceding findings: GroEL and GroES are a pair of chaperonin proteins required for proper folding of many cellular proteins (for reviews, see references 14 and 21). By now, considerable structural and biochemical evidence has established that GroEL functions as a double-ring 14-mer of identical subunits that, together with a 7-mer ring of GroES, facilitates the folding of many specific proteins both in vitro and in vivo. Indeed, in a recent study in vivo, we showed that a severe conditional deficiency of GroEL, involving the mutation E461K (*groEL201*), led to defective biogenesis of a large number of newly translated cellular proteins, many becoming aggregated or degraded (20). We speculated that many but not all cellular proteins require GroEL for proper folding, estimating that perhaps 40 to 50% of bacterial proteins require GroEL action. In another study, Kanemori et al. have shown that steady-state growth under limiting GroE conditions also causes the alteration in concentrations of a large number of proteins (25). In this paper, we present further evidence that GroEL has a broad role in protein folding in the cell by study of the pleiotropic phenotype of a strain with a COOH-terminal deletion-substitution mutation. Both the range of the defects and the nature of the GroEL defect in oligomeric assembly are examined.

MATERIALS AND METHODS

Strains. The bacterial strains used are all derivations of *E. coli* K-12 and are listed in Table 1. Strain constructions with P1*vir* were carried out as previously described (37).

Media. Cultures were grown and plated on Luria broth (37), except when recombinants were selected or growth requirements were tested, in which case supplemented medium 56 buffer was used (34). Sugar utilization was normally tested with MacConkey agar (37).

Growth curves. Overnight cultures were each diluted into 15 ml of Luria broth in 125-ml flasks and shaken gently in water baths at 30, 37, or 42°C. Aliquots were periodically removed for measurement of optical density at 600 nm (OD_{600}) in an LKB Ultraspec II spectrophotometer. Cultures with ODs greater than 1.0 were diluted 10-fold in Luria broth before OD measurement.

Genetic crosses and tests of recombination proficiency. Standard Hfr × F^- and transductional crosses with P1*vir* were carried out as previously described (34, 37). $LacZ^-$ × $LacZ^-$ crosses to test recombination proficiency both by β-galactosi-

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TABLE 1. Bacterial and bacteriophage strains used in this study

Strain	Relevant marker(s)	Other characteristics	Source or reference
Bacteria			
JL495	*recB21 sbcB15 sbcC250*	F⁻ *lacZ813*(Oc) *lacI22 metE70 pro-48 trpA605 gyrA19 rpsL171* λcIind mutant	KL635 (42) cured of cryptic P1 prophage; gift of J. H. Lee
JL1095		F⁻ *lacZ813*(Oc) *lacI22* λcIind	*lac* region from JL495 into MG1655; gift of J. H. Lee
KL432	As JL495 with Ω (Cam^r) 10	As JL495	See Fig. 1
KL433	As JL495 with *groEL202* Ω (Cam^r) 10	As JL495	See Fig. 1
KL434	Ω (Cam^r) 10	F⁻ λ⁻	P1*vir*(KL432) × MG1655→Cam^r
KL435	*groEL202* Ω (Cam^R) 10	F⁻ λ⁻	P1*vir*(KL433) × MG1655→Cam^r
KL482	*dinA1* Ω (Cam^r) 10	As GW1010 (27)	P1*vir*(KL432) × GW1010→Cam^r
KL483	*dinA1 groEL202* Ω (Cam^r) 10	As GW1010	P1*vir*(KL433) × GW1010→Cam^r
KL484	*dinB1* Ω (Cam^r) 10	As GW1030 (27)	P1*vir*(K432) × GW1030→Cam^r
KL485	*dinB1 groEL202* Ω (Cam^r) 10	As GW1030	P1*vir*(KL433) × GW1030→Cam^r
KL486	*dinD1* Ω (Cam^r) 10	As GW1040 (27)	P1*vir*(KL432) × GW1040→Cam^r
KL487	*dinD1 groEL202* Ω (Cam^r) 10	As GW1040	P1*vir*(Kl4333) × GW1040→Cam^r
KL491	Ω (Cam^r) 10	As JL1095	P1*vir*(KL434) × JL1095→Cam^r
KL492	*groEL202* Ω (Cam^r) 10	As JL1095	P1*vir*(KL435) × JL1095→Cam^r
KL493	As JL495 with Ω (Cam^r) 10	As JL495 but *pro*⁺	P1*vir*(MG1655 derivative) × KL432→Pro⁺
KL494	As JL495 with *groEL202* Ω (Cam^r) 10	As JL495 but *pro*⁺	P1*vir*(MG1655 derivative) × KL433→Pro⁺
MG1655		F⁻ λ⁻	15
Phage			
λ *lacZ*⁺	p*lac5* c1857 *S7*		39, 40
λ *lacZ118*	p*lac5 lacZ118* c1857 *S7*		39, 40

dase production and by Lac⁺ colony formation were performed as previously described (38, 39).

UV survival curves. For each strain, 15 ml of exponentially growing cells (37°C, OD_{600} of ~0.2) in Luria broth was resuspended at 4°C into the same volume of 2× minimal medium 56. Five milliliters was placed in a 100-mm-diameter petri dish and irradiated with a Sylvania G15T8 germicidal lamp at a dose rate of 0.5 J/mm²/s, determined with a Latarjet dosimeter. One-milliliter aliquots were removed after each dose, diluted in Luria broth, and plated on Luria plates to determine survival.

Protein analysis. For experiments examining the oligomeric state of GroEL, 600-ml cultures of wild-type or mutant cells were grown to log phase and harvested by centrifugation at 4°C. Cell pellets were typically resuspended in 3 to 4 ml of an ice-cold solution of 50 mM Tris (pH 7.4)–1 mM dithiothreitol–2.5 mM EDTA, and then 5 ml of lysozyme (10 mg/ml) was added. After 5 min, spheroplasts were sonicated and the lysate was centrifuged in a Ti70.1 rotor (Beckman) at 55,000 rpm for 20 min. The supernatant was applied directly to a 10 to 30% (vol/vol) sucrose gradient, which was centrifuged at 25,000 rpm for 18 h at 4°C in an SW27 rotor (Beckman). Fractions were collected, and 35-μl aliquots were solubilized in sodium dodecyl sulfate (SDS) sample buffer and applied to SDS-polyacrylamide gel electrophoresis (PAGE) gels. The gels were blotted to nitrocellulose (16) and incubated with anti-GroEL antiserum. Ornithine transcarbamylase (OTC) activity was determined according to the method of Kalousek et al. (24) with the same amount of total soluble cell protein from mutant and wild-type cells, as determined with a Bio-Rad assay.

RESULTS

Terminal deletions in *groEL*. We constructed and tested several deletions of the COOH terminus of the *groEL* structural gene in order to evaluate the importance of this region in the *E. coli* K-12 chromosome. These deletions are indicated in Fig. 1, which also outlines the earlier constructs made. Deletions of the last 16, 24, or 27 amino acids in the GroEL protein had no noticeable effect on growth or viability. A deletion of the last 16 amino acids was also reported by McLennan et al. (35), who also found that they were dispensable. In contrast, we could obtain no viable clones when the final transformation was done with terminal deletions of 28, 35, or 52 amino acids. (Control transformations normally produced 10 to 30 colonies per 0.1 μg of DNA.) Thus, there appears to be a crucial juncture in GroEL at the −28 to −27 amino acid position. In order to analyze this in more detail, we started with the −27 deletion and engineered several amino acid substitutions in the −28 amino acid (Val-521 in the GroEL sequence [17]). When Val-521 was changed to glutamate or tryptophan, no viable transformants were obtained. When Val-521 was changed to arginine or glycine, slowly growing transformants were obtained at room temperature; these grew ever more slowly at higher temperatures, particularly at 37 or 42°C. The −27 mutant bearing the Val-521→Arg substitution was selected for further analysis and is denoted *groEL202*.

Growth properties of groEL202. As mentioned above, the *groEL202* allele was chosen for further study because of its weak growth on Luria broth medium, particularly at higher temperatures. Growth curves for isogenic *gro*⁺ and *groEL202* strains are shown in Fig. 2. Growth rates at 30°C are fairly similar, except that the *groEL202* strain stops growing at a lower final OD. The weakness of the GroEL202 phenotype is much more apparent at 37 and 42°C, at which both the growth rate and final OD were successively diminished in contrast to those of Gro⁺. The doubling time at 42°C for KL435 (*groEL202*) was about 38 min, as opposed to 18 min for KL434 (*gro*⁺). Similar results were observed when strains KL432 (*gro*⁺) and KL433 (*groEL202*), whose genetic background is considerably different from that of KL434 and KL435 (data not shown), were compared.

When these cultures were plated out during growth in Luria broth, it was found that the *groEL202* strains contained fewer CFU (three- to fivefold) per OD unit than the *gro*⁺ strains.

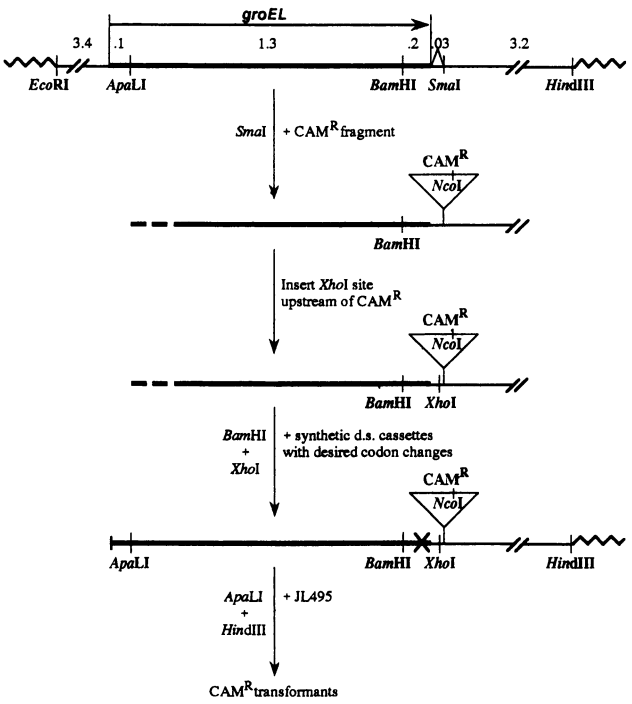

FIG. 1. Construction of *groEL* mutants. Approximate lengths of the wild-type *groEL* gene, obtained from Kohara phage λ649 (28) (top line), are shown in kilobases, as derived from Hemmingsen et al. (17). The chloramphenicol resistance (CAM^R) determinant was derived from plasmid pACYC184 as previously described (20). The large X on the bottom line shows the general region of the deletion endpoints. Complete details of all constructions are available on request. d.s., double stranded.

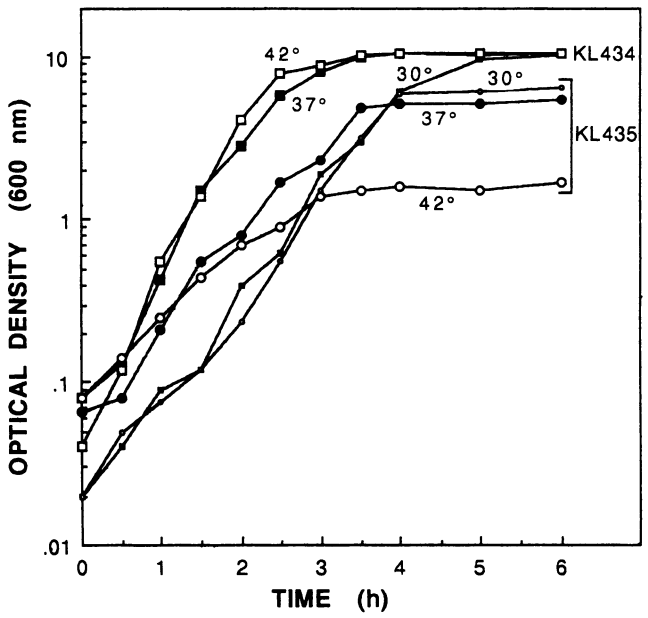

FIG. 2. Growth curves of *gro*$^+$ (KL434) and *groEL202* (KL435) strains in Luria broth at various temperatures.

This was correlated with significant filamentation of the *groEL202* strains (filaments of 3 to 10 cell lengths), particularly at 37 and 42°C (data not shown). In addition, the typically motile swimming behavior in the GroEL$^+$ strains was absent in the *groEL202* strains, even for cells that were of normal length, as observed with the light microscope.

Growth of *groEL202* strains on minimal medium 56 was even more retarded relative to that of *gro*$^+$ strains. At 42°C, for example, it took 6 days to obtain reasonably large colonies of KL433 (*groEL202*), in contrast to 1 1/2 to 2 days for KL432 (*gro*$^+$). The numbers of colonies obtained at 42°C, however, were within a factor of 2 of the number obtained at 30°C (data not shown).

Response of GroEL202 strains to DNA damage. To further develop an understanding of the functions affected by the *groEL202* mutation, we tested some basic parameters of DNA repair and homologous recombination. *groEL202* strains were found to be sensitive to mitomycin when tested by replica plating on Luria agar that contains 0.5 μg of mitomycin per ml, a concentration at which *gro*$^+$ strains grow well. In order to determine whether this indicated a defect in DNA repair, *groEL*$^+$ and *groEL202* strains were tested for sensitivity to UV light. Survival curves are shown in Fig. 3 for two sets of isogenic strains: one set with wild-type *rec* genes and the other in a *recB sbcB sbcC* triple-mutant background (in which the RecF pathway for Hfr × F$^-$ crosses is active [5]). In both cases, the *groEL202* allele is seen to confer considerable sensitivity to UV-induced damage. The 37% survival doses for the *groEL202* strains are approximately 0.3 times the corresponding 37% survival doses for the *gro*$^+$ strains. Similar sensitivity due to *groEL202* was found for cultures exposed to 16 μg of mitomycin per ml for 30 min when resuspended in buffer after exponential growth in Luria broth (data not shown).

A possible defect in SOS inducibility (51) could explain the more UV-sensitive phenotype characteristic of *groEL202*. In order to test this possibility, we introduced the *gro*$^+$ and *groEL202* alleles into each of three SOS-inducible *din*::Mud(Ap^r *lac*) fusion strains of Kenyon and Walker (27) corresponding to *dinA1*, *dinB1*, and *dinD1*. Upon growth and SOS induction of

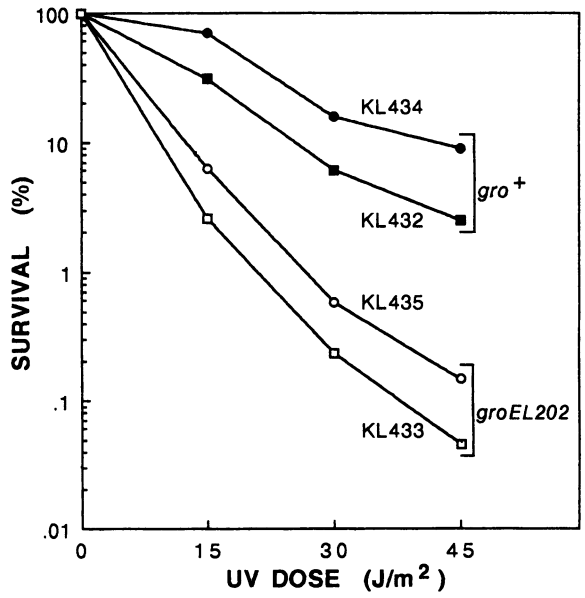

FIG. 3. UV survival curves for *rec*$^+$ *groEL*$^+$ (or mutant) (KL434, KL435) and *recB sbcB sbcC* triple-mutant *groEL*$^+$ (or mutant) (KL432, KL433) strains.

TABLE 2. λ*lacZ* mutant × F⁻ *lacZ* mutant recombination in *gro*⁺ or *groEL202* strains

Cross	β-Galactosidase activity (enzyme units/ml)	Gro⁺/GroEL202 ratio	No. of Lac⁺ colonies/ 10^3 CFU	Gro⁺/GroEL202 ratio
rec⁺ background[a]				
KL491 (*gro*⁺) × λ*lacZ*⁺	4,936.0		1.4	
KL491 (*gro*⁺) × λ*lacZ118*	0.9		0.11	
KL492 (*groEL202*) × λ*lacZ*⁺	3,307.0	1.6	0.38	3.7
KL492 (groEL202) × λ*lacZ118*	0.8	1.1	0.041	2.7
recB sbcB sbcC triple-mutant background[b]				
KL493 (*gro*⁺) × λ*lacZ*⁺	1,502.0		43.0	
KL493 (*gro*⁺) × λ*lacZ118*	4.2		15.0	
KL494 (*groEL202*) × λ*lacZ*⁺	1,494.0	1.0	0.18	240.0
KL494 (*groEL202*) × λ*lacZ118*	3.2	1.3	0.21	72.0

[a] There were 5.0×10^8 CFU/ml for *gro*⁺ and 8.4×10^7 CFU/ml for *groEL202* on glucose-minimal medium.
[b] There were 2.3×10^8 CFU/ml for *gro*⁺ and 1.0×10^8 CFU/ml for *groEL202* on glucose-minimal medium.

these strains with mitomycin (27), we found that the induced β-galactosidase levels in the *groEL202* derivatives were no more than 15% different from those in the *gro*⁺ strains, even for *dinD1*, in which induction ratios of over 10-fold were observed (data not shown). It appears, therefore, that there is no significant block in SOS induction, per se, in the *groEL202* background at 37°C.

One question raised by the findings presented above is whether or not the defect in repair of UV-induced damage in *groEL202* strains is due to a defect in the dimer excision pathway dependent on the UvrABC excinuclease (49) or is due to a different repair pathway such as the *recA*-dependent recombinational repair process, a defect that would be additive to a defect in the *uvrABC*-dependent path, as shown by Howard-Flanders et al. (22). To test this, we constructed isogenic *uvrA*⁺ or *uvrA6* (*rec*⁺) and *groEL*⁺ or *groEL202* strains in all allelic combinations and measured their UV sensitivities as described above for the single *gro* mutants. We found that adding the *groEL202* defect to a *uvrA6* (*rec*⁺) genetic background did not increase the UV sensitivity any more than the presence of the *uvrA6* mutation alone (data not shown). (The *uvrA6* mutation alone resulted in somewhat greater UV sensitivity than the *groEL202* mutation alone.) It is therefore possible that at least one of the effects of the *groEL202* mutation is to partially cripple one or more of the functions in the UvrABC excinuclease repair pathway.

Also possible is an effect of *groEL202* on preventing activity of *umuC* or *umuD* products, in effect, to produce a UmuC⁻ phenotype, which by some reports leads to a slight UV sensitivity (1, 8, 26, 43) although not as high as that for *groEL202*. Since *groEL*⁺ is normally induced by UV, the defect in *groEL202* may result in indirect effects that influence DNA repair capacity in other ways (29).

Tests of homologous recombination. The ability of *groEL202* cells (KL433) to support homologous recombination was found to be either unchanged or defective, depending on the particular types of crosses tested. Hfr × F⁻ crosses, selecting for Trp⁺ [Str^r] recombinants, produced the same numbers of recombinants by using either KL433 (*groE202*) or KL432 (*gro*⁺) cells as recipients (data not shown). Similar recombination proficiency was observed with generalized transduction with bacteriophage P1 (data not shown). In contrast to this, recombination involving a specialized transducing phage (λp*lac*) was severely blocked in a particular way, as shown by the data in Table 2. In these experiments, either λp*lacZ*⁺ or λp*lacZ118* was used to transduce two pairs of *groEL*⁺ or *groEL202* strains and to measure the ability of the incoming *lac* gene to recombine with the chromosomal *lacZ813*(Oc) allele. This recombination can be assayed in two stages, i.e., to measure the level of transcribable *lacZ*⁺ intermediate by assaying β-galactosidase production and to plate out the cells and measure the numbers of viable Lac⁺ recombinants produced (2, 3). As shown in Table 2, the levels of *lacZ* gene expression in the *rec*⁺ strains KL491 (gro⁺) and KL492 (*groEL202*), as measured by β-galactosidase produced after λ*lacZ*⁺ infection (i.e., not requiring recombination before expression) were similar. The recombination proficiency observed in these strains was also affected very little by *groE202*, as seen in both the levels of β-galactosidase produced after the λ*lacZ118* crosses and the numbers of viable Lac⁺ colonies produced from both types of λ*lac* infections. In contrast to this situation in these *rec*⁺ strains, viable Lac⁺ recombinant production in the *recB sbcB sbcC* triple-mutant strain KL494 (*groEL202*) was blocked approximately 72- to 240-fold compared with that in the isogenic *gro*⁺ strain KL493. The ability of these cells to express *lacZ*⁺ (*lacZ*⁺ crosses) and to carry out recombination to the transcribable intermediate stage (β-galactosidase levels in the λ*lacZ118* crosses) was unaffected by *groEL202*. Hence, some late step or steps in viable recombinant production in this system are blocked by *groEL202*. This is reminiscent of another case of a block in late-stage recombinant production in *recB* or *recC* mutant cells (3, 40). The higher levels of β-galactosidase and Lac⁺ colony production in a *recB sbsB sbcC* triple-mutant strain (KL493) than in a *rec*⁺ strain (KL491) have been reported before and are a characteristic of this particular mode (i.e., specialized transduction) of recombination (5, 38). Whatever factors allow the increase in viable (Lac⁺) recombinant production in the *recB sbcB sbcC* triple-mutant background (compared with *rec*⁺) thus appear to be affected adversely by *groEL202*.

Ability to plate bacteriophage λ. As was reported for the classic phenotype of the first *groE* mutants (44, 46), we observed that phage λ was unable to form plaques on strain KL433 (*groEL202*) ($<10^{-3}$ compared with the frequency observed for KL432 [*gro*⁺]).

Utilization of various sugars as carbon sources. Whereas the *groEL202* strains grew normally on arabinose, fucose, galactose, lactose, maltose, mannitol, and xylose, they were unable to grow on rhamnose. Susan Egan (9) has found that rhamnose isomerase activity is uninducible (above background) in strain KL433 (*groEL202*), compared with 40-fold inducibility in KL432 (*gro*⁺). This indicates a failure of production of active *rhaA* (rhamnose isomerase) gene product

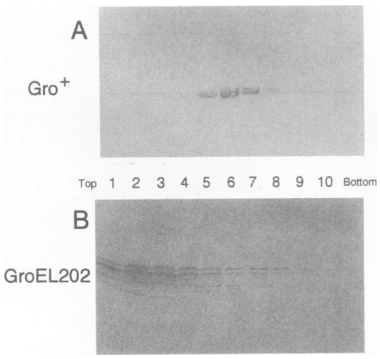

FIG. 4. Distribution of GroEL protein from sucrose gradients, as detected from immunoblots of SDS-PAGE gels. (A) KL432 (*gro*$^+$). (B) KL433 (*groEL202*).

and/or a failure of inducibility of the *rha* operon, which depends on active *rhaR* and *rhaS* gene products.

Defect in production of expressed OTC. In order to determine the effect of *groEL202* on protein biogenesis directly, we measured the activities and protein levels of the product of the human OTC gene cloned and overexpressed on plasmid phOTC (20). This plasmid was introduced into strains KL432 (*gro*$^+$) and KL433 (*groEL202*), and OTC activities and protein levels were determined as previously reported (20). Whereas OTC protein levels in the two strains were equivalent, as determined by immunoblot analysis of equal volumes of solubilized cells with anti-human OTC antiserum, the OTC activity in the *groEL202* strain was not detectable in KL433, as opposed to 20 μg of citrulline per 10 min formed from soluble extract obtained from 2 ml of logarithmically growing KL432 cells. In addition, blot analysis revealed the OTC to be present in the insoluble fraction, in agreement with the behavior observed in the E461K temperature-sensitive mutant (20). Thus, the *groEL202* mutation prevents the proper posttranslational folding of human OTC.

Oligomeric state of GroEL. In order to evaluate the functional defect of GroEL in these strains, cell lysates were prepared and the assembled state of GroEL was analyzed by sucrose fractionation, SDS-PAGE, and immunoblotting with anti-GroEL antiserum. As seen in Fig. 4, the normal assembled state of GroEL as a 20S tetradecamer (Fig. 4A) is altered in the case of GroEL202, whose subunits are mostly present at a lower molecular size in the more lightly sedimenting fractions (Fig. 4B).

DISCUSSION

This report further defines, from study in vivo, features of the structure and action of GroEL as a molecular chaperone. First, from deletion analysis of the COOH terminus of GroEL, we found that a considerable segment, 27 amino acids, can be deleted from the COOH terminus without any observable phenotypic effect. Deletions of 28 amino acids or more, however, are lethal. Interestingly, these observations correlate well with information about the recently obtained crystal structure of GroEL (4). The last residues beyond residue 524 in GroEL cannot be observed in the averaged electron density map, indicating that there is no crystallographic order of the COOH terminus. This could be consistent with a large degree of flexibility predicted for the Gly-Gly-Met tripeptide repeated four times at the tail of the subunit. There seems, on the basis of our deletion studies, to be no essential function for this GGM tail or for the adjoining residues. By contrast, residues 518 to 521 form a β-sheet structure with an invading loop of polypeptide (residues 37 to 41 in the clockwise neighboring subunit in the GroEL ring). This interaction may be important for forming subunit-subunit contact in the rings. Loss of residue 521 is apparently not tolerated, and the substitutions here for Val-521, whose side chain normally forms a local hydrophobic contact, also contribute to the impaired (slow growth) or inadequate (lethal) assembly. The contribution of the residue 521 side chain to the assembled state is significant because when the substitution V-521→R is introduced into the wild-type GroEL, oligomeric assembly is significantly impaired (18).

The second aspect of the present work emphasizes the highly pleiotropic behavior of the particular deletion-substitution mutant *groEL202*, whose DNA alteration affects the crucial residue 521. Although a rather limited range of phenotypes was tested, at least eight diverse defects were observed, some of which—UV sensitivity, selective recombination deficiency, rhamnose nonutilization, and immotility—are new to the growing list of systems that contain proteins dependent on GroEL. These in vivo phenotypes give increased weight to the evidence that GroEL is necessary for the proper folding of a large specific set of polypeptides to active configuration. As in the previous study with a conditional lethal *groEL* mutant (20), we analyzed the proteins from *groEL202* cells by two-dimensional gel electrophoresis and found a large number of altered spots compared with Gro$^+$, indicating once again that there is a major subset of proteins that depend on GroEL to reach an active conformation (19). The weight of in vivo evidence now leads one to interpret all of the various disparate effects of existing *groEL* mutations in terms of a partial (or complete) crippling of the role of GroEL in assisting various proteins, a discrete subset of all proteins, to achieve their native active form.

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