THERMAL EVENTS ASSOCIATED WITH ACTIVE MEMBRANE TRANSPORT IN ESCHERICHIA COLI*

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ABSTRACT

Active transport of non-metabolizable compounds by Escherichia coli resulted in thermogenesis. With substrates of the lactose permease (thiomethyl galactoside, lactose) and of the glucose transport system (α-methylglucoside) the rate of heat production was largest on initial addition, but then decreased. The kinetics of heat production varied with the transport system. For the lactose transport system, more than turnover of the permease was required since heat was not produced in azide treated cells, where facilitated diffusion is known to take place. The lactose permease thermal effects are suggested to reflect operation of the energy coupling system. The thermal effects are considered to represent a useful approach in studying transport energetics and mechanisms.

INTRODUCTION

Although active transport in microorganisms requires energy, the amounts involved have not been determined experimentally. Insight into transport energetics, as well as mechanism, might be obtained through measurement of associated heat effects. Thus far, such measurements have not been made with bacterial cells. The present study investigates the utility of microcalorimetry in providing information about active transport processes. The study was carried out with non-metabolizable substrates of the lactose permease (1, 2) and of the glucose transport system (3) in Escherichia coli.

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MATERIALS AND METHODS

Studies of the lactose permease were carried out with E. coli ML308-225 ($i^-z^-y^+a^+$) and ML30 ($i^+z^+y^+a^+$) grown at 37°C in medium 63 (4) plus 0.4% glycerol. IPTG at a concentration of 0.3 mM was used as inducer with ML30. Studies with the glucose transport system employed ML308-225 grown in medium A (5) supplemented with 0.4% glucose.

Cells were harvested in the logarithmic phase of growth, washed twice and resuspended at a concentration of 2 mg cell protein/ml in either medium 63 salts, pH 7.0, or 50 mM potassium phosphate—0.1 mM $MgSO_4$, pH 7.0, for experiments with the lactose or glucose transport systems, respectively. Chloramphenicol was added to a final concentration of 80 μg/ml. Cells were stored on ice and used within 4 hours after harvesting.

Cell protein was estimated according to the procedure of Lowry et al. (6).

Chromatographically pure α-methyl-D-glucopyranoside was kindly supplied by Dr. M. B. Perry. Methyl 1-thio-β-D-galactopyranoside and lactose were obtained from Sigma Chemical Co.

Calorimetric measurements were made with a Tronac Model 550 Isothermal Calorimeter (Tronac Inc., Orem, Utah, U.S.A.) employing a 4.0 ml stainless steel reaction vessel. This instrument measures the heat flow required to maintain the contents of the reaction vessel at constant temperature, in this case 25°. The instrument was modified to accommodate a second microburette and also to allow gas to be bubbled through the liquid in the reaction vessel, since air has access to the contents but only via a relatively long and narrow path. Air flow, when employed, was at a rate of 2.1 ml/min. Typically, 3 ml of cells (6 mg protein) were placed in the reaction vessel. After thermal equilibration (approximately 10 min) the baseline was run out for 10 min and then substrate in a volume of 54 μl to 120 μl was added.

RESULTS

Lactose Permease

The heat effects produced by adding TMG and lactose to suspensions of E. coli ML308-225 are shown in Figure 1. Air was not bubbled for experiments a to d, and essentially the same results were

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Abbreviations: IPTG = isopropyl 1-thio-β-D-galactopyranoside, TMG = thiomethyl galactoside, α-MG = α-methylglucoside, NEM = N-ethylmaleimide.

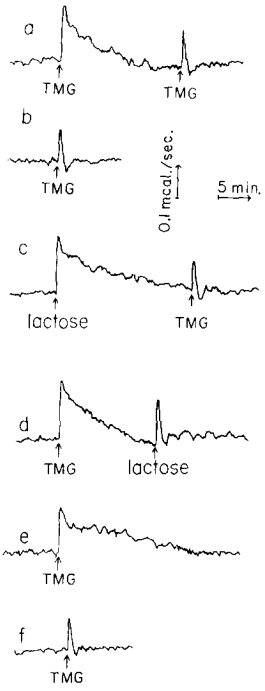

Figure 1. Thermal profiles associated with the lactose transport system. Sample volume was 3 ml. Additions indicated by arrows were 120 μl of 0.24 M TMG or 54 μl of 0.58 M lactose. Experiments (a), (c), and (d) employed ML308-225, (b) suspending buffer, (e) induced ML30, (f) uninduced ML30.

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obtained if this was done. Prior to addition of substrate the baselines were linear, indicating that the rate of endogenous heat production was constant. Addition of 120 μl of TMG solution (final concentration 9.1 mM) initiated thermogenesis, the rate of which decayed over a period of 15-20 min (Figure 1a). The heat evolved in the first 20 min in excess of the endogenous value was ≃ 50 mcal. Further addition of TMG produced a much smaller heat, ≃ 2 mcal, which was comparable to the heat of dilution (Figure 1b). Addition of lactose (54 μl, final concentration 10.3 mM) produced a thermal profile resembling that obtained with TMG (Figure 1c). Prior addition of TMG prevented heat evolution on adding lactose and vice versa (Figures 1c and 1d).

Addition of TMG to uninduced cells of ML30 produced approximately the heat of dilution. However, with induced cells, the response was similar to that with ML308-225, where the lactose permease is constitutive (Figures 1e and 1f).

Sodium azide inhibits active accumulation of β-galactosides, but allows facilitated diffusion (2). The Km for influx of poisoned and untreated cells is the same. When TMG was added to cells in the presence of 30 mM sodium azide the heat evolved was comparable to that for dilution.

Glucose Transport System

The thermal profile obtained by adding α-MG (80 μl, final concentration 5.0 mM) to glucose grown E. coli ML308-225 is shown in Figure 2. Air was bubbled at the rate of 2.1 ml/min. Two different exothermic processes seemed to occur; one peaking shortly after substrate addition, in about 20 seconds; the other after about 3 min. The heat

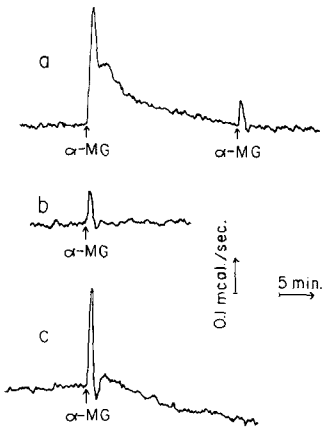

Figure 2. Thermal profiles following addition of α-MG to glucose grown ML308-225. A volume of 80 μl of 0.20 M α-MG was added where indicated by arrows to 3 ml of: (a) cells, (b) suspending buffer, (c) cells plus 30 mM sodium azide.

liberated for the first 15 min in excess of that produced endogenously was approximately 90 mcal. Subsequent addition of more α-MG produced an effect comparable to the heat of dilution (≃ 2 mcal, Figure 2b). Iodoacetate (7) and N-ethyl maleimide (8) inhibit glucose transport in E. coli. Addition of α-MG to cell suspensions containing 4 mM iodoacetate or 0.2 mM NEM gave thermal profiles comparable to the dilution heat of α-MG (data not shown).

Sodium azide has no inhibitory effect on α-MG transport (9). The thermal profile obtained in the presence of 30 mM sodium azide is shown in Figure 2c. A rapid exothermic reaction was observed. However, subsequently heat production declined below the endogenous level observed before addition of α-MG.

DISCUSSION

The data suggest that microcalorimetric measurements may well be useful in elucidating transport mechanisms and energetics. This view depends on the sensitivity of the heat measurements to several parameters. One of the more important is the variation of the thermal profile with the nature of the transport system. The uptake of α-MG occurs via the phosphotransferase system (3) while β-galactoside uptake involves other processes, and the thermal profiles of these systems differ. Thus, the heat effects reflect the chemistry occurring during the uptake process, and hence are a source of data which may aid in elucidation of the events involved.

The nature of the heat generating process is not clear. A main objective of this initial study was to demonstrate that the thermal effects were specifically associated with a transport process, and little effort was directed at identifying the thermogenic processes. The dependence

of thermogenesis on a functional transport system was demonstrated by the induction and inhibition experiments. However, these experiments also provided insight into the nature of the thermogenic process in one instance. The effects of azide on TMG induced heats indicate that more than turnover of the permease is required. It is tentatively suggested that the heats reflect operation of the energy coupling system. That is, the observed heats reflect the energy flow associated with the mass flow resulting in accumulation of substrates of the lactose permease. A point which bears further detailed study in this regard is the larger rate of thermogenesis when substrate is first added, or when the rate of accumulation would be expected to be greatest.

The possibility that the thermal effects reflect operation of the energy coupling system associated with the lactose permease is of interest since it may lead to its identification and to the detailed nature of the processes which occur. In addition, evaluation of the enthalpy of transport per mole of substrate may provide insight into the events taking place during the transport process itself.

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