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Association of Thioredoxin with the Inner Membrane and Adhesion Sites in *Escherichia coli*

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The intracellular localization of thioredoxin in *Escherichia coli* was determined by immunoelectron microscopy and correlated to previous biochemical data which had suggested that thioredoxin resides at inner-outer membrane adhesion sites. Since a considerable amount of thioredoxin was lost during preparation of cells for electron microscopy, we immobilized the protein with the heterobifunctional photoactivatable cross-linker *p*-azidophenacylbromide before the cells were fixed with aldehyde and embedded in Lowicryl K4M. Thin sections were labeled with affinity-purified antithioredoxin antiserum and protein A-gold complexes. Densities of immunolabel in a designated membrane-associated area and in the rest of the cytoplasm were compared and the data were statistically evaluated. Wild-type strain W3110 and strain SK3981, an overproducer of thioredoxin, exhibited increased labeling at the inner membrane and its adjacent cytoplasmic area. In contrast, the more centrally located cytoplasm of both strains showed much lower label density. This label distribution did not change with cell growth or in the stationary phase. Immunolabel was often found at bridges between the inner and outer membranes; this result is consistent with a model which places at least a portion of the thioredoxin at membrane adhesion sites, corresponding to an osmotically sensitive cytoplasmic compartment bounded by a hybrid inner-outer membrane (C. A. Lunn and V. Pigiet, J. Biol. Chem. 257:11424–11430, 1982; C. A. Lunn and V. Pigiet, J. Biol. Chem. 261:832–838, 1986). Specific label was absent in the periplasmic space.

Thioredoxin is a dithiol protein of molecular weight 11,700. The protein is ubiquitous in bacteria, plants, and animal organs (for reviews, see references 8, 11, and 16). Its function is not well established, although roles in protein transmembrane transport and biosynthetic processes such as bacteriophage T7 replication (1, 18) have been suggested (21, 24, 25). Export of filamentous phages, which depends on thioredoxin, has been shown to occur at membrane adhesion sites (3, 12). Thioredoxin has been reported to be localized in all compartments of the bacterial cell, namely the cytoplasm, chromosomal area, periplasm, and also the envelope (20). More recently it was suggested that thioredoxin is localized in a membrane fraction corresponding to the membrane adhesion sites (17); these sites represent domains at which the inner and outer membrane adhere to each other (2). Since thioredoxin contains a highly reactive thiol-disulfide bridge, we wished to examine whether these groups could be used to cross-link thioredoxin to neighboring molecules and stabilize its in vivo distribution. For this purpose we used the heterobifunctional cross-linking agent *p*-azidophenacyl-bromide (pAPA). Cross-linking pAPA was shown by Lunn and Pigiet (17) to concentrate thioredoxin 15 times in the inner membrane as well as in a hybrid membrane fraction of *Escherichia coli* (containing inner membrane and outer membrane) without causing an increase in total membrane-associated protein. The data suggest that a portion of the thioredoxin in the cell is associated with the membrane adhesion sites.

We report here results of an immunoelectron microscopic study in which pAPA photo-cross-linking was used in combination with postembedding labeling of ultrathin sections with antithioredoxin antibody. A characteristic, prevalently membrane-associated distribution of thioredoxin was observed.

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MATERIALS AND METHODS

Materials. pAPA was purchased from Molecular Probes, Junction City, Oreg.; protein A was from Pharmacia Fine Chemicals, Piscataway, N.J.; and Lowicryl K4M was from Polysciences, Warminster, Pa.

Antibodies to thioredoxin were prepared in rabbits as described (15) and purified by DEAE-Affi-Gel Blue cellulose (Bio-Rad Laboratories). Affinity-purified antibodies were obtained by passing the partially purified serum (in 20 mM Tris, 28 mM NaCl, 0.2% [wt/vol] NaN_3, pH 7.5) over a thioredoxin-Sepharose column, eluting the bound antibodies with 0.1 M glycine hydrochloride pH 2.5 (13). The eluate was then neutralized with Tris. The affinity-purified antibodies bound approximately 0.4 pmol of thioredoxin per μl.

Bacterial strain. The construction and characterization of the *E. coli* K-12-derived thioredoxin-minus strain SK3969 and the thioredoxin-overproducing strain SK3981 have been described (14). All mutant strains (Table 1), as well as the wild-type strain W3110, were stored at −70°C as dimethyl sulfoxide-glycerol stocks.

Growth medium. *E. coli* W3110 (Thy^+) was grown at 37°C in Tris minimal medium containing 0.05% (wt/vol) Casamino Acids (vitamin-free; Difco Laboratories, Detroit, Mich.), 0.1% (wt/vol) glucose, and 0.3 mM phosphate (TCG medium [15]). In other experiments, the cells were grown in nutrient broth (0.5% tryptone, 0.5% yeast extract [both from Difco], 0.1% glucose, and 0.5% NaCl). Strains SK3981 and SK3969 were grown in the above medium plus ampicillin (20 μg/ml). Growing cells were fixed at cell densities of 6×10^8 to $8 \times$

TABLE 1. Bacterial constructions used

Strain	Host genotype[a]	Plasmid
SK3969	*trxA1*	pBR325
SK3981	*trxA1*	pBR325 *trx*$^+$

[a] Both strains carried F$^-$ *argE4 his-4 ilvD188 lac mal mtl xyl rpsL supE44*.

10^8 cells per ml. Stationary cells were derived from overnight aerated liquid cultures. Their cell number reached 5×10^9 cells per ml.

Assay for thioredoxin. Thioredoxin was detected by immunotransfer and autoradiography (Western blotting) (6) by modifications of the technique described by Nelson and Lazarides (19). Cell extracts were fractionated by sodium dodecyl sulfate (SDS)–15% polyacrylamide gel electrophoresis. The same cell suspensions from which the extracts were prepared were also used for electron microscopy (see below). Proteins contained within the gel tracks were electrophoretically transferred to a nitrocellulose sheet for 4 h at 0.25 A. The nitrocellulose was then incubated in a 1:1,000 dilution of purified thioredoxin antiserum in buffer (130 mM NaCl, 15 mM Tris hydrochloride, pH 7.5, 5 mM NaN_3, 1 mM EDTA, 0.1% [wt/vol] gelatin, 0.1% [vol/vol] Tween 20) at 37°C overnight. After being washed in six changes of buffer, the sheet was incubated in 10 ml of buffer containing 10 μCi of ^{125}I-labeled protein A (a gift of J. Nelson) per μg. The nitrocellulose was then washed, dried, and subjected to autoradiography.

Cross-linking and preparation for microscopy. For cross-linking, 12-ml cultures of cells were sedimented by centrifugation at 21°C and suspended in 0.5 ml of cold TCG medium containing 1 mM EDTA. The cross-linking agent pAPA (2.5 mg) was freshly dissolved in 10 ml of ethanol in the dark. The reagent was kept in darkness and, when added to the cells, was quickly spread on Falcon tissue culture dishes and maintained at 4 to 6°C for irradiation. The adsorption spectrum of pAPA showed a broad peak between 260 and 320 μm. We tested a number of UV sources, including Mineralight UVSL-25 (Ultra Violet Products, Inc., San Gabriel, Calif.), an unfiltered Hg high-pressure lamp (GEH100 A4/T) without a glass cover, and a number of electronic flashes. Of the latter, only the Zeiss Ukatron UN60 emitted UV in the range of the pAPA adsorption peak. There was no major difference in the labeling pattern after a 4-min UV irradiation between the Hg lamp or the Mineralight (20-cm distance) with an electronic flash. Data obtained by the flash method, however, were more reproducible. For experiments reported here, the Zeiss flash was used without housing at the 60 mW setting 15 cm from the open culture plates. Following irradiation, the cells were fixed for 30 min in 2% formaldehyde in cold growth medium, centrifuged to form a sediment, and enrobed in agar. Many cells in these preparations were slightly plasmolyzed. To increase plasmolysis, we initially exposed the cells to 20% (wt/vol) sucrose shortly before fixation. However, since sucrose-induced plasmolysis did not give new information, we performed most experiments in the absence of sucrose. Dehydration was started in 50% ethyl alcohol at 0°C. All subsequent dehydration steps, the increasing alcohol concentrations as well as the Lowicryl-embedding steps, were performed at temperatures of −20°C in rotating vials. To reduce the tendency of the Lowicryl-embedded bacteria to separate between the cell surface and embedding material, small dehydration steps were used (10% increases in alcohol concentration), and the Lowicryl permeation steps were prolonged to 2 h each (7). The Lowicryl was polymerized under UV light at −70°C as described previously (7). Ultrathin sections were reacted for 20 to 30 min with affinity-purified thioredoxin-specific antibody (from rabbit) in dilutions of 1:50 in 0.01 M phosphate buffer, pH 7.4 (P-buffer). After three subsequent washings in P-buffer, the sections were floated for 45 min on protein A-gold, containing gold particles with an average size of 14 nm. The protein A-gold complexes were prepared by the method of Roth (23). Sections were subsequently stained in 0.05% uranyl acetate to achieve a suitable increase in contrast. Micrographs were taken in Philips 400 and Philips 420 electron microscopes at magnifications of 20,000× to 40,000×; enlargements to 55,000× on paper were used for gold particle counts and area measurements of the sectioned cells.

Quantitative evaluation of electron micrographs. Area determinations of cells were performed with a Hipad electronic digitizer (Houston Instruments) on the photographic prints. Measurements of the entire area of cytoplasm plus membrane were made by following the contour of the inner membrane. The periplasmic space and outer membrane area were not determined. A "membrane-associated" area of the cytoplasm was established by describing with a felt-tipped pen (2 mm wide, corresponding to ca. 38 nm at 55,000× magnification) an area bordering the inner contour of the inner membrane; the ink-covered area was also determined with the Hipad digitizer. For both areas, the membrane-associated area and the (residual) cytoplasmic area, the number of gold particles was counted (see Table 2). With the aid of a computer program (developed by Dr. Litwin of the biostatistics group of the Fox Chase Cancer Center), particle counts per area were used to test the hypothesis that the observed gold bead concentration in the membrane-associated area could have arisen as a result of uniform random particle distribution throughout the cytoplasm. A statistical test based on the Poisson distribution of particle counts allowed a chi-squared test of this hypothesis. In particular, a generalized likelihood ratio test was used to test the null hypothesis that the Poisson parameter lambda 1 (particles per unit area in membrane-associated area) equals lambda 2 (particles per unit area in bulk cytoplasm) versus the alternative that lambda 1 is not equal to lambda 2. The χ^2 values have degrees of freedom equal to the number of sectioned cells observed. The chances of observing a χ^2 larger than those reported are given as P values (see Table 3).

RESULTS

Localization of thioredoxin in non-cross-linked cells. (i) Western blots. Immunotransfer executed on un-cross-linked *E. coli* W3110 (wild type) and the thioredoxin overproducer *E. coli* SK3981 (Trx^{++}) revealed a single labeled species migrating at a position corresponding to 11,700 to 12,000 daltons (Fig. 1). pAPA cross-linking left the position of the monomer in the gel unaltered. As previous studies had shown, no change in the antigenicity of thioredoxin after pAPA alkylation took place (17).

(ii) Electron microscopy. For the immunolocalization of thioredoxin, Lowicryl K4M was used as the embedding medium. This reagent allows embedding to be done at temperatures as low as −40°C, diminishing structural and molecular alterations caused by standard embedding techniques. We tested whether low-temperature embedding (without photo-cross-linking) would be suited to determine the localization of thioredoxin, a protein with weak mem-

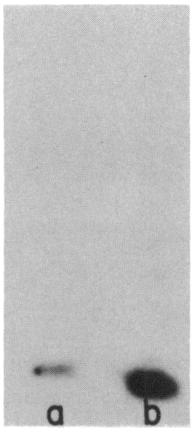

FIG. 1. Western blot of thioredoxin-containing non-cross-linked *E. coli* W3110 (lane a) and SK3981 (lane b). Cells were grown in logarithmic phase to 6×10^8 cells per ml at 37°C. Samples of the cell suspensions were fractionated by SDS-polyacrylamide gel electrophoresis. The separated protein species were transferred to nitrocellulose and assayed for thioredoxin by immunoassay. The native thioredoxin is an 11.7K protein.

brane association (17). However, Lowicryl also introduced disadvantages: aldehyde-fixed and Lowicryl-embedded cells exhibited poor membrane contrast. We were unable to enhance the contrast with prolonged lead-uranyl acetate staining; the staining procedure increased the contrast of all cell structures as well as that of the background. Therefore, quantitation of adhesion sites appeared unfeasible. In contrast, when conventional fixation in aldehyde-OsO_4 was used (micrographs not shown), all strains used in this study (Table 1) showed an ultrastructure characteristic of *E. coli*, with typical membrane adhesion sites.

The localization of (un-cross-linked) thioredoxin of *E. coli* W3110 (wild type), SK3969 (a thioredoxin nonproducer), and SK3981 (Trx^{++}) was achieved by immunolabeling the ultrathin sections and subsequent counting the colloidal gold particles. Thioredoxin-specific labeling is shown for strains W3110 (Fig. 2A) and SK3981 (Fig. 2C). Comparatively little labeling was seen in SK3969 (Fig. 2B). No significant label was observed in samples treated with protein A-gold alone. The data are shown in Table 2 and 3. (In two instances [Table 3] the statistical test indicated a relative overabundance of gold particles in the cytoplasm of the cell, not in the membrane-associated area. The cause for this effect has not been established.)

Thioredoxin in pAPA cross-linked cells. (i) Western blots. For in vivo cross-linking, cells were alkylated with pAPA and cross-linked with the UV flash. When samples of the whole cell extracts were analyzed by immunoelectrophoresis, we observed that the intensity of the 12,000-molecular-weight (12K) thioredoxin band increased significantly after pAPA treatment of both W3110 and SK3981 cells. Considerably more label per cell was detected in cross-linked stationary-phase SK3981 cells (6×10^9 cells per ml) than in cross-linked exponentially growing cultures (6×10^8 cells per ml) (Fig. 3, compare lanes a and b with lane g). This observation is consistent with previous data on the kinetics of thioredoxin overproduction in SK3981 (14). No significant label was observed in extracts from the Trx^- mutant SK3969 (Fig. 3, lanes e, f, and i). In addition to the 12K band, extracts from both SK3981 and W3110 showed bands at higher molecular weights (Fig. 3). One of these bands appeared to correspond to a dimer of thioredoxin (for example, lanes a and b). Plasmolysis was shown to have no effect on the banding pattern (lanes b and d).

(ii) Electron microscopy. When pAPA cross-linking was used to prepare the bacterial cells for Lowicryl embedding and ultrathin sectioning, significantly more gold particles were observed in the cross-linked strains W3110 (Fig. 4A) and SK3981 (Fig. 4C). Quantitation by the number of visible gold particles revealed in both strains W3110 and SK3981 a 2.5- to 3-fold increase in the number of particles associated with the inner membrane over the number of particles in the

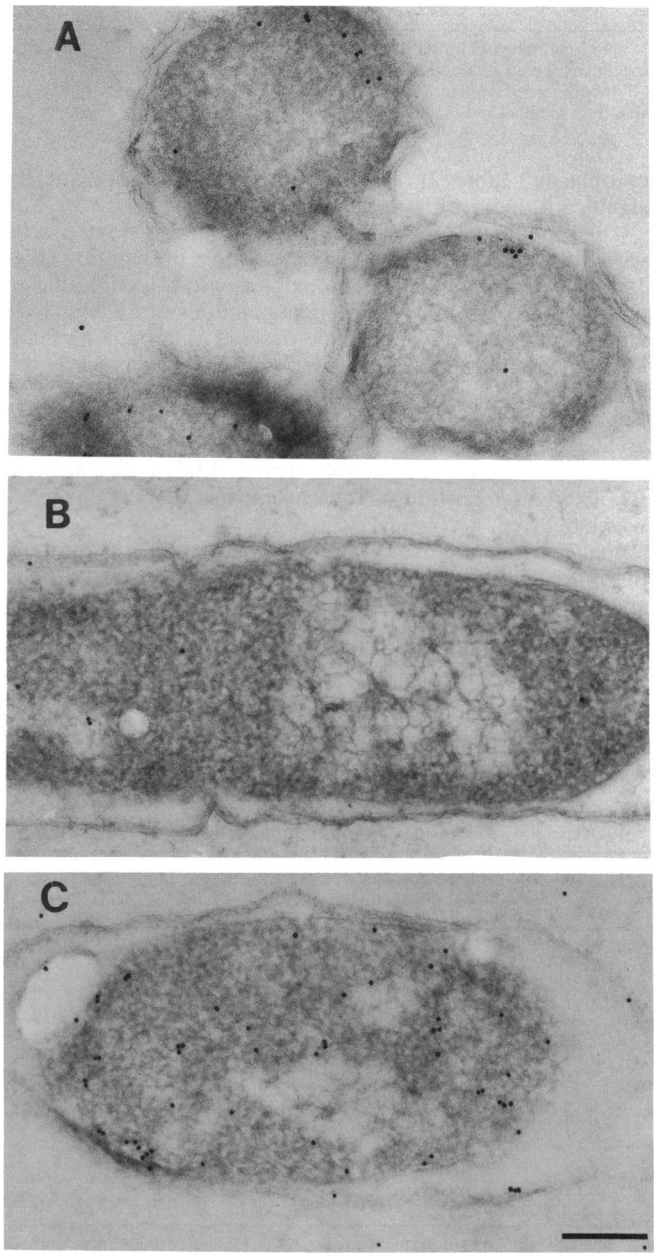

FIG. 2. Localization of thioredoxin in non-cross-linked cells. *E. coli* W3110 (A), SK3969 (B), and SK3981 (C) were embedded in Lowicryl, thin sectioned, treated with antithioredoxin IgG, and labeled with protein A-colloidal gold complexes. Bar, 0.2 μm in this and all subsequent micrographs.

TABLE 2. Effect of cross-linking on distribution of thioredoxin-specific immunolabel

Strain[a]	pAPA and UV treated	No. of cells counted	Label density[b] (no. of gold particles/unit area)	
			IM	Cyto
SK3981 (Trx^{++}) (control)	−	32	0.32	0.48
	+	18	1.10	2.23
SK3981 (Trx^{++})	−	12	2.94	1.83
	+	40	24.65	7.22
W3100 (Trx^{+})	−	6	5.76	3.79
	+	19	3.21	1.28
SK3969 (Trx^{-})	−	6	0.55	0.37
	+	16	1.72	1.92

[a] No immunoglobulin G (IgG) was used with the control. All other specimens were IgG treated.
[b] IM, Inner membrane and membrane-associated area; Cyto, cytoplasmic area (less membrane-associated area).

cytoplasm (Table 2). This result indicates a statistically significant uneven distribution (Table 3).

The amount of label observed in the pAPA-treated cells varied between individual cells in a single preparation (Fig. 5A). Whether this reflects incomplete cross-linking due to uneven illumination or suggests populations of SK3981 cells with varying levels of plasmid-derived thioredoxin is unknown. Membrane-associated thioredoxin was also observed in cells grown to the stationary phase (Fig. 5B). Thioredoxin-specific labeling also occurred in the nucleoid area at a few electron-dense strands (Fig. 5B). These fibrous elements correspond to double-stranded DNA (Fig. 6A and B). The larger portion of the chromosomal area, however, was label free.

In a number of plasmolysed cells, adhesion sites between the outer and inner bacterial membranes were observed in spite of the poor contrast of the Lowicryl embedding. Some of the sites were found to be labeled (Fig. 6, arrows). Occasionally, we observed bridges of label between the inner membrane and the outer membrane, without a structurally identifiable adhesion site (Fig. 6E). This rare event is probably due to the low membrane contrast in the material.

TABLE 3. Probability of nonrandom thioredoxin distribution[a]

Strain	UV-pAPA cross-linking	IgG treatment	χ^2	No. of cells counted	P
SK3981	−	−	37.56	32	2.29×10^{-1}
	+	−	40.35	18	4.49×10^{-3}[b]
	−	+	36.56	12	2.67×10^{-4}
	+	+	285.98	40	$<6 \times 10^{-8}$
W3110	−	−	12.14	6	5.88×10^{-2}
	+	−	41.69	42	4.84×10^{-1}
	−	+	40.9	6	2.98×10^{-7}
	+	+	98.09	19	$<6 \times 10^{-8}$
SK3969	−	+	12.14	6	5.9×10^{-2}
	+	+	44.00	16	1.97×10^{-4}[b]

[a] The probability of nonrandom association of thioredoxin with the inner membrane was determined from quantitative measurements of immunoelectron micrographs (Table 2) using a computer program developed by Dr. Litwin, Institute for Cancer Research. A $P \leq 0.05$ is statistically significant and suggests an affinity of the label for the inner membrane.
[b] These results point toward a somewhat higher label density in the cytoplasmic area (see text).

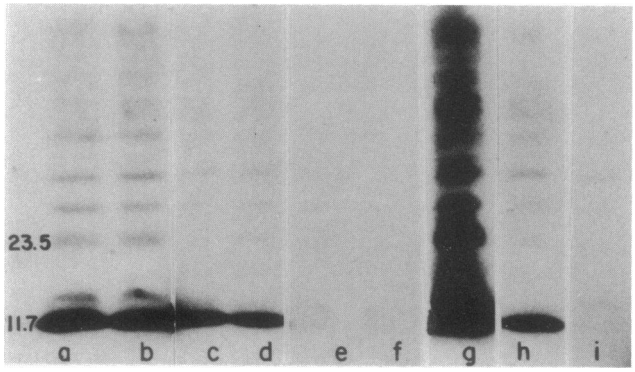

FIG. 3. Western blot of in vivo-cross-linked thioredoxin. *E. coli* SK3981 (lanes a and b), W3110 (lanes c and d), and SK3969 (lanes e and f) were exponentially grown to 6×10^8 cells per ml. Stationary-phase cells of SK3981 (lane g), W3110 (lane h), and SK3969 (lane i) were grown to 6×10^9 cells per ml. Samples in lanes a, c, and e were prepared from unplasmolysed growing cells; those in lanes b, d, and f were from growing cells plasmolysed with 20% sucrose. The migration positions of proteins with molecular weights (in thousands) of native thioredoxin (11.7K) and a thioredoxin dimer (23.5K) are indicated.

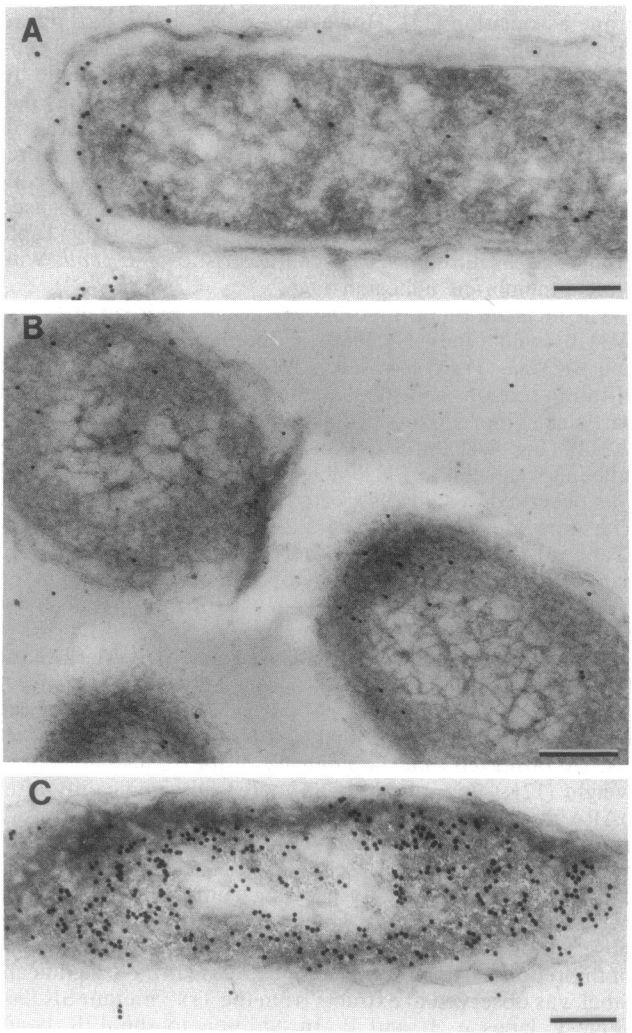

FIG. 4. Localization of thioredoxin on sections of pAPA-cross-linked *E. coli* W3110 (A), SK3969 (B), and SK3981 (C).

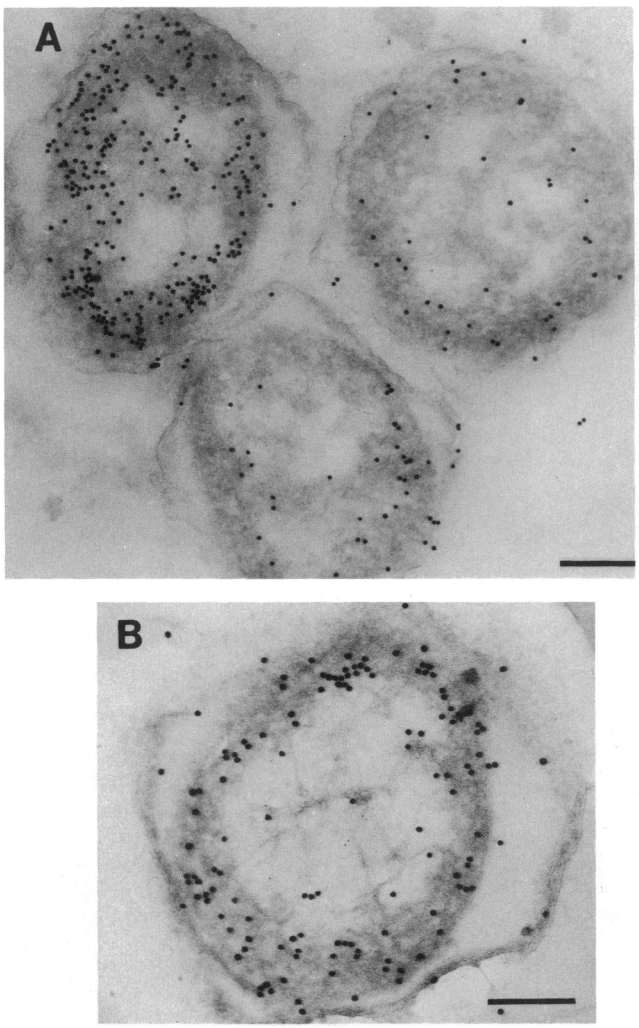

FIG. 5. Localization of thioredoxin on sections of growing and nongrowing SK3981 after pAPA cross-linking. UV-crosslinked *E. coli* SK3981 cells from logarithmically growing cultures (A) and stationary-phase cultures (B). Note the variation in label density in panel A.

Our results indicate that at least a portion of the thioredoxin in the cell is associated with membrane adhesion sites. Micrographs (Fig. 6B and D) also showed that not all adhesion sites were labeled with thioredoxin antibody, a result that could be generated by several factors, such as inaccessibility of the deeper parts of a section and the existence of a subpopulation of membrane adhesion sites containing no thioredoxin. In all the strains examined, the periplasmic space was not observed to accumulate significant amounts of label.

DISCUSSION

In the present study, we sought to determine the cellular localization of thioredoxin. This protein exhibits broad chemical specificity in vitro. However, viable mutants lacking thioredoxin have been reported (18), and no reactions involving the known thioredoxin chemistry have been demonstrated to occur in vivo in bacteria. The only known in vivo activities for this protein involve bacteriophage replication (thioredoxin is a subunit of the T7 DNA polymerase [1]), as well as bacteriophage assembly and export of filamentous phages (24). We hoped that our studies would define the cytological domains that exhibit antibody-binding sites for thioredoxin. The newly developed flash cross-linking procedure as well as the fixation and postembedding regimens described here considerably improved the ability to realistically localize a low-molecular-weight protein such as thioredoxin. We found the antigen present at a variety of sites and in different concentrations; most of the thioredoxin was localized towards the inner surface of the cytoplasmic membrane. Membrane-associated thioredoxin was also present at some of the membrane adhesion sites, which correspond to bridges connecting the inner membrane with the outer membrane and which have been shown to be involved in a number of export and import activities (2). In the relatively rare cases when we observed a labeled "bridge" without an obvious membrane structure associated with it, the general features of the envelope structure at these sites (such as indentation of the outer membrane and extension of the inner membrane) suggested in most instances the presence of an adhesion site. We therefore interpret these labeled sites as the result of the generally severe lack of membrane contrast in the Lowicryl sections.

Our data enabled us to address a number of questions about thioredoxin localization. Thioredoxin, which behaves as a soluble protein in terms of classical cell fractionation techniques, has more recently been described as being associated with the cell membrane (17). Is it possible that the use of a thiol-specific cross-linker and the subsequent fixation regimen could create the observed localization of thioredoxin? Several data sets argue against this possibility. The cross-linker appears to be efficient, as indicated by the Western blot data. The ability to observe cytoplasmically localized thioredoxin, especially in cells overproducing thioredoxin (Fig. 4 and 5), suggests that the reagent does not attach most or all of the available thioredoxin to the membrane. Instead, one can assume that some of the thioredoxin exists in the cytoplasm. A similar observation (but without cross-linking before fixation) has been made by immunoelectron microscopy of *E. coli* mutants which overproduced the protein FhuA. This membrane protein and its precursor were also localized in the cytoplasm as well as in association with the inner membrane (10). Previous biochemical studies with pAPA (17) also showed that the reagent does not generally cross-link other proteins to the cell membrane, suggesting that the cross-linking activity occurs at thioredoxin sites. Since the electronic flash produced a peak output in less than 1/500 s, we hypothesize that any directional diffusion of the excited thioredoxin toward the membrane will take considerably more time than the lifetime of an activated molecule—i.e., its binding to a neighboring molecule or deactivation. A noticeable shift of thioredoxin localization during subsequent treatment, such as fixation, dehydration, and embedding, therefore appears unlikely. Un-cross-linked wild-type W3110 cells revealed a label distribution very similar to that of the cross-linked cells; similarly, the un-cross-unlinked overproducing cells (SK3981) were labeled in a way that generated a nonrandom probability (Tables 2 and 3). Cross-linking was highly effective in both strains in revealing the membrane-associated label distribution.

We observed in our Western blot studies that cell envelopes prepared by French pressure cell (5) showed, in addition to the major (11.7K to 12K) thioredoxin band, some minor bands very similar to those found in whole-cell preparations (data not shown). However, while whole-cell

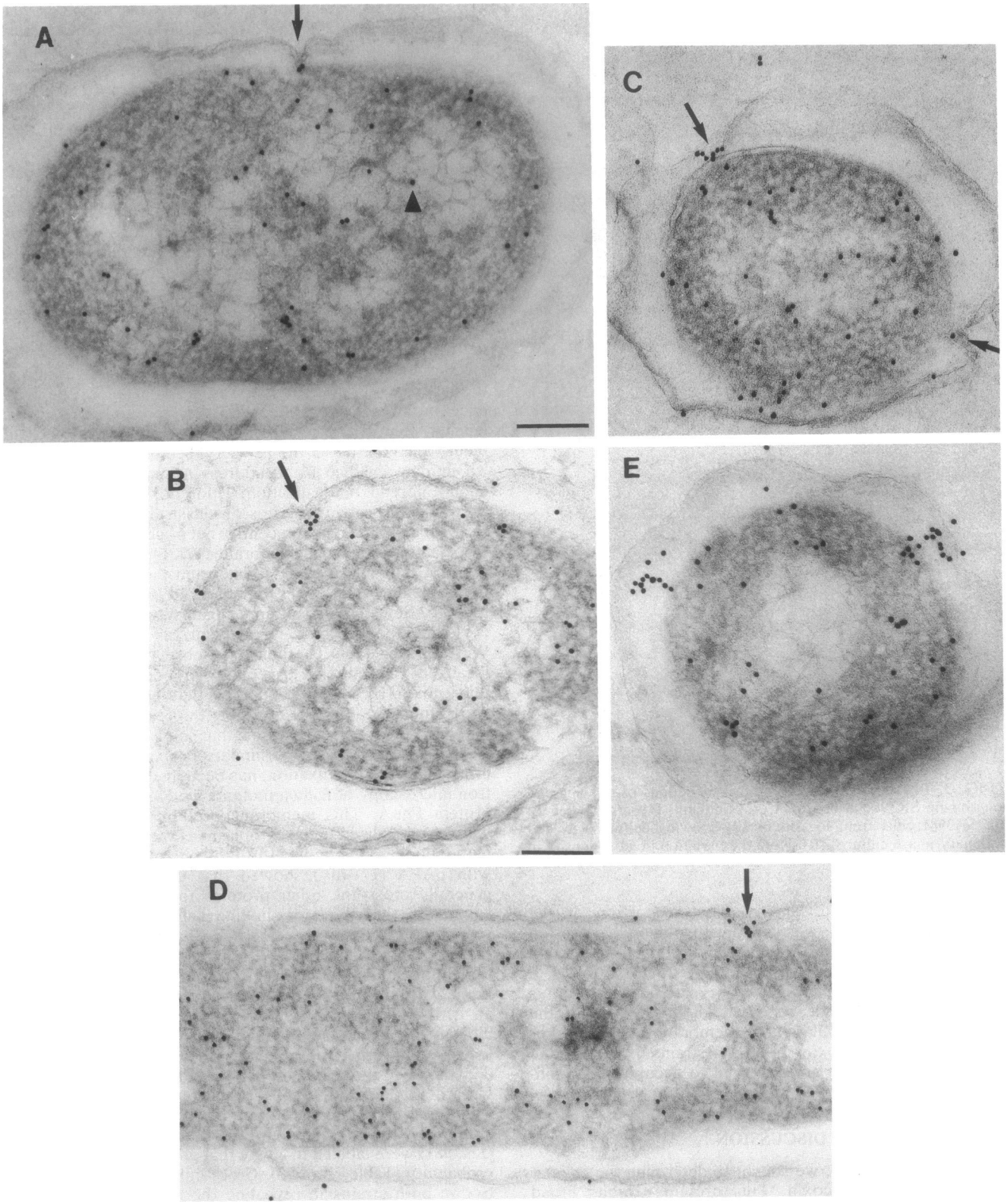

FIG. 6. Localization of thioredoxin at membrane adhesion sites. (A, B, C) Cross-sections revealing one or two labeled sites. (D) Longitudinal section with one of several visible adhesion sites labeled. (E) Cells exhibiting bridge of gold label, with membrane portion of adhesion site either vaguely defined or invisible. Arrows indicate labeled membrane adhesion sites. Arrowhead (A) indicates gold particle localized over DNA strands.

preparations revealed a relatively pronounced band in a position where thioredoxin "dimers" would be expected (23.5K to 24K) (Fig. 3), the isolated cell envelope fractions showed pronounced bands mainly of trimer and tetramer molecular weights. One could therefore tentatively propose that the envelope contains higher multimers, possibly trimers and tetramers of thioredoxin.

In a previous study, another laboratory (20) reported the localization of thioredoxin by means of immunoelectron microscopy. These authors concluded that thioredoxin resides in the periplasm and that it is also associated with the cell nucleoid. We have been unable to confirm these observations. It is important to note that periplasmic localization of the thioredoxin would require either that the protein be synthesized as a protein precursor (with an amino-terminal signal peptide) or that the protein contain an internal signal peptide. There is at present no evidence that thioredoxin contains either of these structures.

We have also been unable to observe relatively massive localization of thioredoxin in the nucleoid. Instead, we found that occasionally immunolabel may bind to very short stretches of the fibrous component within the nucleoid (Fig. 6A). Such fibers can be interpreted as DNA (9). We have obtained data which support this interpretation: electron-dense fibers of the nucleoid (in Lowicryl-embedded cells) interact with gold-labeled DNA intercalators in the same way as double-stranded isolated DNA does (M. E. Bayer, D. Weed, and S. Haberer, submitted for publication). However, the association of thioredoxin label with the fibers appears to be infrequent. Nonspecific binding of the label to the elements of the nucleoid cannot be excluded without quantitative evaluation of the labeling intensity of fibers versus background.

In previous work (15), it was concluded that osmotic rupture of membrane extrusions (associated with adhesion sites) was the critical factor in extraction of thioredoxin from whole cells. Work on the release of colicin E2 from cells suggests an additional factor. Pugsley and Schwartz (22) have shown that release of colicin E2 is coincident with the generation of lysophosphatidylethanolamine by the detergent-resistant phospholipase A. This phospholipase can be induced by a number of factors, including EDTA treatment, a treatment used during the osmotic shock procedure to release thioredoxin (15). This fact, coupled with the observation that phospholipases are present in the hybrid membrane fraction (4, 5), suggests that the detergent effect of lysophospholipids may be important in releasing the contents of the osmotically sensitive cytoplasmic compartment.

We conclude from our present study that (i) use of the photo-cross-linker pAPA is a significant improvement in retaining the low-molecular-weight protein thioredoxin in the cell during the procedures of aldehyde fixation and embedding, (ii) thioredoxin is largely associated with the area of the inner membrane, (iii) overproducing cells exhibit considerable amounts of thioredoxin in the cytoplasm as well, (iv) thioredoxin is present in a number of membrane adhesion sites, and (v) the periplasmic space appears to lack the protein.

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