Does the lactose carrier of *Escherichia coli* function as a monomer?

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The purified lactose carrier of *Escherichia coli* (product of the *lacY* gene) is shown to be a monomer in detergent micelles of dodecyl-*O*-β-D-maltoside. The negative-dominant phenotype of mutant carriers (*lac*Y^{-d} mutants) could not be verified by measurements of the rate of galactoside transport in *lac*Y^{+}/Y^{-d} diploid strains. It is proposed that the membrane-embedded carrier functions as a monomer in galactoside–H^+ symport.

Lactose carrier M_r-*value* *Detergent micelle* lacY^{-d} *mutant* *Negative dominance*

1. INTRODUCTION

The lactose:H^+ carrier (lactose permease, product of the *lacY* gene) is an integral protein of the cytoplasmic membrane of *Escherichia coli*, which functions as a galactoside–proton symporter [1–3], (review [4]). It has been shown by a combination of DNA and protein sequencing techniques [5,6], (K. Beyreuther cited in [4]) as well as by gel filtration experiments of the denatured protein [7] that the product of the *lacY* gene is a highly hydrophobic protein of M_r 46500 (417 amino acid residues). Using overproducing strains [8], the lactose carrier has been purified and reconstituted into lipid vesicles [9–12]. The purified and reconstituted carrier is fully functional in terms of substrate binding, the first step in the translocation cycle [12]. However, under optimized conditions the rate constant, $k_{cat}^{lactose}$, for active lactose transport in reconstituted vesicles is about 10-times smaller (4.3 s^{-1}, [12]) than in cells (50 s^{-1}) but similar to that in cytoplasmic membrane vesicles (2.9 s^{-1}) derived from *E. coli* strain ML308-225 [13]. Thus, while all carrier molecules isolated recognize substrate, transport rates in proteoliposome preparations are considerably smaller than in cells. The reason for this discrepancy is unknown.

This communication addresses the question of whether functional lactose carrier is a monomer or oligomer of the M_r 46500 polypeptide chain. The possibility of oligomerization has been considered in several recent publications. The first argument comes from studies on the so-called $K_D \longrightarrow K_T$-shift observed for some permease substrates; e.g., lactose (details in [13–19]). The experimental observation is that the dissociation constant, $K_D^{lactose}$ = 14 mM [13], as well as the half-saturation constant for facilitated diffusion ($K_T^{lac} \triangleq$ ^{tose} = 24 mM) in the absence of an electrochemical proton gradient, $\Delta\tilde{\mu}_{H^+}$, are about 100-times larger than the half-saturation constant for active transport ($K_T^{lactose}$ = 0.1–0.3 mM) in the presence of $\Delta\tilde{\mu}_{H^+}$ [13,15,16]. To interpret their investigations of this effect, Robertson et al. [17] suggested that:

Abbreviations: DodOMalt, dodecyl-*O*-β-D-maltoside; NpβGal, *o*-nitrophenyl-β-D-galactopyranoside; GalSGal, β-D-galactosyl-1-thio-β-D-galactopyranoside

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'the *lac* carrier may exist in two forms, monomer and dimer, that the monomer catalyzes facilitated diffusion and the dimer active transport, and, finally, that $\Delta\tilde{\mu}_{H^+}$ promotes aggregation of monomers to dimers'.

Secondly, Mieschendahl et al. [20] described a class of *lacY* mutants, designated *lacY*$^{-d}$, which conferred a negative-dominant phenotype in a *lacY*$^+$ background. The negative-dominant phenotype might be explained by the formation of transport-defective heterodimers or higher oligomers from *lacY*$^{-d}$ and *lacY*$^+$ gene products implying that wild-type carrier is likewise functional as a dimer or higher oligomer.

Here, we show that the isolated carrier in detergent micelles is a monomer and that the negative dominance of *lacY*$^{-d}$ mutations is not observable by transport measurement in cells.

2. MATERIALS AND METHODS

2.1. *Determination of* M_r

The lactose carrier was purified from the overproducing strain T206 [12]. When the amount of detergent bound to the carrier was determined, DodOMalt [1-^{14}C]Ac (46.4 MBq/mol), prepared as in [21], was used throughout the preparation and the ECTEOLA-column was equilibrated with buffer containing only 0.2 mg DodOMalt/ml. Purified carrier (1.3 mg protein/ml) was loaded on a 2.5 × 50 cm Sephadex G-100 column equilibrated with 10 mM Tris, 20 mg glycerol/ml, 1 mM NaEDTA, 0.1 mg DodOMalt [1-^{14}C]Ac/ml (pH 7.5). The carrier-containing micelles were only partially resolved from the protein-free micelles. The leading fraction contained 87 ± 19 mol DodOMalt/mol carrier and the average for the first three carrier-containing fractions was 121 ± 17 mol DodOMalt/mol carrier. These values correspond to δ_D = 0.95 and 1.32 g DodOMalt/g carrier. The carrier concentration was determined by traces of MalN[^{3}H]Et-labelled carrier which had been added to the original vesicle preparation in which the carrier content had been determined by substrate binding [12,13].

The weight-average M_r was determined by sedimentation equilibrium in the analytical ultracentrifuge as detailed in [22]. The M_r is calculated from the equilibrium distribution, A_{280} profile as a function of the radius (fig.1) by:

$$M = \frac{2RT}{\omega^2}\,\frac{d\ln A_{280}}{dr^2}\cdot\frac{1}{1-\bar{\nu}_c\varrho+\delta_D(1-\bar{\nu}_D\varrho)} \qquad (1)$$

where:

ω = the anfular velocity (1571 rad/s);

$\bar{\nu}_c$ = the partial specific volume of the carrier (0.759 cm^3/g, calculated from the amino acid composition [5]);

δ_D = the amount of detergent bound to the carrier (0.95–1.32 g DodOMalt/g carrier);

$\bar{\nu}_D$ = the partial specific volume of the detergent (0.81 ± 0.01 cm^3/g, determined by densitometry and sedimentation velocity in H_2O–D_2O mixtures); and

ϱ = the solvent density (1.015 g/cm^3).

The quantity $d\ln A_{280}/dr^2$ was determined by the best fit (linear correlation coefficient 0.99941) to the digitalized data.

2.2. *Transport measurements*

Cells were either grown in Cohen-Rickenberg medium [23] in the presence of 0.5% glycerol, 0.3% casamino acids and thiamin (10 μg/ml) for strains K12YMel through MMO7 *Y*$^{-d4}$ (cf. table 1) or in 1% tryptone, 0.5% yeast extract, 1% NaCl for strains HB1 and 71-56-10 or this same medium plus 20 μg ampicillin/ml for strains HB2, HB3, and 71-56-10 *Y*$^{-d3}$. Cells were induced for 2–3 generations with 0.5–1 × 10^{-3} M isopropyl-1-thio-β-D-galactopyranoside. Transport of NpβGal and GalSGal and β-galactosidase activity was determined as in [24,25].

3. RESULTS

3.1. *Determination of detergent-solubilized carrier* M_r*-value*

The lactose carrier is isolated as protein–detergent complex [12]. The M_r of the protein moiety of this complex at sedimentation equilibrium can be calculated if the number of detergent molecules bound to the carrier and their partial specific volume are determined (eq. (1), [22]). Gel filtration experiments show that the number of DodOMalt molecules/carrier is at least 87 ± 19 and on the average 121 ± 17. The inability to completely separate the carrier–detergent complex from the pure micelles suggests that both aggregates have similar Stoke's radii. The aggregation number of DodOMalt under these conditions

is about 94. Therefore, these numbers suggest that the purified carrier is located in a micelle. From the equilibrium distribution (fig.1) the carrier has M_r 42000 for 0.95 g DodOMalt/g carrier or M_r 36000 for 1.32 g DodOMalt/g carrier. Both values are close to the value of M_r 46500 expected for the amino acid sequence [5,6]. Therefore, it is concluded that the carrier is a monomer in DodOMalt micelles.

3.2. *Galactoside transport in* lacY$^+$/Y^{-d} *diploid strains*

If an oligomeric structure is necessary for the function of the carrier, the replacement of some functional subunits by non-functional mutant subunits might result in the impairment of the activity of the complex. The genetic approach taken in [20] led to the isolation of 4 phenotypically *lacY*$^-$ mutants which were designated Y^{-d} because they had a *trans* dominant effect on an unlinked *lacY*$^+$ allele. The mutations were mapped at 3 different positions in the *lacY* gene: Y^{-d1} (deletion group V), Y^{-d2} (deletion group XXI) and Y^{-d3} and Y^{-d4} (both in deletion group XXVIII). Y^{-d1} maps near the end of the gene corresponding to the N-terminus of the carrier while the 3 other mutations map in positions corresponding to the middle of the carrier sequence. It was subsequently shown by DNA sequencing that mutations Y^{-d3} and Y^{-d4} are identical (Gly^{262} to Asp^{262}; H. Bocklage and B. Müller-Hill, cited in [4]). The Y^{-d} mutations are present on an F′*lacI*^{q}Z^{M330b}Y^{-d} episome (strains HB Y^{-d1} and MMO5 Y^{-d2} through MMO7 Y^{-d4});

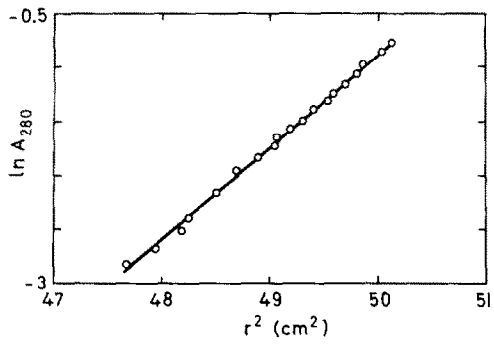

Fig.1. Distribution of the lactose carrier in DodOMalt micelles at sedimentation equilibrium. The data are linearized by plotting ln $A_{280\,nm}$ against the square of the distance (d) from the center of rotation (r^2).

Y^{-d3} has also been incorporated into a recombinant plasmid (strains HB3 and 71-56-10 pY^{-d3}).

The results in table 1 are expressed relative to the prototrophic wild-type strain K12YMel. Three groups of strains are investigated. The strains in the first group (MMO4 through MMO7 Y^{-d4}) all carry the promotor mutation p^{L8} on the chromosomal *lac* operon. Under our conditions, this mutation reduces the expression of the adjacent structural genes to 1–3% of the wild type as judged by the level of β-galactosidase. The relevant control strain in this group is strain MMO3. MMO3 carries on the episomal *lac* operon the polar mutation Z^{U118} which completely abolishes the expression of the adjacent *Y* gene. The level of *Y* gene expression in this strain as judged by NpβGal transport is thus due to the chromosomal Y$^+$ gene. Replacement of F′*lacI*^{q}Z^{U118}(Y$^+$) in strain MMO3 by F′*lacI*$^+$Z^{M330b}Y$^+$ (cf. strain MMO4, the parent of strains HB Y^{-d} through MMO Y^{-d4}) which allows normal expression of the *Y* gene [24] leads to a 19-fold increase in transport rate. This is a lower estimate since in this strain the reduced level of β-galactosidase may limit the rate of NpβGal hydrolysis in vivo. The ratio of the *lacY* gene product derived from the episomal to that of the chromosomal operon is, therefore, at least 18 (rate MOO4 – rate MOO3/rate MOO3). This ratio is also expected for the Y$^+$/Y^{-d} heterodiploid strains HB Y^{-d1} through MMO7 Y^{-d4}. Comparison of the transport rates of these strains with strain MMO3 shows that the product of the mutant Y^{-d} genes do not cause a reduction of the transport rate of wild-type carrier, derived from the chromosomal Y$^+$ gene. Therefore, the negative-dominant phenotype of these strains (cf. table 1) cannot be confirmed by the determination of transport rates. The reason for the discrepancy between growth rate and transport rate is not known. A likely explanation is that the generation time of the Y$^+$/Y$^-$ diploid strains is somewhat longer than control strains such as strain MMO3.

The negative dominance of mutation Y^{-d3} at the level of transport likewise cannot be demonstrated in the two other groups of strains (HB1 through HB3 or 71-56-10 and 71-56-10 pY^{-d3}) as shown for both NpβGal and GalSGal transport. The hybrid plasmid carrying the *lacY*$^{-d3}$ gene does not reduce the activity of the Y$^+$ gene located on the F′ factor (compare strains HB3 and HB1 or 71-56-10 pY^{-d3}

Table 1

Transport in phenotypically negative dominant *lacY* mutants

Strain	*lac* Genotype			Lac phenotype	NpβGal-transport (%)	GalSGal-transport (%)	β-Galacto-sidase (%)
	Chromosome	*F'*	Plasmid				
K12YMel	$I^+Z^+Y^+$	–	–	+	100	(100)	100
MMO4	$I^qP^{L8}Z^+Y^+$	$I^+Z^{M330b}Y^+$	–	+	32	n.d.	3
MMO3	$I^qP^{L8}Z^+Y^+$	$I^qZ^{U118}(Y^+)$	–	+	1.7	n.d.	1.1
HBY^{-d1}	$I^qP^{L8}Z^+Y^+$	$I^+Z^{M330b}Y^{-d1}$	–	–	1.9	n.d.	1.0
MMO5Y^{-d2}	$I^qP^{L8}Z^+Y^+$	$I^+Z^{M330b}Y^{-d2}$	–	–	2.7	n.d.	1.3
MMO6Y^{-d3}	$I^qP^{L8}Z^+Y^+$	$I^+Z^{M330b}Y^{-d3}$	–	–	3.2	n.d.	1.6
MMO7Y^{-d4}	$I^qP^{L8}Z^+Y^+$	$I^+Z^{M330b}Y^{-d4}$	–	–	2.3	n.d.	1.2
HB1	Δ(*lac*)	$I^qZ^+Y^+$	–	+	20	27	26
HB2	Δ(*lac*)	$I^qZ^+Y^+$	Δ(*I*)Δ(*Z*)Y^+	+	48	30	26
HB3	Δ(*lac*)	$I^qZ^+Y^+$	Δ(*I*)Δ(*Z*)Y^{-d3}	+	34	29	48
71-56-10	Δ(*lac*)	I^qZY^+	–	+	14	16	0.6
71-56-10 pY^{-d3}	Δ(*lac*)	I^qZY^+	Δ(*I*)Δ(*Z*)Y^{-d3}	–	14	16	0.72

Strains MMO3, MMO4, HBY^{-d1}, MMO5Y^{-d2}, MMO6Y^{-d3} and MMO7Y^{-d4} were described in [20]. Strains HB1 and 71-56-10 were constructed by transferring the episomes of BMH611Z$^+$ (Δ (*lac pro*) *thi/F'lac* $I^qZ^+Y^+$ *pro*$^+$) and BMH71-56-10 (Δ (*lac pro*) *thi/F'* I^qZY^+ *pro*$^+$), respectively, into strain DP54-1 (Δ (*lac pro*) *thi strA rif recA*. The construction of strains HB2, HB3, and 71-56-10 pY^{-d3} has been achieved by transformation of HB1 and 71-56-10 with plasmids pHBY^+ and pHBY^{-d3}, respectively. The construction of these plasmids is described in [26]. These plasmids are unrelated to the *lacY*-carrying plasmids described in [8]. The Lac phenotype refers to growth on mineral salts–lactose plates. The negative-dominant phenotype of the Y^{-d3} mutation is not observable in a *F'lac* $Y^qZ^+Y^+$ background (compare strains HB3 and HB1). 100% activity refers to 0.25 μmol.min^{-1}.mg cell protein^{-1} for NpβGal transport (substrate conc. 2 mM), 6 nmol.min^{-1}.mg cell protein^{-1} for GalSGal transport (substrate conc. 10 μM), and 15.6 μmol.min^{-1}.mg cell protein^{-1} for β-galactosidase

and 71-56-10). In these strains the expected ratio of Y^{-d3} to Y^+ gene products is 1.4 and 2.4, respectively. Within each group, GalSGal is accumulated to the same extent.

4. DISCUSSION

The M_r-value of the lactose carrier in DodOMalt micelles is close to the M_r of the polypeptide chain (monomer) expected from the DNA sequence of the *lacY* gene. DodOMalt, like other non-ionic detergents, is not expected to disrupt protein–protein interactions. Indeed, lactose carrier in DodOMalt micelles binds galactoside with a 1:1 stoichiometry (J.K. Wright, unpublished). The three-dimensional structure of the protein in micelles of this detergent must be retained to a great extent. Furthermore, the results presented here are in agreement with the absence of any cooperative effects in substrate binding when the carrier is embedded in the cytoplasmic membrane of *E. coli* [13] or after reconstitution [11].

The determination of transport rates in *lacY*$^+$/Y^- diploid strains failed to confirm the suggestion [20] that the lactose carrier functions as a dimer or higher oligomer. On the other hand, these experiments cannot rule out the existence of other *lacY* mutants which might exhibit negative dominance with respect to growth and where this negative dominance can be verified at the level of transport. In the case of an outer membrane protein of *E. coli*, maltoporin, a trimeric structure has been confirmed by the analysis of negative-dominant mutations [27].

The suggestion in [17] that the carrier dimerizes only in the presence of an electrochemical proton gradient, $\Delta\tilde{\mu}_{H^+}$, is neither confirmed nor refuted by the demonstration that the carrier is a monomer in detergent micelles. However, several points should be borne in mind:

(1) The monomer–dimer hypothesis is presented [17] in connection with the change in apparent affinity of the carrier for galactosides in going from the active transport mode to the facilitated diffusion mode. This effect is only observed for some substrates (e.g., lactose) but not for others; e.g., GalSGal [13]. Both types of substrates are actively transported. Therefore, the change in affinity is an ancillary effect unrelated to the intrinsic mechanism of energy coupling. We had given two possible interpretations of this effect within the framework of a classical carrier model [13].

(2) The transport measurements were conducted in the presence of $\Delta\tilde{\mu}_{H^+}$. Because a negative-dominant effect of the Y^{-d} mutations could not be demonstrated by the measurement of transport rates, these data do not support a $\Delta\tilde{\mu}_{H^+}$-dependent oligomerization.

(3) Although the formation of an oligomer from parallel-oriented monomers in response to $\Delta\tilde{\mu}_{H^+}$ remains possible although, in our view, rather unlikely, the existence of subunits oriented antiparallel has been excluded [28].

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