THE UPTAKE OF ACETATE

By

Escherichia coli w

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SUMMARY: The uptake of acetate by intact cells of Escherichia coli w has been studied. Acetate uptake is inhibited competitively by propionate, but not butyrate. The uptake process exhibits biphasic saturation kinetics. The Km for the first phase is 6.0 μM and the Km for the second phase is 0.74 mM. These results suggest two separate processes are involved in acetate uptake. No free acetate was detected in the cells. The relationship of the processes measured by acetate uptake studies to acetokinase activity is discussed.

The transport of nutrients into cells has been the subject of considerable study for many years, and in a large number of cases it has been found that the uptake of these nutrients is not a result of simple diffusion, but due to specific transport systems. Much of this work has been carried out using bacteria and other microorganisms. This recently has been the subject of a number of reviews (1,2). The types of compounds which have been shown to utilize specific transport systems include amino acids, sugars and inorganic ions. More recently, several studies have appeared which have been concerned with the uptake of organic acids (3,4). In contrast with the aforementioned, the transport of fatty acids by microorganisms has received very little attention and the question of whether these compounds are also transported by specific permeation systems is therefore of considerable interest. As a model for fatty acid transport we have chosen the uptake of acetate by Escherichia coli. The work presen-

ted in this report describes some of the general characteristics of acetate uptake by E. coli w $13a^{(-)}$.

MATERIALS AND METHODS

Growth of the Organism: E. coli w $13a^{(-)}$ was used throughout these studies (obtained from Dr. H. Wiesmeyer). Liquid cultures were grown in basal medium (5) containing either glucose or acetate at a concentration of 0.5%. The cells were grown at 37° with vigorous aeration. Growth was measured as absorbance at 540 nm. An absorbance of 0.8 was equivalent to 380 µg dry weight per ml.

Chemicals: I^{14}-C-sodium acetate was obtained from the New England Nuclear Corp. All other chemicals were obtained from commercial sources and were the highest quality available.

Uptake of Acetate: Cells were harvested during the logarithmic growth phase (absorbance 0.6-0.85), washed once with the basal medium and then resuspended in the basal medium to an absorbance of 1.6 (equivalent to 760 µg dry wt/ml). Incubations were routinely carried out in a solution which consisted of 0.4 ml of the basal growth medium (minus substrate); 10 µmoles of glucose (unless specifically omitted) radioactive acetate and 0.2 ml of the suspension of cells in a total volume of 1.0 ml to give a final concentration equivalent to 157 µg dry wt/ml. Where larger volumes were required, multiples of this formula were used. The mixture was incubated in a small beaker and stirred rapidly with a magnetic stirrer at 25°C. The cells were routinely incubated for 5 minutes before addition of ^{14}C-acetate (2µCi per µmole).

Samples were removed at appropriate intervals and diluted into 40 volumes of ice-cold basal growth medium. This was mixed and rapidly filtered through a Millipore filter (0.45 µ pore size). The filter was washed twice with 5 ml of ice-cold basal medium. The filter was air-dried and the radioactivity counted by placing it in a scintillation vial containing 10 ml of Bray's (6) scintillation fluid.

The zero time samples were obtained by adding the radioactive acetate to the incubation mixture after it had been diluted into 40 volumes of ice-cold

basal medium. Correction for adsorbed acetate was made by subtracting the zero time values from the incubated samples.

Measurement of Acetate Pool: The procedure used by Kay and Kornberg for this measurement of the dicarboxylic acid pool in E. coli (4) was adapted for the measurement of the acetate pool. After extraction of the cellular contents as described in (4) carrier acetate was added and the volatile fatty acids were collected and chromatographed on a celite column (7). The radioactivity in the acetate peak was measured in the scintillation counter.

RESULTS

Rate of Acetate Uptake: The rate of uptake of I-^{14}C-acetate by washed cells of E. coli is shown in Fig. 1. It can be seen that the rate shown by acetate-grown cells was greater than that shown by glucose-grown cells. The uptake of acetate by acetate-grown cells was linear for the first 20 minutes. When glu-

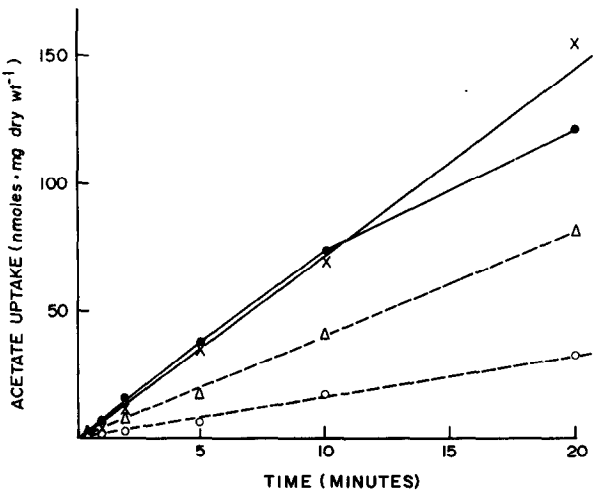
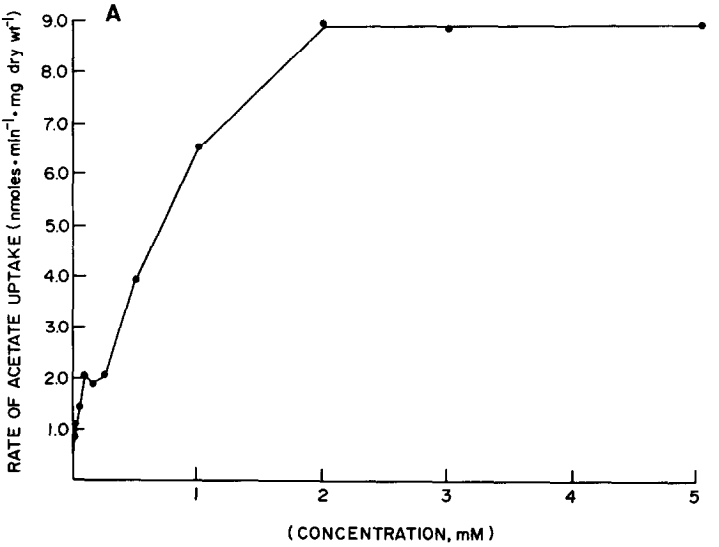


Figure 1. Time course of acetate incorporation by washed cells of E. coli. The uptake of ^{14}C-acetate was measured in glucose-grown (----) and acetate-grown (——) cells as described under Materials and Methods. The incorporation was measured in the presence and absence of 10^{-2} M glucose. ●——● indicates acetate-grown cells incubated in the presence of glucose. X——X indicates acetate-grown cells incubated in the absence of glucose. O----O indicates glucose-grown cells incubated in the presence of glucose. Δ----Δ indicates glucose-grown cells incubated in the absence of glucose. The concentration of acetate was 0.5 mM (2μCi per μmole).

cose was included in the uptake system there was no significant increase in the rate of acetate uptake; however, the rate was linear for only 10 minutes and then decreased. It is possible that during extended incubation in the presence of both glucose and acetate, acetate-grown cells may begin to adapt to glucose utilization. It is known that E. coli excretes acetate during growth on glucose (8) and this decreased rate of acetate uptake in the presence of glucose may be a result of dilution of the radioactive acetate by acetate derived from glucose after adaptation to glucose has taken place. This is supported by the observations made using glucose-grown cells. These cells presumably have the capacity to excrete acetate from the very beginning of the experiment. It may be for this reason that the rate of uptake of radioactive acetate by glucose-grown cells was less in the presence of added glucose than in its absence. An alternative explanation is that the intracellular acetate concentration is probably higher in glucose-grown cells (which leads to the acetate excretion), and it is this intracellular acetate which directly or indirectly inhibits the uptake of extracellular acetate.

Saturation and Specificity of Sites Involved in Acetate Uptake: When the initial rate of acetate uptake was measured at varying substrate concentrations complex kinetics were observed. A 1000-fold range of substrate concentration was used and the data are presented in the conventional manner in Fig. 2A. Fig. 2B depicts an Eadie-Hofstee plot of the same data. Included in Fig. 2B are the results obtained when either propionate or butyrate was included during the uptake experiments. In the absence of propionate or butyrate, the saturation curve (Fig. 2A) for acetate uptake shows two distinct regions which are shown more clearly in Fig. 2B. The sharp break in the curve indicates at least two separate processes are involved in acetate uptake. The first process is active under very low concentrations of acetate and has an apparent Km of about 6.0 μM. As the concentration of acetate is increased the dip in the curve of Fig. 2A (which is quite reproducible) may represent a region which is subject to substrate inhibition. Upon further increases in acetate concentration



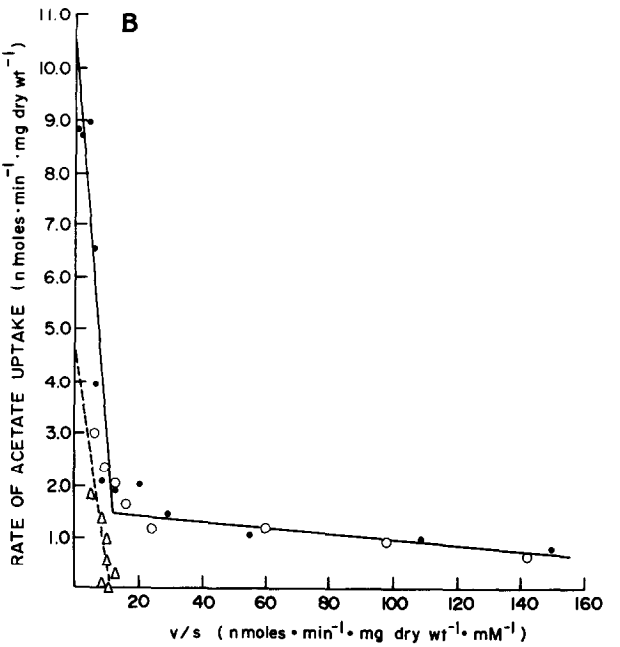


Figure 2A. The effect of acetate concentration upon the rate of acetate uptake. Uptake by acetate-grown cells was measured in the presence of added glucose as described under Materials and Methods. Acetate uptake was measured during the initial one-minute period of incubation.

Figure 2B. Eadie-Hofstee plot relating the rate of acetate uptake to the acetate concentration in the absence (●) of other acids and in the presence of 5.0 mM propionate (Δ) or 10.0 mM butyrate (O). All other details are the same as Fig. 2A.

another saturation process was seen. This has an apparent Km of about 0.74 mM.

The data presented in Fig. 2B also shows that the uptake of acetate was inhibited in the presence of propionate. No inhibition was seen in the presence of butyrate. The inhibition exhibited by propionate was competitive with acetate in that it was reversed in the presence of high acetate concentrations, but it is difficult to determine from the present data whether only the uptake process active at low acetate concentrations is inhibited by propionate or whether the process with a Km of 0.74 mM is inhibited by propionate as well.

Evidence for Absence of an Acetate Pool: The size of the free acetate pool was determined by permitting cells to take up radioactive acetate at a concentration of 5 mM for 5, 10, and 20 min., as described under Materials and Methods. There was no detectable amount of free acetate present under these conditions, after correction was made for the radioactive acetate associated with a zero time control to correct for adsorbed material.

DISCUSSION

The data presented above suggest that a specific system exists for the uptake of acetate by E. coli w. This system shows saturation kinetics, is inhibited competitively by propionate and is more active in acetate-grown than in glucose-grown cells. Our data also indicate that acetate transport does not involve simple "active transport" in that there is no detectable free acetate within the cell.

Klein et al. (9) have investigated the uptake of long chain and medium chain length fatty acids in E. coli K_{12}. They showed that the specificity of fatty acid uptake was correlated with the specificity of fatty acid oxidation and suggested that the acylation of fatty acids is an integral part of the transport process. They proposed a term, "vectorial acylation" to describe the uptake process, but left open the question of whether another protein was involved in the transport of acetate across the cell membrane before acylation occurred.

Salanitro and Wegener (10,11) have presented evidence derived from studies

carried out with mutants of E. coli K_{12} which indicates that there are separate uptake systems for short chain (C_4 and C_5), medium chain (C_6 to C_{11}) and possibly for long chain (C_{12} to C_{18}) fatty acids. The uptake of acetate and propionate was not carried out by the short chain fatty acid uptake system. Our studies (Fig. 2B) on the effect of propionate and butyrate upon the uptake of acetate also indicate that acetate and propionate, but not butyrate, share a common uptake system.

The data obtained from substrate saturation kinetics (Fig. 2; A and B) clearly have a biphasic character. Similar biphasic substrate saturation curves have been observed in studies on the transport of amino acids in Salmonella typhinurium by Ames (12), and in Mycobacterium smegmatis by Yabu (13). In each of these cases the results were attributed to the operation of two separate unrelated processes involved in the uptake of the particular amino acids; one having a higher affinity than the other for the amino acid. It seems that in the present case the results obtained for the uptake of acetate are also best explained in terms of two separate uptake systems; one having a Km of 6.0 μM and another having a Km of 0.74 mM. Since no free acetate is found within the cell it is possible that acetate uptake is essentially similar to the uptake of medium and long chain fatty acids involving a "vectorial acylation" process. The enzyme usually considered to be responsible for acetate activation is acetokinase. However, the Km reported for acetokinase from E. coli (14) is 0.3 M. This value is several orders of magnitude higher than the values obtained for either of the processes associated with acetate uptake. It is difficult to understand, therefore, how acetokinase can be responsible for the initial step in the acylation of acetate. During their studies on the growth of E. coli on acetate, Kornberg, Phizackerly, and Sadler (15) considered only the role of the enzymes of the glyoxalate cycle. The enzymes thought to be responsible for the formation of acetyl CoA (i.e., acetokinase and phosphotransacetylase) were not investigated at that time. The resolution of this discrepancy is currently under investigation.

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