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Effects of Fatty Acids on Growth and Envelope Proteins of *Bacillus subtilis*

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Fatty acids of different chain lengths were added to cultures of *Bacillus subtilis* growing in nutrient sporulation medium, and the effects of these fatty acids on growth, oxygen uptake, adenosine triphosphate (ATP) concentration, and membrane protein composition were examined. All fatty acids inhibited growth, the effect being reduced in the presence of glycolytic compounds and reversed by transfer to medium without fatty acids. The inhibition of growth was correlated with a reduction in both the rate of oxygen consumption and the concentration of ATP per cell. The concentration required to obtain a certain degree of inhibition increased with decreasing molecular weight of the fatty acid. However, the reduced nicotinamide adenine dinucleotide oxidation system of cell envelope preparations (i.e., the electron transport system) was not inhibited. Submaximal growth inhibition was accompanied by the relative increase of a membrane protein band revealed by urea-acetic acid gel electrophoresis. This increase was blocked by actinomycin or chloramphenicol. All of the above changes could also be produced by 2,4-dinitrophenol. The inhibition results are best explained by assuming that the fatty acids reversibly react with the cell membrane or proteins in it; they could either alter the membrane structure or uncouple the electron transport chain from two types of proteins, those used for ATP regeneration and others needed for the transport of certain compounds into the cells.

During investigations in this laboratory on the effect of acetate and other fatty acids on the sporulation of *Bacillus subtilis* mutants (6, 18), it was noticed that acetate at concentrations higher than 0.04 M inhibited the growth of cells in nutrient sporulation medium (NSMP), growth resuming only after a lag of 2 hr or more (6). A similar inhibitory effect of fatty acids was observed in *Escherichia coli* by Weeks et al. (22). They found that the nonmetabolizable 0.1% *n*-butyrate, *n*-hexanoate, or *n*-octanoate inhibited growth on amino acid media, even in strains constitutive for the enzymes of fatty acid β-oxidation, whereas the metabolizable long-chain fatty acids were used as carbon sources for growth. The growth inhibition of *Lactobacillus casei* by potassium acetate differs from the effect reported here because it can be reversed by Na^+ or Li^+ ions and by long-chain fatty acids (3).

In this paper, we show that all fatty acids inhibit growth, oxygen consumption, and adenosine triphosphate (ATP) synthesis of *B. subtilis*. The concentration required for inhibition increases with decreasing molecular weight of the fatty acids. In addition, we show that under conditions of partial growth inhibition a particular membrane protein increases in relative amount.

MATERIALS AND METHODS

Bacteria. Both strains 60009 and 60015 are transformable sporulating strains of *B. subtilis*, derived from strain 168 of Spizizen. Strain 60009 is prototroph, whereas strain 60015 requires both L-methionine and L-tryptophan for growth.

Media. Tryptose-blood-agar base (TBAB) plates, NSMP, and synthetic medium N have been described (5). All media were supplemented with 25 μg of L-tryptophan per ml and 10 μg of L-methionine per ml.

Aqueous solutions of formic, acetic, propionic, *n*-butyric, *n*-pentanoic, and *n*-hexanoic acids as well as lactic, succinic, and malic acids were prepared as 2.5 M solutions, adjusted to *p*H 7.0 with KOH (or NaOH where stated). *n*-Octanoic, *n*-decanoic, and *cis*-9,*cis*-12-octadecadienoic (linoleic) acids as well as 2,4-dinitrophenol (DNP) were prepared as 0.25 M ethanolic solutions. All solutions were sterilized by membrane filtration (pore size 0.45 μm).

Growth. An overnight culture on a TBAB plate was inoculated into NSMP medium at an absorbancy at 600 nm (A_{600}) of 0.05, and the suspension was incubated at 37 C in a water bath shaker reciprocating at 120 strokes per min. The culture volume was less than 20% of that of the Erlenmeyer flask. To determine growth inhibition, the culture was grown to A_{600} = 0.5, samples were transferred to prewarmed Erlenmeyer flasks containing the fatty acids, and the A_{600} was followed.

ATP determination. In preliminary studies, 0.5-ml samples were withdrawn 5 min after transfer to acetate-containing flasks and every 15 min thereafter for 2 hr; ATP was extracted and assayed as described by Klofat et al. (8), but using a spring-loaded Hamilton syringe (model CR 700) to inject 10 μliters of the formic acid extract directly into the buffered luciferase solution. The value of ATP/A_{600}, characteristic for each experimental condition, remained approximately constant during 2 hr of incubation after transfer. Therefore, the concentration of ATP per cell was determined on samples withdrawn at 20 min after transfer to the flasks containing fatty acids.

Oxygen uptake. After growth of strain 60015 to A_{600} = 0.5 in NSMP, 2-ml cultures were transferred to an oxygen electrode chamber (Gilson Medical Electronics, Inc., Middleton, Wis.), and the rate of oxygen uptake was followed polarographically at 37 C. Upon establishment of a constant rate, the fatty acid solution was injected into the electrode chamber by using a Hamilton spring-loaded syringe, and the decrease in the uptake rate was recorded.

Isolation of cell envelopes. The growth of cells was terminated by the addition of chloramphenicol (100 μg/ml) and crushed ice. The cells were centrifuged at 0 C and washed twice with ice-cold 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer, *p*H 7.5. (In our early isolation procedure, 100 μg of chloramphenicol per ml was included in the Tris-hydrochloride buffer. Omission of this compound from the buffer did not alter the gel electrophoretic profile of envelope proteins). The washed cells were suspended in Tris buffer to an A_{600} of 20 to 40 and incubated at 37 C with lysozyme (200 μg/ml) for 40 min, followed by deoxyribonuclease (10 μg/ml) and ribonuclease (20 μg/ml) for 20 min. The mixture was diluted four times in Tris buffer, to assure maximal lysis, and centrifuged at 3,000 $\times$ *g* for 10 min to remove intact cells. The fraction of intact cells was negligible for exponential-phase cultures, but it increased to nearly 10% for cells harvested several hours after the cessation of growth in NSMP. Prolonged incubation did not increase the extent of lysis. Cell envelopes were collected by centrifugation at 40,000 $\times$ *g* for 20 min and washed in Tris buffer. Initially, the supernatant fluid was mixed after each washing with an equal volume of 10% trichloroacetic acid and examined for the amount of acid-precipitable protein present. Protein was assayed by the Lowry method (12). The amount of protein removed in the fourth washing was less than 0.5% of the protein present in the envelope pellet. Consequently, the envelopes were washed routinely four times. *B. subtilis* membranes prepared similarly have been shown to maintain their components (16). The envelopes were stored at −20 C or lyophilized.

Isolation of cell membranes. Membranes were prepared from protoplasts of washed cells as described by Konings and Freese (9) and stored at −20 C or lyophilized.

Acrylamide gel electrophoresis. The method of Takayama et al. (21) as modified by Kahane and Razin (7) was used for electrophoresis. The gel solution containing 7.5% (w/v) acrylamide, 0.25% (v/v) ethylene diacrylate, 35% (v/v) acetic acid, and 5 M urea was filtered through Whatman no. 1 filter paper and kept at 4 C in a dark bottle. Ammonium persulfate, 0.36% (w/v), and *N,N,N',N'*-tetramethylethylenediamine, 0.48% (v/v), were mixed with the gel solution just before use. Each glass tube (5 by 76 mm) was filled with 1.25 ml of gel solution, and 0.1 ml of 35% acetic acid was layered on top. Polymerization was carried out at 37 C for 1 hr.

Fresh or lyophilized membranes or envelopes were solubilized in a phenol-acetic acid-water mixture (2:1:0.5, w/v/v), and the clear solution was centrifuged at 40,000 $\times$ *g* for 20 min, which produced a trace amount of insoluble pellet. The supernatant fluid was mixed with two-thirds of its volume of 40% sucrose in 50% acetic acid, and 20 to 50 μliters of this mixture, containing 50 to 120 μg of protein, was placed on top of the gel. A 0.15-ml amount of 75% (v/v) acetic acid was layered over the sample-sucrose mixture, and the tube was filled up with 10% (v/v) acetic acid. Both the upper and the lower baths of the Canalco electrophoretic apparatus (Canal Industrial Corp., Rockville, Md.) were filled with 10% acetic acid. Electrophoresis was carried out at room temperature for 3.5 hr with a constant current of 3 ma per gel tube. The electrode in the lower chamber served as cathode.

The gels were stained with 1% Amido black 10B in 7% acetic acid or with 0.25% Coomassie blue in 7% acetic acid for 90 min. Destaining was carried out at room temperature either by incubating the gels with several changes of 7% acetic acid for 2 days or by incubating only overnight followed by 2 hr of destaining in a Canalco electrophoretic quick gel destainer.

Protein patterns of the gels. The stained gels were traced by using a model IIIB microdensitometer (Joyce Loebl & Co., Gateshead, England) with a 16-mm lens. The gels were placed into screw-topped spectrophotometric glass tubes which were filled up with 7% acetic acid. The tubes were then secured on a holder for scanning. All major bands visible in the gels, even when closely adjacent, were separated as peaks in the tracing. Minor bands, especially those adjacent to a major band, were not well represented. They showed up either as a small shoulder or not at all in the resulting tracing.

Quantitative comparison of protein bands. The microdensitometer peak heights of the major gel bands were regarded as a measure of the amount of protein present. The linearity of peak height versus the amount of protein applied to the gels was examined for envelope preparations containing between

25 and 250 μg of protein; approximate linearity was obtained for protein concentrations up to 60 μg per gel. Therefore, the relative amount of protein in different bands was determined in gels containing not more than 50 μg of protein. With this amount of protein, good resolution was obtained; higher amounts revealed no additional bands but showed higher background staining and larger accumulation on the top of the gel.

Determination of radioactivity of protein bands. The gels were sliced into 1-mm discs, by using a modified hand microtome (American Optical Co., model 880). Description of this slicer will be published elsewhere. Each slice was transferred into a scintillation vial, and 0.5 ml of 25% (w/w) ammonia was added. The slices were completely solubilized after 2 to 3 hr of incubation at room temperature with occasional mixing. One milliliter of NCS solubilizer (Amersham/Searle, Des Plaines, Ill.) was then added, and the mixture was left overnight. Next day, 10 ml of scintillation fluid containing toluene-Triton X-100 (4:1), 55 mg of 2,5-diphenyloxazole, and 1.25 mg of 1,4-bis-2-(5-phenyloxazolyl)-benzene was added to each vial; the mixture was shaken and allowed to equilibrate in a Packard Tri-Carb liquid scintillation spectrophotometer (Packard Instrument Co., Downers Grove, Ill.), and the radioactive disintegrations were counted.

RESULTS

Inhibition of growth. The growth of our standard strain (60015) in NSMP was inhibited by K-acetate, the degree of inhibition depending on the concentration of acetate (Fig. 1). Different concentrations of Na-acetate each inhibited to the same extent as K-acetate. A concentration of 0.2 M acetate was chosen for further studies, since this level of acetate produced strong inhibition but allowed resumption of growth within a reasonable period of time. The inhibition was not limited to our standard strain because other *B. subtilis* mutants, e.g., the prototroph 60009, were inhibited by 0.2 M K-acetate to the same extent. Li-acetate at 0.2 M inhibited as effectively as K- or Na-acetate, whereas 0.2 M Li-, Na-, or K-chloride had no effect.

The growth inhibition by acetate was reduced but not eliminated in the presence of glycolytic compounds (0.2%). Glucose or fructose were most effective, whereas glycerol, glucosamine, gluconate, or ribose were moderately effective. Oxaloacetate or pyruvate also reduced the inhibition slightly, but malate, citrate, or amino acids had no effect. The inhibition was reduced (in the presence of glucose) to the same extent (not more) for the prototroph 60009.

Other fatty acids also inhibited growth in NSMP (Fig. 2), whereas 0.2 M K-malate, K-succinate, or K-lactate showed no significant effect. The longer the carbon chain of the fatty acid, the lower was the concentration required to produce maximal growth inhibition. For comparison, the uncoupling agent 2,4-dinitrophenol was as effective, inhibiting growth maximally at concentrations above 1 mM (Fig. 2). At sufficiently high concentration, each fatty acid caused a decrease in turbidity, presumably owing to cell lysis, resulting in more than 100% "inhibition" as calculated here. In synthetic N medium containing 0.5% malate or glucose as sole carbon source, acetate and other fatty acids also inhibited growth but less effectively than in NSMP. For example, 0.2 M K-acetate inhibited the growth of both strains, 60009 and 60015, by 55%.

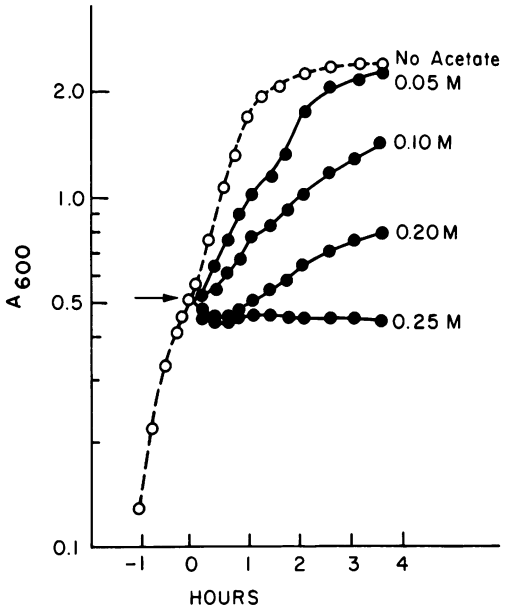

FIG. 1. *Effect of K-acetate on the growth of strain 60015 in NSMP. The arrow indicates the A_{600} at which samples of cultures were mixed with acetate (●, concentrations in M). The same results were obtained with Na-acetate and with 0.2 M Li-acetate. Use of 0.2 M LiCl, NaCl, or KCl did not inhibit growth.*

The reversibility of the growth inhibition was examined for cells that had been grown in NSMP and exposed for 2 hr to 200 mM acetate, 0.8 mM *n*-decanoate, or 0.3 mM linoleate; they were collected on membrane filters, washed with fresh NSMP at 37 C, and reincubated in NSMP at 37 C at a low cell density (A_{600} = 0.1). The treated cells grew without lag at the same rate as nontreated control cells, indicating that they had not lost any component required for immediate growth.

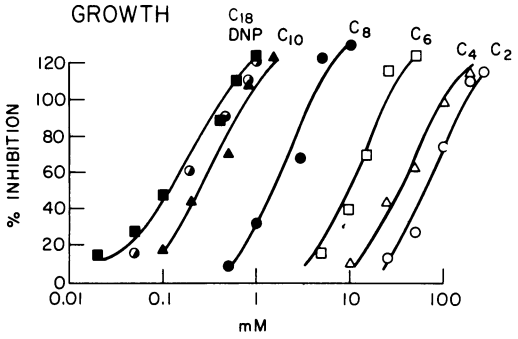

FIG. 2. *Relative inhibitory activities of fatty acids and 2,4-dinitrophenol (DNP) on the growth of strain 60015 in NSMP. Compounds were added to cultures at A_{600} = 0.5, incubation was continued for 1 hr, and the A_{600} of each culture was measured. Percent inhibition was calculated as 100 (C[1] − E[1])/(C[1] − C[0]), where C(t) = A_{600} of NSMP control culture at time t and E(t) = A_{600} of experimental culture containing the compound at time t. The growth inhibition of formic acid (C_1) was less than that of acetic acid (C_2), that of propionic acid (C_3) was between that of C_2 and n-butyric acid (C_4), and that of n-pentanoic acid was between that of C_4 and n-hexanoic acid (C_6). Symbols: ◑ = linoleic acid (C_{18}), ■ = DNP.*

Inhibition of oxygen consumption and ATP production. The growth studies using various glycolytic compounds had suggested the possibility that the regeneration of ATP via the electron transport system, but not via glycolysis, might be inhibited by the fatty acids. In fact, the addition of fatty acids to cells grown in NSMP caused a decline in the amount of ATP per A_{600} (ATP/A_{600}) which continued for about 5 min, whereafter the ATP/A_{600} remained constant. This constant value decreased with increasing fatty acid concentration (Fig. 3). Figure 3 also shows that the rate of oxygen consumption by cells grown in NSMP was similarly reduced by the fatty acids. The same inhibitory effects were observed with DNP.

However, the inhibition of oxygen consumption by whole cells was not paralleled by an inhibition of reduced nicotinamide adenine dinucleotide (NADH) oxidation in envelope preparations of NSMP-grown cells. Neither fatty acids nor DNP inhibited NADH oxidation measured polarographically.

Envelope proteins of Bacillus subtilis grown in NSMP. The cell envelope proteins were first examined for cells grown in NSMP alone and harvested at different stages of growth and spore development. The prepared and freeze-dried envelopes were dissolved in a phenol-acetic acid-water mixture, and their proteins were separated by acrylamide gel electrophoresis. They showed remarkably similar protein patterns at different stages of growth, with the exception of one band (designated D for development) which increased in amount toward the end of growth and during the developmental period (Fig. 4).

The gradual increase of the D band during late growth and development was quantitatively determined by measuring the peak height of this band relative to that of another band (designated R for reference), whose amount (per milligram of envelope protein) showed minimal variation throughout. The D/R ratio increased up to fourfold when the band patterns were stained with either Coomassie blue or Amido black 10B (Fig. 5). The fact that the D band contained protein was verified by labeling with L-[^{3}H]lysine (Fig. 6).

A diffuse band running at the front was present in all preparations. Its intensity varied from preparation to preparation. The material was not extractable by chloroform-methanol mixtures. Comparison of microdensitometer tracings and radioactivity profiles showed that this front band was not labeled by either L-valine or L-lysine. Since its gel position corre-

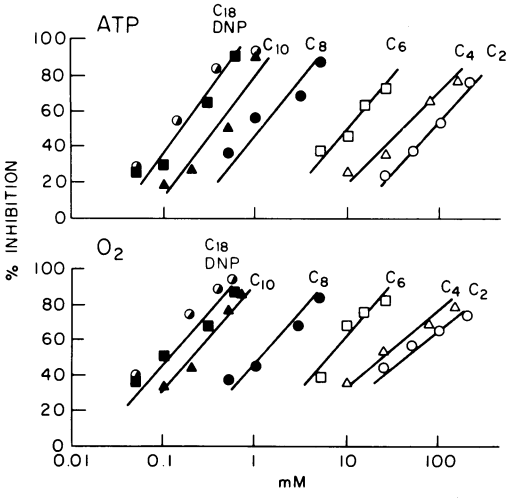

FIG. 3. *Effect of fatty acids and 2,4-dinitrophenol on oxygen uptake and ATP concentration of strain 60015 grown in NSMP medium. Without inhibitors the oxygen uptake rate of whole cells was 293 nmoles of O_2/(min × A_{600}), and the concentration of ATP was 7.3 μM ATP/A_{600}. In the presence of inhibitors, oxygen still was consumed at a constant but reduced rate. The cellular concentration of ATP declined rapidly after addition of inhibitors to a value characteristic for each inhibitor concentration. Symbols same as in Fig. 2.*

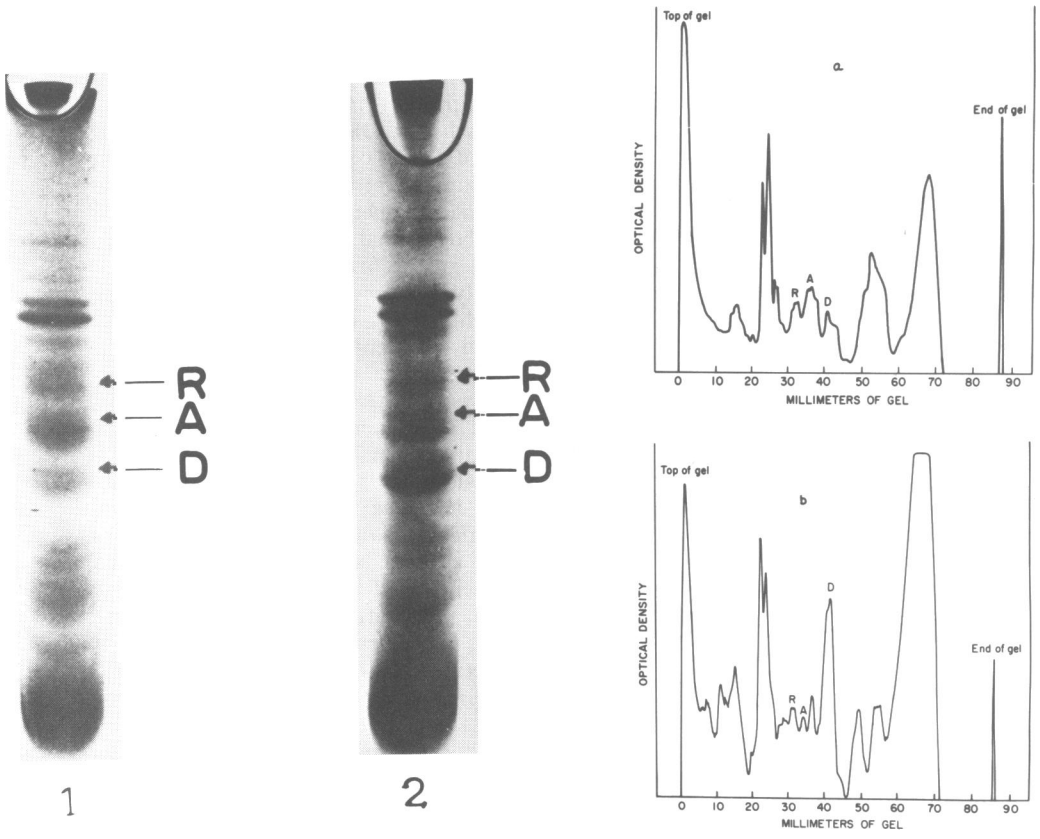

FIG. 4. *Acrylamide gel profiles of envelope proteins. Envelopes containing 100 μg of protein were applied to urea-acetic acid gels. The gels were exposed to electrophoresis for 3.5 hr at 3 ma per tube and stained with Coomassie blue. Left figures: stained gels. Gel 1, exponentially growing cells harvested at A_{600} = 0.7; gel 2, developmental-phase cells at T_6 (6 hr after growth had declined from the exponential rate), A_{600} = 2.2. Right figures: microdensitometer tracings of gel 1 (upper figure) and gel 2 (lower figure). Three protein bands were designated R, A, and D, respectively.*

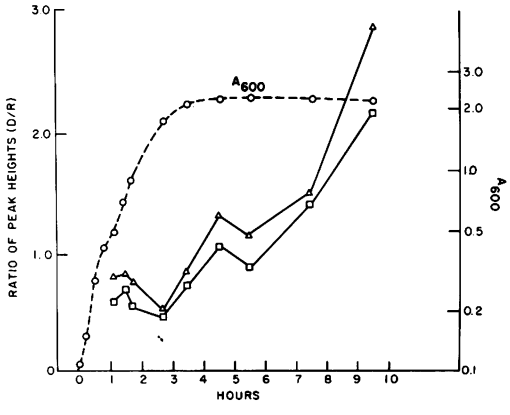

FIG. 5. *Change of the D band relative to the R band in the envelopes of strain 60015 during growth and development. Symbols: △ = gels stained with Amido black 10B and □ = gels stained with Coomassie blue.*

sponds to that of lysozyme, the band probably represents contaminating lysozyme used for cell lysis, as already suggested by Patterson and Lennarz (14). After electrophoresis, some portion of the sample was left on top of the gel. Such incomplete migration of membrane proteins (4) or of commercially available pure proteins (19) has been reported. Other workers have solubilized the accumulated proteins and separated them on a second gel; they found the same protein pattern as in the first gel (4, 19). Therefore, conclusions based on the protein patterns of urea-acetic acid gels can be considered valid.

The possible significance of the D protein for sporulation was investigated by using several sporulation mutants of *B. subtilis* blocked at a very early stage of sporulation. The same gradual increase of the D/R band ratio was observed for the mutants, indicating that the D band increase is independent of sporulation.

Change in envelope proteins during growth inhibition. The effect of acetate on envelope proteins was determined by growing strain 60015 in NSMP to A_{600} = 0.5, adding 200 mM acetate, harvesting cells at 30-min intervals, and preparing their envelopes. Gel electrophoresis of the envelope proteins in the urea-acetic acid system showed an increase in a particular band, called A band (named for the effect of acetate which was observed first) (Fig. 7). The peak height of the A band relative to that of the reference (R) band increased gradually while growth was inhibited by acetate and later decreased again when growth resumed (Fig. 8).

The fact that the A band contained protein was established by adding, 10 min after the transfer to 200 mM acetate, L-[^{14}C]valine and harvesting the cells after 3 hr of incubation. Examination of the envelope fractions in urea-acetic acid gels showed the incorporation of radioactivity into the A band (Fig. 9).

When chloramphenicol (100 μg/ml) or actinomycin D (2 μg/ml) were added at the same time as acetate, all growth was stopped and the A_{600} of the cultures declined slowly. The envelope fractions isolated 3 hr later contained only the normal amount of A protein.

Other fatty acids had a similar effect as acetate. Since 200 mM acetate produced the maximal increase of the A band during 2 to 4 hr of incubation and this level of acetate inhibited 70 to 80% of ATP production, similar conditions were employed for the other fatty acids

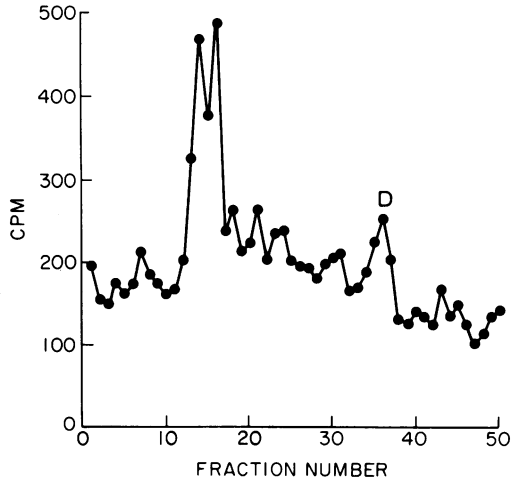

FIG. 6. *Radioactivity profiles of envelope proteins of cells grown in NSMP to T_4 and labeled with* L-[^{3}H]*lysine (5 μCi/ml). NSMP already contains 0.25 mM lysine which is not used up during growth and sporulation (20). Envelopes containing 50 μg of proteins were applied to urea-acetic acid gels, and after electrophoresis the radioactivity of 1-mm gel slices was determined.*

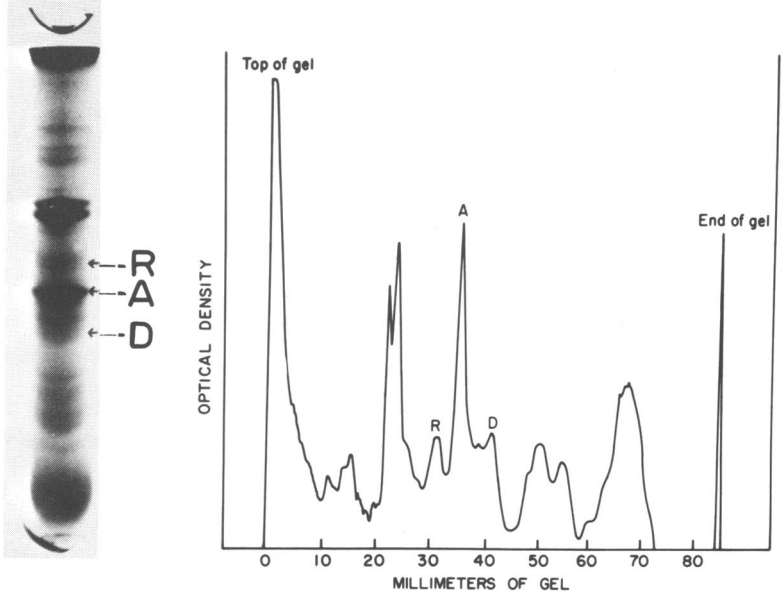

FIG. 7. *Acrylamide gel profile of envelope proteins from cultures incubated with 200 mM acetate in NSMP for 3 hr. Envelopes containing 100 μg of proteins were applied to urea-acetic acid gels. The gels were exposed to electrophoresis and stained with Coomassie blue. Left figure: stained gel. Right figure: microdensitometer tracing of the gel.*

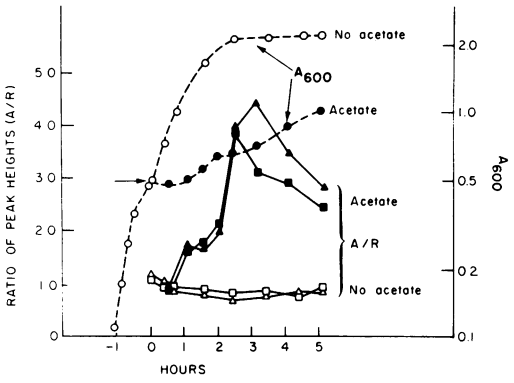

FIG. 8. *Changes of A protein in the envelopes of strain 60015 during 5 hr of incubation with 200 mM acetate in NSMP. Envelopes containing 50 μg of proteins were applied to urea-acetic acid gels. The gels were exposed to electrophoresis, stained with Coomassie blue (△, ▲) or Amido black 10B (□, ■), and traced with a microdensitometer.*

and DNP. These compounds were added at concentrations sufficient to inhibit 70 to 80% of ATP production (*see* Fig. 3), and envelopes were isolated after 2 to 4 hr of incubation. The A band produced during growth inhibition by the different compounds is shown in Fig. 10.

Localization of the A protein on protoplast membranes. To ascertain that the A band material found in cell envelope fractions was actually located on the membranes, protoplast membranes of strain 60015, grown with and without 200 mM acetate, were prepared as described above. In the urea-acetic acid gel electrophoresis system, the band pattern of the membranes was the same as that of the envelope preparation for all major bands, with only slight variations for the minor bands. In the membranes of cells grown with acetate, the prominent A protein band was present.

Electrophoresis of freshly prepared or lyophilized membrane preparations showed the same A protein band in both cases, indicating that it is not the product of proteolysis. Removal of lipid from the membrane by a chloroform-methanol (2:1) mixture did not alter the protein band profiles in gel electrophoresis.

Senior and MacLennan (19) established in urea-acetic acid gels a linear relationship between the mobility of proteins and the logarithm of the molecular weights, with occasional exceptions arising from protein aggregation. Several commercially available proteins were run in our system and their migrations were found to be in good agreement with their respective molecular weights. By comparison, the molecular weight of the A protein was estimated to be about 38,000.

DISCUSSION

The concentration of fatty acid causing maximal growth inhibition decreased with increasing chain length such that long-chain fatty acids were as effective as DNP. When the molecules contained additional hydrophilic groups, no inhibition was observed, as shown for lactate, succinate, or malate.

Since the growth inhibition in NSMP could be reduced by glycolytic compounds, especially glucose and fructose, but only slightly or not at all by citric acid cycle intermediates or amino acids, the fatty acids seem to interfere with the uptake or oxidation of compounds that reduce the electron transport system but not (or less) with sugar uptake or ATP production via glycolysis. The growth inhibition by

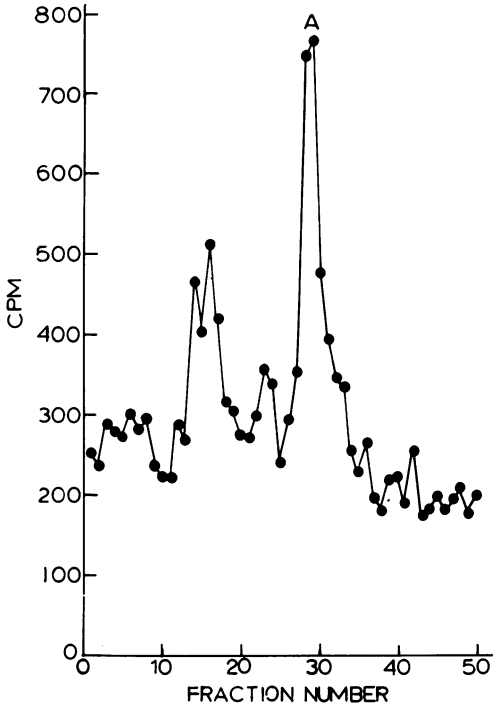

FIG. 9. *Radioactivity profile of envelope proteins labeled with L-[^{14}C]valine. Acetate (200 mM) was added to the cultures at A_{600} = 0.5 in NSMP. L-[^{14}C]valine (UL specific activity 260 mCi/mM) was added 10 min later to a final concentration of 0.5 μCi/ml, and incubation was continued for 3 hr. Envelopes containing 50 μg of protein were applied to urea-acetic acid gels, and after electrophoresis the radioactivity of gel slices was determined.*

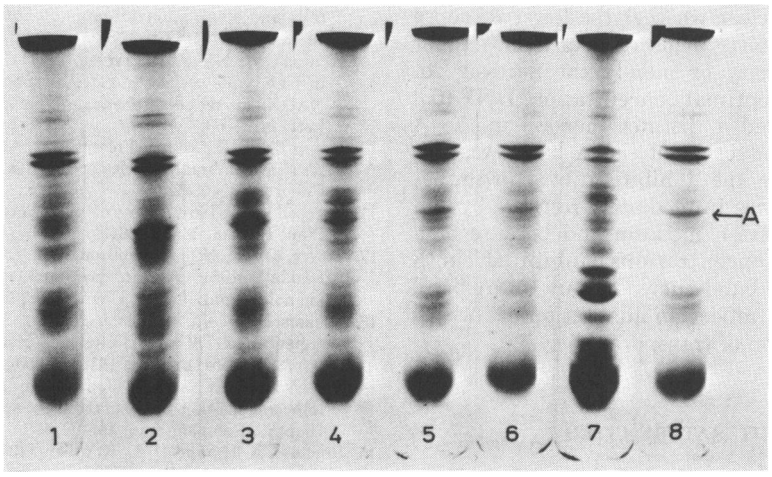

FIG. 10. *Acrylamide gel profiles of envelope proteins from cultures incubated with fatty acids or 2,4-dinitrophenol in NSMP medium for 2 to 4 hr. Envelopes containing 50 μg of proteins were applied to urea-acetic acid gels, and after electrophoresis the gels were stained with Coomassie blue. The following media were used: (1) no addition; (2) 200 mM acetate; (3) 100 mM n-butyrate; (4) 25 mM n-hexanoate; (5) 3 mM n-octanoate; (6) 0.8 mM n-decanoate; (7) 0.3 mM linoleic acid; (8) 0.3 mM DNP.*

the fatty acids was less pronounced when the cells were grown in synthetic (N) media, with malate or glucose as sole carbon source, in which the cells grew even without inhibitor only at a low rate; i.e., rapidly growing cells (in NSMP) were inhibited more effectively than slowly growing cells (in N). Presumably, the intracellular concentration of some compound, whose production or uptake was inhibited, became more easily growth rate-limiting in the rich (NSMP) than in the synthetic (N) medium. The intracellular concentration of neither tryptophan nor methionine, both of which are required for the growth of the standard strain, became growth-limiting in any of the above media because the same extent of inhibition was observed for a prototroph strain (60009).

Fatty acids also reduced the rate of oxygen consumption and the amount of ATP per cell. But in cell envelope preparations they did not inhibit the NADH oxidation which is inhibited by KCN or 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide, typical inhibitors of the electron transport system (10). This shows that fatty acids inhibited either the uptake (transport) or the metabolic production of reducing compounds, which could be oxidized by the still normally functioning electron transport system.

Other authors have found that high concentrations of acetate inhibit the uptake of sugars in *Aspergillus* (15) and that short-chain fatty acids (2, 17) as well as DNP (2, 11, 13) inhibit the uptake of phosphate by yeast.

These results as well as our studies suggest that fatty acids reversibly react with the cell membrane or proteins in it. They may either alter the membrane structure (e.g., reduce its fluidity) or uncouple the connection between the electron transport chain and two types of proteins, those required for ATP regeneration and other (carrier) proteins required for the transport of various compounds into the cell. (For example, transport of amino acids apparently is coupled to the electron transport chain either directly or through cation flux, without the involvement of ATP [10].) Since the affinity of the fatty acids to the membrane should increase with increasing lipophilic portion of the molecules, it is not surprising that the concentration of fatty acids, required to inhibit growth, oxygen consumption, or ATP synthesis to a certain degree, decreases with increasing chain length. Long-chain fatty acids are known to uncouple oxidative phosphorylation in mitochondria (1).

While growth in NSMP was partially inhibited, a particular envelope protein (A band) increased relative to other membrane proteins. It reached up to four times the vegetative level after (2 to 4 hr) exposure to 200 mM acetate. Generally, C_1-C_4 *n*-fatty acids appeared to be more effective in producing a strong A band than longer fatty acids. But the A band increase depended critically on the fatty acids concentration; too high fatty acid concentrations may inhibit protein synthesis too much to allow an A protein increase. It is,

therefore, not clear whether the lower response to long-chain fatty acids reflects their smaller inducing capacity or merely the necessity of employing an optimal concentration. DNP (0.3 mM) also caused a distinct increase in the A band. The production of the A band was not responsible for the inhibition of growth because growth was immediately restored by cell suspension in fresh medium and because high fatty acid concentrations inhibited both growth and A band increase. But the A band increase might reflect an adaptation of the cell to the inhibition of transport.

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