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Regulation of Biotin Transport in *Saccharomyces cerevisiae*[1]

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The metabolic control of biotin transport in *Saccharomyces cerevisiae* was investigated. Nonproliferating cells harvested from cultures grown in excess biotin (25 ng/ml) took up small amounts of biotin, whereas cells grown in biotin-sufficient medium (0.25 ng/ml) accumulated large amounts of the vitamin. Transport was inhibited maximally in cells grown in medium containing 9 ng (or more) of biotin per ml. When avidin was added to biotin-excess cultures, the cells developed the ability to take up large amounts of biotin. Boiled avidin was without effect, as was treatment of cells with avidin in buffer. Avidin did not relieve transport inhibition when added to biotin-excess cultures treated with cycloheximide, suggesting that protein synthesis was required for cells to develop the capacity to take up biotin after removal of extracellular vitamin by avidin. Cycloheximide did not inhibit the activity of the preformed transport system in biotin-sufficient cells. The presence of high intracellular free biotin pools did not inhibit the activity of the transport system. The characteristics of transport in biotin-excess cells (absence of temperature or *p*H dependence, no stimulation by glucose, absence of iodoacetate inhibition, independence of uptake on cell concentration, and nonsaturation kinetics) indicated that biotin entered these cells by diffusion. The results suggest that the synthesis of the biotin transport system in *S. cerevisiae* may be repressed during growth in medium containing high concentrations of biotin.

The uptake of many substances by microorganisms is mediated by specific permeation systems (18). Since the transport of a compound across the cell membrane may be considered the first step in its metabolism, it would not seem unreasonable to expect that transport processes would be subject to regulatory control mechanisms. Reports have appeared demonstrating that various permeation systems may be regulated by induction (4, 14, 17, 18, 20, 23, 29), feedback inhibition (8, 13, 17, 18, 33, 34), catabolite repression (4–6, 10, 24), and repression (7, 11, 16, 31). Other mechanisms of control, such as rapid turnover of transport components (32), have also been reported.

In the preceding paper (28), it was established that biotin uptake in *Saccharomyces cerevisiae* occurs by active transport. It was of interest to determine whether there is some mechanism for controlling the levels of intracellular biotin in the yeast. Since this strain of *S. cerevisiae* is auxotrophic for biotin, it was expected that if such a mechanism does exist it would operate at the level of transport. The purpose of this investigation, therefore, was to determine whether vitamin transport in *S. cerevisiae* is subject to some type of regulatory control.

[1] Taken from a dissertation submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree in Microbiology.

MATERIALS AND METHODS

The methods and organism used were described in the preceding paper (28), except that the biotin concentration of the growth medium was varied.

Avidin (12 units/mg) was purchased from Worthington Biochemical Corp., Freehold, N.J. One unit of avidin was defined as that amount of protein which binds 1 μg of *d*-biotin. Avidin solutions were sterilized by filtration. Cycloheximide was purchased from Calbiochem, Los Angeles, Calif.

Biotin uptake was measured at 30 C as described previously (28). Unless indicated otherwise, the composition of the uptake reaction mixture was 0.5 or 1 mg of dry cells/ml, 0.05 M potassium phosphate (*p*H 4), 1% glucose, and *d*-biotin.

RESULTS

During the course of an investigation of biotin uptake in *S. cerevisiae*, it became evident that the biotin transport system is subject to some sort of metabolic control mechanism (T. O. Rogers and H. C. Lichstein, Bacteriol. Proc.,

p. 106, 1966). Certain results indicated that biotin controls its own uptake by influencing the system involved in its transport into the yeast cell. The following experiments were undertaken to investigate the control of biotin transport in *S. cerevisiae*.

Influence of biotin in the growth medium on the cellular biotin content of S. cerevisiae. The first evidence suggesting that the biotin transport system might be subject to metabolic control came from a simple experiment. The intracellular free- and bound-biotin content of yeast cells was determined after 24 hr of growth in media containing increasing amounts of biotin. Cellular biotin was plotted against the $\log_{10}$ of the biotin concentration of the growth medium (Fig. 1). Except for the lowest biotin concentration, growth was essentially the same at each vitamin concentration. As has been observed by other workers (1, 2, 21, 22), cellular biotin became saturated with increasing amounts of biotin in the growth medium. The bound-biotin content of the cells did not increase until the concentration of biotin in the medium exceeded that needed for maximal growth. Free-biotin levels began to increase when bound biotin was nearing saturation. At saturation levels (>50 ng of biotin per ml of medium), the cellular free-biotin level was very small in relation to the biotin concentration of the medium. At these high biotin concentrations, only a small percentage of extracellular vitamin was taken into the cells.

Biotin uptake by nonproliferating cells grown in sufficient biotin and excess biotin. Biotin-sufficient cells (28) were compared to biotin-excess cells for their ability to take up the vitamin (Fig. 2). Biotin-excess cells took up very little biotin, whereas biotin-sufficient cells took up large amounts of the vitamin. Since cellular biotin binding sites became saturated during growth in excess biotin, no increase in bound biotin was observed during incubation in the reaction mixture. These cells contained a small free-biotin pool (ca. 2 ng/mg of dry cells), and the small amount of biotin taken up resulted from a slight increase in free biotin above the 0 time or endogenous level. When the yeast-*Lactobacillus* differential assay was employed to measure vitamin uptake, essentially 100% recoveries of added biotin were obtained. Thus, biotin-excess cells were not degrading biotin or converting it to biotin vitamers. From these results, it is clear that when yeast cells are grown in high levels of biotin their ability to take up the vitamin is severely impaired.

Effect of biotin concentration in the growth medium on biotin transport. It was of interest to

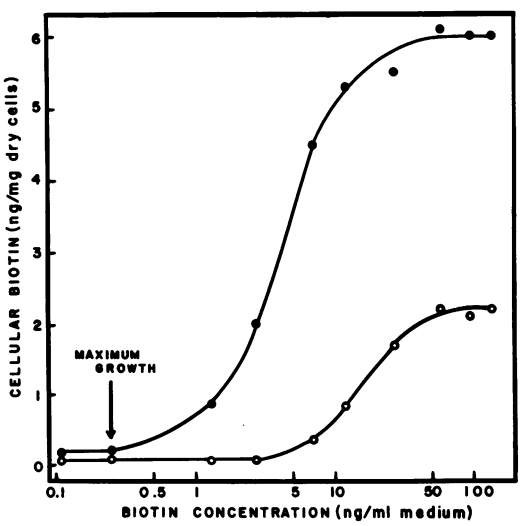

FIG. 1. *Intracellular free- and bound-biotin content of S. cerevisiae after 24 hr of growth in Hertz medium containing different concentrations of d-biotin. Free biotin (○) and bound biotin (●) were determined by microbiological assay with S. cerevisiae. The data are plotted as the intracellular biotin concentration versus the $\log_{10}$ of the biotin concentration of the medium.*

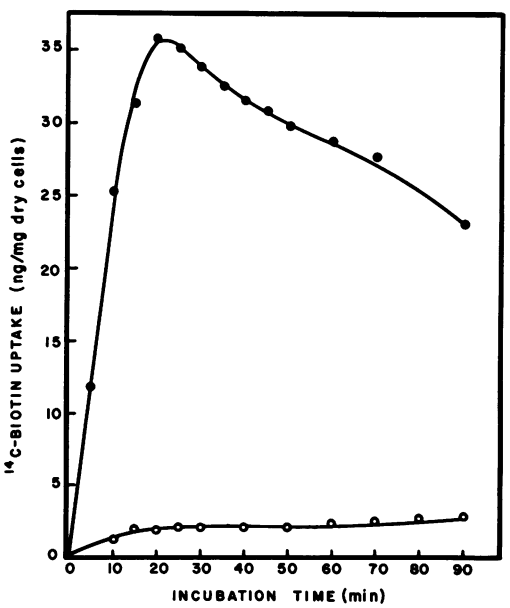

FIG. 2. *^{14}C-biotin uptake by nonproliferating cells after growth in biotin-sufficient (0.25 ng/ml) or biotin-excess (25 ng/ml) media. Cells equivalent to 0.5 mg (dry weight)/ml were incubated in reaction mixtures containing 50 ng of ^{14}C-biotin (57.5 mc/mmole) per ml. The uptake of radioactive biotin was determined at the indicated times. Symbols: ●, cells grown in sufficient biotin; ○, cells grown in excess biotin.*

determine what concentration of biotin in the growth medium would inhibit the subsequent uptake of the vitamin by nonproliferating cells. Free-biotin transport was measured in cells incubated in reaction mixtures after growth in media containing increasing amounts of biotin (Fig. 3). When the concentration of biotin exceeded that needed for maximum growth (0.25 ng/ml), the ability of cells to take up biotin decreased as the concentration of the vitamin in the medium increased. At a concentration of 9 ng/ml, transport was inhibited maximally. These data support the view that biotin controls its own transport in some manner.

Parameters of biotin transport in nonproliferating cells grown in excess biotin. The inability of biotin-excess cells to take up the vitamin might reflect the need for optimal conditions different from those required by biotin-sufficient cells (28). However, we found that the inability of biotin-excess cells to take up biotin was not due to unfavorable conditions of temperature or *p*H. No clear-cut dependence of vitamin uptake on these parameters was observed. Glucose did not stimulate free-biotin uptake. Iodoacetate, at concentrations which inhibited transport in biotin-sufficient cells (28), had no effect on the small amount of free biotin taken up by biotin-excess cells. In contrast to results with biotin-sufficient cells (28), there was no linear relationship between cell mass and biotin uptake by biotin-excess cells. The amount of free biotin entering these cells was constant over the cell concentration range tested. These results suggest that biotin enters biotin-excess cells by a diffusion mechanism. The following kinetic data support this conclusion.

Kinetics of biotin transport in cells grown in excess biotin. Biotin uptake by cells grown in the presence of excess biotin was measured at increasing concentrations of the vitamin in the reaction mixture (Fig. 4). The rate of uptake increased in a linear fashion with increasing biotin concentration. Saturation kinetics were not observed over the concentration range that had been used to demonstrate that the rate of vitamin uptake in biotin-sufficient cells increased according to Michaelis-Menten kinetics (28). Similar results were obtained by using unlabeled biotin and microbiological assay to measure transport.

Biotin transport during growth in biotin-excess medium. Hertz medium containing biotin at a concentration of 25 ng/ml was inoculated with a heavy suspension of cells grown in the presence of excess biotin as described previously (28). Samples were removed during growth, and biotin uptake was determined by incubating washed cells in reaction mixtures. The amount of biotin remaining in the growth medium was also determined. Growing cells removed biotin from the medium, as reflected by a decrease in the amount

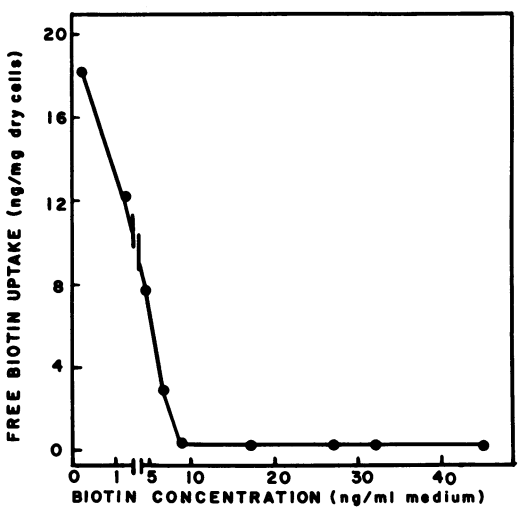

FIG. 3. *Effect of the biotin concentration in the growth medium on the uptake of free biotin by nonproliferating cells. After 18 hr of growth in Hertz medium containing the indicated biotin concentration, cells equivalent to 1 mg (dry weight)/ml were incubated for 30 min in reaction mixtures containing 25 ng of d-biotin per ml. Free biotin was determined by microbiological assay with S. cerevisiae.*

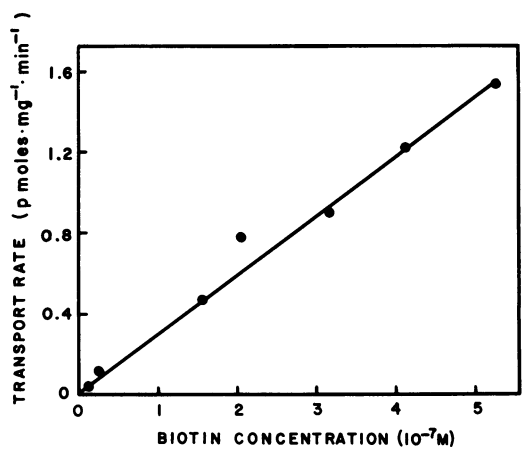

FIG. 4. *Nonsaturation kinetics of free-biotin uptake in nonproliferating cells grown in excess biotin. Yeast cells [equivalent to 0.5 mg (dry weight)/ml] grown in Hertz medium containing 25 ng of d-biotin per ml were incubated in reaction mixtures containing ^{14}C-biotin (57.5 mc/mmole) at the indicated concentrations. Radioactivity was determined after 7 min of incubation. Each experimental point represents the average of duplicate determinations.*

of extracellular biotin remaining in the medium (Fig. 5). Most of the biotin taken up during growth appeared intracellularly in the bound form; therefore, at any time during growth in excess biotin, the cells were bound biotin-saturated and contained a small free-biotin pool. Cells harvested from the biotin-excess medium did not possess the ability to take up significant amounts of biotin. When these cells were incubated in biotin reaction mixtures, there was little or no increase in the level of cellular free biotin. In contrast, the biotin transport system in cells growing in biotin-sufficient medium functioned throughout the growth cycle, although the amount of biotin taken up during the stationary phase was reduced (28). These results made it clear that the reduced capacity of biotin-excess cells to take up the vitamin is a stable characteristic not influenced by the age of the cells used to measure biotin uptake.

Effect of avidin on biotin transport. Avidin is a biotin-binding (9), basic glycoprotein having a molecular weight of 60,000 to 70,000 (12) which forms biologically inactive complexes with biotin (25), making the extracellular vitamin unavailable to cells. Pai and Lichstein (27) found that the synthesis of the biotin biosynthetic enzymes in *Escherichia coli* is derepressed when avidin is added to cultures growing in the presence of biotin. This observation prompted the design of an experiment to determine whether avidin would influence biotin transport when added to yeast cells growing in medium containing excess biotin. *S. cerevisiae* was grown in biotin-excess medium, and free biotin uptake was measured at various time intervals by incubating washed cells in biotin reaction mixtures. Avidin was added to the cultures at the mid-log phase of growth (11.5 hr). Upon the addition of avidin, the concentration of biotin in the medium decreased immediately to an undetectable level (Fig. 6). The presence of the biotin-binding protein did not affect the growth rate of the cells. It has been shown that avidin has as much affinity for enzyme-bound biotin as for the free vitamin (25); therefore, the absence of any growth inhibition in the presence of avidin indicated that the protein did not enter the cells. Since these cells were bound biotin-saturated, they did not require the presence of extracellular biotin for growth. Concurrent with the addition of avidin, the cells developed the capacity to take up large amounts of biotin against a concentration difference. The rate of biotin uptake decreased somewhat when the culture approached the stationary stage of growth. A similar decrease was observed earlier in cells growing in biotin-sufficient medium (28).

To characterize further the effect of avidin, biotin-excess cells were incubated in phosphate buffer with and without avidin. Samples were removed after various times of exposure to avidin, and biotin uptake was measured. It was found that incubation of nonproliferating biotin-excess

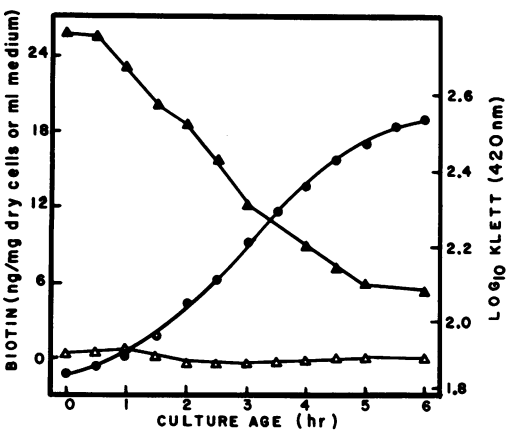

FIG. 5. *Free-biotin uptake during the growth cycle of S. cerevisiae in medium containing excess biotin (25 ng/ml). Growth conditions were as described. Growth (●) was followed turbidimetrically. At the indicated times, samples were removed and biotin uptake was measured by incubating washed cells [1 mg (dry weight)/ml) for 25 min in reaction mixtures containing 30 ng of d-biotin per ml. Free biotin (△) and biotin remaining in the medium (▲) were determined by microbiological assay with S. cerevisiae.*

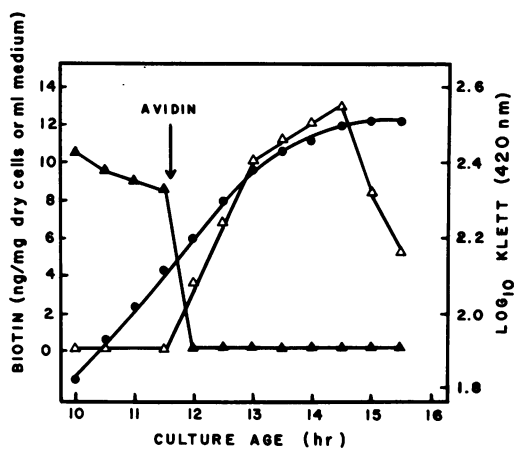

FIG. 6. *Effect of avidin on biotin uptake by cells growing in medium containing excess biotin. After 10 hr of incubation in Hertz medium containing 25 ng of d-biotin per ml, samples were removed and growth (●) and biotin uptake (△) were measured as in Fig. 5. The amount of biotin remaining in the growth medium (▲) was also determined. At 11.5 hr, avidin was added to a final concentration of 0.045 units/ml of medium.*

cells with avidin did not increase the capacity of the cells to take up biotin.

Avidin is a heat-labile protein (9). Pai and Lichstein (26) found that, when avidin solutions are boiled for 30 min, 97% of their biotin-binding activity is destroyed. The addition of boiled avidin to *S. cerevisiae* growing in medium containing excess biotin had no effect on the ability of the cells to take up the vitamin.

Effect of cycloheximide on biotin transport. It was of interest to determine whether avidin would influence biotin uptake by cells growing in biotin-excess medium if such cells were treated with an inhibitor of protein synthesis. The inhibitor of choice was cycloheximide, an antibiotic which has been shown to be an inhibitor of protein synthesis in yeast (19). The effect of cycloheximide on biotin uptake in yeast cells exposed to avidin is shown in Fig. 7. The organism was grown in medium containing excess biotin, and samples were removed at various times to determine biotin uptake and vitamin remaining in the medium. After 11.5 hr of incubation, cycloheximide was added to give a final concentration of 5 μg/ml. In the presence of the antibiotic, growth was inhibited. Thirty minutes later avidin was added. Upon the addition of avidin, the concentration of biotin in the medium decreased to 0; however, the cells did not develop the ability to take up biotin. This was in contrast to the effect of avidin when added to cells growing in biotin-excess medium in the absence of the antibiotic (Fig. 6). These results suggest that protein synthesis is required for biotin-excess cells to acquire the capacity to take up biotin after removal of excess extracellular vitamin by avidin.

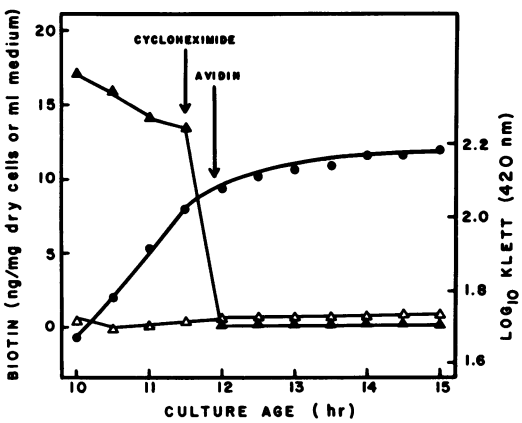

FIG. 7. *Effect of avidin on biotin uptake when added to biotin-excess cells in the presence of cycloheximide. Procedures and symbols as in Fig. 6, except that cycloheximide was added to the medium (5 μg/ml) at 11.5 hr. Thirty minutes later avidin was added.*

It is pertinent that cycloheximide did not inhibit the activity of the preformed transport system in biotin-sufficient cells (Fig. 8). The presence of the antibiotic, even in fivefold excess of that used previously, did not significantly influence biotin uptake when added to nonproliferating, biotin-sufficient cells. This result supports the idea that cycloheximide prevents the synthesis of the biotin transport system but has no effect on biotin uptake per se.

Effect of biotin transport of the addition of excess biotin to cells growing in biotin-sufficient medium. Biotin uptake was measured in cells growing in biotin-sufficient medium to which excess biotin was added (Table 1). Before the addition of biotin, cells harvested from the medium were capable of taking up considerable amounts of the vitamin. Upon the addition of biotin, the cellular, free-biotin content of growing cells increased 200-fold (0 time value for 3-hr sample). These cells were still capable of taking up normal amounts of biotin during incubation in the reaction mixture, even though they contained a large, free-biotin pool. During continued incubation, the ability to take up biotin decreased in growing cells as well as in nonproliferating cells

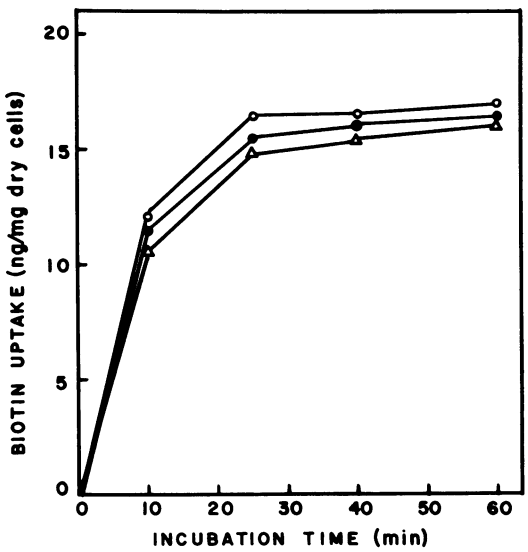

FIG. 8. *Effect of cycloheximide on free-biotin uptake by cells grown in sufficient biotin. After 18 hr of growth in Hertz medium containing 0.25 ng of d-biotin per ml, washed cells equivalent to 1 mg/ml were incubated in reaction mixtures containing 25 ng of d-biotin per ml. Free biotin was determined by microbiological assay with S. cerevisiae. Symbols: ○, no cycloheximide; ●, 5 μg of cycloheximide per ml; △, 25 μg of cycloheximide per ml.*

TABLE 1. *Effect on biotin transport of the addition of excess biotin to cells growing in biotin-sufficient medium*[a]

Culture age (hr)	Turbidity (Klett units)[c]	Free biotin (ng/mg of dry cells)		
		0 time	25 min	Uptake[b]
0	80	0.1	25.0	24.9
2.5	185	0.1	22.3	22.2
3	226	21.5	43.0	21.5
3.5	257	17.0	37.2	19.3
4.5	294	12.5	29.1	16.6
5.5	320	4.3	7.7	3.4

[a] Conditions as in Fig. 5, except that the initial concentration of biotin in the growth medium was 0.25 ng/ml and 10 min before the 3-hr sample biotin was added to the culture to a final concentration of 25 ng/ml of medium.

[b] Free biotin uptake was calculated by subtracting the 0-time value from the 25-min value.

[c] Turbidity attained by the culture at the stated age; 1 Klett unit = 0.0036 mg of dry cells/ml, with blue filter no. 42 (400 to 450 nm).

prepared from the culture. At 5.5 hr, the cells accumulated only small amounts of biotin. These results suggest that the activity of the transport system is not inhibited by high intracellular levels of free biotin and support the idea that the synthesis of the transport system may be inhibited in the presence of high concentrations of biotin.

DISCUSSION

A most interesting aspect of the study of biotin transport in *S. cerevisiae* is the discovery that the synthesis of the biotin transport system is apparently controlled by the level of vitamin in the growth medium. Oniy a few reports have appeared in the literature describing this type of regulation of transport (11, 16). In other studies, the synthesis of a transport system for a particular compound, namely sulfate (7) or acetylornithine (31), was repressed by the end product of the biosynthetic pathway in which the compound served as a precursor. The synthesis of several transport systems has been shown to be controlled by catabolite repression (4–6, 10, 24). No evidence has been published, as yet, to indicate that the synthesis of vitamin transport systems is controlled in any manner.

At any particular time during growth in medium containing excess biotin, yeast cells lacked the capacity to accumulate the vitamin against a definite concentration gradient. This observation, combined with the finding that glucose and iodoacetate were without effect on biotin uptake in these cells, rules out the existence of an active transport system for biotin in such cells. In this respect, biotin-excess cells were markedly different from cells grown in biotin-sufficient medium, which have the capacity to accumulate the vitamin by an active mechanism (28).

In several microorganisms, active transport systems can be selectively inhibited by conditions which still allow the uptake of solutes by facilitated diffusion (30, 33). It would not seem unreasonable, therefore, to expect that growth in excess biotin repressed the synthesis of, or inhibited the activity of, the biotin-active transport system; but the cells would still be capable of taking up the vitamin by facilitated diffusion. Since facilitated diffusion has been shown to be mediated by carrier systems displaying saturation kinetics (3), the demonstration that vitamin uptake in biotin-excess cells proceeded by a nonsaturable system argues against the presence of such a diffusion mechanism in these cells. The linear kinetics of biotin uptake resemble those of β-galactoside uptake in a cryptic mutant of *E. coli* (15). The possibility does exist that biotin-excess cells possess a carrier which is saturated only at extremely high vitamin concentrations.

The mechanism of control acting at the level of biotin permeation appears to involve a repression of the synthesis of some component(s) involved in biotin uptake, thus eliminating the capacity of the cells to transport biotin by a carrier mechanism. The addition of avidin to a culture growing in excess biotin caused a rapid increase in the capacity of the cells to accumulate the vitamin against a concentration gradient. This effect depended on the capacity of avidin to complex with extracellular biotin, thus making it unavailable to the cells. Other transport systems subject to repression control are synthesized when the concentration of the compound causing repression is decreased in one way or another (7, 11, 16, 31).

The development of an active transport system for biotin after exposure of *S. cerevisiae* to avidin depended upon cell growth and probably de novo protein synthesis. This was evident from the following observations. (i) The transport system of biotin-excess cells was not affected by incubation with avidin in buffer. (ii) The addition of cycloheximide, an inhibitor of protein synthesis in yeast (19), to cells growing in excess biotin before the addition of avidin prevented the formation of the biotin transport system, even though avidin had complexed with extracellular biotin. The second result is similar to the effect of chloramphenicol on the derepression of the potassium transport system in *E. coli* incubated in potassium-deficient medium (11).

Several transport systems are thought to be controlled by feedback inhibition (8, 13, 17, 18,

33, 34). The activity of the biotin transport system in *S. cerevisiae* did not appear to be controlled by a biotin-mediated feedback inhibition. When excess biotin was added to a culture growing in biotin-sufficient medium, there was an immediate 200-fold increase in the level of cellular free biotin. Cells harvested from the growth medium after the addition of biotin were still capable of taking up as much vitamin as cells harvested before biotin addition. Thus, the presence of high levels of free biotin did not appear to inhibit the activity of the transport system. However, the biotin uptake capacity of yeast cells was reduced during growth after the addition of excess biotin. These results suggest that the addition of biotin repressed the synthesis of the transport system. The transport system was then diluted out in the culture by growth, as evidenced by the decreased ability of the cells to take up the vitamin.

Chang and Peterson (2) found that many biotin-requiring yeasts become bound biotin-saturated during growth in high concentrations of the vitamin, but in none did they observe an accumulation of large, free-biotin pools. In an adenine-tryptophan auxotroph of *S. cerevisiae*, biotin transport is severely limited after growth in excess biotin (Becker, *personal communication*). The ability of nonproliferating cells of *L. plantarum* to accumulate free biotin is reduced greatly after growth in high levels of the vitamin (*unpublished data*). It is tempting to speculate that in many biotin-requiring organisms the level of cellular free biotin may be controlled by a mechanism similar to that observed in *S. cerevisiae* 139.

In view of our results, it may be profitable to inspect permeation systems for amino acids and sugars in cells grown in the presence of various concentrations of these substrates, especially excessive amounts. Perhaps the level of substrate present during growth influences the nature of the transport system(s) synthesized by the cells. Such a phenomenon would be quite significant physiologically and may also help to explain some of the complexities in the literature on permeation.

ACKNOWLEDGMENTS

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