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Uptake of *N*-acetylneuraminic acid by *Escherichia coli* K-235

Biochemical characterization of the transport system

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Kinetic measurement of the uptake of *N*-acetyl[4,5,6,7,8,9-^{14}C]neuraminic acid by *Escherichia coli* K-235 was carried out *in vivo* at 37 °C in 0.1 M-Tris/maleate buffer, pH 7.0. Under these conditions uptake was linear for at least 30 min and the K_m calculated for sialic acid was 30 μM. The transport system was osmotic-shock-sensitive and was strongly inhibited by uncouplers of oxidative phosphorylation [2,4-dinitrophenol (100%); NaN_3 (66%)] and by the metabolic inhibitors KCN (84%) and sodium arsenate (76%). The thiol-containing compounds mercaptoethanol, glutathione, cysteine, dithiothreitol and cysteine had no significant effect on the sialic acid-transport rate, whereas the thiol-modifying reagents *N*-ethylmaleimide, iodoacetate and *p*-chloromercuribenzoate almost completely blocked (> 94%) the uptake of this *N*-acetyl-sugar. *N*-Acetylglucosamine inhibited non-competitively the transport of *N*-acetylneuraminic acid, whereas other carbohydrates (hexoses, pentoses, hexitols, hexuronic acids, disaccharides, trisaccharides) and *N*-acetyl-sugars or amino acid derivatives (*N*-acetylmannosamine, *N*-acetylcysteine, *N*-acetylproline and *N*-acetylglutamic acid) did not have any effect. Surprisingly, L-methionine and its non-sulphur analogue L-norleucine partially blocked the transport of this sugar (50%), whereas D-methionine, D-norleucine, several L-methionine derivatives (L-methionine methyl ester, L-methionine ethyl ester, L-methionine sulphoxide) and other amino acids did not affect sialic acid uptake. The *N*-acetylneuraminic acid-transport system is induced by sialic acid and is strictly regulated by the carbon source used for *E. coli* growth, arabinose, lactose, glucose, fructose and glucosamine being the carbohydrates that cause the greatest repressions in this system. Addition of cyclic AMP to the culture broth reversed the glucose effect, indicating that the *N*-acetylneuraminic acid-uptake system is under catabolic regulation. Protein synthesis is not needed for sialic acid transport.

INTRODUCTION

Poly[α-(2,8)-*N*-acetylneuraminic acid], so-called 'colominic acid' (Barry & Goebel, 1957; Barry, 1958) is a capsular homopolymer of *N*-acetylneuraminic acid (sialic acid; Neu5Ac) produced by several species of bacteria (Barry *et al.*, 1962; Barry 1965). Its physiological significance is not still well known, although it seems to be related to the invasiveness, colonization and pathogenicity of these bacterial strains (Rohr & Troy, 1980). Several polysialyl molecules with α-(2,8) and α-(2,9) ketosidic linkages (Liu *et al.*, 1977; Egan *et al.*, 1977) have been found as pathogenic determinants in *Neisseria meningitidis*, *Salmonella toucra*, *S. arizona* and *Citrobacter freundii* serogroups (Liu *et al.*, 1971; Bhattacharjee *et al.*, 1975, 1976) and in *Escherichia coli* capsular antigens (Troy, 1979; Silver *et al.*, 1981; Jann & Jann, 1983). In spite of the biosynthesis and structure of these polysaccharides having received considerable attention (Barry & Goebel, 1957; Barry, 1958; Kimura, 1966; Troy, 1979; Schauer, 1982; Kundig *et al.*, 1971), little is known about the regulation and metabolic effectors involved in their biosynthesis. Some approaches in this field have been made by Troy and co-workers (Troy, 1979; Troy & McCloskey, 1979; Rohr & Troy, 1980; Whitfield *et al.*, 1984; Vimr & Troy, 1985), who established the influence of exogenous sialic acid on the biosynthesis of colominic acid by *E. coli*. Although several lines of evidence support the uptake of Neu5Ac into intact tissues (Harms *et al.*, 1973; Hirschberg & Yeh, 1977), very little work has dealt with the transport of sialic acid in bacteria. Vimr & Troy (1985) showed that, like many Gram-positive bacteria (Nees & Schauer, 1974), Neu5Ac could be used by *E. coli* and *S. typhimurium* as the sole carbon source. This involves the induction of two enzymic activities that are co-ordinately regulated and both repressed by glucose: a sialic acid-specific transport activity and an aldolase activity specific for cleaving sialic acid.

Here we present a biochemical study of the Neu5Ac-uptake system of *E. coli* K-235 when this bacterium was grown in a chemically defined medium ideal for colominic acid production. An attempt was also made to establish a model of its catabolic regulation.

MATERIALS AND METHODS

Chemicals

N-acetylneuraminic acid, D- and L-methionine, D- and L-norleucine, *N*-acetylglucosamine, colominic acid, D-xylose, resorcinol, L-proline and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). *N*-Acetyl[4,5,6,7,8,9-^{14}C]neuraminic acid

Abbreviations used: Neu5Ac, *N*-acetylneuraminic acid; CA, colominic acid; CAP, chloramphenicol; Ery, erythromycin; St, streptomycin; 2,4-DNP, 2,4-dinitrophenol.

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(350 mCi/mmol) was from Amersham (Bucks., U.K.). Other reagents used were of analytical quality.

Strains

Escherichia coli K-235 (A.T.C.C. 13027) was obtained from the type collection and a culture was also kindly supplied by Professor F. A. Troy (Department of Biological Chemistry, University of California, Davis, CA, U.S.A.). The strain was kept freeze-dried or in liquid N_2 (gas phase).

Culture media and growth conditions

E. coli K-235 was maintained on Trypticase Soy Agar (Difco) and grown slants (8 h at 37 °C) were used to inoculate liquid media. Each 250 ml Erlenmeyer flask containing 62.5 ml of medium H (see below) was inoculated with 1.0 ml of a bacterial suspension ($A_{540} = 1.0$). Incubation was carried out in a rotary shaker (250 rev./min) at 37 °C for 19 h ($A_{540} = 4.0$) or for the time required in each experiment.

Media

The medium used for the growth of *E. coli* was chemically defined (medium H) with the following composition ($g \cdot l^{-1}$): NaCl, 1.2; K_2SO_4, 1.1; $MgSO_4,7H_2O$, 0.15; $CaCl_2,6H_2O$, 0.02; $FeSO_4,7H_2O$, 0.001; $CuSO_4,5H_2O$, 0.001; Na_2HPO_4, 10.8; KH_2PO_4, 0.5; D-xylose, 8.4 and L-proline, 8.7. In this medium, *E. coli* produces 1400 μg of colominic acid/ml over 72 h.

When necessary, the carbon source was replaced by other sugars, the carbon concentration being maintained constant.

N-Acetylneuraminic acid uptake in whole cells

Cells grown in medium H (see above) were harvested at different times, washed twice with sterile distilled water and resuspended in 0.1 M-Tris/maleate buffer, pH 7.0. Cell concentrations were adjusted to an A_{540} of 0.5 and incubated at the assay temperature for 15 min in the presence of unlabelled Neu5Ac (116 $nmol \cdot ml^{-1}$) before adding [^{14}C]Neu5Ac (0.15 $nmol \cdot ml^{-1}$). When required (induction experiments), unlabelled Neu5Ac and [^{14}C]Neu5Ac were added together. Agitation was carried out in a thermostatically controlled bath at 150 $strokes \cdot min^{-1}$. Portions (1 ml) were taken from the uptake mixture at various time intervals, filtered through Millipore filters (0.45 μm pore size) and washed twice with 10 ml of cold buffer. The whole procedure was completed in 45 s. The filters were dissolved in 10 ml of toluene/Triton X-100 (2:1, v/v) scintillation fluid, containing (l^{-1}) 3.3 g of PPO and 0.125 g of dimethyl-POPOP. The radioactivity of the samples was counted in a Beckman 1800 LS liquid-scintillation counter. The amount of [^{14}C]Neu5Ac adhering to the bacterial cells was measured by using cells pretreated with formaldehyde (Koch, 1964; Lo *et al.*, 1972). This quantity was substracted from the experimental values. When required, several compounds (sugars, amino acids, metabolic inhibitors, thiol-containing or thiol-modifying reagents) were added to the uptake mixture. In these cases the effector was added 2–3 min before the radiolabelled Neu5Ac. Protein determinations were carried out by the method of Lowry *et al.* (1951). Bovine serum albumin was used for establishing the standard curves. Neu5Ac uptake is given in units (nmol of Neu5Ac incorporated/15 min per ΔA_{540}) or in Neu5Ac uptake/A_{540} (nmol of Neu5Ac incorporated/ΔA_{540}) when different uptake periods were employed.

Cell-shocking procedure

Cells were subjected to osmotic shock as described by Neu & Heppel (1965). A 50 ml portion of bacterial suspension grown in medium H for 19 h was centrifuged (10000 *g*, 10 min), washed with sterile distilled water and suspended in 50 ml of 20% (w/v) sucrose/0.2 M-Tris (pH 7.2)/0.005 M-EDTA and incubated in a rotary shaker (250 rev./min) at 37 °C. After 5 min the cells were harvested by centrifugation (15000 *g*, 10 min) in a coldroom (4 °C). The supernatant fluid was removed and the well-drained pellet was rapidly mixed with 50 ml of cold sterile distilled water (0 °C). The suspension was mixed in an ice bath on a rotary shaker for 10 min, and the supernatant fluid was removed. Cells were resuspended in 0.1 M-Tris/maleate buffer, pH 7.0, adjusted to an A_{540} of 0.5 and used for the uptake experiments. Control cells were treated in a similar way, but without osmotic shock. Viability was determined on serial dilutions of cells in Trypticase Soy Agar (Difco).

Colominic acid determinations

Cells grown as above were separated by centrifugation (10000 *g*, 10 min) and the clear supernatant, once decanted, was dialysed (Medicell International LTD dialysis tubing; exclusion size < 10000 M_r) against 100 vol. of sterile distilled water (4 °C, 24 h). A 1 ml portion of the dialysed liquid was employed for the determination of colominic acid, the resorcinol methodology of Svennerholm (1958) being employed.

RESULTS AND DISCUSSION

Time course of the appearance of the *N*-acetylneuraminic acid-uptake system

To attempt to characterize fully the sialic acid-transport system in *Escherichia coli* K-235 it was necessary to grow the bacteria on a defined medium. We used a xylose/proline medium (H), which was ideal for the production of colominic acid (CA) (Fig. 1*a*) and one that readily allowed growth of *E. coli* K-235 on a wide variety of sugars or sugar derivatives as the sole carbon source (ribose, arabinose, glucose, fructose, mannose, galactose, lactose, maltose, threalose, glucosamine, *N*-acetylglucosamine, glycerol, pyruvic acid). The time-course studies indicate (Fig. 1*b*) that the enzyme (or enzymic system) appears during the first hours of growth, reaching maximal levels at 10 h ($A_{540} = 0.3$). From thereafter up to 72 h the quantity of enzyme continuously decreased. These results indicate that maximal transport activity is found in the lag phase of growth, decreasing along the exponential phase and remaining constant (at very low levels) in the stationary phase. These kinetics of synthesis, which are the opposite of those followed by CA accumulation, suggest a negative effect of this polymer or some of its derivatives [oligo(sialic acid) or free sialic acid] on the uptake of [^{14}C]Neu5Ac. Vimr & Troy (1985) indicated that a 10-fold molar excess of sialic acid inhibited the uptake by more than 95%. According to these results the oligosialyl molecules or free sialic acid spontaneously generated by hydrolysis of the colominic acid accumulated in the broth (Troy & McCloskey, 1979) could inhibit the activity of the permease involved in

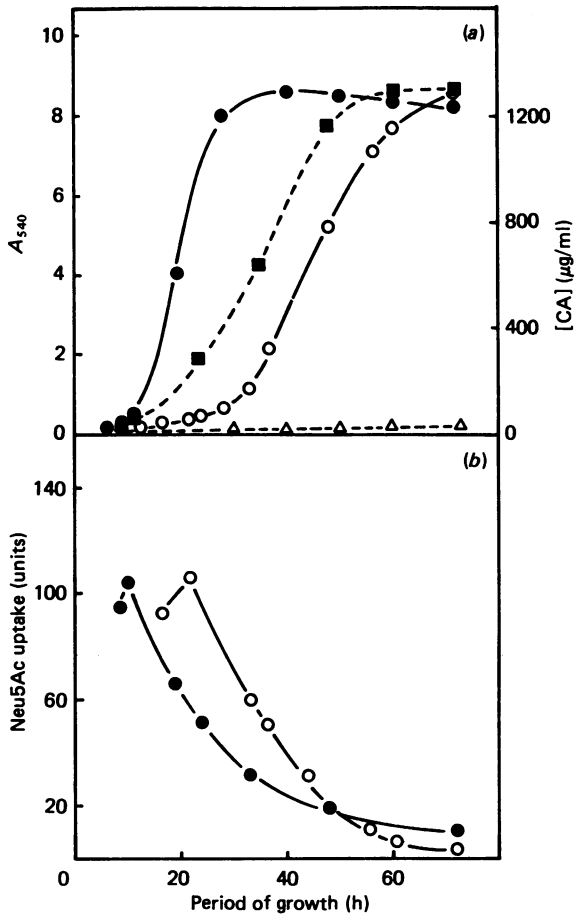

Fig. 1. (*a*) Growth of *E. coli* K-235 at 37 °C (●) and 20 °C (○) and production of CA at 37 °C (■) and at 20 °C (△) and (*b*) time course of appearance of sialic acid permease when *E. coli* K-235 was grown at 37 °C (●) and at 20 °C (○)

sialic acid uptake. In order to clarify whether the levels of permease shown in Fig. 1(*b*) corresponded to the true quantity of enzyme or to these decreased by the inhibitory effect of free sialic acid, Neu5Ac uptake was studied in cells grown at 20 °C. At this temperature no colominic acid is produced (Fig. 1*a*), and therefore neither oligosialyl nor free sialic acid can be present in the broth. The kinetics of the time course of appearance in this case were similar to those described at 37 °C, but were some what delayed, owing to the lower specific rate of growth at this temperature (Fig. 1). These results indicate that colominic acid or its extracellular derivatives do not affect the level of permease and suggest that the genes for Neu5Ac uptake are efficiently transcribed during the first hours of growth.

Characteristics of Neu5Ac transport

To determine whether sialic acid transport in *Escherichia coli* is an active process, the kinetic properties of the uptake were examined. Before the experiments could be carried out, it was necessary to establish the time period over which initial rates of transport could be estimated (Christensen, 1975). Although sialic acid transport was a linear function of time for at least 30 min (Fig. 2*a*), 15 min assays were routinely used. Assuming that the volume of cell water is 2.7 μl/mg dry weight (Winkler & Wilson, 1966), it can be readily calculated that, after 15 min, the concentration of sialic acid inside the cell is 126 mM when the outside concentration is between 0.06 and 0.083 mM. Thus, sialic acid is taken up against a concentration gradient, which implies an active process.

Another argument for the uptake of Neu5Ac as being active comes from the results obtained when the efflux of Neu5Ac was measured in presence of the protonophore 2,4-dinitrophenol (2,4-DNP). Cells were incubated under standard conditions and, after 15 min of uptake, 2,4-DNP was added. No efflux of labelled Neu5Ac was detected even after 1 h of incubation, suggesting that there is not a passive Neu5Ac-transport system in *E. coli* K-235. A study, by paper chromatography, of the labelled material accumulated inside the cells revealed that Neu5Ac is transported without chemical modification (results not shown). Similar results have been previously described by Vimr & Troy (1985) using an aldolase-negative mutant.

The uptake of sialic acid was maximal at pH 7.0 in both 0.1 M-phosphate buffer and 0.1 M-Tris/maleate buffer (Fig. 2*b*), the uptake rates from pH 6.5 to 7.5 being very similar. In order to avoid the possible effect of variation in ionic strength (*I*), the molarity of the buffer was modified (from 0.01 to 0.15 M). The variations in transport rates clearly indicate that the best conditions are reached at *I* 0.1 (Fig. 2*c*). Under the above conditions (0.1 M-Tris/maleate buffer, pH 7.0, at 37 °C for 15 min), initial rates of uptake were measured at different concentrations of Neu5Ac. This is illustrated in

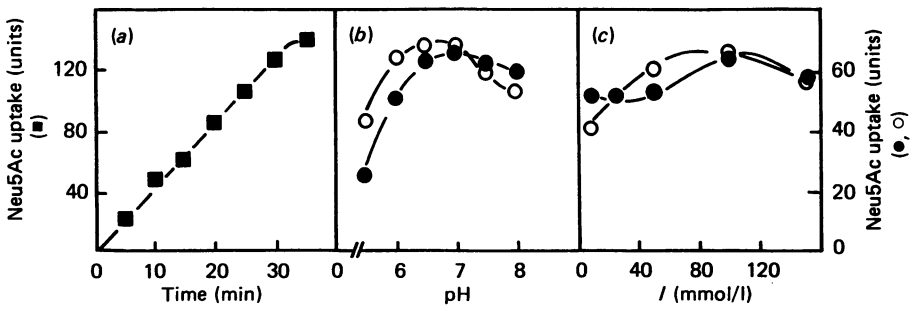

Fig. 2. Effect of (*a*) the time of incubation (■), (*b*) pH and (*c*) variation in ionic strength (*I*) (●, Tris/maleate buffer; ○, phosphate buffer) on the Neu5Ac-uptake system

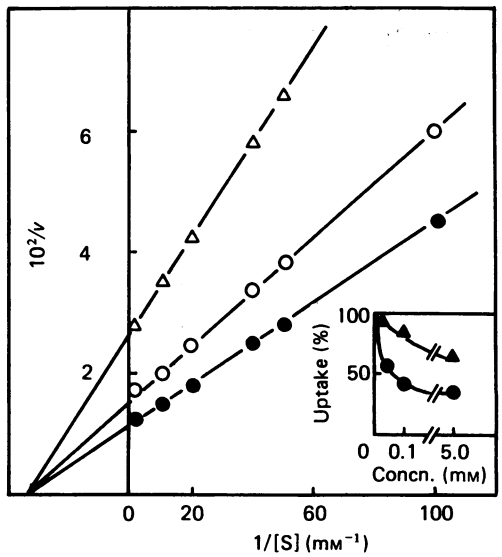

Fig. 3. Kinetics of [^{14}C]Neu5Ac transport by *E. coli* K-235 cells

A plot of $1/v$ versus 1/[S], where v is sialic acid uptake (units), is shown. ●, control; ○, plus 0.01 mM-*N*-acetyl-D-glucosamine; △, plus 0.1 mM-N-acetyl-D-glucosamine. The inset shows the inhibition in uptake (%) caused by different concentrations of *N*-acetyl-D-glucosamine (●) and D-glucosamine (▲). Other monosaccharides (glucose, fructose, mannose, galactose, xylose, arabinose and ribose), disaccharides (lactose, melibiose and threalose) and sugar derivatives (galactitol, mannitol and sorbitol) or sialic acid precursors (*N*-acetylmannosamine, pyruvic acid, phosphoenolpyruvic acid) did not cause any effect.

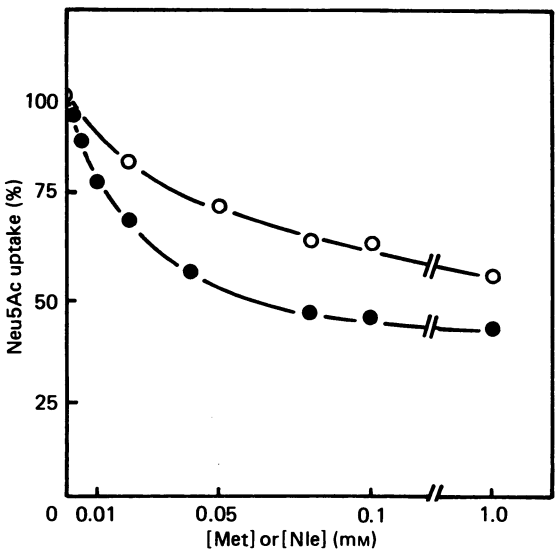

Fig. 4. Effect of different concentrations of L-methionine (●) and L-norleucine (Nle) (○) on the uptake of Neu5Ac

Other L-amino acids (Trp, Phe, Cys, Thr, Ser, Ile, Leu, Val, Ala, Lys, Gly, Arg, His, Asp, Glu, Gln, Asn, Pro), D-amino acids (Met, Nle) or aminoacyl derivatives (L-Met methyl ester, L-Met ethyl ester, L-Met sulphoxide, *N*-acetyl-Cys, *N*-acetyl-Glu, *N*-acetyl-Pro) tested did not cause any effect.

Fig. 3 which shows that the K_m for sialic acid is 30 μM. This value, which is very similar to those previously reported for the arabinose-binding protein from *E. coli* (Hogg & Englesberg, 1969) and succinate entry in *E. coli* (Lo *et al.*, 1972) and *Rhizobium leguminosarum* (Finan *et al.*, 1981), is 10-fold lower than that reported by Vimr & Troy (1985), who described a K_m range of 0.2–0.6 mM. It is not clear why these different values of K_m have been obtained, although it could be attributable to the different conditions of assay employed in both cases.

The specificity of the Neu5Ac-uptake system was examined by adding various compounds to the uptake mixture. Neither monosaccharides (glucose, fructose, mannose, galactose, xylose, arabinose and ribose) nor disaccharides (lactose, melibiose and threalose), sugar derivatives (galactitol, mannitol and sorbitol) or sialic acid precursors (*N*-acetylmannosamine, pyruvic acid and phosphoenolpyruvic acid), even at a concentration of 5 mM, caused any variations in the transport efficiency (Fig. 3). However, *N*-acetylglucosamine and glucosamine caused a marked decrease in Neu5Ac uptake (Fig. 3, inset), suggesting that sialic acid and these two sugars are transported by a common carrier. However, the kinetic study of the inhibition caused by *N*-acetylglucosamine (Fig. 3) showed that it corresponds to a non-competitive model which precludes a common system for the transport of both *N*-acetylamino sugars.

In spite of the fact that the addition of different amino acids to the uptake mixture did not affect the sialic acid-transport rates (Fig. 4), L-methionine and its non-sulphur analogue (L-norleucine) inhibited such transport by more than 50% (Fig. 4), whereas the D-analogues (D-methionine and D-norleucine) and other methionine derivatives (L-methionine methyl ester, L-methionine ethyl ester and L-methionine sulphoxide) had no significant effect.

The lack of interference in the transport of sialic acid by other sugars suggests that Neu5Ac is transported inside the cells by a very specific uptake system modulated by other compounds (*N*-acetylglucosamine, glucosamine, L-methionine and L-norleucine) and thus modifying its catalytic efficiency.

Effect of thiol-containing and thiol-modifying reagents on Neu5Ac transport

The effect of exogenously added monothiol compounds (GSH, mercaptoethanol and cysteine) and dithiols (dithiothreitol and cysteine) on the uptake of sialic acid was tested. None of these (Table 1) had any significant effect on the rate, or on the efficiency, of transport. These results suggest that an extracellular environment containing reduced thiols might not be necessary for the transport of sialic acid. This implies that maintenance of membrane-associated disulphide groups (which must be present in an oxidized state) are not necessary for the bioactivity of the transport system (Morgan *et al.*, 1985). However, the thiol-modifying reagents *N*-ethylmaleimide (Riordan & Vallee, 1967), iodoacetate and *p*-chloromercuribenzoate (Lo *et al.*, 1972) almost completely block (> 94%) the uptake of sialic acid (Table 1). Similar results have been described for other transport systems (Carter *et al.*, 1968; Kennedy, 1970) and in the inhibition of the catalytic activity of proteins containing reactive thiol groups (Friedmann *et al.*, 1949; Tsao & Bailey, 1953; Alexander, 1958; Riordan & Vallee, 1967; Lo *et al.*, 1972).

Table 1. Effect of energy poisons, thiol reagents and protein synthesis inhibitors on Neu5Ac transport

The results shown here, in Table 2 and in the Figures are averages of at least four different determinations. Further abbreviations: PCMB, *p*-chloromercuribenzoate; NEM, *N*-ethylmaleimide; IAc, iodoacetate.

Inhibitor	Concn.	Inhibition (%) of Neu5Ac transport
Energy poisons		
NaN_3	1.0 mM	31.8
	5.0 mM	66.4
Na_3AsO_4	1.0 mM	53.7
	5.0 mM	75.6
KCN	1.0 mM	72.7
	5.0 mM	83.5
2,4-DNP	1.0 mM	97.6
	5.0 mM	100.0
Thiol reagents*		
PCMB	1.0 mM	96.0
NEM	1.0 mM	94.3
IAc	1.0 mM	95.2
Protein-synthesis inhibitors		
Ery	250 $\mu g \cdot ml^{-1}$	46.1
CAP	250 $\mu g \cdot ml^{-1}$	56.0
St	250 $\mu g \cdot ml^{-1}$	44.8

* The thiol-containing compounds mercaptoethanol, GSH, cysteine, dithiothreitol and cystine had no significant effect on sialic acid transport.

Effect of metabolic inhibitors

NaN_3, KCN and sodium arsenate inhibited Neu5Ac uptake by more than 66% (Table 1), whereas the protonophore 2,4-DNP inhibited uptake by about 97%. It is not surprising that these energy poisons (DNP, N_3^- and CN^-) decrease uptake, since they inhibit oxidative phosphorylation by lowering ATP pools (Dills *et al.*, 1980). These results are in full agreement with the inhibition caused by arsenate, which also decreases ATP levels (Klein & Boyer, 1972; Parnes & Boos, 1973), and suggest that sialic acid uptake in living *E. coli* K-235 cells takes place via an active transport system dependent on an energized membrane or on a high-energy phosphate intermediate (Glenn *et al.*, 1980).

These results, allied to the fact that *E. coli* K-235 partially loses the Neu5Ac transport system when osmotically shocked (27% of the non-shocked control cells), suggest that the uptake of sialic acid in this bacterium is an active transport energized directly by phosphate-bond energy (direct chemical-energy coupling) and that, in addition to the membrane-bound permease, requires the participation of a binding protein located in the periplasmic space (Romano, 1986).

When the inhibitors of protein synthesis chloramphenicol (CAP) erythromycin (Ery) and streptomycin (St) were added (250 $\mu g \cdot ml^{-1}$) to the uptake mixture after 15 min of preincubation in the presence of 0.116 mM-Neu5Ac (see the Materials and methods section), an inhibition lower than 15% was detected (Fig. 5*d*). However, if these antibiotics were supplied at the same time as Neu5Ac addition, the uptake of sialic acid decreased by about 50% (Fig. 5*a* and Table 1). Moreover, if CAP was added a few minutes later, different uptake rates were observed (Fig. 5). These results clearly indicate that: (*a*) 50% of the permease for Neu5Ac uptake is present in *E. coli* when grown in medium H (basal level) (Fig. 5*a*); (*b*) the level of enzyme is increased after Neu5Ac induction; (*c*) almost all the permease is induced after 15 min of preincubation in the presence of 0.116 mM-Neu5Ac; and (*d*) protein synthesis is not required for transport but rather for induction.

Effect of glucose and other carbohydrates on the control of sialic acid uptake

The study of the effect of the carbon source (used for *E. coli* growth) on the uptake of Neu5Ac showed that this transport was dependent on the sugar employed Fig. 6 shows that, when arabinose, lactose, glucose, fructose

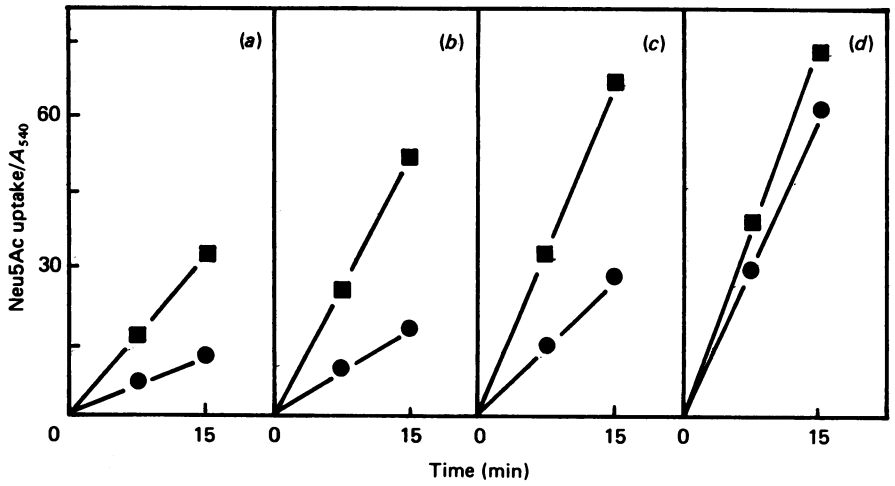

Fig. 5. Induction of Neu5Ac permease

Cells grown for 19 h (A_{540} = 4.0) in medium H were suspended in the uptake mixture plus 0.116 mM unlabelled Neu5Ac, and [^{14}C]Neu5Ac (■) or [^{14}C]Neu5Ac plus CAP (250 $\mu g \cdot ml^{-1}$) (●) were added at: (*a*) zero time; (*b*) 5 min; (*c*) 10 min; and (*d*) 15 min. Similar results were obtained when Ery or St (250 $\mu g \cdot ml^{-1}$) were used.

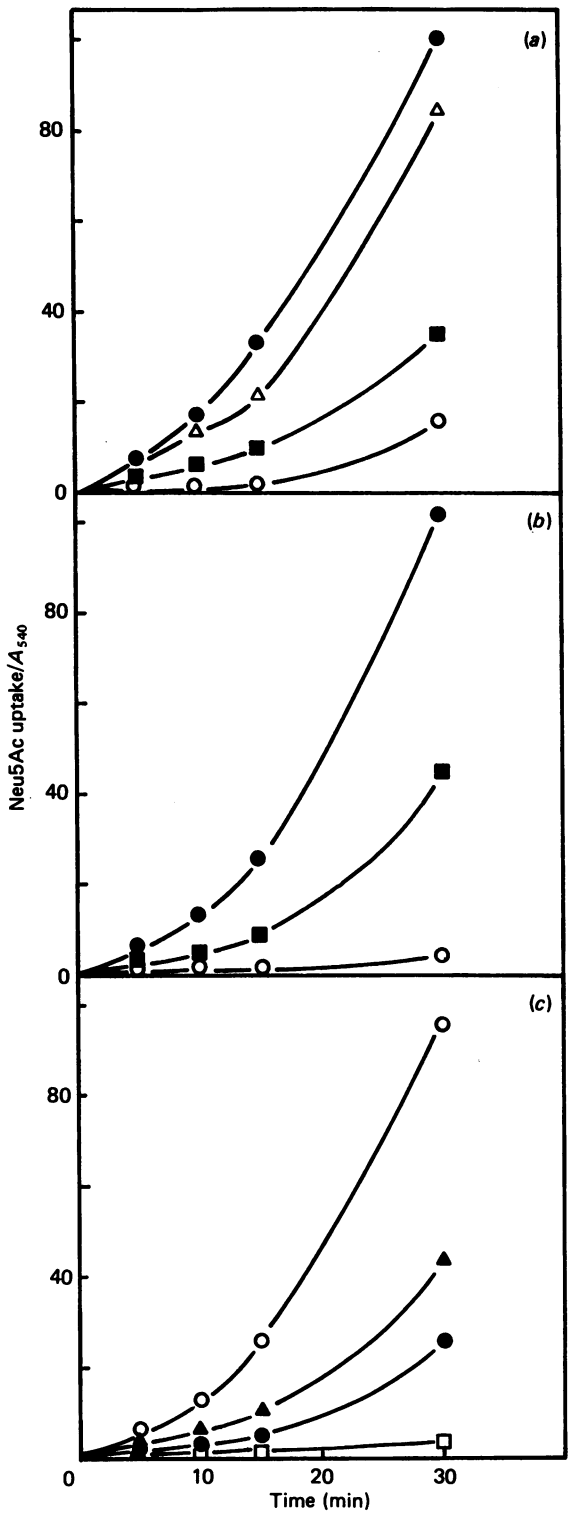

Fig. 6. Uptake of [^{14}C]Neu5Ac in *E. coli* when grown (A_{540} = 4.0) on different carbon sources

(*a*): ●, Xylose; △, mannose; ■, glucosamine; ○, glucose; (*b*): ●, galactose; ■, threalose; ○, lactose; (*c*): ○, ribose; ▲, glycerol; ●, fructose; □, arabinose. In all these experiments, unlabelled Neu5Ac and [^{14}C]Neu5Ac were added at the same time.

glucosamine were used as the carbon source, a considerable decrease in the uptake of sialic acid occurred. Other sources, such as glycerol and threalose,

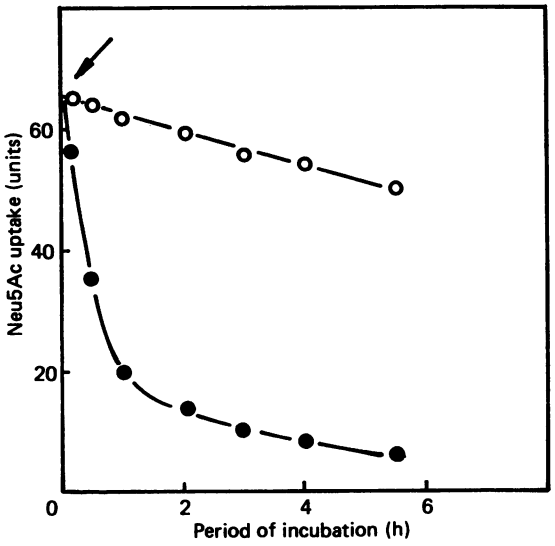

Fig. 7. Repression of the Neu5Ac-uptake system when *E. coli* K-235 was grown for 19 h (A_{540} = 4.0) in medium H and glucose (0.3%) was added at this time

○, Control (xylose); ●, xylose+glucose. The arrow indicates the time at which glucose was added.

Table 2. Repression of Neu5Ac uptake by D-glucose and reversal of the effect by cyclic AMP

Cells of *E. coli* K-235 were grown in medium H for 19 h (A_{540} = 4.0) and, at this time, 0.1 ml were used to inoculate two 50-ml Erlenmeyer flasks containing the minimal medium H in which xylose was replaced by glucose (0.3%) or glucose (0.3%) plus cyclic AMP (5 mM). Incubations were carried out for 3 h.

Addition(s)	Uptake (%)
D-Glucose (0.3%)	100.0
D-Glucose (0.3%)+cyclic AMP (5 mM)	350.0

caused a smaller effect, but mannose, ribose and galactose caused no effect at all. Furthermore, when glucose was added to an *E. coli* culture growing in medium H (with xylose as carbon source), the rate of transport greatly decreased in a short time (Fig. 7). The 'glucose effect' was reversed by the addition of cyclic AMP (Table 2), suggesting that it could be due to a lowering of intracellular cyclic AMP levels and indicating that Neu5Ac uptake is subject to catabolic regulation control (Magasanik, 1961; Perlman & Pastan, 1968; Pastan & Adhya, 1976). Similar results have been described for other transport systems in *Rhizobium leguminosarum* (Glenn *et al.*, 1980), *Pseudomonas putida* (Dubler *et al.*, 1974), *Azotobacter vinelandii* (Reuser & Postma, 1973) and *Bacillus subtilis* (Willecke *et al.*, 1973). Additionally, the repressive effect of glucose was reversed by sialic acid, although the induction of permease activity was delayed by 15–20 min after Neu5Ac had been added to the uptake mixture (Fig. 8). Similar data were reported by Nees & Schauer (1974), working with *Clostridium perfringens*. Several experi-

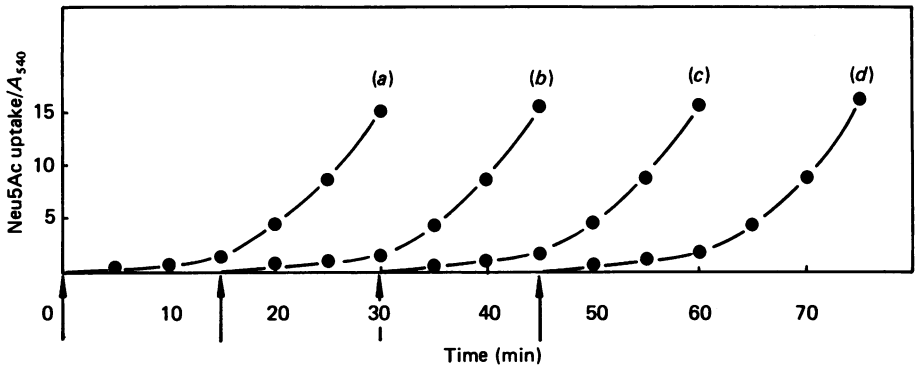

Fig. 8. Induction of sialic acid-transport system when cells grown in medium H with glucose were incubated for different times before Neu5Ac addition

(*a*) Zero time; (*b*), 15 min; (*c*) 30 min and (*d*) 45 min.

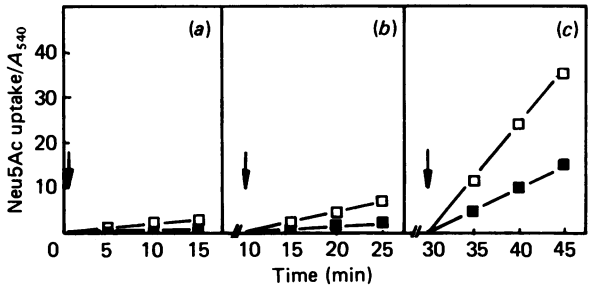

Fig. 9. Effect of CAP on glucose repression of Neu5Ac uptake

Cells, grown until $A_{540} = 4.0$ in medium H with glucose as carbon source, were suspended in the uptake mixture plus 0.116 mM unlabelled Neu5Ac and [^{14}C]Neu5Ac (□) or [^{14}C]Neu5Ac plus CAP (250 $\mu g \cdot ml^{-1}$) (■) were added at: (*a*) Zero time; (*b*) 15 min; (*c*) 30 min and (*d*) 45 min.

ments were performed in order to establish whether the lag phase required for induction was due to the presence of a repressor that necessarily must be diluted either by stopping its synthesis in the absence of glucose (uptake medium) or due to the effect of the inductor just added (0.1 mM-sialic acid). Fig. 8 shows that, when Neu5Ac was added at different times to the uptake mixture, the lag phase of induction was almost similar in all the cases (even after 45 min of preincubation), suggesting that the synthesis of permease is not due to glucose depletion but rather to the induction caused by sialic acid. Indeed, when CAP was added to the uptake mixture, the repression caused by glucose was not reversed, even though Neu5Ac was present (Fig. 9). This implies that, in cells grown in glucose (unlike cells grown in xylose), no available permease is present. However, when chloramphenicol was added after a period of induction the quantity of permease increases as the period of induction progressed (Fig. 9).

These results, together with the fact that the repressive effect caused by glucose can be reversed by the addition of cyclic AMP (Table 2) seem to indicate that the permease(s) for sialic acid uptake (which is induced by Neu5Ac, Fig. 5) is under positive control, implying the activation of the transcription by a regulatory protein (complex–cyclic AMP–protein), thus suppressing the repression caused by a catabolite (Makman & Sutherland, 1965; Perlman & Pastan, 1968).

On the other hand, the strong repression of sialic acid uptake when *E. coli* was grown in arabinose or lactose as the sole carbon source (Figs. 6*b* and 6*c*) seems to indicate that induction of some of the system under catabolic regulation (Pastan & Adhya, 1976; Postma & Lengeler, 1985) could cause the overrepression of other inducible systems, which are also regulated by a similar control mechanism.

Further experiments should be directed to attaining a better understanding of these regulatory aspects.

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