FOLATE TRANSPORT IN *LACTOBACILLUS CASEI:* SOLUBILIZATION AND GENERAL PROPERTIES OF THE BINDING PROTEIN

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SUMMARY: Intact cells of *Lactobacillus casei* grown in a medium containing a low level (5 nM) of folate have the capacity to bind (at 4°) appreciable quantities (0.35 nmoles/10^{10} cells) of the vitamin. Folate binding is rapid, saturable (K_d = 36 nM), insensitive to sulfhydryl reagents, and has a broad pH optimum. A folate-binding protein has been solubilized in high yield by sonic disruption of lysozyme-treated cells in the presence of [^{3}H]folate and Triton X-100. The protein-folate-Triton complex (MW 230,000 by filtration through Sephadex G-150) is stable to dialysis but dissociates upon heating (50% loss of bound folate after 5 min at 49°). Evidence is presented to suggest that the binding protein functions as the carrier of folate during its transport into the cells.

Transport of folate compounds into *Lactobacillus casei* is concentrative, saturable, pH-dependent, and temperature-dependent (1-3). Intact cells utilize glucose as the energy source for folate transport, but cells rendered more permeable by treatment with lysozyme can also use several glycolytic intermediates for this purpose (4). The present report describes the general properties of a folate-binding protein, a component of the transport system that has been solubilized from lysozyme-treated cells.

The presence of this component in the cell envelope of *L. casei* was first detected by binding studies that were performed at 4° to suppress transport. Under these conditions, association of [^{3}H]folate with the cells was complete within 5 min and exhibited saturation kinetics (Fig. 1). A double-reciprocal plot of these data (inset, Fig. 1) was linear, indicating that a single component (0.35 nmoles/10^{10} cells; K_d = 36 nM) was responsible for the binding of folate. Inhibitors of folate transport, such as iodoacetate (10 mM), *p*-chloromercuriphenylsulfonate (1 mM), arsenate (10 mM) and hydroxylamine (10 mM), had no effect upon binding of the vitamin. The amount of bound

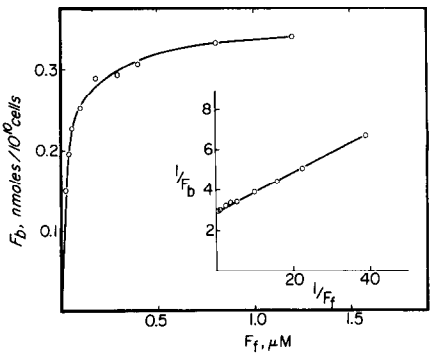

Fig. 1. Folate binding to intact cells of *L. casei*. Assay mixtures contained 0.8 ml of a cell suspension (10^9/ml in 0.05 M potassium phosphate, pH 6.8) and the indicated concentrations of [^{3}H]folate in a total volume of 1.0 ml. After standing for 5 min at 4°, samples were diluted to 10 ml with ice-cold buffer and centrifuged at 4,000 x *g* for 10 min to remove unbound folate. Cell pellets were suspended in 0.5 ml of water and their radioactivity determined in a liquid scintillation counter as described previously (2). Results are expressed as nmoles of folate per 10^{10} cells. F_b (nmoles/10^{10} cells) and F_f (μM) represent bound and free folate, respectively. Inset, plot of $1/F_b$ vs. $1/F_f$.

folate did not vary over the pH range of 4.0 to 7.2 (0.1 M Tris-phosphate buffer). At higher pH values, binding declined; a 50% reduction was observed at pH 8.4. The pH profile for folate transport was essentially the same, except that the latter process also decreased at pH values below 4.5 (2).

The folate-binding component was readily solubilized by sonic disruption of lysozyme-treated cells in the presence of excess [^{3}H]folate and Triton X-100 (Table I). The total amount of bound folate in the Triton extract (22.8 nmoles) was determined by Sephadex G-25 chromatography and represented 83% of the original folate-binding capacity of the cells. Lower yields of the binding component were obtained (Table I) when the standard procedure was varied as follows: (a) Intact cells were used in place of lysozyme-treated cells; (b) Triton X-100 was omitted; or (c) [^{3}H]Folate was added after sonication.

The specific activity of the solubilized binding component was 0.08 nmole of bound folate/mg protein*. The size of this component was estimated

* Evidence that this component is a single protein will be presented elsewhere.

by filtration of the Triton extract through a calibrated column of Sephadex G-150. Bound [^{3}H]folate appeared in the effluent as a single, symmetrical peak, and the apparent molecular weight of the complex was ca. 230,000. The actual size of the folate-binding protein is undoubtedly less than this value, since membrane proteins are known to bind considerable amounts of Triton X-100 (5).

Stability of the protein-[^{3}H]folate-Triton complex was examined under various conditions. There was no decrease in bound radioactivity when the preparation was: (a) stored for one week at -20° or 4°; (b) dialyzed exhaustively against 0.05 M potassium phosphate, pH 6.8; or (c) treated with a 50-fold excess of unlabeled folate. Repeated freezing and thawing, however, led to a breakdown of the complex. The preparation was also sensitive to elevated temperatures (Fig. 2); 50% reduction in the level of bound folate

TABLE I. Solubilization of the Folate-Binding Protein from *L. Casei*

Conditions	Bound folate in supernatant
	nmoles
Lysozyme-treated cells	22.8
Intact cells	5.8
Triton X-100 omitted	7.2
Folate added after sonication	0.6

L. casei cells, grown on a medium (2) that contained 5 nM folate, were treated with lysozyme, as described elsewhere (4). Under standard conditions, lysozyme-treated cells (10 g wet weight; total binding capacity of 27.2 nmoles of folate) were suspended in 30 ml of 0.05 M potassium phosphate, pH 6.8, that also contained 5 μM [^{3}H]folate (27,000 cpm/nmole) and 5% Triton X-100. The suspension was sonicated for 5 min at 4° and then centrifuged at 24,000 x *g* for 30 min to remove cell debris. To assay for the folate-binding protein, an aliquot (1.0 ml) of the supernatant was applied to a 1.8 x 30 cm column of Sephadex G-25 and was eluted with 0.05 M potassium phosphate, pH 6.8, containing 0.05% Triton X-100. Bound [^{3}H]folate, which appeared in the void volume, was measured in a liquid scintillation counter.

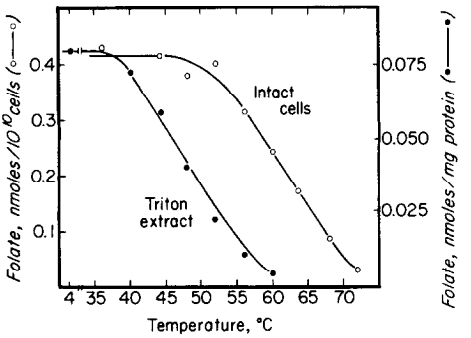

Fig. 2. Heat stability of the folate-binding protein in intact cells (o——o) and in Triton extracts (●——●). A. Intact cells. *L. casei* cells (pretreated with 10 mM iodoacetate to block folate transport) were suspended in 0.05 M potassium phosphate, pH 6.8, containing 5 μM [^{3}H]folate. Aliquots (1.0 ml) were heated for 5 min at the indicated temperatures and then transferred to an ice bath. Bound folate was determined by the centrifugation method described in Fig. 1. B. Triton extracts. Aliquots (1.0 ml) of the solubilized binding protein (prepared as described in the legend for Table I) were treated in the above manner; bound folate was then determined after separation from free folate with the use of Sephadex G-25 (see Table I).

resulted from exposure to 49° for 5 min. In contrast, a higher temperature (62°) was required for 50% destruction of the folate-binding component in intact cells. The folate-binding component thus appears to be destabilized when it is transferred from the cell membrane to an aqueous-detergent environment.

A relationship between the solubilized folate-binding protein and the folate transport system has been established by the following experiment. Cells were grown on various levels of folate and, after being harvested and washed thoroughly, were tested for their ability to transport the vitamin. As shown in Fig. 3, transport which was maximal in cells grown on 2 nM folate declined progressively at higher concentrations and was totally repressed at 1 μM folate. Nearly identical responses were obtained when these cells were examined for their ability to bind [^{3}H]folate at 4° or for the level of solubilized folate-binding protein.

The detergent-solubilized, folate-binding protein of *L. casei* is distinctly different from the folate binders of cow milk (6,7), hog kidney

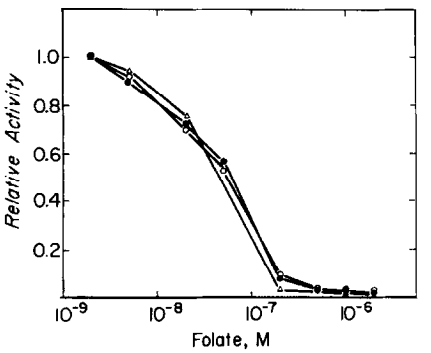

Fig. 3. Effect of folate concentration in the growth medium upon folate transport (●——●) and folate binding (○——○) by intact cells and upon folate-binding in Triton extracts (△——△). Folate transport was determined as described previously (2); folate binding by cells and by Triton extracts was measured by the procedures described in Fig. 1 and Table 1, respectively. Results in each case are expressed as activity relative to the binding or transport exhibited by cells grown in the presence of 2 nM folate.

(8) and human serum (9). These latter proteins are water-soluble, whereas the *L. casei* component is associated with the cell envelope and has properties similar to other bacterial binding proteins (e.g., the shock-resistant amino acid binders of *Escherichia coli* (10) which require detergent for solubilization). The biochemical role of the water-soluble binders is still not clear (11), but they do not appear to be involved in the transport of folate into cells. The *L. casei* binding protein, however, appears to be a functional component of the folate transport system, as established by the following lines of evidence presented in this paper and elsewhere (2,4): (a) The folate-binding component is located in the cell envelope and requires detergent for solubilization; (b) Folate transport and folate binding have a similar pH profile; (c) A parallel repression of folate transport by cells, folate binding by cells, and folate-binding activity in Triton extracts is observed as the level of folate in the cellular growth medium increases (Fig. 3), and (d) A folate transport mutant has been isolated in which both the transport and binding of folate to cells are stimulated to the same extent (2- to 3-fold) by the addition of thiols (4).

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