albumin when the pH drops to slightly below 3, and the charge certainly should exceed +50 at pH 2 where the protein has acquired its full complement of 96 protons[6]. Nevertheless, $[\alpha]_D$ in 0.005 M detergent solution never reaches even $-77°$, the rotation for albumin in water at pH 3, let alone approach $-87°$ as observed for the protein in water at pH 2. If the magnitude of $[\alpha]_D$ were merely a reflection of the net cationic charge (and any consequent expansion) of the albumin molecule, one would expect substantially more negative values at pH's near 2 in dodecyl sulfate solutions than are actually observed (Fig. 1). It seems possible, therefore, that levorotation in acid solutions of this protein is sensitive to the presence of some cationic residues in the charged state and that their influence may be removed when they are complexed with anions.

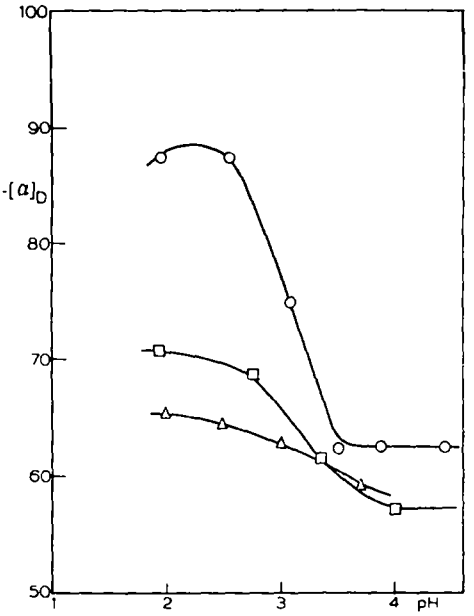

Fig. 1. Optical rotations of bovine serum albumin ($1.45 \cdot 10^{-4} M$) in aqueous solutions at various pH's: ○, only added HCl; △, in presence of $2.2 \cdot 10^{-3} M$ sodium dodecyl sulfate plus added acid; □, in presence of $4.5 \cdot 10^{-3} M$ sodium dodecyl sulfate plus added acid.

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The site of galactoside-permease activity in *Escherichia coli*

It has been shown that the bacterium *Escherichia coli* can accumulate galactosides intracellularly[1,2,3]. Under certain conditions the cellular concentration of a galactoside may be several hundred times that in the medium. In the case of thiomethyl-β-D-galactoside (TMG), a substance not metabolized by *E. coli*, the intracellular accumulation may attain 3–5% of the bacterial dry weight. The system responsible for this intracellular accumulation of galactosides has all the characteristics of an inducible enzyme and has been termed galactoside-permease.

The location of the galactoside-permease in *E. coli* is not known. However, the very nature of its function, that of transport, suggests that it may be associated with either the cell wall or the plasma membrane of the organism, although an intra-cytoplasmic site cannot be excluded.

Techniques have been developed for the production of viable protoplasts by removal of the bacterial cell wall under conditions that conserve the osmotic integrity of the cell (*e.g.*[4,5,6]). It is possible then to determine whether the galactoside-permease is associated with the cell wall or the protoplast. In this communication, it is demonstrated that the permease is associated with the protoplast.

E. coli, strain W-2244, the Lac_i^- mutant of K-12[7], was employed in these experiments. This strain cannot form the enzyme β-galactosidase and consequently does not metabolize lactose. However, cultivation of W-2244 in the presence of a galactoside will induce formation of the galactoside-permease. It is therefore possible to measure lactose accumulation in this organism.

Cultures were grown on the mineral medium "56" as previously described[3] with 0.4% glycerol as carbon source. TMG at a concentration of $5 \cdot 10^{-4} M$ served as permease inducer. Protoplasts were prepared by the method of REPASKE[6], modified as follows: bacteria in the exponential phase of growth were harvested by sedimentation and washed twice with distilled water. They were

then resuspended at a density of $5 \cdot 10^8$ to $3 \cdot 10^9$ bacteria per ml in 0.05 M tris(hydroxymethyl)-aminomethane, pH 8, containing 17 g sucrose (0.5 M final concentration), 108 mg versene and 68 mg lysozyme (Armour's crystalline product) per 100 ml. This suspension was agitated at room temperature for 12 min, sedimented, and washed twice with growth medium containing 0.5 M sucrose. Clumps consisting of lysing protoplasts and debris were removed by centrifugation at low speed for 2 min. Microscopic examination (stained preparation) of the supernatant fluid showed typical spherical protoplasts. Protoplast preparations were stable (insignificant lysis) for 12 h, if kept at 2° C, but at room temperature slow lysis occurred. The rate of lysis at room temperature was highly variable. Ten-fold dilution of protoplast preparations in water resulted in the instantaneous lysis and clearing of suspensions. Preparations containing more than 5% residual rod-shaped cells were rejected as incompletely converted.

Protoplasts and intact cells were exposed to $^{14}C_1$-labeled lactose (National Bureau of Standards) at a concentration of $2.5 \cdot 10^{-4}$ M under conditions as described earlier for the measurement of TMG uptake[3]. 0.5 M sucrose served as osmotic stabilizer and glycerol replaced succinate as carbon source. Microscopic examination and dilution into water of the protoplast preparations after their exposure to lactose demonstrated that no regeneration of the cell wall had taken place. Results of some representative experiments are shown in Table I. An intracellular accumulation of 50 mμmoles lactose/10^9 bacteria corresponds to an approximately 200-fold concentration gradient between bacteria and medium when the medium concentration is $2.5 \cdot 10^{-4}$ M and when a cellular volume of 10^{-12} ml per bacterium is assumed. It will be noted that the lactose accumulation by protoplasts ranged from 33 to 100% of that measured in the intact cells. This variability is assumed to reflect slow lysis of protoplasts during exposure to the lactose at 35° C.

TABLE I

UPTAKE OF LACTOSE $^{14}C_1$ BY *E. coli*, STRAIN W-2244 INTACT BACTERIA AND PROTOPLASTS

Experiment number	*mμmoles lactose accumulated by 10^9 bacteria**	*mμmoles lactose accumulated by 10^9 protoplasts**
1	48	34
2	51	51
3	33	11

* Contamination of cell and protoplast pellets by passively adsorbed lactose was found to be considerable (up to 30% of total radioactivity of pellet) in media containing a high sucrose concentration. An accurate measure of this contamination was obtained by the addition of sodium azide ($2 \cdot 10^{-2}$ M) to control suspensions. This concentration blocks active galactoside uptake completely[3] and any radioactivity associated with the bacteria or protoplasts in the azide controls was taken to be the result of passive contamination. The values presented have been corrected by subtraction of the contamination control values from the total radioactivity measured.

It has been shown that the affinity of the galactoside-permease for galactosides can be expressed as a constant K[3]. K, by analogy with the dissociation constant K_s conventionally employed in the description of enzyme affinities for their substrates, is taken to be a measure of the dissociation of the "bacterium-galactoside" complex. The dissociation constant K observed for TMG and several *E. coli* strains, including W-2244, is approximately $4.5 \cdot 10^{-4}$ M TMG[3]. The constant K for both intact cells *and* protoplasts of strain W-2244 with respect to lactose is approximately $9 \cdot 10^{-5}$ M lactose.

The fact that the affinity of intact bacteria and protoplasts for lactose is the same suggests that the same mechanism may be responsible for galactoside accumulation in the intact bacterium as in the bacterium which has been deprived of its cell wall. This observation also suggests that the protoplast, and not the cell wall, is the site of galactoside-permease activity.

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