The transient kinetics of uptake of galactosides into *Escherichia coli*

Malcolm G. P. PAGE* and Ian C. WEST†

Department of Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, U.K.

(Received 2 May 1984/Accepted 17 July 1984)

The uptake of galactosides into *Escherichia coli* via the lactose permease was studied in the time range 0.01–10 s by rapid mixing and quenched flow. An initial transient was observed under two conditions. Firstly, a lag in the approach to the steady state was observed at low galactoside concentrations ($<K_m$). Secondly, a burst of uptake was observed when anaerobic cell suspensions were mixed with aerobic substrate solutions. However, the cause of the burst of uptake appears to be a burst in the rate of respiration. The rate of galactoside uptake during this phase is 10-fold greater than during the steady state.

The process of galactoside uptake into *Escherichia coli* by the lactose permease (Rickenberg *et al.*, 1956) has been extensively studied by both thermodynamic (Ahmed & Booth, 1981) and steady-state kinetic (Bentaboulet & Kepes, 1981; Page & West, 1981, 1982) investigations. Although steady-state kinetics has provided essential information about the behaviour of the permease, far more information about the mechanism of uptake is potentially available from the study of the transient, or pre-steady-state, phase of uptake. We have examined the kinetics of formation of internal galactosides during the first 500 ms of uptake as a preliminary step in the investigation of the transient kinetics of the lactose permease.

Steady-state kinetic studies (Kaczorowski & Kaback, 1979; Page & West, 1981; Wright *et al.*, 1981) suggest a minimum model of the following form:

where C^- denotes the permease, H^+ a proton and S a galactoside, the subscripts e and i denote external and internal species respectively. Product formation (increasing S_i) would normally be expected to go through a lag phase in the approach to the steady state as the intermediates accumulate. In the model shown in eqn. (1) above, the lag phase would become shorter as $[H_e^+]$ and $[S_e]$ are

increased, since the time taken to accumulate the intermediates will decrease. Indeed, in this simple case the lag would disappear as $[H_e^+]$ and $[S_e]$ approach saturation. If there were an additional first-order step between $CH_e S_e$ and $CH_i S_i$, for example if the permease were to form an intermediate in which the proton and galactoside were occluded, namely:

or, if proton and galactoside translocation were not concerted, namely:

then it is possible for this lag phase in the approach to the steady state to persist with increasing substrate concentration. Thus, in eqn. (2) above, if k'_3 were rate-limiting and the reverse steps negligible,

there would be a constant lag of $1/(k_3 + k'_3)$ when substrates are saturating.

If the rate-limiting step occurred late in the cycle, for example if deprotonation on the inside of the membrane (k_5) or re-orientation of unloaded carrier (k_6) were rate-limiting, as has been suggested by Kaczorowski and colleagues (Kaczorowski & Kaback, 1979; Kaczorowski *et al.*, 1979), then the first molecules of substrates would combine rapidly with the permease to form a stoichiometric amount of internal product (S_i), but subsequent turnovers of the carrier would be slow because of the rate-limiting step. This would lead

* Present address: European Molecular Biology Laboratory, Postfach 10.2209, Meyerhofstrasse 1, 6900 Heidelberg, Federal Republic of Germany.

† Present address: Glynn Research Institute, Bodmin, Cornwall PL30 4AU, U.K.

to a burst in the appearance of internal galactoside, stoichiometric with the amount of carrier present, followed by a slower steady-state rate of uptake.

We have not found evidence of a lag in the formation of internal galactoside in the time range 10–1000 ms except at very low external concentrations of galactoside. There is evidence of a burst in galactoside uptake, but this is not stoichiometric with the amount of permease thought to be present in the cell suspension. The burst only occurs if suspensions are able to generate a protonmotive force, and we ascribe it to a burst of respiration that occurs as the anaerobic suspension mixes with aerobic substrate solution. However, during that burst the turnover number of the carrier approach 2000 s^{-1} , 10–20 times higher than steady-state turnover numbers.

Methods

Quenched flow

Two types of apparatus were used for the experiments discussed below.

One machine was home-built, and used a $\frac{1}{4}$ h.p. (200 W) electric motor to drive four 5 ml syringes via a gear-box and electrically operated clutch. Flow tubes were 1.6 mm-internal-diameter rigid-walled Teflon tubing (Lee Products, Harrow, Middx., U.K.). The mixing chambers were constructed from polythene blocks (Lee Products) adapted to give four-jet mixing chambers for both initial mixing of reactants and the subsequent mixing of reaction mixture with quench solution. One pair of syringes was used to hold the reactants, and the second pair to hold quench solution so that equal volumes of reaction mixture and quench solution were mixed at the second stage. Flow occurred at approx. 2 m/s through the system, and the lengths of tubing between the two mixing chambers was varied to give different reaction times. Usually 2 ml of quenched solution was discharged before collection of a 1 ml sample for assay.

The second machine was constructed by Hi-tech Ltd., Salisbury, Wilts., U.K. (model SF multi-mixing stopped-flow spectrophotometer).

Both machines were tested by following the time course of hydrolysis of 2,4-dinitrophenyl acetate in aq. NaOH (Gutfreund, 1969). Quench times of 8 ± 3 ms and 12 ± 3 ms were found for the home-built and the Hi-tech machines respectively, with various concentrations of dinitrophenyl acetate and NaOH (Fig. 1).

Uptake of galactosides was monitored by using either (i) galactosides that release a coloured or fluorescent molecule when hydrolysed by internal β -galactosidase or (ii) radioactive galactosides. With the former method the quenched solution was

Fig. 1. Testing the quench-time of the mixing apparatus 2,4-Dinitrophenyl acetate was mixed with aq. NaOH and then after an appropriate time with 4M-HCl. The quenched samples were immediately mixed with saturated aq. sodium acetate and the absorption at 380 nm was measured. ●, 0.7 mM-2,4-Dinitrophenyl acetate mixed with 0.5 M-NaOH; ○, 1.4 mM-2,4-dinitrophenyl acetate mixed with 0.5 M-NaOH; ■, 0.5 mM-2,4-dinitrophenyl acetate mixed with 0.5 M-NaOH; □, 1.0 mM-2,4-dinitrophenyl acetate mixed with 0.5 M-NaOH. The points denoted by circles (● and ○) were obtained with the home-built apparatus and those denoted by squares (■ and □) with the Hi-Tech multi-mixing module. The temperature was $20 \pm 0.1^\circ\text{C}$.

centrifuged and the concentration of the hydrolysis product in the supernatant was assayed. With the latter method the quenched solution was centrifuged and the radioactivity in the pellet was assayed. In both cases the quench solution was 0.1 M-sodium phosphate buffer, pH 7.0, containing 10 mM-HgCl₂. The concentration of HgCl₂ could be decreased to 1 mM without affecting the results.

Although quite high pressures are expected to develop in the mixing chamber during flow, there was no sign of damage to cells (assayed as an increase in the rate of non-permease-mediated hydrolysis of 2-nitrophenyl β -galactoside) at the highest flow rates used in these studies.

Control runs in which the cell suspension had been pre-treated with 5 mM-HgCl₂ were routinely performed with radioactive substrates, and the amount of radioactivity in the pellet was deduced from the corresponding time point in the uptake

experiment. Experiments performed with thiodi-galactoside or 4-nitrophenyl α -galactoside present together with substrate in the substrate syringe (5 mM and 2 mM final concentrations respectively) were also used as controls, and these produced results identical with those obtained with pre-treatment of the cells with HgCl_2 . Mixing with 4-nitrophenyl α -galactoside was used in preference to HgCl_2 when the hydrolysis assay was to be used, since pretreatment with HgCl_2 inhibits β -galactosidase. All experiments were performed at pH 7.0 and 20°C.

Stopped flow

Stopped-flow experiments were performed with the Hi-tech multi-mixing device set up in the appropriate mode. Absorbance changes were monitored with a Perkin-Elmer 156 dual-wavelength spectrophotometer interfaced to the mixing device by silica light-guides. Fluorescence changes were followed by using the same apparatus but with a 100 W mercury arc lamp as light-source and a Wratten no. 8 filter. The signal was recorded with a Gould OS1400 oscilloscope.

Galactosides

2-Nitrophenyl β -galactoside and lactose were purchased from BDH Chemicals (Poole, Dorset, U.K.) and 4-methylumbelliferyl β -galactoside from Koch-Light Laboratories (Colnbrook, Bucks., U.K.). All other galactosides were purchased from Sigma Chemical Co. Tritiated galactosides were synthesized by following the method of Barnett (1967). NaB^3H_4 (12.6 GBq/mmol) was purchased from Amersham International. Much of the thio[6,6'- ^3H]digalactoside used in this study was generously given by Dr. P. Overath (Tübingen, Germany). Galactosides were used at specific radioactivities of 37–370 GBq/mol.

Bacteria

Two strains of *Escherichia coli* were used in these experiments: strain ML308 (i^- , z^+ , y^+ , a^+) is a wild-type with respect to the *lac* operon except that it is constitutive; strain ML35TR contains a multicopy plasmid carrying the *y* gene and a tetracycline-resistance marker and was constructed as follows. *E. coli* ML35 (i^- , z^+ , y^- , a^-) was transformed with the multicopy plasmid vector containing the *lac y* gene extracted from strain T206 by following the method of Birnboim & Doly (1979). Strain T206 was kindly provided by Dr. P. Overath; for genetic details see Teather *et al.* (1980). The resulting strain was routinely cultured on minimal-medium/agar plates containing 0.5% glucose+0.5% fructose to suppress expression of the *lac y* gene and 10 μg of tetracycline/ml to select for retention of the plasmid. For growth, an overnight culture in

liquid minimal medium M9 (Anderson, 1946) supplemented with 0.5% glucose+0.5% fructose and 10 μg of tetracycline/ml was used to inoculate a 2-litre conical flask containing 500 ml of M9 medium supplemented with 0.5% glycerol. The culture was grown for approx. 6 h (1.5–2 generations). The uptake activity of the glucose-grown cells (assessed by uptake and hydrolysis of 2-nitrophenyl β -galactoside) was slightly higher than in a similar culture of strain ML308, whereas the uptake activity of the glycerol-grown culture was about 6-fold higher than that of a comparable suspension of strain ML308. Membrane vesicles prepared from the glycerol-grown cultures by the method of Futai (1974) had about as much permease-specific dansylaminoethylthiogalactoside binding as vesicles prepared from fully induced strain T206, whereas binding by the permease in strain-ML308 vesicles was undetectable at the protein concentrations used. Cytoplasmic membranes of strain ML35TR, prepared by the method of Osborn & Munson (1974), bound 2 nmol of 4-nitrophenyl α -[6- ^3H]galactoside/mg of protein.

All cell suspensions were treated with EDTA in accordance with the method of Leive (1968), to make the outer membrane more permeable both by galactosides and by ionophoric antibiotics. The time course of galactoside uptake in EDTA-treated and in untreated cell suspensions was identical except at low galactoside concentration (<0.1 mM) and in the presence of ionophoric antibiotics.

Results

At the cell densities used in these experiments (50–100 mg dry wt./ml final density) the suspensions were essentially anaerobic until mixed with the reagent solution. Mixing of the suspension with the reagent solution initiated a burst of respiration, which could be followed as a change in internal pH, by using either Neutral Red or Acridine Orange as indicator, and as a change in NADH oxidation, by using the intrinsic fluorescence of the cell suspension. The change in pH indicated by Neutral Red could be abolished or decreased by pre-incubating the cell suspension with 40 μM -carbonyl cyanide *m*-chlorophenylhydrazine, or with 5 mM-KCN, or by gassing the suspension with CO (Fig. 2). These respiration-induced changes of pH and intrinsic fluorescence were independent of the presence or absence of galactosides in the reagent solution.

All the galactosides examined (Table 1) showed essentially the same time course of uptake, and that time course showed the same dependence on the metabolic state of the cells. When a cell suspension

Fig. 2. Changes in the energization of cell suspensions of *E. coli* ML308 during the first few seconds after mixing. Cell suspensions containing 0.4–0.6 g wet wt. of cells/ml were mixed with 0.1 M-sodium phosphate buffer, pH 7.0, at $20 \pm 1^\circ\text{C}$. In each case both syringes contained the appropriate conditions. (a) Spectrophotometric record of changes in internal pH. The indicator was 1 mM-Neutral Red. (b) Fluorimetric record of changes in internal pH. The indicator was 10.0 μM -Acridine Orange. (c) Changes in intrinsic cell fluorescence (largely NADH oxidation). (d) Effects of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (40 μM), KCN (5 mM) and CO on the pH excursion observed with Neutral Red. Carbonyl cyanide *m*-chlorophenylhydrazone and KCN were added 30 min before the experiment was due to start. Gassing with CO was for 5 min. All solutions were stored in glass syringes.

that had not been poisoned or treated with ionophore was used, there was a short (<0.5 s) burst of rapid uptake, which soon slowed to an apparently linear phase lasting for at least 5 s (Fig. 3). The slower, apparently linear, phase of uptake is characterized by kinetic parameters (apparent

K_m and V_{max} .) similar to those obtained in conventional assays with lower cell densities and longer assay times (Table 1). In addition, uptake in this phase was competitively inhibited by alternative galactoside substrates (Fig. 4) with approximately the same inhibition constants that are

Table 1. Kinetic parameters of uptake of galactosides into *E. coli* ML308

Uptake was followed by measuring the concentration of 4-methylumbelliferone (for 1) or 2-nitrophenol (for 2) in supernatants or by measuring the radioactivity in pellets (for 3–6) after centrifuging the quenched samples. All experiments were performed in 0.1 M-sodium phosphate buffer, pH 7.0, at 20 ± 0.2°C. The additions were present in both syringes for 30 min before the experiment was commenced. Final cell density was 50 mg dry wt./ml. —, Information not available; N.D., this phase of uptake not observed.

Substrate	Additions	$V_{app.}^{max.}$ (nmol/s per mg dry wt.)			$K_m^{app.}$ (mM)		
		Initial	Steady state	Lit.*	Initial	Steady state	Lit.*
(1) 4-Methylumbelliferyl β -galactoside	None	17	2	—	0.4	0.5	—
(2) 2-Nitrophenyl β -galactoside	None	23 ± 10	4.7 ± 3.1	5	0.3 ± 0.1	1.2 ± 0.3	0.9
	30mM-NaN ₃	28	0.2	—	>5	7.5	—
(3) Lactose	None	15 ± 7	3.1 ± 2.1	3	0.5 ± 0.1	0.7 ± 0.2	0.27
(4) Thiodigalactoside	None	3.4 ± 2.6	0.45 ± 0.35	0.2	0.17 ± 0.05	0.08 ± 0.03	0.04
	Gramicidin	N.D.	0.035	—	N.D.	0.20	—
(5) Melibiose	None	27	3.0	—	0.15	0.4	0.5
	Gramicidin	N.D.	0.2	—	—	0.95	—
(6) 4-Nitrophenyl α -galactoside	None	4	0.17	0.11	<0.05	0.02	0.02
	Gramicidin	N.D.	0.02	—	N.D.	0.04	—

* Literature values taken from Wright *et al.* (1981) and references cited therein.

Fig. 3. Uptake of galactosides into *E. coli* ML308

Cell suspensions containing 0.4–0.6 g wet wt. of cells/ml were mixed with 0.1 M-sodium phosphate buffer pH 7.0, containing galactosides at the appropriate concentration. (a) 2-Nitrophenyl β -galactoside (hydrolysis assay) at 0.7 mM (○) and 0.1 mM (●). (b) 4-Nitrophenyl α -[6-³H]galactoside uptake at 0.1 mM.

observed in conventional assays (Page & West, 1982). We therefore equate this phase with ‘steady-state’ turnover of active transport. The rapid burst phase can be characterized by three features: an initial rate, an exponential decay to the eventual linear ‘steady-state’ rate and an amplitude (the value on the ordinate to which the linear, ‘steady-state’, portion of the time course extrapolates back to zero time). Both the maximum amplitude and the time constant of the

exponential decay are about the same for all galactosides tested (Table 2). If the burst phase were to be ascribed to a single rapid turnover of the permease with subsequent rate-limitation by a slow step occurring after galactoside release, the calculated number of permease molecules would have to be 50-fold greater than the carrier content of the cells as estimated from α -galactoside binding, to membrane vesicles (see above; and Wright *et al.*, 1981).

Fig. 4. Inhibition of 2-nitrophenyl β -galactoside uptake into *E. coli* ML308 by thiodigalactoside (a) A cell suspension containing 0.3 g wet wt. of cells/ml was mixed with 0.1 M-sodium phosphate buffer, pH 7.0, containing 0.8 mM-2-nitrophenyl β -galactoside and the appropriate concentration of thiodigalactoside. Uptake was assayed by measuring 2-nitrophenol in the supernatant after centrifugation. (b) Effect of thiodigalactoside on the steady-state rate of uptake of 2-nitrophenyl β -galactoside.

Table 2. Parameters of the apparent burst in galactoside uptake into *E. coli* ML308
Conditions were those described in the legend to Table 1.

Substrate	Concn. (mM)	Addition	Amplitude of burst (nmol/mg dry wt.)	Decay rate (s ⁻¹)
2-Nitrophenyl β -galactoside	5	None	0.85	12
Thiodigalactoside	1	None	0.80	12
		Nigericin	0.50	10
		Valinomycin	0.30	4.5
		None	0.85	11
Melibiose	1	None	0.85	11

Fig. 5. Effects of ionophores and inhibitors on the uptake of thiodigalactoside into *E. coli* ML308 (a) Cell suspensions (0.3–0.6 g wet wt./ml) were pre-treated with the antibiotic at 10 μ g/ml for 30 min before the experiments. The buffer was 150 mM-KCl/50 mM-sodium phosphate, pH 7.0, at 20°C. (b) Conditions were as described in the legend to Fig. 2(d). The final thiodigalactoside concentration was 1 mM.

Starving the cells in the presence of 30mM- NaN_3 (Page & West, 1980) or pre-treating the suspension with 40 μM -carbonyl cyanide *m*-chlorophenylhydrazine or either nigericin or valinomycin + 150mM-KCl all lowered the steady-state rate, lowered the amplitude of the burst and (to a variable extent) slowed the exponential decay to the steady state (Fig. 5).

Pre-treating the cell suspension with 10mM-NaCN or gramicidin completely abolished the burst and greatly lowered the steady-state rate of uptake (Fig. 5). There was a significant increase in the steady-state K_m for lactose and 2-nitrophenyl β -galactoside uptake in the presence of gramicidin (>5-fold increase), whereas there was little change in the K_m of uptake for thiodigalactoside and melibiose (Table 1).

Pre-loading non-poisoned cells with unlabelled thiodigalactoside resulted in a doubling of the final steady-state rate of uptake of radioactive thiodigalactoside, without significantly altering the other parameters of the burst (amplitude and decay) (Fig. 6). Pre-loading gramicidin-treated cells caused a 3-fold increase in steady-state rate but caused no restoration of the burst (Fig. 6).

Use of the strain with amplified permease content caused a 6-fold increase in the steady-state rate of uptake of thiodigalactoside, and the exponential decay was apparently much more rapid, but there was no significant change in the amplitude of the burst phase (Fig. 7).

No lag in the uptake of galactoside could be detected, except at low concentrations of external galactoside (Fig. 8), where unstirred-layer effects, caused by rate-limiting diffusion through the outer membrane, are expected to affect the results (West & Page, 1984). The rather low values for the galactoside association constant derived from these experiments (5×10^5 – $4 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$) strongly suggest that this is occurring.

Discussion

Although the time course of uptake resembles that outlined in the introduction for a mechanism where an internal intermediate accumulates during turnover, it does not seem likely that this is what is happening. The amplitude of the burst is too great and was independent of the permease content of the cells.

Several alternative explanations of the burst kinetics have to be considered. A second protein component, present in 50-fold excess over normal permease contents and obligatorily involved in a late slow step of active transport, could give the observed kinetics. However, that seems very unlikely, as there is no good evidence for the involvement of any protein other than the permease in uptake via this system (Newman *et al.*, 1981).

A rapid increase in internal galactoside to a steady-state concentration would be a possible

Fig. 6. Effect of pre-loading with unlabelled substrate on the rate of uptake of thiodigalactoside into *E. coli* ML308. Cell suspensions (0.3–0.5 g wet wt./ml) were pre-treated with (a) EDTA, 1 mM-thiodigalactoside and, in (b), 10 μg of gramicidin/ml as well for 60 min before the experiments. For the curves denoted by $\bigcirc$, thiodigalactoside was omitted from the pre-incubation medium.

Fig. 7. Time-course of thiodigalactoside uptake into *E. coli* ML35TR

Cell suspensions (0.3–0.4 g wet wt./ml) were mixed with 1 mM-thio[6,6'- ^3H]digalactoside. ○, Uptake at 20.4°C; □, uptake at 1°C; ●, uptake at 20.4°C in the presence of 10mM-NaCN.

explanation for those galactosides that are hydrolysed rapidly by β -galactosides (2-nitrophenyl β -galactoside, lactose, 4-methylumbelliferyl β -galactoside), but not for those galactosides that are not hydrolysed rapidly and that continue to be accumulated (thiodigalactoside, melibiose, 4-nitrophenyl α -galactoside). The time courses of uptake of both types of galactoside are essentially the same.

Galactoside-induced depolarization of the membrane does not seem to be a sufficient explanation either. Cells treated with nigericin (which collapses the pH gradient) and valinomycin in the presence of KCl (which collapses the membrane potential) still show burst kinetics. The results obtained with Neutral Red and acridine dyes indicate that the internal pH is still increasing during the experiments and that there is no significant effect of galactosides on this change.

It has been reported that, in mitochondrial suspensions, respiration occurs at a rate approx. 10-fold greater than the normal steady-state rate for the first 0.5 s (Penniston, 1973; Papa *et al.*, 1973, 1974, 1975). *E. coli* appears to show a similar burst in respiration, and the rapid initial rate of active transport via the permease (turnover numbers of up to 2000 s^{-1} , or 10–20 times the maximum steady-state turnover number) would appear to be due to this transient burst of proton-pumping and electron transport through the intermediates of the respiratory chain.

In conclusion, we have been unable to identify a kinetic transient due to the interaction of galactoside and permease. But the unexpected observation that net turnover during active transport can

Fig. 8. Approach to steady-state uptake into *E. coli* ML308

(a) Uptake of 4-nitrophenyl α -[6- ^3H]galactoside (●, 0.1 mM; ○, 0.02 mM). The cell density was 0.4 g wet wt./ml.
(b) Re-plot of the apparent lag against substrate concentration for 4-nitrophenyl α -[6- ^3H]galactoside.

transiently occur very much more rapidly than previously supposed is extremely interesting.

This work was carried out with support from the Science and Engineering Research Council. We are indebted to John Ferguson for help in interfacing the Perkin-Elmer 156 spectrophotometer with the Hi-Tech rapid mixer.

References

- Ahmed, S. & Booth, I. R. (1981) *Biochem. J.* **200**, 583–589
- Anderson, E. H. (1946) *Proc. Natl. Acad. Sci. U.S.A.* **32**, 120–128
- Barnett, J. E. G. (1967) *Carbohydr. Res.* **4**, 267–269
- Bentaboulet, M. & Kepes, A. (1981) *Eur. J. Biochem.* **117**, 233–238
- Birnboim, H. C. & Doly, J. (1979) *Nucleic Acid Res.* **7**, 1513–1523
- Futai, M. (1974) *J. Membr. Biol.* **15**, 15–28
- Gutfreund, H. H. (1969) *Methods Enzymol.* **16**, 229–249
- Kaczorowski, G. J. & Kaback, H. R. (1979) *Biochemistry* **18**, 3691–3697
- Kaczorowski, G. J., Robertson, D. E. & Kaback, H. R. (1979) *Biochemistry* **18**, 3697–3704
- Leive, L. (1968) *J. Biol. Chem.* **243**, 2373–2380
- Newman, M. J., Foster, D. L., Wilson, T. H. & Kaback, H. R. (1981) *J. Biol. Chem.* **256**, 11804–11808
- Osborn, M. J. & Munson, R. (1974) *Methods Enzymol.* **31A**, 642–653
- Page, M. G. P. & West, I. C. (1980) *FEBS Lett.* **120**, 187–191
- Page, M. G. P. & West, I. C. (1981) *Biochem. J.* **196**, 721–731
- Page, M. G. P. & West, I. C. (1982) *Biochem. J.* **204**, 681–688
- Papa, S., Guerrieri, F., Simone, S., Lorusso, M. & Lavossa, D. (1973) *Biochim. Biophys. Acta* **292**, 20–38
- Papa, S., Guerrieri, F. & Lorusso, M. (1974) *Biochim. Biophys. Acta* **357**, 181–192
- Papa, S., Lorusso, M. & Guerrieri, F. (1975) *Biochim. Biophys. Acta* **387**, 425–440
- Penniston, J. T. (1973) *Biochemistry* **12**, 650–655
- Rickenberg, H. V., Cohen, G. N., Butlin, G. & Monod, J. (1956) *Ann. Inst. Pasteur Paris* **91**, 829–857
- Teather, R. M., Bramhall, J., Riede, I., Wright, J. K., Fürst, M., Aichele, G., Wilhelm, U. & Overath, P. (1980) *Eur. J. Biochem.* **108**, 223–231
- West, I. C. & Page, M. G. P. (1984) *J. Theor. Biol.* **110**, 11–19
- Wright, J. K., Riede, I. & Overath, P. (1981) *Biochemistry* **20**, 6404–6415