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Kinetics of Accumulation of a Photodynamically Induced Cell-Surface Polypeptide in a Species of *Arthrobacter*

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Cells of a species of *Arthrobacter* were incubated in the light with methylene blue, a dye that sensitizes photooxidative reactions by the production of singlet oxygen. An early and major response by the cells to these conditions was stimulation of synthesis of a single cell-surface polypeptide, 21,000 daltons in mass. The rate of synthesis of this polypeptide reached a maximal level about 30 min after the start of illumination. As a consequence, the amount of this polypeptide increased at least 10-fold during a period of 5 h. The presence of histidine or methionine, scavengers of singlet oxygen, markedly diminished synthesis and accumulation of this polypeptide. Concomitant with the accumulation of this polypeptide on the cell surface was the appearance of an extensive array of pili.

A set of environmental conditions has been found that causes induction of synthesis of a bacterial cell-surface polypeptide (14). This induction, observed with a species of *Arthrobacter*, requires light, oxygen, and a photosensitizing dye. Rose bengal, riboflavin, chlorophyllin, and methylene blue, although differing in chemical structure, cause stimulation of synthesis of the same polypeptide. These dyes are known to promote oxidative reactions in the presence of light (27).

Dye-sensitized photooxidation may occur by at least two pathways. The type I pathway is characterized by the formation of an intermediate complex between an excited dye molecule and the substrate. A free radical form of the substrate, which results from this interaction, then reacts with ground-state oxygen to give oxidized products. Type II reactions, on the other hand, involve quenching of the excited, triplet state of the dye by molecular oxygen. In this process the energy absorbed by the dye as light is transferred to oxygen, elevating the energy of the oxygen molecule from the triplet, ground state to the singlet state ($^1O_2$, $^1\Delta_2$) (9, 18, 40). Apparently any compound that contains unsaturated bonds is susceptible to attack by singlet oxygen. Whether the type I or type II mechanism prevails depends upon the dye and the experimental conditions.

To study in more detail the process of induction of the cell-surface polypeptide, and to determine whether singlet oxygen was a common intermediate in the action of the various dyes, methylene blue was chosen as the sensitizing dye. An extensive literature exists which documents that methylene blue sensitizes photooxidation reactions via the generation of $^1O_2$ (2, 10, 12, 26, 34, 39). Methylene blue-sensitized photooxidation of proteins proceeds almost exclusively via $^1O_2$ (25). Also, kinetic analyses (2, 26) showed that the rate of quenching of triplet states of methylene blue by molecular oxygen (the type II pathway) exceeds the rate of quenching by readily oxidized substrates, tryptophan, and histidine (the type I pathway) by a factor of 10^2 to 10^3.

The results of the study with methylene blue, reported in this paper, strongly suggest that $^1O_2$ is involved in stimulation of synthesis of the cell-surface polypeptide. The consequences of this stimulation are, chemically, a marked accumulation of this polypeptide and, morphologically, the development of an extensive array of pili.

MATERIALS AND METHODS

Growth of cells. *Arthrobacter* cells were maintained on agar containing a defined medium in association with *Chlamydomonas* cells, as described previously (14). After transfer of the mixture to agar medium supplemented with 0.1% yeast extract, the bacterial cells were isolated as the result of their more rapid rate of growth. For liquid cultures, the bacterial cells, free of *Chlamydomonas*, were introduced into flasks containing the defined medium supplemented with 4 g of casein hydrolysate, 1 mg of thiamine, and 0.1 mg of biotin per liter in place of the yeast extract. Cells were grown in the dark, collected from liquid culture by centrifugation at 12,000 × *g* for 10 min, and washed two times with a carbon-free medium (pH 7.8) containing 1 mM Na_2HPO_4, 0.2 mM KH_2PO_4, 1 mM $(NH_4)_2SO_4$, 1 mM $MgCl_2$, and 0.03 mM $CaCl_2$. Finally, the cells were suspended in the deficient medium

supplemented with 15 mM sodium glutamate. The density of the cell suspension was adjusted to give an optical density at 450 nm of 5.

Analysis of cell walls. Cells collected from liquid cultures were washed two times by centrifugation with 25 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.6), containing 5 mM ethylenediaminetetraacetate, and suspended in this medium. Lysozyme was added to a concentration of 0.5 mg/ml, and the suspension was incubated for 1 h at 37°C. The cells were again collected by centrifugation, suspended in the tris(hydroxymethyl)aminomethane-hydrochloride–ethylenediaminetetraacetate buffer, and sonically disrupted for five 1-min periods at 70 W (model W185 Sonifier, Heat Systems Ultrasonics, Inc.) on ice, interspersed with 1-min cooling periods. The nearly clear sample was centrifuged at 12,000 × *g* for 10 min at 2°C to remove unbroken cells. A solution (25%, wt/vol) of sodium dodecyl sulfate (SDS) was added to the supernatant fluid to provide a final SDS concentration of 1%, and the mixture was heated in boiling water for 1 min. The sample was centrifuged at 100,000 × *g* for 1 h at 20°C, and the pellet was washed two times with 0.15 M NaCl containing 0.1 M ethylenediaminetetraacetate (pH 8.0) and then an additional two times with water.

The amino acid and amino sugar composition of the cell wall preparation was analyzed with a Beckman model 119 automatic amino acid analyzer after hydrolysis with 6 N HCl in evacuated, sealed vials at 108°C for 6, 24, or 48 h. Glucosamine and an unidentified amino sugar were quantitated using a color value determined experimentally for glucosamine. Purified muramic acid was dissolved in 6 N HCl, and a color value and the rate of degradation were obtained by relating the release of ammonia to the loss of the amino sugar during incubation at 108°C.

Isomers of diaminopimelic acid were separated by thin-layer chromatography on glass-backed cellulose plates (0.10-mm layer thickness, EM Laboratories, Inc.) which were developed two times with the solvent system of Rhuland et al. (28). Sugars were released from the cell wall by hydrolysis with 2 N HCl in evacuated, sealed vials for 2 h in boiling water and identified by thin-layer chromatography on silica gel plates (0.25-mm thickness, EM Laboratories, Inc.) using a mixture of *n*-butanol, acetic acid, and water (5:4:1, vol/vol/vol) as the developing solvent (16). Sugars were located on chromatograms after spraying with α-napththol-sulfuric acid (16). Total sugar content of the wall was estimated with the phenol-sulfuric acid assay (6) with galactose as the standard. Phosphate was measured after ashing as described by Ames and Dubin (1). A portion of the wall preparation was dried at 110°C to constant weight.

Assay of photodynamic induction. Two milliliters of the cell suspension, prepared as above (optical density of 5 at 450 nm), were placed in 50-ml Erlenmeyer flasks and exposed to light (15 J/m^2 per s from cool-white fluorescent lamps, Westinghouse) on a rotating platform at 25°C. Methylene blue was added to a final concentration of 2×10^{-7} M, unless otherwise stated, and other additions were made as indicated in legends to the figures. Newly synthesized proteins were labeled by adding a mixture of ^{14}C-amino acids (1.25 μCi/ml). The labeling period was stopped by addition at room temperature of 1 ml of 50% (wt/vol) trichloroacetic acid and then 2 ml of water. Cells were placed on ice, collected by centrifugation, and washed, once with 3 ml of 5% trichloroacetic acid and finally with 3 ml of water.

Electrophoresis. Electrophoresis on slab gels of polyacrylamide in the presence of SDS and radioautography of dried gels were performed as described previously (14). Stained gels and radioautographs were scanned with a Gilford gel scanner. The relative area under the peak for each band was determined with an Autolab Minigrator (Spectra-Physics) connected to the recorder.

Electron microscopy. Unfixed samples of the cultures were placed on Formvar-carbon-coated copper grids (400 mesh) and negatively stained with a saturated aqueous solution of uranyl acetate. Cells were examined in a Siemens Elmiskop IA electron microscope with an instrumental magnification of 20,000.

Materials. Casein hydrolysate, histidine, methionine, thiamine, biotin, glucosamine, and muramic acid were obtained from Sigma Chemical Co. Lysozyme (egg white, 3× crystallized) was purchased from Schwarz/Mann. The mixture of ^{14}C-amino acids was obtained from Amersham/Searle. Acrylamide (Eastman Chemical Co.) was crystallized from chloroform. SDS was obtained from Pierce Chemical Co.

RESULTS

Cell wall composition. The organism used in this investigation previously was designated as a species of *Arthrobacter* (14), although its identification remained tentative. Thus the composition of the cell wall was analyzed to obtain additional taxonomic information. The walls were prepared from populations of predominantly rod-shaped cells collected at the late log phase of growth. The results of this analysis are shown in Table 1. Alanine, glutamic acid, glycine, and LL-2,6-diaminopimelic acid were found in a ratio of 2:1:1:1. Other amino acids were present at levels less than 2% that of glutamic acid. The amount of ammonia recovered after acid hydrolysis of the wall was approximately equal to the extent of destruction of amino sugars. Thus, no amide groups occur in the peptidoglycan.

The amount of muramic acid in the cell wall was equivalent to that of glutamic acid. Two other amino sugars, glucosamine and an unidentified component, were present in amounts exceeding that of muramic acid. Glucosamine apparently resides within the peptidoglycan in an alternating sequence with muramic acid, both sugars as their *N*-acetyl derivatives, as occurs in other bacterial cell walls. The additional glucosamine, in excess of the amount of muramic acid, was approximately equal to the amount of the unidentified component. Another polysaccharide possibly occurs in the wall that contains

TABLE 1. *Composition of the cell wall*

Component	Concn (nmol/mg, dry weight)	Ratio to glutamic acid
Amino acids		
Glutamic acid	193	(1.00)
Glycine	206	1.07
Alanine	384	1.99
LL-Diaminopimelic acid	189	0.98
Amino sugars[a]		
Muramic acid	190	1.0
Glucosamine	630	3.3
Unidentified	360	1.9
Carbohydrate[b]	1,700	8.8
Phosphate	374	1.94

[a] Amino sugars were determined after hydrolysis in 6 N HCl for 6 h at 108°C. The amount of muramic acid was corrected for a 27% loss during hydrolysis, whereas the amounts of glucosamine and the unidentified component were corrected for losses of 15%.

[b] Chromatographic analysis revealed galactose and rhamnose as the only sugars in this fraction.

these two amino sugars along with the neutral sugars. No other amino sugars were detected in the preparation.

The unidentified component was quantitated by its reaction with ninhydrin. It was eluted from the column of the amino acid analyzer, operated with a single-column program, at 212 min, just before the elution time for histidine, whereas glucosamine was eluted at 172 min, between the elution times for leucine and tyrosine. This unidentified component gave a positive reaction with the Elson-Morgan reagent (5). It was eluted with 0.33 N HCl from a column of Dowex 50-X8, 1 by 50 cm (38), in an effluent volume 1.7 times that for glucosamine. These properties are characteristic of a 2-amino-2,6-dideoxyhexose (38, 41).

Two sugars, identified as galactose and rhamnose, were found after hydrolysis of the cell wall. No other sugars were detected by the chromatographic procedure. Total carbohydrate was estimated to be about 9 mol of sugars per mol of glutamic acid, assuming that all sugars were hexoses.

The amount of phosphate found after ashing corresponded to nearly 2 mol per mol of glutamic acid. No free phosphate was detected before ashing. Material extracted from the preparation with 0.6 N $HClO_4$ at 80°C for 20 min did not absorb light of wavelengths 250 to 300 nm, indicating an absence of nucleic acids. Whether the phosphate was part of teichoic acids was not determined.

Methylene blue-sensitized stimulation of synthesis of a 21,000-dalton polypeptide. When the bacterial cells were exposed to light in the presence of a dye, synthesis of a polypeptide 21,000 daltons in mass (hereafter referred to as the 21K polypeptide) was markedly stimulated (14). As a result, this polypeptide became a major product of total cellular protein synthesis (35). When cells were treated with SDS (1 to 2%), a group of polypeptides, including the 21K polypeptide, was extracted (14). Electrophoresis of the SDS-extracted proteins resulted in the patterns shown in Fig. 1 (lanes a and b). In this experiment, a suspension of cells was incubated in the light or dark for 3 h with 2×10^{-7} M methylene blue. The major change in the pattern of polypeptides caused by light was an increase in the amount of the 21K polypeptide, whose position is marked by the arrow.

The incorporation of ^{14}C-amino acids by illuminated cells into the SDS-extractable polypeptides was examined by radioautographic analysis of a gel after electrophoresis. Figure 1 shows that only one band was significantly labeled, which corresponded to the 21K polypeptide (lane c). Concentrations of methylene blue above 2×10^{-7} M resulted in a decline in the rate of protein synthesis, whereas the dye was effective in causing synthesis of the polypeptide at 10^{-9} M (the lowest concentration tested). Chloramphenicol (100 μg/ml) strongly inhibited the incorporation of ^{14}C-amino acids into the polypeptide (lane d). Other inhibitors of protein synthesis, such as erythromycin, streptomycin, and kanamycin, also strongly inhibited synthesis of the 21K polypeptide (data not shown).

When cells were washed with 2 M NaCl, essentially the same group of polypeptides were removed as with 2% SDS. Radioautography of a gel after electrophoresis of the NaCl-extracted polypeptides again revealed only the one significantly labeled polypeptide (data not shown). These results support the previous suggestion (14) that the 21K polypeptide resides on the cell surface. Therefore, its synthesis can be studied without interference from the incorporation of labeled amino acids into intracellular proteins.

Kinetics of photoinduction. The kinetics of methylene blue-sensitized photoinduction of synthesis of the 21K polypeptide were determined by labeling cells during 5-min periods of incubation. After a lag period of about 5 min, the rate of synthesis increased markedly (Fig. 2). By 30 min of incubation, a maximal rate was reached, which was maintained for at least several hours. No increase in the low rate of synthesis was observed in the dark, nor was the rate of synthesis significantly increased during the

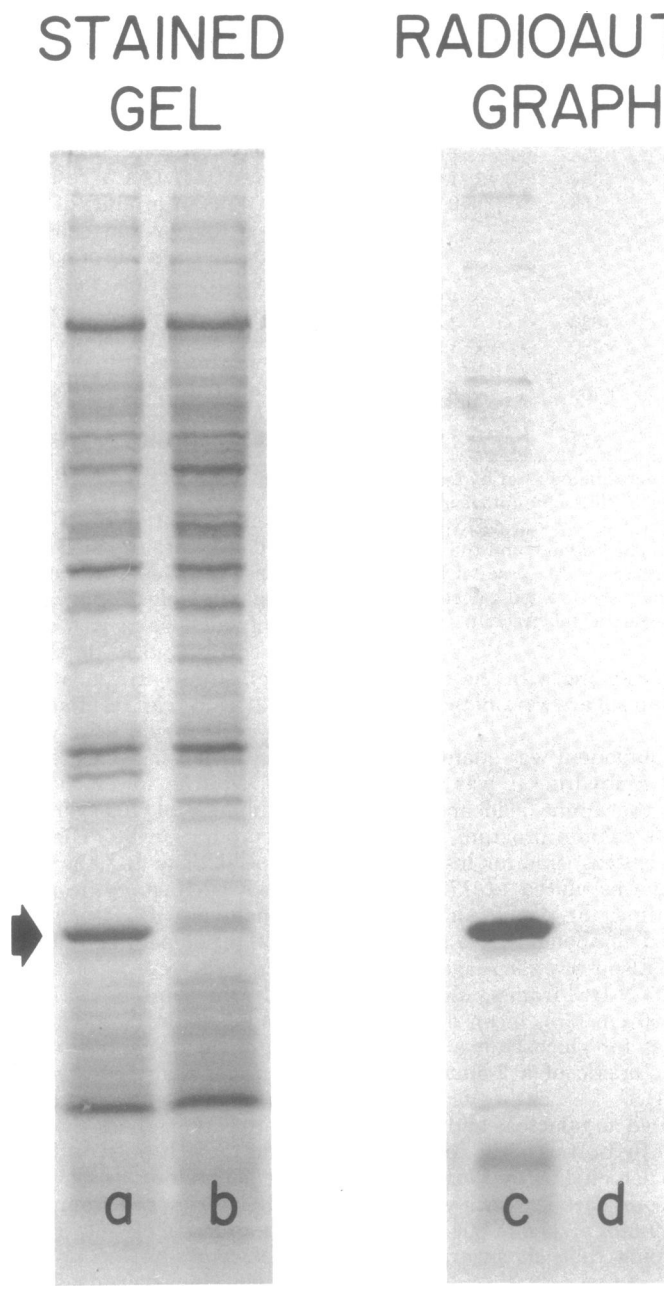

FIG. 1. *Electrophoretic analysis of surface proteins extracted with SDS from cells incubated with methylene blue. The pattern of protein stain is shown for cells incubated for 3 h (a) in the light or (b) in the dark. Lanes c and d show a radioautograph of a gel containing proteins from cells incubated in the light for 2 h and then labeled for 30 min with ^{14}C-amino acids (c) in the absence or (d) in the presence of 100 μg of chloramphenicol per ml.*

first hour in the light in the absence of dye, as shown previously (14).

An experiment was run to test whether the lag in labeling (Fig. 2) resulted from time required for transport of the polypeptide from the site of synthesis to an exposed position on the cell. Cells were incubated in the light with methylene blue for 1 h to achieve the maximal rate of

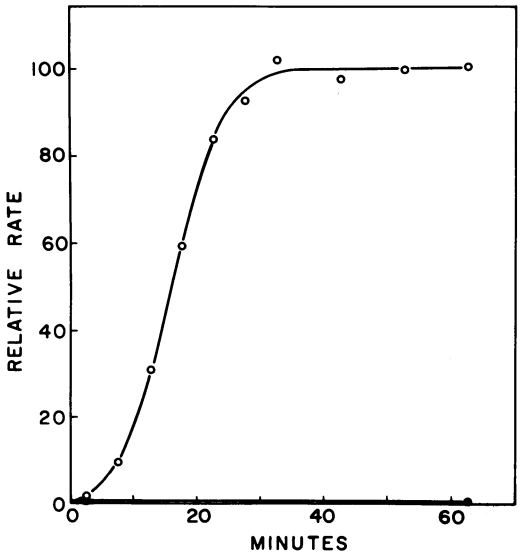

FIG. 2. *Kinetics of photoinduction of synthesis of the 21K polypeptide in the presence of 2 × 10^{-7} M methylene blue. At the times indicated after the start of illumination, cells were labeled for 5-min periods by the incorporation of ^{14}C-amino acids. After electrophoresis, radioautographs of gels were scanned, and darkening of the film was quantitated by integrating peaks in densitometric traces. For the figure, an integration value for the peak corresponding to the 21K polypeptide was plotted at the midpoint of the labeling period. Cells were incubated either* (○) *in the light or* (●) *in the dark.*

synthesis of the polypeptide. ^{14}C-amino acids then were added, and the incorporation of ^{14}C into the polypeptide was measured. In this experiment, radioactivity appeared in the polypeptide at a linear rate during the first 10 min of labeling, indicating that time of transport was not a significant factor in the data presented in Fig. 2. Other factors, such as time required to generate a sufficient concentration of photoproducts to initiate induction or the mechanism of induction itself, therefore may contribute to the lag period.

In other experiments, the rate of synthesis of the 21K polypeptide was examined after the light was turned off. During the subsequent period in the dark, the high rate of synthesis was maintained for about 1 h, after which the rate gradually declined. After 2.5 to 3 h in the dark, the level of incorporation of ^{14}C-amino acids into this polypeptide again was similar to that with cells that had not been exposed to light (data not shown).

Accumulation of the 21K polypeptide on the cell surface. Figure 3 shows scans of stained gels after electrophoresis of SDS extracts of cells that were incubated in light with methylene blue for 0, 2, and 5 h. Over this period of time, increases were not observed in the amounts of most of the polypeptides recovered in this fraction. However, the amount of the 21K polypeptide (at 8.5 cm from the origin and marked in Fig. 3 by the solid arrowhead) increased substantially until it became the major component of the fraction. The relative amount

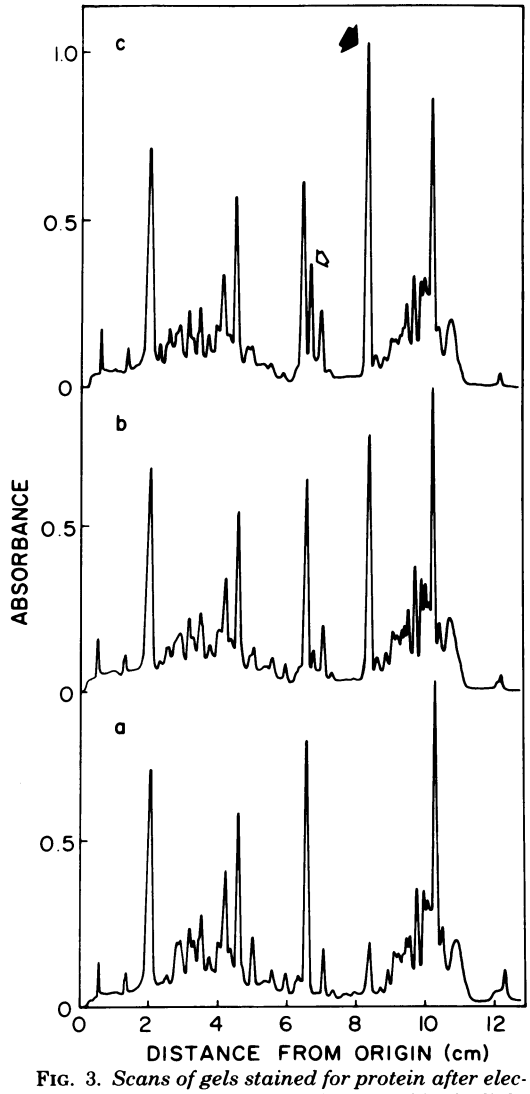

FIG. 3. *Scans of gels stained for protein after electrophoresis of dark-grown cells incubated in the light with methylene blue for (a) 0, (b) 2, and (c) 5 h. The 21K polypeptide migrated 8.5 cm from the origin and is marked by the solid arrowhead. A 28,000-dalton polypeptide that appeared only after about 2 h of illumination (see text) is marked by the open arrowhead.*

of this polypeptide was quantitated by integrating the area under the peak, corresponding to this polypeptide, during a scan of the gel. After electrophoresis of a series of samples, the time course of accumulation was obtained by plotting an integration value versus the time of incubation in the light. After an initial slow phase, corresponding to the time required to reach the maximal rate of synthesis, the amount of the polypeptide increased rapidly (Fig. 4). In the absence of a dye, but in the presence of light, the polypeptide also accumulated, but at a much slower rate after an extended lag period of about 1 h. Apparently an endogenous sensitizer was present in the cells that weakly promoted synthesis of the 21K polypeptide in the light. No increase in the amount of the polypeptide was observed with cells kept in the dark.

Appearance of a 28,000-dalton polypeptide. SDS extracts of cells incubated in the light for 2 h or more with 2×10^{-7} M methylene blue contained a new polypeptide of about 28,000 daltons in mass (open arrowhead in Fig. 3). By 5 h of illumination, the amount of this polypeptide had achieved a level 20 to 25% of that of the 21K polypeptide. When ^{14}C-amino acids were added to cell suspensions exposed to light, the 28,000-dalton polypeptide became labeled, but only after 1.5 h of illumination. The rate of labeling of this polypeptide increased to a maximal level between 2 and 2.5 h in the light, and thereafter declined.

The 28,000-dalton polypeptide did not appear if cells were incubated in the light with 2×10^{-8} M methylene blue or with chlorophyllin, another dye that causes photoinduction of synthesis of the 21K polypeptide (14). Thus, this 28,000-dalton polypeptide appears only under relatively strong photooxidative conditions and does not seem to be related to the 21K polypeptide.

Involvement of singlet oxygen. Photoreactions sensitized by methylene blue generally proceed by a pathway which involves the generation of $^1O_2$ (25, 26). To test further if $^1O_2$ is involved in stimulation of synthesis of the 21K polypeptide, scavengers of $^1O_2$, which should diminish synthesis of this polypeptide, were added to the medium. A variety of scavengers of $^1O_2$ are known, including substituted furans, tertiary amines, NaN_3, and carotenes (10, 21, 22, 32). However, in the experiments described here histidine (26, 37) and methionine (36) were chosen for this purpose to preclude inhibition of protein synthesis by the scavengers themselves and because of the high solubility of the amino acids in water.

Figure 5 shows that addition of 50 mM histidine or methionine to the incubation medium

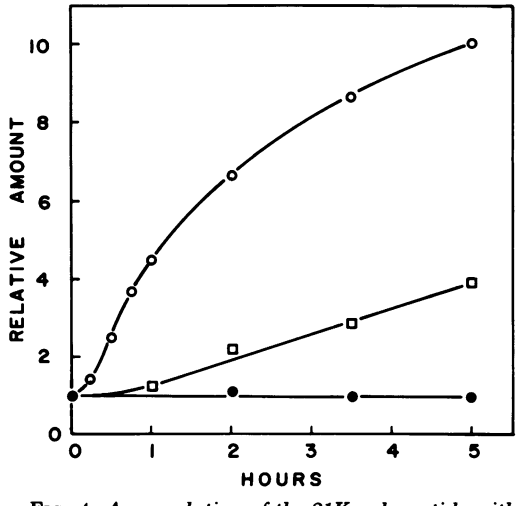

FIG. 4. *Accumulation of the 21K polypeptide with time in the light. Dark-grown cells were exposed to light in the presence of methylene blue. After electrophoresis of samples taken at various times of incubation (see Fig. 3), the relative amount of the 21K polypeptide was determined by integrating the peak corresponding to this polypeptide during a scan of each sample lane on the gel. The figure shows the changes in amount of the polypeptide* (○) *in the light with methylene blue,* (□) *in the light but without methylene blue, and* (●) *in the dark with methylene blue.*

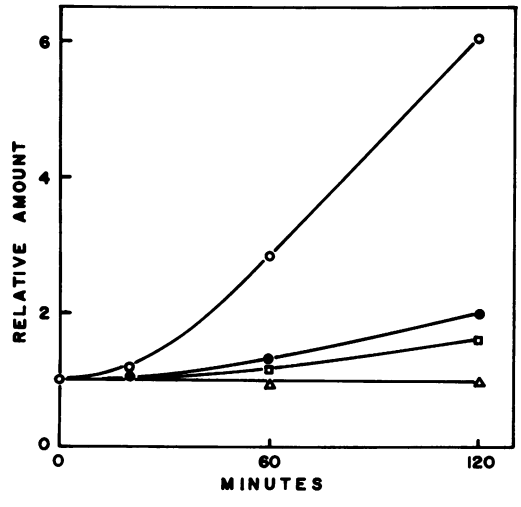

FIG. 5. *Effect of 50 mM histidine and methionine on the accumulation of the 21K polypeptide. In this experiment the concentration of methylene blue was* 2×10^{-8} *M and the light intensity was 7.5 J/m*^{2} *per s. Cells were incubated* (○) *without histidine or methionine,* (●) *with histidine,* (□) *with methionine, or* (△) *with histidine but without dye.*

containing 2×10^{-8} M methylene blue strongly suppressed the dye-sensitized accumulation of the 21K polypeptide. Furthermore, histidine completely prevented an increase in this polypeptide during incubation of the cells in the light but in the absence of an exogenous dye (refer to Fig. 4). In contrast, 50 mM glycine, alanine, or glutamate, which are not readily attacked by $^1O_2$, slightly stimulated accumulation of the polypeptide (data not shown).

In another experiment, similar to that shown in Fig. 5, the incorporation of ^{14}C-amino acids was tested after cells were illuminated for 30 min in the presence of 50 mM histidine. For a control, histidine was added to cells after the 30 min of light, along with the ^{14}C-amino acids, to compensate for dilution of the ^{14}C resulting from metabolism of the histidine. In this experiment, histidine decreased the dye-stimulated incorporation of ^{14}C-amino acids into the 21K polypeptide by about 80%, a value similar to that observed for the suppression of accumulation (Fig. 5). Histidine was less effective at lower concentrations or in the presence of higher concentrations of methylene blue. Since the addition of methionine resulted in severe dilution of the ^{14}C-amino acids, its effect on labeling of the polypeptide was not determined.

Production of pili. Cells were examined by electron microscopy to determine whether the increase in amount of the 21K polypeptide altered the appearance of the cell surface. For these experiments, chlorophyllin was used as the sensitizing dye because it did not cause stimulation of synthesis of the 28,000-dalton polypeptide. Chlorophyllin also did not prevent growth of the cells during long-term experiments in the light (14). Thus, cells were grown in the light in the presence of 4×10^{-6} M chlorophyllin overnight and then placed in the dark for 4 h before being prepared for electron microscopy. From other experiments it was learned that synthesis and accumulation of the 21K polypeptide continued for 2 to 3 h after transfer of cells to the dark.

Figure 6 shows typical micrographs of negatively stained cells from these experiments. Cells that were exposed to light and chlorophyllin contained an extensive array of pili. In contrast, cells grown in continuous darkness were only sparsely populated with pili. The major difference in the cell surface polypeptides between light- and dark-grown cells is the amount of the 21K polypeptide. Since this polypeptide is similar in mass to pilins from other bacterial species (3, 11, 24, 29, 30), these results suggest that the polypeptide whose synthesis is induced by mild photooxidative conditions is the subunit of pili.

DISCUSSION

The genus *Arthrobacter* comprises a heterogeneous group of gram-positive (or gram-variable), aerobic organisms whose major distinction is that they undergo a morphological change from rod-shaped to coccoid cells with aging of the culture (19). Although there is no uniform composition of the cell wall among these organisms, the variations in the wall composition of most arthrobacters fit into a few related groups (4, 8, 20, 31). The composition of the peptidoglycan of the organism used in these experiments (Table 1) resembles the type designated A3γ by Schleifer and Kandler (31). The A3γ type of structure contains LL-diaminopimelic acid and one or more glycine residues as the connecting bridge between adjacent peptides. The group of organisms known to contain this type of peptidoglycan includes mainly arthrobacters and aerobic actinomycetes (31).

The presence of an accessory polysaccharide containing galactose, rhamnose, and perhaps glucosamine and the unidentified amino sugar also was useful in determining a classification for the organism. Relatively few species that contain the A3γ type of peptidoglycan also contain these sugars, although the composition shown in Table 1 matches that for a group of coryneform bacteria, referred to as group B, found by Keddie et al. (20). However, the unidentified amino sugar, a distinguishing feature of the analysis shown in Table 1, was not reported by Keddie et al. (20). It also was not reported to occur in the carbohydrate fraction of cell walls of other species of *Arthrobacter* that have been examined (4, 7, 23). The structure of this amino sugar was not determined, but its properties are similar to those of a 2-amino-2,6-dideoxyhexose (38, 41).

These data on the composition of the cell wall, in conjunction with the morphological and nutritional characteristics of the bacterium used in these experiments (14), are compatible with its designation as a species of *Arthrobacter*. Although the composition of the cell wall does not completely match those reported for named species of this genus, it is similar to the cell wall composition of *Arthrobacter simplex* (4, 31).

The dyes used in these experiments are known to sensitize oxidation reactions by generating singlet oxygen (12, 26, 33, 39, 40). Although most agents that are used to verify the involvement of $^1O_2$ in the mechanism of oxidation (22, 25, 26) are not applicable for use in cultures of whole cells, the characteristics of the photoinduction of synthesis of the 21K polypeptide by the dyes, and its inhibition by histidine or methionine,

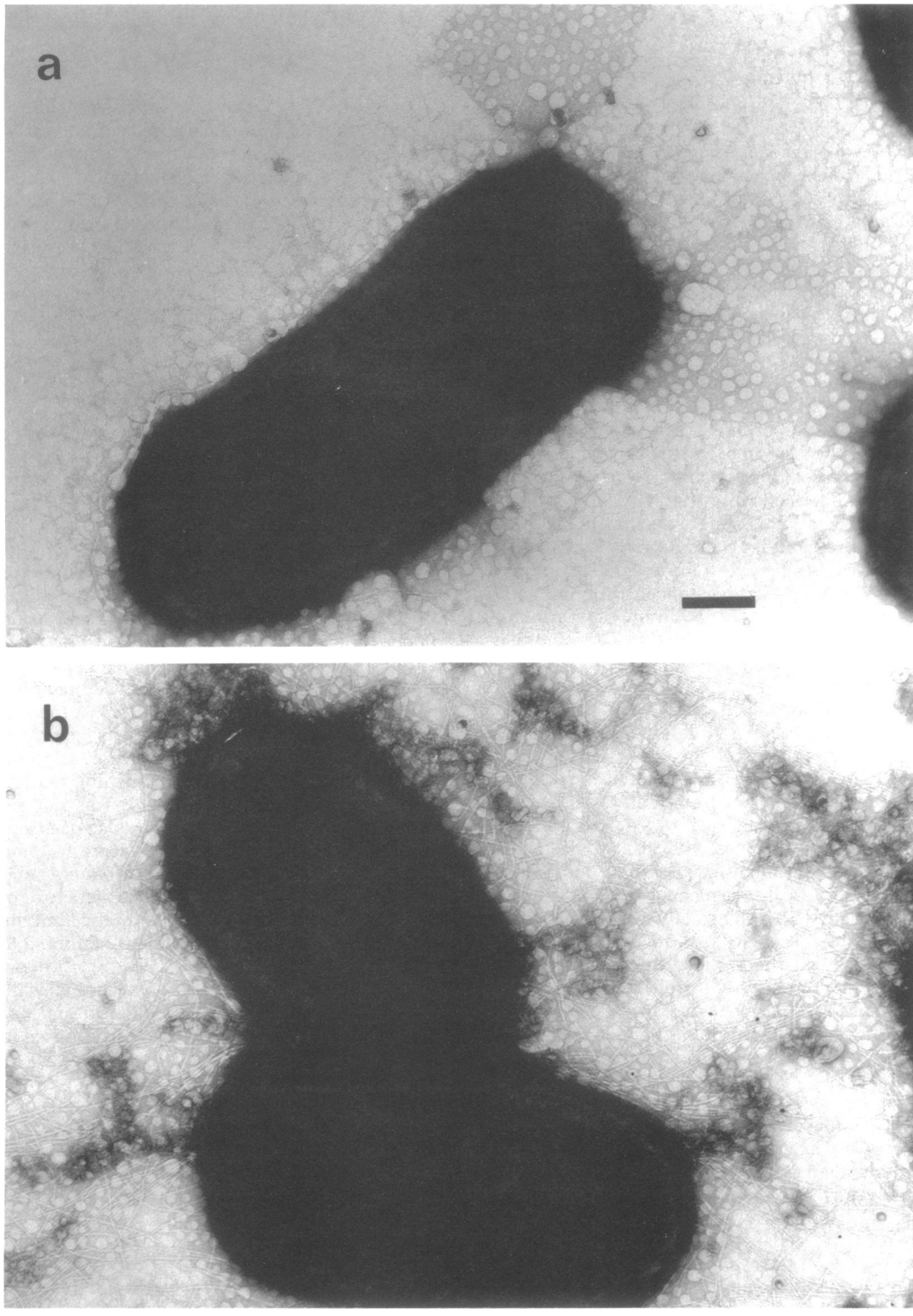

FIG. 6. *Increase in piliation of cells grown under photooxidative conditions. Cells were grown in the light in the presence of* 4×10^{-6} *M chlorophyllin but were allowed to stand for 4 h in the dark before examination by electron microscopy. Samples were taken from cultures grown (a) continuously in the dark or (b) in the light with chlorophyllin. The bar represents 180 nm.*

strongly suggest that $^1O_2$ is involved. The cellular component(s) affected by this excited species of oxygen is not known. Also, additional work must be done to determine whether the dye must enter the cells to initiate the induction. Although methylene blue does not seem to penetrate yeast cells (15), this dye causes degradation of DNA (17) and mutagenesis (33) within bacterial cells. Photooxidative conditions also cause damage to the cell envelope of bacteria, as demonstrated by an increased sensitivity of *Proteus mirabilis* to lytic agents after only a few seconds of treatment (17). However, cells of *P. mirabilis* were able to repair sublethal damage in DNA and the cell envelope within minutes after the light was turned off.

A major and consistent response by the *Arthrobacter* cells to photooxidative conditions was a rapid production and accumulation of a single cell-surface polypeptide. A high rate of synthesis of this polypeptide was maintained as long as cells were exposed to light, but the rate declined to the uninduced level within 2 to 3 h after transfer to the dark. Since the effects of these conditions were reversible, the stimulation of synthesis of this polypeptide may be the result of an attempt by the cells to replace protein molecules damaged by oxidation.

Concomitant with the accumulation of the cell surface polypeptide was the formation of an extensive array of pili. Although this evidence suggests that the 21K polypeptide is pilin, further information on the properties of pili from these cells is necessary before a definitive identification of the polypeptide can be made. However, pili have been observed with several species of the genus *Corynebacterium* (13, 42), to which *Arthrobacter* is taxonomically related (19). Whether the presence of pili on gram-positive organisms is limited to the coryneform type remains to be determined. Also, it is of interest to determine whether stimulation of synthesis of pilin by mild photooxidative conditions is a general phenomenon.

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