Amino acid deprivation leads to the emergence of System A activity and the synthesis of a specific membrane glycoprotein in the bovine renal epithelial cell line NBL-1

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1. Amino acid deprivation of confluent monolayers of the bovine renal epithelial cell line NBL-1 causes a stimulation of Na⁺-dependent alanine transport. 2. This stimulation is mediated by a protein-synthesis-dependent induction of 2-(methylamino)isobutyric acid (methyl-AIB)-sensitive alanine transport activity (System A), which was not previously present in these cells. 3. Induction was prevented by the addition of methyl-AIB, alanine or glutamine. 4. Tunicamycin prevented the induction of alanine transport activity. 5. Induction of System A activity was accompanied by incorporation of [³H]mannose into a single membrane protein band of molecular mass 113–140 kDa. 6. These results are consistent with the possibility that induced System A activity in confluent NBL-1 cells is mediated by the synthesis of a 113–140 kDa membrane glycoprotein.

INTRODUCTION

The Na⁺-dependent uptake of neutral amino acids by many mammalian cell types is mediated by two major transport systems with overlapping specificity, which are termed System A and System ASC. System A but not System ASC transports N-methylated substrates. The amino acid analogue 2-(methylamino)isobutyric acid (methyl-AIB) is widely used as a specific substrate for System A, and transport via System A is often taken as that component of total Na⁺-dependent amino acid uptake which can be inhibited by an excess of methyl-AIB. It has been recognized for many years that amino acid deprivation of cells in suspension or in culture causes an increase in amino acid transport activity which is mediated by the specific induction of System A (for reviews see Guidotti *et al.*, 1978; Shotwell *et al.*, 1983; Christensen, 1990). The molecular identity of System A has not yet been established.

Brush border membranes of epithelial cells contain a system for Na⁺-dependent neutral amino acid uptake which is different from Systems A and ASC. This system, which has been termed B^o (Doyle & McGivan, 1992), has been characterized in bovine renal brush border membranes vesicles (Lynch & McGivan, 1987). System B^o has a broad specificity, catalyses the transport of branched-chain and neutral amino acids and is insensitive to methyl-AIB. A somewhat similar broad-specificity system has been described in rabbit (Stevens *et al.*, 1984) and guinea pig (Satoh *et al.*, 1989) intestinal brush border membrane vesicles.

A number of renal epithelial cell lines have been employed as model systems to study the regulation of amino acid transport. It has been reported that Na⁺-dependent transport in non-confluent cells of the MDCK cell line from canine kidney is mediated by the A and ASC systems (Boerner & Saier, 1982). In contrast, the LLC-PK1 cell line from pig kidney did not express System A activity (Sepulveda & Pearson, 1982), while in the opossum kidney cell line (OK) the uptake of a wide range of amino acids, including phenylalanine and valine, was Na⁺-dependent and electrogenic, and not characteristic of System A activity (Schwegler *et al.*, 1989). However, no complete character-

ization of neutral amino acid transport in these cell lines has yet been performed.

We have recently characterized amino acid transport in the bovine renal epithelial cell line NBL-1. This cell line does not possess Na⁺-dependent glucose transport or γ -glutamyltransferase activity and, like the well-studied MDCK cell line, is presumably of distal tubular origin (Doyle & McGivan, 1992). In this study it was shown that confluent NBL-1 cells express a Na⁺-dependent amino acid transport activity which is insensitive to inhibition by methyl-AIB and has a broad specificity which is similar to that of System B^o characterized in bovine renal brush border membrane vesicles (BBMV). It therefore appeared that this cell line could constitute an appropriate model system for the study of the regulation of system B^o activity.

In this paper we report that amino acid deprivation of NBL-1 cells causes stimulation of Na⁺-dependent alanine transport activity, but that this is mediated by the *de novo* synthesis of System A rather than by induction of the System B^o already present. Induction of System A was accompanied by the incorporation of mannose into a specific membrane glycoprotein.

MATERIALS AND METHODS

Cell culture

NBL-1 cells were obtained from Flow Laboratories (McLean, VA, U.S.A.) and cultured in Ham's F-12 medium supplemented with 10% foetal calf serum (FCS), 2 mM-L-glutamine, 100 units of penicillin G/ml, 0.1 mg of streptomycin/ml, 0.25 μ g of amphotericin B/ml and 50 μ g of gentamycin/ml, at 37 °C in a gas phase of 5% CO₂/95% air. Cells were grown in 75 cm² flasks for continuous culture and 35 mm-diam. Petri dishes for experiments. Cells were treated with trypsin once a week, seeded into Petri dishes and fed every other day until confluent (5–7 days), when they were used for experiments. In experiments where cells were deprived of amino acids, the Ham's F-12 supplemented medium was removed and replaced by a medium containing only the inorganic salt components of Ham's F-12 medium plus 10 mM-glucose and 0.5% Phenol Red and supplemented with

Abbreviations used: BBMV, brush border membrane vesicles, methyl-AIB, 2-(methylamino)isobutyric acid; FCS, foetal calf serum.

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0.1% BSA and antibiotics as above (amino-acid-free medium). In some experiments, where shown, single amino acids at 5 mM concentration were added to this medium.

Measurement of transport

Transport was measured using a modification of the method of Quamme *et al.* (1989). Briefly, cells were washed in the following transport medium without substrate: 137 mM-NaCl, 5.4 mM-KCl, 1.8 mM-CaCl₂, 1.2 mM-MgSO₄ and 10 mM-Hepes, adjusted to pH 7.4 with Tris. Amino-oxyacetate (0.5 mM) was also present to inhibit alanine metabolism. Amino acid uptake was initiated by adding 1 ml of the above medium containing 0.1 mM ³H-labelled amino acid (1 µCi/ml) at 20 °C. The uptake was terminated by removing the transport medium and washing with 3 × 2 ml aliquots of ice-cold 137 mM-NaCl/10 mM-Tris/Hepes, pH 7.4. Plates were drained and 0.5 ml of 0.5% Triton X-100 was added. Cells were then detached from the plates with a cell scraper and dissolved. Samples of 200 µl were added to 10 ml of scintillation fluid for counting of radioactivity. Protein was measured using the method of Bradford (1976). Na⁺-independent uptake was performed using 137 mM-choline chloride instead of NaCl in the transport medium.

In some experiments, cells were grown on permeable membrane supports (Transwell-COL; Costar, Cambridge, MA, U.S.A.) until confluent. Transport was measured by replacing the medium on either the apical or the basolateral side with medium containing radioactive substrates as described above. Cells were removed from the support with a cell scraper and dissolved in Triton X-100 as above.

Labelling experiments using [³H]mannose

Cells were incubated for 24 h in Ham's F-12 medium or the amino acid-free medium described above in the presence or absence of tunicamycin (0.025 µg/ml). [³H]Mannose (5–25 µCi/ml) was also present. Cells were then washed five times in 50 mM-mannose/10 mM-Tris/HCl, pH 7.4 (homogenization buffer), and removed from the dishes into this medium. A cell membrane fraction was isolated as follows. The suspension was homogenized by five passes through an 18-gauge needle and centrifuged at 100 000 *g* and 4 °C for 30 min. The supernatant was discarded and the pellet was resuspended in 5 ml of homogenization buffer and centrifuged again at 100 000 *g*. This procedure was repeated once more and the final pellet was dissolved in 2.5% Triton X-100 at a protein concentration of 3–5 mg/ml.

SDS/PAGE

The protein fractions prepared above were run on SDS/10% -PAGE gels under reducing conditions (Laemmli, 1970). Gels were stained using Coomassie Brilliant Blue R and then destained to reveal the protein bands. Gels were cut into 5 mm slices and dissolved in 0.15 ml of 70% HClO₄ plus 0.15 ml of 30% H₂O₂ at 80 °C for 1 h in plastic vials with the lids firmly closed to prevent loss of ³H₂O. Vials were allowed to cool at 4 °C for 1 h before opening to add 15 ml of scintillation fluid. The vials were left several hours in the dark prior to counting of radioactivity, to allow chemiluminescence to decay.

Materials

FCS, antibiotic/antimycotics (10 000 units of penicillin G, 10 mg of streptomycin/ml and 25 µg of amphotericin B/ml), tunicamycin, cycloheximide and Ham's F-12 medium were obtained from Sigma (Poole, Dorset, U.K.). Gentamycin (50 mg/ml) was from Gibco (Paisley, Scotland, U.K.). ³H-labelled amino acids and [³H]mannose were obtained from Amersham. All other chemicals were of analytical grade.

RESULTS

The time course of the uptake of L-alanine (0.1 mM) into confluent monolayers of NBL-1 cells is shown in Fig. 1. As has been reported previously (Doyle & McGivan, 1992), the uptake was linear over the first 5 min and was stimulated in the presence

Fig. 1. Time course of alanine uptake into confluent monolayers of NBL-1 cells

Confluent cell monolayers were incubated overnight in control medium or amino-acid-free medium, and L-alanine transport (0.1 mM) was measured in the presence and the absence of an inward gradient of Na⁺ as described in the Materials and methods section. ●, Control cells, Na⁺ medium; ○, control cells, choline medium; ■, amino-acid-starved cells, Na⁺ medium; □, amino-acid-starved cells, choline medium.

Fig. 2. Time course of the induction of Na⁺-dependent alanine transport into confluent monolayers of NBL-1 cells in the presence and the absence of cycloheximide

Confluent cell monolayers were incubated in the presence or the absence of amino acids for up to 48 h. The initial rate of L-alanine transport (0.1 mM) was measured in the presence or absence of Na⁺ over 3 min. The values shown are those of the Na⁺-dependent rate, i.e. the rate in the presence of Na⁺ minus that in the absence of Na⁺. Values are expressed as percentages of the rates in uninduced cells, i.e. cells incubated in the presence of amino acids throughout. The mean rate in uninduced cells was 1.71 ± 0.06 nmol of alanine/3 min per mg. ●, No further additions; ○, +cycloheximide (10 µg/ml). Results are means ± S.E.M. of at least four different cell monolayers.

Fig. 3. Inhibition by methyl-AIB of alanine transport in control and amino-acid-starved NBL-1 cells

Cells were incubated overnight in the presence (a) or the absence (b) of amino acids. Alanine transport (0.1 mM) was measured in the presence or absence of 5 mM-methyl-AIB. Results are the means $\pm$ S.E.M. of 3–5 experiments. $\bullet$, Na^+ medium, no methyl-AIB; $\circ$, choline medium, no methyl-AIB; $\blacksquare$, Na^+ medium, methyl-AIB; $\square$, choline medium, methyl-AIB.

Table 1. Inhibition of Na^+ -dependent L-alanine transport by different amino acids

Alanine uptake (0.1 mM) into confluent monolayers of NBL-1 cells was measured in the presence or absence of Na^+ over 3 min, as described in the Materials and methods section. The Na^+ -dependent rate is the rate of uptake in the presence of Na^+ minus the rate in its absence. Induced uptakes are the rates in amino-acid-starved cells minus the rates in control cells. Uptake rates are expressed as nmol of L-Ala/3 min per mg of protein. Values are the means $\pm$ S.E.M. of four different experiments. ** $P < 0.01$; *** $P < 0.001$ versus additions (Student's t test).

Additions (5 mM)	Uptake in control cells		Uptake in amino-acid-starved cells		Induced uptake (nmol/3 min per mg)
	(nmol/3 min per mg)	(% of control)	(nmol/3 min per mg)	(% of control)	
None	1.73 ± 0.14	100	3.26 ± 0.10	100	1.53
Methyl-AIB	1.66 ± 0.13	97.6 ± 2.7	$1.85 \pm 0.06^{***}$	56.8 ± 1.3	0.19
Pro	1.41 ± 0.08	83.0 ± 4.7	$1.76 \pm 0.11^{***}$	53.3 ± 3.2	0.26
Gln	$0.21 \pm 0.04^{***}$	12.4 ± 2.1	$0.47 \pm 0.05^{***}$	14.2 ± 1.0	0.35
Leu	$0.64 \pm 0.02^{***}$	39.0 ± 1.3	$1.84 \pm 0.05^{***}$	56.5 ± 1.6	1.20
Phe	$0.95 \pm 0.07^{**}$	56.7 ± 4.1	$2.14 \pm 0.12^{***}$	65.5 ± 3.7	1.19
Lys	1.67 ± 0.20	99.8 ± 10.3	3.03 ± 0.02	93.0 ± 0.5	1.36

of an inward gradient of Na^+ . Incubation of the cells in an amino-acid-free medium for 30 h caused an increase in the rate of uptake of alanine in the presence but not in the absence of Na^+ .

The increase in the rate of transport on incubation of cells in amino-acid-free medium was significant after 6 h, half-maximal after 20 h and maximal after 30 h (Fig. 2). When cycloheximide was present initially in the amino-acid-free medium no increase in transport activity was apparent, showing that the induction of the transport activity was a protein-synthesis-dependent phenomenon.

Na^+ -dependent alanine transport in these cells when grown in Ham's-F12 medium is catalysed by a broad-specificity transport system (System B^o), which is insensitive to inhibition by methyl-AIB (Doyle & McGivan, 1992). The results in Fig. 3 confirm that the basal rate of alanine transport was insensitive to methyl-AIB. However, the component of Na^+ -dependent alanine uptake which was induced on amino acid starvation was completely sensitive to inhibition by 5 mM-methyl-AIB. On the other hand, deprivation of amino acids did not induce leucine transport (results not shown). Leucine, like alanine, is a substrate for System B^o and system L in these cells (Doyle & McGivan, 1991), but is known not to be a substrate for System A. These results clearly indicate that amino acid starvation of confluent cell monolayers

causes specific induction of System A in cells which did not previously express this activity.

Table 1 shows the inhibition of Na^+ -dependent alanine (0.1 mM) uptake in control and amino-acid-starved NBL-1 cells by other amino acids and by methyl-AIB added at 5 mM to the transport assay. In control cells the initial rate of alanine transport was inhibited by glutamine, leucine and phenylalanine, substrates for System B^o. Methyl-AIB and proline, specific and preferred substrates respectively for System A, did not cause a significant inhibition. In contrast, in amino-acid-starved cells, proline, methyl-AIB and glutamine (which is also a substrate for System A in many mammalian cells) inhibited the induced component of transport. Leucine and phenylalanine, which are not transported by System A, were only poor inhibitors of the induced component of transport. Lysine, which is a substrate for neither System A nor System B^o, did not inhibit alanine uptake in control or amino-acid-starved cells.

The induction of System A in these cells was further characterized (Table 2). Cells were incubated overnight in the presence of single amino acids at a concentration of 5 mM. The cell monolayers were then washed three times to remove the added amino acid and the initial rate of Na^+ -dependent uptake of 0.1 mM-alanine was measured. The presence of a single substrate of System A (alanine, methyl-AIB or glutamine) abolished the

Table 2. Effect of the addition of a single amino acid to the amino-acid-free medium on the induction of Na⁺-dependent alanine transport in NBL-1 cells

Confluent monolayers were exposed overnight to control (Ham's F-12) or amino-acid (Aa)-free medium supplemented or not with different amino acids (5 mM). Na⁺-dependent alanine transport was measured as described in the Materials and methods section. Results are the mean $\pm$ S.E.M. of 4–6 different cell monolayers. ** $P \leq 0.01$ versus control (Student's *t* test).

Medium	Additions (5 mM)	Na ⁺ -dependent uptake	
		(nmol/3 min per mg of protein)	(% of basal rate)
Control	–	1.93 $\pm$ 0.08	100
Aa-free	–	3.01 $\pm$ 0.19**	156
Aa-free	L-Ala	1.84 $\pm$ 0.18	95
Aa-free	Methyl-AIB	1.84 $\pm$ 0.08	95
Aa-free	L-Gln	1.85 $\pm$ 0.03	96
Aa-free	L-Leu	2.53 $\pm$ 0.10**	131
Aa-free	L-Phe	3.60 $\pm$ 0.43**	186

induction of System A activity. Leucine and phenylalanine did not abolish induction. In control experiments (results not shown), cells were incubated overnight with a single amino acid and were then incubated in amino-acid-free medium with cycloheximide (10 μ g/ml) for 1 h before the transport assay, in order to completely deplete the cells of amino acids and abolish further protein synthesis. The results were very similar to those in Table 2. This indicates that the washing procedure used was adequate to deplete the cells of internal amino acids and that none of the effects described above were due to trans-inhibition or trans-stimulation.

In order to determine the localization of the induced System A transport in confluent cell monolayers, cells were grown on permeable supports so that both the basolateral and apical membranes were accessible to the growth medium. The uptake of alanine from both the apical and the basolateral sides was measured in control cells and in cells exposed overnight to amino-acid-free medium (Table 3). As shown previously (Doyle & McGivan, 1992), in control cells the rate of Na⁺-dependent

alanine transport across the basolateral membrane was twice that across the apical membrane, and transport across both membranes was insensitive to methyl-AIB. In starved cells, alanine transport was induced in both basolateral and apical membranes and the increase was completely sensitive to inhibition by methyl-AIB in each case, showing induction of System A in both membranes. The percentage increase in the apical membrane was somewhat higher than that in the basolateral membrane.

In liver, the protein responsible for System A transport has been shown to be a glycoprotein by direct reconstitution of transport activity from concanavalin A-binding fractions of liver plasma membranes (Quesada & McGivan, 1988). Induction of System A activity in isolated hepatocytes is inhibited by tunicamycin, a specific inhibitor of protein glycosylation (Barber *et al.*, 1983). Fig. 4 shows that the induction of Na⁺-dependent alanine transport by amino acid starvation was abolished by the presence of tunicamycin at concentrations which did not affect the basal rate of transport. These results are similar to those obtained in hepatocytes and indicate that the induced System A in NBL-1 is a glycosylated protein.

In order to identify membrane glycoproteins which are synthesized during amino acid starvation, cell monolayers were incubated with ³H-labelled mannose overnight in the presence and absence of amino acids and of tunicamycin. In amino-acid-starved cells a single peak of radioactivity was obtained, and this consistently occurred in slice 6 (five separate induction experiments; Fig. 5a). Incorporation of radioactive mannose into this slice was abolished by tunicamycin (Fig. 5b). In control cells no specific incorporation of [³H]mannose into any slice was observed in either the absence (Fig. 5c) or the presence (Fig. 5d) of tunicamycin. The range of molecular mass covered by slice 6 was 140–113 kDa.

DISCUSSION

It has been shown previously that NBL-1 cells in confluent culture do not express System A activity, as judged by the lack of inhibition of alanine transport by an excess of methyl-AIB (Doyle & McGivan, 1991). Na⁺-dependent amino acid transport in these cells is mediated by a broad-specificity system similar to that in bovine BBMV (Lynch & McGivan, 1987). The results in

Table 3. Membrane localization of induced transport activity after amino acid deprivation

Cells were grown on permeable supports until confluent. In some experiments, control medium was replaced overnight by amino-acid-free medium. Alanine uptake (0.1 mM) was measured over a 3 min time period. The percentage increase in transport is the rate of Na⁺-dependent transport in amino-acid-starved cells compared with that in control cells. Values shown are the averages of determinations from two separate monolayers, and agreed to within 10%.

Cells	Inhibitor (5 mM)	Membrane	Presence of Na ⁺	Total uptake (nmol/3 min per mg)	Na ⁺ -dependent (nmol/3 min per mg)	Inhibition by methyl-AIB (%)	Increase due to starvation (%)
Control	–	Apical	+	0.81	0.58		
	Methyl-AIB	Apical	+	0.80	0.57	3	
	–	Apical	–	0.23	–		
Amino-acid-starved	–	Apical	+	1.44	1.19		105
	Methyl-AIB	Apical	+	0.88	0.63	53	
	–	Apical	–	0.25	–		
Control	–	Basolateral	+	1.29	1.07		
	Methyl-AIB	Basolateral	+	1.39	1.18	0	
	–	Basolateral	–	0.22	–		
Amino-acid-starved	–	Basolateral	+	2.05	1.83		70
	Methyl-AIB	Basolateral	+	1.38	1.16	64	
	–	Basolateral	–	0.22	–

Fig. 4. Effect of tunicamycin dose on Na^+ -dependent alanine uptake in NBL-1 cells

Confluent cell monolayers were incubated for 24 h in the presence (●) or the absence (○) of amino acids, together with various concentrations of tunicamycin as shown. Na^+ -dependent alanine transport was measured as described in the legend to Fig. 2. Values are the means $\pm$ S.E.M. of four different experiments.

the present paper clearly establish that amino acid deprivation of these cells results in the protein-synthesis-dependent induction of a previously absent System A-like activity, with an unchanged activity of System B^o. This conclusion follows from the observations that the induced alanine transport activity was completely sensitive to inhibition by an excess of methyl-AIB and by proline, specific substrates of System A in a variety of cells. Leucine transport was not increased on amino acid starvation, and leucine is not known to be a substrate for System A in any cell type.

System A activity has been shown to be induced by amino acid starvation in many types of cells. Some of the characteristics of induction shown in NBL-1 cells are similar to those observed previously in other cell types (see Shotwell *et al.*, 1983). In hepatocytes, as in NBL-1 cells, induction is inhibited by cycloheximide and by tunicamycin and does not occur in the presence of single substrates of System A (see Kilberg *et al.*, 1985). The time course of induction in NBL-1 cells is however slower, with a longer lag period than in hepatocytes (Barber *et al.*, 1983) or fibroblasts (Gazzola *et al.*, 1981). In all of these previously studied cell types, the response to amino acid deprivation is brought about by an increased synthesis of a transport system which is already active in the cell membrane. In confluent NBL-1 cells in amino-acid-containing medium, Na^+ -dependent amino acid transport is already well provided for by the existence of System B^o. Amino acid starvation results in the induction, not of this pre-existing system, but of System A, which was previously totally repressed. These findings suggest that these two transport systems are differently regulated at the gene level in these cells.

Adaptive regulation of methyl-AIB uptake has been reported in the MDCK renal epithelial cell line (Boerner & Saier, 1985). In that study non-confluent cells were used, and it was noted that little induction occurred in confluent monolayers. The exact identity of the transport system induced was not clearly defined. In MDCK cells the rate of methyl-AIB uptake (0.1 mM) was only 5% of that of 0.1 mM-alanine. Methyl-AIB uptake was abolished by an excess of alanine, but alanine uptake (0.1 mM) was not inhibited by an excess of methyl-AIB (Boerner & Saier, 1982).

Fig. 5. Incorporation of [^3H]mannose into membrane glycoproteins in NBL-1 cells

Cells were incubated for 24 h in amino-acid-free or control medium in the presence or absence of 0.025 μg of tunicamycin/ml. Membrane samples were processed and fractionated by SDS/PAGE as described in the Materials and methods section. The values shown are the percentages of total radioactivity recovered from the gel that were found in each slice. Values are the means $\pm$ S.E.M. of five different cell preparations. The gels for each experiment were closely similar; details of the gels are as follows. The stacking gel/separating gel interface was at the origin. A 100 μg sample of protein was added to each track. This corresponded to 22000–27000 c.p.m. for (b), (c) and (d), and 40000–45000 c.p.m. for (a). The counts were corrected for background (approx. 30 c.p.m.). The gels were calibrated using proteins of known molecular mass. The molecular mass values of the markers and their location in a typical gel were as follows: 200 kDa, slice 2; 116 kDa, slice 6; 97 kDa, slice 7; 66 kDa, slice 11; 44 kDa, slice 15; 35 kDa, slice 17. The dye front is located just after slice 20. (a) Amino-acid-starved cells, no tunicamycin; (b) amino-acid-starved cells + tunicamycin; (c) control cells, no tunicamycin; (d) control cells + tunicamycin. The molecular mass range of the slice containing the labelled band in (a) had a mean value of 113–140 kDa.

The relationship of these findings to the present results is not clear.

Various approaches have been used to attempt to identify the protein responsible for System A transport activity. System A activity has been reconstituted into artificial proteoliposomes after extraction from Ehrlich ascites cells (McCormick *et al.*, 1984) and liver plasma membranes (Bracy *et al.*, 1987; Quesada & McGivan, 1988; Fafournoux *et al.*, 1989). System A transport activity has been expressed in *Xenopus* oocytes after injection of mRNA fractions from rat liver (Tarnuzzer *et al.*, 1990; Palacin *et al.*, 1990), but the molecular identity of the protein has not been established. McCormick & Johnstone (1988) isolated a protein fraction with a major component of 120–130 kDa from Ehrlich ascites cells which exhibited System A activity on reconstitution. Polyclonal antibodies to this 120–130 kDa protein fraction were shown to immunoprecipitate System A activity from solubilized membranes. Recently it was shown that radiation inactivation of System A activity in Ehrlich cell plasma membranes could be correlated with fragmentation of a 120–130 kDa protein component (McCormick *et al.*, 1991). It

was concluded that this peptide is a component of System A activity. However, this tentative identification has not so far been confirmed in other membranes.

Induction of System A activity in NBL-1 cells was inhibited by tunicamycin, indicating that the protein responsible for this activity is a glycoprotein, as is the case in liver (Barber *et al.*, 1983; Quesada & McGivan, 1988). The fact that System A activity is induced from an undetectable level in confluent NBL-1 cells suggests that induction of System A activity should be accompanied by detectable incorporation of labelled mannose into the newly synthesized membrane transport proteins. This approach has recently been used to identify the phosphate transport system of NBL-1 cells as a 55 kDa glycoprotein (Helps & McGivan, 1991).

The results in the present paper show that the major single membrane protein labelled with [³H]mannose on amino acid starvation has a molecular mass in the range 113–140 kDa. Although there is no direct evidence that this glycoprotein is responsible for System A transport activity, the results are consistent with the identification of System A in NBL-1 cells as a glycoprotein with a molecular mass within this range. This agrees very well with the previously suggested identity of the System A protein in Ehrlich ascites cells (McCormick & Johnstone, 1988).

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