Protein Aggregation in a Mutant Deficient in YajL, the Bacterial Homolog of the Parkinsonism-associated Protein DJ-1

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YajL is the closest prokaryotic homolog of the parkinsonism-associated protein DJ-1 (40% sequence identity and similar three-dimensional structure), a protein of unknown function involved in the cellular response to oxidative stress. We report here that a yajL mutant of Escherichia coli displays an increased sensitivity to oxidative stress. It also exhibits a protein aggregation phenotype in aerobiological cells, but not in anaerobiosis or in aerobic cells overexpressing superoxide dismutase, suggesting that protein aggregation depends on the presence of reactive oxygen species produced by respiratory chains. The protein aggregation phenotype of the yajL mutant, which can be rescued by the wild-type yajL gene, is not by the corresponding cysteine 106 mutant allele, is similar to that of multiple mutants deficient in superoxide dismutases and catalases, although intracellular hydrogen peroxide levels were not increased in the yajL mutant, suggesting that protein aggregation in this strain does not result from a hydrogen peroxide detoxification defect. Aggregation-prone proteins included 17 ribosomal proteins, the ATP synthase β subunit, flagellin, and the outer membrane proteins OmpA and PAL; all of them are part of multiprotein complexes, suggesting that YajL might be involved in optimal expression of these complexes, especially during oxidative stress. YajL stimulated the renaturation of urea-unfolded citrate synthase and the solubilization of the urea-unfolded ribosomal proteins S1 and L3 and was more efficient as a chaperone in its oxidized form than in its reduced form. The mRNA levels of several aggregated proteins of the yajL mutant were severely affected, suggesting that YajL also acts at the level of gene expression. These two functions of YajL might explain the protein aggregation phenotype of the yajL mutant.

The gene encoding YajL has close homologs in many prokaryotes and eukaryotes. YajL is a member of the DJ-1/YajL/Pfpl superfamly which includes peptidases (1), chaperones (2), and the Parkinson disease protein DJ-1 (3, 4). All members of this superfamly share a common domain with a nucleophilic elbow displaying an important cysteine residue (Cys$^{106}$ in YajL), which is part of a Cys, His, Glu/Asp catalytic triad in several members of the superfamily, such as the peptidases Pfpl and Hsp31 (5). In other members of the family, such as DJ-1, this residue plays an important role in oxidative stress resistance by undergoing oxidation to sulfenic or sulfinic acid (3, 4).

The crystal structures of YajL and DJ-1 have been solved (6, 7) and are remarkably similar, with essentially identical backbone structures (0.9 Å root mean square deviation). Both YajL and DJ-1 lack the Cys, His, Asp/Glu catalytic triad of Pfpl or Hsp31, and their “nucleophilic elbow” cysteine is oxidized in crystals to cysteine sulfenic or sulfinic acid (6, 7). This conserved cysteine is mandatory for the protective function of DJ-1 against oxidative stress (3). Many biochemical functions have been proposed for DJ-1. It has been suggested to function as a weak protease (4), an oxidative stress-activated chaperone for synuclein, preventing its aggregation (8, 9), an atypical peroxidase that scavenges H₂O₂ (10), an apoptosis inhibitor via its interaction with Daxx (11), a stabilizer of the antioxidant transcriptional regulator Nrf2 (12), and a transcriptional or translational (4, 13) regulator of gene expression.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Media**—Bacteria were grown aerobiologically at 37 °C in LB or M63 medium supplemented with 0.4% glycerol or glucose (1). The yajL mutant JW5057 (yajL::kan referred to as yajL or ΔyajL), its parent BW25113, and the YajL expression strain containing pCA24N-yajL were kindly provided by Dr. H. Mori (14). For complementation studies of the yajL mutant, pCA24N-yajL was extracted from the YajL expression strain (14) and used for transformation of strain JW5057. For construction of the yajL C106A and C106D mutants, Cys$^{106}$ was substituted for alanine or aspartate by in vitro site-directed mutagenesis of the appropriate codon in the pCA24N-yajL plasmid (Stratagene QuikChange kit). The katEkatGhpx (hpx) and sodAssodBkatEkatG (skx) mutants and the psodA plasmid were kind gifts from Dr. S. Dukan (15). Oxidative, thermal, osmotic, UV, and pH stress studies were performed as described previously (16).

**Preparation and Analysis of Protein Aggregates**—Bacteria were grown at 37 °C in LB medium to midexponential phase. Cells were harvested by centrifugation and resuspended in 50 mM Tris, pH 7.4, 5 mM EDTA, 20% sucrose, 1 mg/ml lysozyme, and the mixture was incubated for 10 min at 23 °C, after which it was diluted into 5 volumes of 30 mM Tris, pH 7.4, at 23 °C. Following brief sonication (20 s), MgCl₂ was added to a final

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1 The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S7.

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concentration of 10 mM, and the mixture was treated with DNase I. Intact cells were removed by centrifugation at 2,000 × g for 2 min in a Beckman microcentrifuge. Triton X-100 was added to 0.5%, and the mixture was incubated for 15 min at 20 °C. The insoluble cell fraction was isolated by subsequent centrifugation for 15 min at 8,000 × g in a microcentrifuge (15), and then pellets were analyzed by SDS-PAGE or two-dimensional gel electrophoresis as described previously (1). Oxidative stress was induced by the addition of hydrogen peroxide (0–40 mM) to the bacterial cultures 20 min before harvesting.

Detection of Protein Carboxyls—Protein aggregates were prepared as described above and then analyzed by using an OxylBlot carbonyl detection kit, as described previously (16). Proteins were analyzed by dot-blotting (1 μg) and SDS-PAGE (10 μg).

YajL Expression and Purification—The YajL expression strain was grown in LB medium (16) to an A600 = 0.6 and then induced with 1 mM isopropyl-β-D-galactopyranoside for 2 h. Bacteria were lysed by ultrasonic disruption, and the 200,000 × g supernatant was used for YajL purification. YajL was first purified on a DEAE-Sephasel (Pharmacia) column equilibrated in 30 mM Tris, pH 8, 20 mM NaCl, 0.5 mM EDTA, 1 mM dithiothreitol at 20 °C. Protein was eluted with a linear gradient of 20–400 mM NaCl in equilibration buffer. YajL was then purified on a hydroxyapatite column (Bio-Gel HTP from Bio-Rad) equilibrated in 30 mM Tris, pH 8, 20 mM NaCl, 0.5 mM EDTA, 1 mM dithiothreitol at 20 °C. Protein was eluted with a linear gradient of 0–50 mM sodium phosphate, pH 8, in the same buffer. YajL was dialyzed for 2 h against 30 mM Tris, pH 8, 20 mM NaCl, 0.5 mM EDTA, 1 mM dithiothreitol at 20 °C, and stored at −80 °C in the same buffer until use.

Preparation of Reduced and Oxidized YajL—40 μl of YajL (15 mg/ml) was incubated for 30 min with 10 mM dithiothreitol and isolated by gel filtration through a Bio-Gel P10 column (2-ml bed volume, Bio-Rad) equilibrated with oxygen-free 0.1 mM potassium phosphate, pH 6.8, 1 mM EDTA. Oxidized YajL was obtained by incubating reduced YajL for 1 h at 37 °C in the presence of 5 mM hydrogen peroxide, as described for DJ-1 (9). For chaperone experiments in the presence of oxidized YajL, oxidized YajL was separated from hydrogen peroxide by filtration through a Bio-Gel P2 gel permeation column (Bio-Rad).

Refolding of Citrate Synthase, and Ribosomal Proteins S1 and L3—Denaturation, renaturation, and activity determination of citrate synthase were carried out at 23 °C as described previously (17). The ribosomal proteins S1 and L3 were prepared as described previously (18, 19). S1 and L3 were unfolded in 8 M urea and then diluted 50-fold in 20 mM Tris, pH 7.4, in the absence or in the presence of YajL. Their appearance in 15,000 × g pellets or supernatants was monitored by SDS-PAGE. YhbO and DnaK were prepared as described previously (16, 17); DnaJ and GrpE were obtained from Stressgene.

RESULTS

Oxidative Stress Sensitivity of the yajL Mutant—The doubling time of the yajL mutant was similar to that of its parental strain in all media tested, which suggested that bacterial metabolism in the mutant was not significantly affected. This result is in agreement with the absence of any clear mitochondrial defect in DJ-1 mutants (4). The yajL mutant was more sensitive to hydrogen peroxide than the parental strains (Fig. 1A). In contrast, sensitivity to heat, osmotic, UV, acid, or alkaline stress was similar in the mutant and parental strains (data not shown). The sensitivity of the yajL mutant to hydrogen peroxide stress is reminiscent of that of DJ-1 mutants (4) and suggests that YajL is involved in the bacterial response to oxidative stress but not to other environmental stresses.

Protein Aggregation in the yajL Mutant—Wild-type Escherichia coli exhibited a low level of aggregated proteins, similar to previous reports (15, 23), whereas the yajL mutant displayed relatively higher levels of protein aggregates (Fig. 1B). Several aggregated proteins could be identified, including ribosomal protein S1, flagellin (Flc), and the ATP synthase β subunit. In the upper part of the gel and ribosomal proteins S2, S3, L1, L2, L3, and L4 and the outer membrane protein OmpA in the lower part of the gel. We analyzed the aggregates by two-dimensional gel electrophoresis at alkaline pH (8–11 pH range, 15% polyacrylamide; Fig. 1C). Increases in spot intensities in the mutant extracts were reproducibly detected for ribosomal proteins S5, S8, S10, S16 and L1, L3, L5, L6, L10, L11, and L25. We also analyzed protein aggregates

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![Graph showing hydrogen peroxide concentration (mM) vs. log CFU/mL](image)

**FIGURE 1.** A, increased sensitivity of the ΔyajL mutant to hydrogen peroxide stress. Bacteria (wild-type strain, filled symbols; ΔyajL mutant, open symbols) were incubated for 30 min in the presence of the indicated hydrogen peroxide concentrations, and viable cells were counted. Results are the averages ± S.E. (error bars) of three experiments. B, protein aggregates of the ΔyajL mutant. Insoluble proteins were separated by SDS-PAGE, detected by Coomassie Blue, staining and characterized by N-terminal sequencing. wt, wild-type. C, insoluble proteins were separated by two-dimensional gel electrophoresis as described in Ref. 16, detected by Coomassie Blue staining, and characterized by mass spectrometry. D, 8,000 × g pellet (lane 1) from the ΔyajL mutant, lysed in the absence of detergent, resuspended in either 50 mM Tris, pH 8, 5 mM MgSO₄, or the same buffer supplemented with 1% Triton X-100 or 5% SDS and incubated for 30 min at 22 °C. The 8,000 × g supernatants of the three samples (buffer alone, lane 2; buffer supplemented with 1% Triton X-100, lane 3; buffer supplemented with 5% SDS, lane 4) were analyzed by SDS-PAGE. ImageQuant software (Molecular Dynamics) was used to quantify proteins.

**TABLE 1**

Aggregated ribosomal proteins

| Protein | Molecular mass | pI | Soluble/Insoluble | Peripheral/Central | Translational repressor |
|---------|---------------|----|------------------|--------------------|------------------------|
| S1      | 72            | 4.7| S                | P                  | Yes                    |
| S2      | 26            | 6.7| I                | P                  | Yes                    |
| S3      | 25            | 12.0| I               | P                  | Yes                    |
| S5      | 17            | 10.6| I               | Yes                |
| S6      | 16            | 4.9 | I                | Yes                |
| S8      | 14            | 9.1 | I                | Yes                |
| S10     | 12            | 7.9 | S                | Yes                |
| S16     | 9             | 11.0| S                | Not regulated      |
| L1      | 24            | 9.2 | I                | Yes                |
| L2      | 30            | 9.1 | I                | Yes                |
| L3      | 22            | 9.7 | I                | Yes                |
| L4      | 22            | 7.6 | I                | Yes                |
| L5      | 20            | 9.4 | I                | Yes                |
| L6      | 18            | 10.7| ND               | Yes                |
| L7      | 17            | 7.5 | ND               | YES                |
| L10     | 17            | 10.1| ND               | Yes                |
| L11     | 11            | 9.4 | I                | ND                 |
| L25     | 11            | 9.4 | I                | ND                 |

* ND, not determined.

by two-dimensional gel electrophoresis (3–10 pH range, 15% polyacrylamide) (supplemental Fig. S1), which confirmed that large amounts of flagellin, Atp-β, and OmpA were aggregated in the yajL mutant and enabled reproducible detection of peptidoglycan-associated lipoprotein PAL, which, together with OmpA, is involved in maintaining outer membrane integrity.

The levels of ribosomes and polysomes (isolated as described previously) (24) in the yajL mutant were slightly lower than in the parental strain, whereas the levels of Atp-β and OmpA in 8,000 × g supernatants (detected by two-dimensional gel analysis) were not significantly different (data not shown). In contrast, flagellin was undetectable in the supernatants of wild-type and mutant strains and in the pellet of the wild-type strain but was present in huge amounts in the pellet of the yajL mutant (Fig. 1B and supplemental Fig. S1), which suggested that it was overexpressed in mutant cells and underwent massive aggregation. Thus, with the exception of flagellin, protein aggregation in the yajL mutant did not appear to be the result of a marked increase in the cellular levels of the aggregation-prone proteins.

Ribosomal and nonribosomal aggregated proteins are listed in Tables 1 and 2, respectively. These aggregated proteins are different from those identified in a dnaK mutant, which are enriched in high molecular weight proteins but not in ribosomal proteins (23). Given the role of DnaK in ribosome biogenesis, we confirmed that this chaperone was not depleted in the yajL mutant (25) (supplemental Fig. S2). The aggregated proteins in the yajL mutant are also distinct from those of the Hsp33 mutant (26). Protein aggregation is a key pathological characteristic of Parkinson disease, but the involvement of DJ-1 in preventing the formation of Lewy bodies has not been clearly established, and DJ-1 rarely labels Lewy bodies (4). It has, however, been found to be associated with intraneuronal inclusions formed by the microtubule-binding protein tau (4), and it prevents the aggregation of α-synuclein in vitro (9).

**Detergent Solubility of Protein Aggregates**—The 8,000 × g pellet of wild-type cells was barely visible, whereas a dense precipitate was recovered from the same number of mutant cells (data not shown). Moreover, when 8,000 × g pellets of mutant cells were resuspended in 20 mM Tris, pH 7.4, protein aggregates could be visualized by light scattering at
600 nm, suggesting that they included large aggregates (supplemental Fig. S3).

To investigate the detergent solubility of these aggregates, cells were lysed in detergent-free buffer as described under “Experimental Procedures,” and then the 8,000 × g pellets were resuspended and incubated for 30 min in Tris buffer alone or Tris buffer supplemented with either 1% Triton X-100 or 5% SDS. Samples were analyzed and quantified by SDS-PAGE (Fig. 1D). 17% of total aggregated protein (lane 1) was soluble in Tris buffer (lane 2), 22% was soluble in Tris-Triton X-100 buffer (lane 3), and >70% was soluble in Tris-SDS buffer (lane 4). Bacterial inclusion bodies and eukaryotic protein aggregates are insoluble in mild detergents such as Triton X-100 (15, 23) and display variable solubilities in SDS-containing buffers (4, 15, 23, 27, 28).

TABLE 2
Aggregated nonribosomal proteins

| Protein                      | Molecular mass | Function                  |
|------------------------------|----------------|---------------------------|
| Flagellin                    | 51 kDa         | Flagella main component   |
| ATP synthase β subunit       | 50 kDa         | ATP synthesis             |
| OmpA                         | 33 kDa         | Outer membrane protein A  |
| PAL                          | 19 kDa         | Outer membrane lipoprotein|

Complementation of the yajL Mutant by Wild-type yajL, but Not the Corresponding C106A or C106D Alleles—The protein aggregation phenotype of the yajL mutant was suppressed by wild-type yajL gene (expressed from the expression vector pCA24N, under the control of the pTrs-lac promoter) (Fig. 2A), but not by the C106A yajL allele (cysteine 106 replaced by alanine), or the C106D allele (cysteine 106 replaced by aspartate) (Fig. 2A). Loss of viability of the yajL mutant under hydrogen peroxide stress was also suppressed by wild-type yajL gene but not by either of the mutant alleles (supplemental Fig. S4). These results suggested that in vivo, to prevent both protein aggregation and loss of viability, YajL requires cysteine 106. This result is in agreement with the critical role of cysteine 106 in DJ-1. In contrast to cysteine 106, cysteines 53 and 46 of DJ-1 are dispensable for function in vivo (4). These residues are either absent from YajL (Cys106) or not conserved (Cys106) in several YajL variants.

Increased Protein Aggregation in the yajL Mutant under Endogenous or Exogenous Oxidative Stresses—When the yajL mutant and parental strains were grown at 37 °C in LB medium in the absence of oxygen, both strains displayed the same low basal level of protein aggregates (Fig. 2B), suggesting that protein aggregation in the yajL mutant in aerobic conditions depends on the presence of reactive oxygen species produced by respiratory chains (29). Consistent with this result, protein aggregation in aerobic conditions was lower in a yajL mutant transformed with an expression plasmid for SodA, a superoxide dismutase (15) (Fig. 2C).

To compare the formation of protein aggregates in wild-type and mutant bacteria under hydrogen peroxide stress, midexponential phase bacteria were incubated for 20 min

FIGURE 2. A, insoluble proteins extracted from bacteria and analyzed by SDS-PAGE: ΔyajL mutant (lane 1); ΔyajL mutant carrying pCA24N-yajL (lane 2), pCA24N-yajLC106A (lane 3), or pCA24N-yajLC106D (lane 4). The amount of protein aggregate in each fraction (quantified using ImageQuant software) is indicated below each lane and is expressed as the percentage of total protein. B, protein aggregation in anaerobic cells. Cultures were grown under anaerobiosis until an A600 = 0.5 was reached, and aggregated proteins were analyzed by SDS-PAGE. C, decrease in protein aggregation in the aerobically grown ΔyajL mutant carrying plasmid psodA. Insoluble proteins extracted from the ΔyajL mutant and the ΔyajL mutant carrying plasmid psodA were analyzed by SDS-PAGE. D, exponential phase cells incubated for 20 min in the presence of increasing hydrogen peroxide concentrations ranging from 0 to 40 mM. Protein aggregates were analyzed by SDS-PAGE. The amount of protein aggregates (expressed as a percentage of total protein) is indicated below each lane. E, protein aggregates from the wild-type strain (filled symbols) and ΔyajL mutant (open symbols) (quantified by using ImageQuant software (Molecular Dynamics)) presented as a function of hydrogen peroxide concentration. Results are the averages ± S.E. (error bars) of three experiments.
in the presence of increasing concentrations of hydrogen peroxide, lysed, and then analyzed for protein aggregate formation. Hydrogen peroxide increased protein aggregation in wild-type E. coli, as reported previously (15), and the yajL mutant was more sensitive to oxidative stress than its parent (Fig. 2, D and E). The levels of aggregated proteins in the parental and mutant strains rose from 0.2% and 2.8%, respectively (relative to total protein), before oxidative stress to 4.2% and 8.8% after exposure to 5 mM hydrogen peroxide. On the other hand, protein aggregation in the mutant did not increase under heat stress (43 °C) (supplemental Fig. S5). These results suggest that protein aggregation in the yajL mutant results from (or is potentiated by) hydrogen peroxide stress and are in accordance with the sensitivity of DJ-1 mutants to oxidative stresses, but not to other types of stress (4).

**Increased Protein Carbonylation in the yajL Mutant**—Protein carbonylation is a biomarker of oxidative damage to proteins and occurs in Alzheimer and Parkinson diseases (30). In E. coli, carbonylation increases in stationary phase cells and in cells deficient in chaperones, proteases, oxidative stress defenses, or translational accuracy (30). The carbonyl levels of crude extracts of mutant cells were higher than those of parental cells and were further elevated in the presence of increasing concentrations of hydrogen peroxide (Fig. 3A). Several ribosomal proteins (S1, L4) and nonribosomal proteins (flagellin and Atp-β) were more heavily carbonylated in the yajL mutant than in the parental strain (Fig. 3B). Thus, hydrogen peroxide stress increased protein carbonylation levels in the yajL mutant.

**Comparison of Protein Aggregates in yajL Mutant with Those in Superoxide Dismutase and Catalase Mutants**—Protein aggregates in the yajL mutant were more abundant than those in the skx mutant, which is deficient in the two cytoplasmic superoxide dismutases SodA and SodB and in catalases KatE and KatG, but lower than in the hpx mutant, which is deficient in the two E. coli catalases and alkyl hydroperoxide reductase AhpC, the three main hydrogen peroxide-detoxifying enzymes in E. coli (31) (Fig. 4). The intracellular hydrogen peroxide concentrations of the yajL, skx, and hpx mutants and their two parental strains were 0.18 μM, 0.33 μM, 1.21 μM, 0.27 μM, and 0.24 μM, respectively (supplemental Fig. S6). These values were similar to those reported previously (30, 31). Thus, in contrast to the skx and hpx mutants, the yajL mutant exhibited a protein aggregation phenotype, even though the intracellular hydrogen peroxide level in this strain was lower than in the parental strain. This result suggests that the YajL defect results in protein aggregation without enhancing the formation of hydrogen peroxide, the main protein-insulting reactive oxygen species (29). It was somewhat unexpected, given that DJ-1 has been reported to function as a hydrogen peroxide scavenger (10). After 10 min in the presence of 2 mM hydrogen peroxide, the level of protein aggregates in the yajL mutant was higher than in the skx strain and similar to the hpx strain (Fig. 4).

**Chaperone Activity of YajL**—In light of the protein aggregation phenotype of the yajL mutant and the reported function of DJ-1 as an α-synuclein chaperone (8, 9), we examined the chaperone properties of YajL. Because DJ-1 and the oxidative stress chaperone Hsp33 are activated upon oxidation (9, 26), we investigated the chaperone functions of both reduced and oxidized YajL.

Purified YajL was reduced by dithiothreitol or oxidized by hydrogen peroxide as described under “Experimental Procedures,” and then its abilities to facilitate the renaturation of a classical chaperone substrate, citrate synthase (refolding of which is facilitated by GroEL, DnaK, Hsp31, and small Hsps) and the solubilization of ribosomal proteins S1 and L3 (which aggregate in the yajL mutant) were tested. The refolding yield of urea-unfolded citrate synthase increased from 8% in the absence of added proteins to 26% in the presence of 2 μM reduced YajL, 28% in the presence of 2 μM DnaK, and 36% in the presence of the DnaK/DnaJ/GrpE/ATP chaperone machine (Fig. 5A). In contrast to YajL, YhbO, which also belongs to the DJ-1/YajL/PfpI family and is very similar to PfpI (16), did not stimulate the renaturation of citrate synthase (Fig. 5A) (16). The solubilization yields of urea-unfolded ribosomal proteins S1 and L3 (1.6 μM) increased severalfold in the presence of reduced YajL (Fig. 5C). Thus, YajL, like DJ-1 and Hsp31, functions as a bona fide chaperone in vitro.

The maximal refolding yield of 0.1 μM citrate synthase was 28% in the presence of either reduced or oxidized YajL (4 μM), whereas half-maximal reactivation occurred with concentrations of 0.48 μM and 0.15 μM reduced and oxidized YajL,
FIGURE 5. Chaperone properties of YajL. A, citrate synthase renaturation. Citrate synthase was denatured in urea and then renatured for 20 min by dilution as described under “Experimental Procedures,” at a concentration of 0.1 μM, either in the absence of additional protein (control) or in the presence of 2 μM reduced YajL, 2 μM DnaK, 1 μM DnaJ/0.4 μM DnaJ, 0.4 μM GrpE, 0.2 mM ATP, and 2 mM MgCl₂, or 2 μM YihD O. B, dependence of citrate synthase renaturation on the concentrations of reduced or oxidized YajL. Citrate synthase (0.1 μM) was renatured in the presence of reduced (filled circles) or oxidized (open circles) YajL at the indicated concentrations. C, dependence of ribosomal proteins S1 and L3 solubilization on reduced or oxidized YajL. S1 (circles) and L3 (squares) were denatured in urea and subsequently diluted for 20 min in buffer containing reduced (filled symbols) or oxidized (open symbols) YajL at the indicated concentrations. Samples were centrifuged for 10 min at 5,000 × g, and supernatants and pellets were analyzed by SDS-PAGE. The amounts of S1 and L3 in the supernatant fractions are shown. Ribosomal protein L3 in supernatants and pellets (silver-stained) from the solubilization experiment performed with oxidized YajL (open squares) is shown above the figure.

FIGURE 6. mRNA levels in the yajL mutant. Northern blot analysis of OmpA (A), FliC (B), L1 (C), and S1 (D) mRNAs prepared from the wild-type strain (filled symbols) and the yajL mutant (open symbols), grown in LB medium at 37 °C to midexponential phase. Hybridization detected the 1041-nucleotide OmpA mRNA, the 1497-nucleotide FliC mRNA, the 2352-nucleotide bicistronic cmk rpsA mRNA, and the 112-nucleotide bicistronic rplK rplA mRNA (supplemental Fig. S7). We quantified the mRNA bands on Northern blots using National Institutes of Health 1.62 software and plotted the mRNA levels as a function of the time elapsed after exposure of bacteria to 0.3 mM hydrogen peroxide. A ssrA-specific probe (tmRNA) was used as a control for the normalization of each sample. The results are normalized to the amounts (before oxidative stress) of the ompA, rplK rplA (L1), cmk rpsA (S1) mRNAs in the wild-type strain and fliC mRNA in the mutant strain. The results are the mean values of three independent experiments. Results are the averages ± S.E. (error bars) of three experiments.

respectively (Fig. 5B). The solubilization yields of S1 and L3 were higher in the presence of oxidized YajL than in the presence of reduced YajL (Fig. 5C). Thus, YajL is active in both its reduced and oxidized states, and it is more active in its oxidized form, in contrast to DJ-1 and Hsp33, which are active in their oxidized states only (9, 26).

We also examined whether YajL prevented the aggregation of S1 exposed to hydrogen peroxide. Native S1 (1 μM), either alone or in the presence of 2 μM YajL, was incubated for 1 h in the presence of 40 μM hydrogen peroxide. 42% of S1 was aggregated in the control sample (8,000 × g pellet), versus 29% in the YajL-containing sample (data not shown). This modest difference suggests that YajL is more efficient in promoting protein folding than in protecting proteins against stress-induced denaturation. Nevertheless, these results are consistent with a mechanism of YajL-mediated protection of proteins in response to oxidative stress because misfolded proteins, which are more abundant in the absence of YajL, are more sensitive to oxidative stress than native proteins (30, 43).

Messenger RNA Levels in the yajL Mutant—Because most of the proteins that aggregated in the yajL mutant are finely regulated at the level of expression (32–34) we measured the mRNA levels of OmpA, flagellin, and ribosomal proteins S1 and L1 by Northern blot analysis of mRNA extracted from yajL mutant and parental strains before and after exposure to hydrogen peroxide stress. As shown in Fig. 6 (A–D) and supplemental Fig. S7, with the exception of OmpA mRNA levels, which were similar in both strains and were not significantly affected by oxidative stress, the mRNA levels of flagellin, S1, and L1 were strikingly different in the yajL mutant and the parent strain. The mRNA levels of flagellin were 30-fold higher in the yajL mutant than in the wild-type strain, which suggested that flagellin expression is deregulated in mutant cells. S1 mRNA (cmk rpsA mRNA) levels were similar in both strains before hydrogen peroxide stress, but decreased more rapidly in the mutant after hydrogen peroxide stress. L1 mRNA (rplK rplA mRNA) levels were lower in the mutant, both before and after hydrogen peroxide stress. Although lower levels of L1 and S1 mRNAs and increased L1 and S1 aggregation might appear to be contradictory, lower mRNA levels for these two proteins, particularly after oxidative stress, could be a result of higher levels of free ribosomal proteins. Proteins that are not incorporated into ribosomal particles and are aggregation-prone could negatively regulate the translation of ribosomal operons (32), resulting in a decrease in ribosomal mRNA stability and levels (see “Discussion”).

DISCUSSION

Similarly to DJ-1 (3), YajL protects cells against oxidative stress, and the presence of cysteine 106 is required for this protection. In aerobiosis, the yajL mutant displays a protein aggregation phenotype, which is increased by exposure to hydrogen peroxide stress. The main group of aggregation-
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proteins comprises several ribosomal proteins, Atp-β, flagellin, OmpA, and PAL. This set of aggregated proteins is different from those of a dnaK mutant (23) and a Hsp33-deficient mutant (26). Several aggregated proteins (S1, OmpA, Atp-β) belong to a set of 23 proteins for which carbonylation increases in stationary phase (35).

Like bacterial inclusion bodies, the aggregates of the yajL mutant are for the most part insoluble in Triton X-100 (15, 23). On the other hand, they are mainly soluble in SDS. Bacterial and eukaryotic protein aggregates display various solubilities in SDS-containing buffers (4, 15, 23, 27, 28).

The aggregated ribosomal proteins that we identified are involved in both early and late stages of ribosome biogenesis (32). S1, S2, S3, and S10 belong to the tertiary binding protein class, (i.e., proteins involved in late interactions with 30 S subunit components); S5, S6, and S16 belong to the secondary binding protein class, and S8 belongs to the primary binding protein class (i.e., proteins that interact directly with 16 S RNA) (32). L3 and L4 are essential for the first stage of 50 S subunit biogenesis (32) whereas L6 is involved in the late stages of its biogenesis.

Most of the aggregated ribosomal proteins of the yajL mutant belong to the set of ribosomal proteins that are insoluble when overexpressed in bacteria (18, 36), whereas this set comprises only 50% of the ribosomal proteins. A small amount of aggregated proteins with 22% ribosomal protein content has been found in exponentially growing Escherichia coli cells (15), suggesting that ribosomal proteins aggregate under many physiological conditions.

Seven of the aggregated ribosomal proteins (S1, S2, S6, S8, L1, L4, and L10) belong to the set of the 11 translational repres- sors of ribosomal protein operons (32), and seven others belong to operons regulated by S8, L1, and L4, which suggests that a translational regulation defect might be involved in the aggre- gation of ribosomal proteins. Biogenesis of the eukaryotic ribo- some is more complex than that of the bacterial ribosome. rRNAs and ribosomal proteins are transcribed by three different RNA polymerases. Furthermore, 220 nonribosomal pro- teins and 70 small nucleolar rRNAs are involved in ribosome biogenesis. Ribosomal defects have not been reported in par- kinsonism. Ribosomal proteins, however, are overrepresented in the array of proteins associated with soluble α-synuclein and/or with DJ-1 in cells treated with the parkinsonian toxicant rotenone (37), which suggests that ribosomal protein defects might be involved in parkinsonism. Moreover, the mitochon- drial ribosomal protein S6 has been identified as a possible candidate gene in Parkinson disease (38).

In yeast, the Tsa1 peroxiredoxin has been characterized as a ribosome-associated antioxidant, and ribosomal protein aggrega- tion is observed in a Tsa1-deficient strain (39). Tsa1 does not have any sequence similarity with YajL, but its function sug- gests that sensitivity of the translation apparatus to oxidative stress might be more widespread than previously recognized.

Several nonribosomal proteins also aggregate in the yajL mutant. Flagellin is the main component of the flagellum prop- erner. Its N- and C-terminal residues are in a disordered state and are essential for its self-assembly. Moreover, it can form abnormal fibrillar structures similar to disease-associated amy- loids such as prions or α-synuclein (40).

ATP synthase comprises three F0 and five F1 subunits. The stoichiometric incorporation of the F0 and F1 subunits in the F0F1 complex is regulated at the translational level (33). The nonstoichiometric expression of α and β subunits results in the aggregation of whichever one is overexpressed (41). Eukaryotic ATP synthase is encoded by both mitochondrial and nuclear genes, and its biogenesis is also regulated at the translational level (41). Low levels of ATP synthase have been documented in the substantia nigra in Parkinson disease (42).

OmpA and PAL are involved in maintaining the integrity of the outer membrane. PAL is part of the Tol-Pal system composed of TolA, TolQ, and TolR inner membrane proteins, the periplasmic protein TolB, and the outer membrane protein PAL. Defects in PAL trigger the release of periplasmic proteins and of outer membrane vesicles. PAL and OmpA interact with each other and with peptidoglycan to maintain the structure of the perituberculosis wall and outer membrane. The synthesis of TolB, PAL, and OmpA are closely linked (34).

In the yajL mutant, in contrast to the skx and hpx mutants, protein aggregation occurs in the presence of near wild-type levels of hydrogen peroxide. This result suggests that the YajL defect triggers protein aggregation without enhancing the for- mation of reactive oxygen species, and it contradicts somewhat the proposed role of DJ-1 as a mitochondrial hydrogen peroxide scavenger in mice (10). It is possible, and has been suggested by Nyström et al. for mistranslated proteins (30, 43), that oxida- tion of misfolded proteins by reactive oxygen species is required for aggregation in the yajL mutant. In support of this hypothe- sis, the protein aggregation defect of the yajL mutant was sup- pressed in anaerobically growing cells or in aerobically growing cells that overexpressed the superoxide dismutase SodA.

YajL exhibited chaperone properties, increasing severalfold the renaturation of citrate synthase and the solubilization of ribosomal proteins S1 and L3 after denaturation in urea. The stimulation factors of citrate synthase renaturation and the concentration of YajL required for half-maximal renaturation (below 1 μM) were similar to those of Hsp90, DnaK, thiore- doxin, and small Hsps (16), which suggests that YajL is a bona fide chaperone. Oxidized YajL displayed moderately higher chaperone activity than the reduced form. In contrast, oxidized DJ-1 and Hsp33 are much more active than their reduced forms, which are almost totally inactive (9, 26). DJ-1 has been shown to function as a chaperone for synuclein in vitro, but the physiological relevance of this activity has not been established. Furthermore, it rarely labels Lewy bodies (4). Our results provide evidence that a chaperone function of YajL is involved (at least in part) in protecting cells against protein aggregation. YhbO, which belongs to a different subclass (the YhbO, PfpI subclass) of the DJ-1/Hsp31/PfpI superfamily (44), does not display chaperone properties (16 and this study).

The mRNA levels of several aggregated proteins were severely affected by the yajL mutation, suggesting that the absence of YajL alters gene expression. Flagellin mRNA levels were 30-fold higher in the yajL mutant than in the parental strain, which is consistent with the higher levels of flagellin and flagellin aggregation in mutant cells. Conversely, the mRNA levels of two of the ribosomal proteins (S1 and L1) were lower in the yajL mutant (both before and after oxidative stress). These low mRNA levels might be due to
the presence in the mutant of higher levels of free ribosomal protein, which can negatively regulate translation (32). The underlying mechanisms of the gene expression defects in the yajL mutant might involve the regulation of transcription, translation (as reported for DJ-1) (4, 13, 45) or mRNA stability by YajL. Such functions might involve either a direct interaction between YajL and nucleic acids or an interaction between YajL and protein complexes involved in these processes. GroEL and DnaK regulate RNA and DNA metabolism (46–50), although, in several cases, it is not clear whether this regulation involves a direct interaction with RNA or DNA or classical protein chaperone activity. For example, DnaK/DnaJ has been shown to regulate RNA degradation through interactions with RNaseE (48) and controls a DNA replication through interactions with protein components of the primosome (46).

Protein aggregation may result from defects in chaperones, proteases, environmental stress resistance, translation accuracy, mutations that render a protein aggregation-prone, an imbalance between the cellular chaperone capacity and the production of dangerous protein species, or from an imbalance in the levels of subunits in a multiprotein complex. The aggregation-prone proteins of the yajL mutant belong to multiprotein complexes, whose formation requires regulation at multiple levels, including transcription, translation, folding, and stoichiometric incorporation into the complex. Consequently, aggregation of these proteins may result from expression defects leading to the accumulation of lone subunits. Moreover, the expression of ribosomal proteins (32), ATP synthase subunits (33), flagellin (51), and OmpA (52) is regulated at the translational level as well. Interestingly, DJ-1 has been implicated in the translational regulation of several mitochondrial genes including components of respiratory complex I (13), although such a translational regulation has not been related to protein aggregation. Mutations associated with Parkinson disease, such as those in α-synuclein, can trigger the formation of inclusion bodies, whereas others (in parkin, DJ-1 or PINK1) induce neuronal cell loss without any apparent protein aggregation pathology (4). Protein expression or solubility defects leading to an apparent protein aggregation phenotype in one cellular species, such as in yajL E. coli mutants, may in another species simply over-crowd the chaperone and proteasome pathways, thereby affecting many cellular functions, without any apparent protein aggregation pathology. Ribosomal proteins or mitochondrial multienzyme complexes such as respiratory complex I, which is often deficient in Parkinson disease (4) and depends on finely tuned interactions between the nuclear and the mitochondrial genome for its expression, might be preferred targets of such protein expression or solubility defects.

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