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Stimulation of hexose transport in L6 rat myoblasts by antibody and by glucose starvation

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Treatment of glucose-grown L6 rat myoblasts with rabbit or sheep anti-(L6-rat myoblast) antibody for 35 min or glucose starvation for at least 8 h results in a 2-fold increase in the $V_{max.}$ of 2-deoxy-D-glucose (dGlc) and 3-*O*-methyl-D-glucose uptake. In both cases, apparent transport affinities were not affected. Furthermore, once stimulation has occurred, further increases in hexose uptake could not be produced. Assays of antibody binding to whole cells suggested that the antibody is not internalized but remains bound on the cell surface. To elucidate the site and mechanism of antibody action, plasma-membrane vesicles from L6 cells were prepared. Anti-L6 antibody was found to cause a time- and dosage-dependent stimulation of dGlc transport in these vesicles. Maximum activation was achieved after 30 min exposure. This antibody-mediated activation could be inhibited by treatment of vesicles with various proteinase inhibitors. Treatment of vesicles with trypsin was also found to activate dGlc transport to levels observed with antibody. These results are virtually identical with those obtained with whole cells and suggest that antibody-mediated activation of hexose transport results from interaction of antibody with a specific membrane component(s).

INTRODUCTION

The hexose-transport system of the L6 muscle-cell line has been the object of recent investigations (Klip *et al.*, 1982; Lo & Duronio, 1984*a*,*b*; D'Amore & Lo, 1986*a*,*b*). Hexose transport, not phosphorylation, was demonstrated to be the rate-limiting step of hexose uptake into L6 myoblasts (Klip *et al.*, 1982; D'Amore & Lo, 1986*a*). Furthermore, our results suggest that two hexose-transport systems may be operating in these cells (D'Amore & Lo, 1986*a*,*b*; D'Amore *et al.*, 1986). 2-Deoxy-D-glucose (dGlc) was demonstrated to be transported by both high- and low-affinity systems, whereas 3-*O*-methyl-D-glucose (MeGlc) was transported predominantly by the low-affinity system.

We have previously observed that hexose transport in glucose-grown L6 rat myoblasts can be activated by treatment of cells with anti-L6 antibody; however, treatment with pre-immune IgG has no effect (Lo & Duronio, 1984*a*). This elevated transport activity is due to an increase in transport capacity. Furthermore, this increase in transport capacity occurs within 15 min after the addition of antibody and is independent of protein synthesis. Antibody treatment does not result in changes in cell volume, membrane permeability, hexokinase activity or in hexose-transport specificity and affinity (Lo & Duronio, 1984*a*). In addition, treatment of whole cells with IgG fragments and with various inhibitors suggests that dimerization of cell-surface receptors, functioning of microfilaments, transglutaminase and membrane-associated proteinases may be involved in the activation process (Lo & Duronio, 1984*b*).

Glucose starvation has also been shown to activate hexose transport in L6 myoblasts (D'Amore & Lo, 1986*a*), as well as in other cell lines (Ullrey *et al.*, 1975; Christopher *et al.*, 1976*a*; Rapaport *et al.*, 1979; Gay & Hilf, 1980). This increased rate of hexose transport is due to an increase in the number of hexose carriers operating in the plasma membrane (D'Amore & Lo, 1986*a*; Christopher *et al.*, 1976*a*,*b*). Furthermore, this activation was found to require protein synthesis (Christopher *et al.*, 1976*a*,*b*).

In the present paper we compare the effect of antibody treatment and glucose starvation on the kinetics of hexose transport in L6 cells. Furthermore, in an attempt to elucidate the mechanism of antibody stimulation, the effect of specific antibody on hexose transport in plasma-membrane vesicles will also be described.

MATERIALS AND METHODS

Materials

2-Deoxy-D-[G-^{3}H]glucose (8.3 Ci/mmol) and 3-*O*-[*methyl*-^{3}H]methyl-D-glucose (60–90 Ci/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). HP/b scintillation fluid was from Beckman Instruments (Irvine, CA, U.S.A.). All other chemicals were obtained from commercial sources and were of the highest available purity.

Methods

Cell line and culture media. Yaffe's L6 rat skeletal myoblast line (Yaffe, 1968) was maintained in Alpha medium (Flow Laboratories) supplemented with 10%

Abbreviations used: dGlc, 2-deoxy-D-glucose; MeGlc, 3-*O*-methyl-D-glucose; PBS, phosphate-buffered saline (150 mM-NaCl/80 mM-Na_2HPO_4/15 mM-KH_2PO_4/30 mM KCl, pH 7.4; Tos-Lys-CH_2Cl, tosyl-lysylchloromethane ('TLCK'); PMSF, phenylmethanesulphonyl fluoride.

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(v/v) horse serum and 50 μg of gentamycin/ml as previously described (Lo & Duronio, 1984*a*). Transfers were made every 3 days (before fusion) by using 0.1% trypsin to dissociate attached cells. Cells were counted by using a Coulter counter. In experiments in which cells were grown under glucose-free conditions, the same medium was prepared without glucose and supplemented with horse serum that had been dialysed extensively against PBS. The medium was also supplemented with 0.1% fructose.

Preparation of antibody. Rabbit or sheep anti-L6 rat myoblast antibody were prepared against whole cells of L6 and IgG isolated as described previously (Lo & Duronio, 1984*a*). Rabbit IgG was also prepared from a pre-immunized animal as described previously (Lo & Duronio, 1984*a*).

Transport studies in whole cells. Cells were grown for 2 days in six-well Costar plates (35 mm × 15 mm) seeded with 10^5 cells per well [yielding about (2–2.5) × 10^5 cells per well]. Medium was aspirated and each well was washed with 10 ml of PBS. A 900 μl portion of uptake buffer (PBS containing 1 mg of bovine serum albumin/ml) was added to each well. Transport studies were carried out at 23 °C and were initiated by addition of 100 μl of radioactive substrate. After appropriate times, the uptake was terminated by washing the cells twice with ice-cold PBS containing 1 mM-$HgCl_2$ to prevent the efflux of free sugar. Unless otherwise stated, 1 min uptake assays were performed as described previously (D'Amore & Lo, 1986*a*). L6 myoblasts were treated with specific antibodies for 35 min before the transport assays as described elsewhere (Lo & Duronio, 1984*a*).

Transport in plasma-membrane vesicles. Plasma-membrane vesicles were isolated and purified from L6 cells by a previously described method (Cheung & Lo, 1984). Hexose transport into vesicles was studied by the flow-dialysis method as described previously (Cheung & Lo, 1984). Briefly, the apparatus consisted of a Perspex (Lucite) block containing two chambers separated by a dialysis membrane. The upper chamber contained 0.5 ml of the running buffer (50 mM-sodium phosphate, pH 7.0) and the lower chamber was filled with running buffer (1 ml). Exactly 30 s after the addition of radioactive substrate to the top chamber, buffer was pumped through the lower chamber at a constant flow rate of 4 ml/min. Fractions (0.55 ml) were collected directly into scintillation vials. At the beginning of fraction 20, 20 μl of membrane vesicles (0.5–7.5 mg of protein/ml) were added to the upper chamber and 15 more fractions collected. Changes in free substrate concentration in the top chamber were continuously monitored by measuring the radioactivity in the lower chamber. Transport rates and the amount of non-specific binding were determined as described previously (Cheung & Lo, 1984).

Antibody-binding assay. Cells were grown in six-well plates as described for the transport studies. The cells were washed twice with ice-cold PBS and 900 μl of PBS was added to each well. The plates were kept at 4 °C throughout the assay. A 0.1 ml portion of rabbit anti-L6 antibody (0.5 mg of protein) was then added to each well and allowed to incubate. At various time intervals, the buffer was aspirated off and the cells washed twice with PBS. The cells were then incubated with 1 ml of iodinated protein A (1.5 × 10^5 c.p.m.) in PBS at 4 °C for 30 min. The cells were washed again with PBS (three times) and released from the plates by incubating with 1 ml of trypsin for 5 min. Radioactivity bound per well was determined by counting a portion in a scintillation counter. Background radioactivity was determined by incubating the cells with the same amount of rabbit IgG taken from a pre-immunized animal. This background level was subtracted in order to obtain the amount specifically bound.

Antibody internalization assay. Rabbit anti-L6 antibody was allowed to bind to cells grown in six-well plates at 4 °C for 30 min. The plates were then washed and regular growth medium was added back. The cells were incubated for various lengths of times at 37 °C, after which they were washed three times with cold PBS. Iodinated protein A was added to the cells and allowed to incubate for 30 min at 4 °C. The amount of radioactivity in the wells and the background radioactivity were then determined as described in the binding assays.

Protein determination. Protein determinations were made by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

RESULTS

Stimulation of hexose uptake by glucose starvation

Stimulation of hexose uptake as a result of glucose starvation has been reported in a number of systems (Ullrey *et al.*, 1975; Christopher *et al.*, 1976*a*; Rapaport *et al.*, 1979; Gay & Hilf, 1980; D'Amore & Lo, 1986*a*). In order to monitor the time required for this increase in transport activity to occur in L6 cells, the growth medium was changed from normal to glucose-free. Preliminary studies indicated that an increase in transport occurred within 2 h, as observed in other systems (Rapaport *et al.*, 1979; Gay & Hilf, 1980). In these previous studies, however, the medium from the control cells was not changed at the same time as were those receiving the glucose-free medium.

In order to eliminate the effect of simply adding fresh medium, the control experiment included replacing the medium with normal (glucose-containing) medium instead of glucose-free medium. Fig. 1 shows the difference in transport activity in glucose-free and normal media. The results indicate that increases in transport activity due to glucose starvation do not occur until at least 8 h after the removal of glucose. The previous observation that stimulation was observed as early as 2 h is likely due to the presence of fresh serum, which has been shown to stimulate the growth of L6, as well as other, cells (Florini *et al.*, 1977).

Antibody activation of hexose uptake

We have previously demonstrated that treatment of glucose-grown L6 cells with sheep anti-L6 antibody also activates dGlc uptake, whereas treatment with pre-immune IgG has no effect on this transport process (Lo & Duronio, 1984*a*,*b*). Antibody activation occurs within 15 min and reaches a maximum value after 35 min. Furthermore, no apparent morphological changes could be detected up to 4 h after treatment. Studies have now

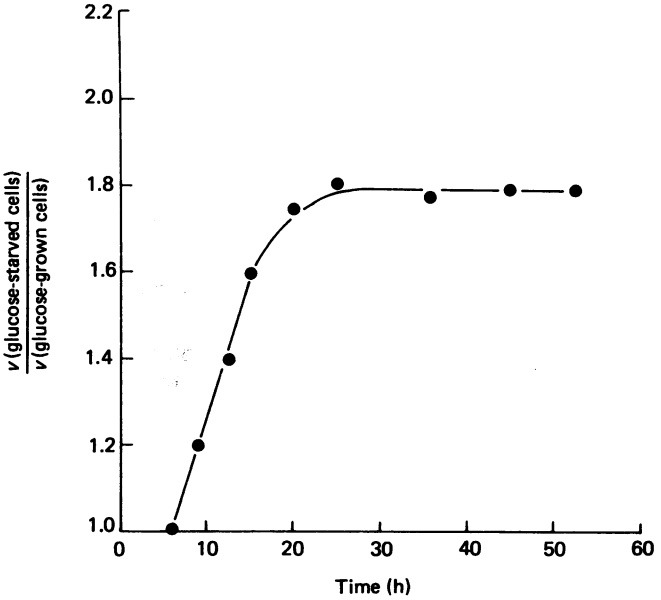

Fig. 1. Effect of glucose starvation on dGlc uptake in rat myoblasts

Transport studies were carried out with 0.06 mM-dGlc (specific radioactivity 8.4 mCi/mmol) as described in the Materials and methods section. Six-well Costar plates were seeded with 10^5 cells per well in normal growth medium and grown for 2 days. At various times before transport assays were performed, the medium was changed to normal medium or glucose-free medium supplemented with 0.1% fructose. The Figure is a plot of the rates of dGlc uptake in glucose-grown cells versus the time after media change.

been extended to determine the effect of anti-L6 antibody on the kinetics of dGlc and MeGlc uptake in both glucose- and fructose-grown cells. Since hexose transport, not phosphorylation, was demonstrated to be the rate-limiting step of the uptake process, kinetics of hexose uptake could be investigated (D'Amore *et al.*, 1986*a*,*b*). It is evident from Table 1 that antibody treatment significantly increases the $V_{max.}$ of dGlc and MeGlc uptake in glucose-grown cells. In fact, the transport capacity for both sugars is approximately doubled. On the other hand, $V_{max.}$ values from fructose-grown cells are not significantly altered. In all cases, the apparent transport affinities were not affected by antibody. It should be noted that the concentration range used for dGlc uptake reflects the high-affinity system, whereas the concentration range used for MeGlc uptake reflects the low-affinity system (D'Amore & Lo, 1986*a*).

Table 1 also shows that the $V_{max.}$ values for dGlc and MeGlc uptake are increased in glucose-starved cells (i.e. fructose-grown). The ratio of the $V_{max.}$ values in fructose- and glucose-grown cells is virtually constant (1.6–1.8) and is similar to the extent of activation by antibody. Again, the apparent transport affinities remain the same. Thus treatment of L6 cells with specific antibody or glucose starvation have similar effects of stimulating hexose transport.

Antibody binding to whole cells

The specific binding of rabbit anti-L6 antibody to whole cells is shown in Fig. 2(*a*). This binding was determined with iodinated protein A, which does not penetrate the membrane. It is apparent that binding occurs very rapidly and reaches a maximum level within 10 min. This experiment was carried out at 4 °C to ensure that there was no internalization of antibody. This was found to be unnecessary, since it was observed that antibody was not internalized into whole cells (Fig. 2*b*). The first point in Fig. 2(*b*) is at 1 min after the addition of growth medium and represents the amount of antibody present at the cell surface after the initial 30 min preincubation. At times up to 3 h after the addition of medium, the amount of antibody present at the cell surface was virtually the same. Thus the antibody is most likely bound to determinants on the cell surface. Similar results were observed with rabbit anti-(L6 plasma-membrane-vesicle) antibody (results not shown).

Antibody activation of hexose transport in membrane vesicles

To determine the site of antibody activation, the effect of antibody on hexose transport in plasma-membrane vesicles was also investigated. Vesicles were treated with a fixed amount of specific antibody for various lengths of time and the rates of hexose influx were determined. Fig. 3(*a*) shows that stimulation of dGlc influx occurred within 10 min. Furthermore, the transport rates by

Table 1. Effect of antibody on dGlc and MeGlc uptake in glucose- and fructose-grown L6 cells

Cells were grown in six-well Costar plates for 2 days [yielding (2–2.5) × 10^5 cells/well]. Cells were washed and then incubated with 1 ml of PBS with or without sheep anti-(L6 myoblast) IgG (0.5 mg of protein) for 35 min. Wells were then washed with 10 ml of PBS and transport studies carried out as described in the Materials and methods section. Concentrations ranges used were 0.06–1.0 mM for dGlc uptake and 1.0–10 mM for MeGlc uptake. The results represent averages for four independent trials.

Uptake substrate	Growth substrate	Antibody treatment	K_m (mM)	$V_{max.}$ (pmol/min per 10^5 cells)
dGlc	Glucose	–	0.6	235
		+	0.5	456
	Fructose	–	0.6	396
		+	0.7	427
MeGlc	Glucose	–	3.5	357
		+	4.2	714
	Fructose	–	3.6	596
		+	4.4	680

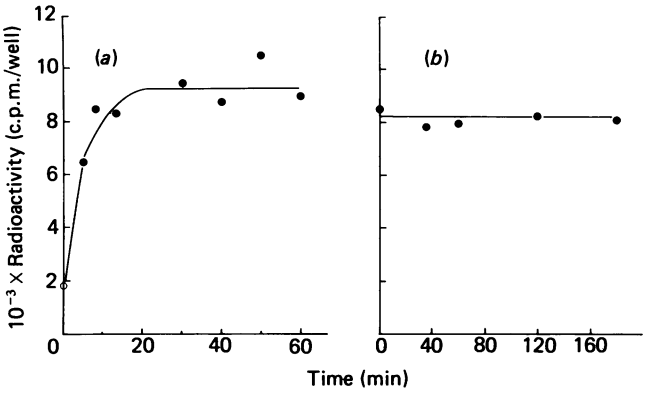

Fig. 2. Antibody binding to whole cells

Cells were grown in six-well Costar plates on normal medium for 2 days. The cells were washed twice with ice-cold PBS and 900 μl of PBS was added to each well. A 0.1 ml portion of rabbit anti-L6 antibody (0.5 mg of protein) was then added to each well and allowed to incubate at 4 °C. (*a*) At various time intervals, the buffer was aspirated off and the cells washed twice with PBS. (*b*) After 30 min incubation, the plates were washed with PBS and regular growth medium was added back. The cells were incubated for various lengths of time at 37 °C, after which they were washed three times with cold PBS. The amount of IgG binding in both cases was determined by adding 1 ml of iodinated protein A (1.5×10^5 c.p.m.) in PBS to each well and incubating at 4 °C for 30 min. Background radioactivity was determined by incubating the cells with the same amount of IgG taken from a pre-immunized animal.

antibody-treated vesicles reached a maximum level of about twice that by control vesicles or vesicles treated with pre-immune IgG after 30 min exposure. To determine the relationship between dGlc transport and the dosage of antibody, vesicles were exposed for 35 min to various amounts of antibody before assaying for transport activity. Fig. 3(*b*) show that there is a direct correlation between the rate of dGlc transport and the amount of antibody added. Maximum stimulation occurred when 14 μg of protein of vesicles was treated with about 70 μg of protein of antibody. Again, virtually no effect was observed if cells were incubated for 35 min in buffer or with pre-immune IgG. A similar pattern of activation was observed in whole cells (Lo & Duronio, 1984*a*,*b*).

Effect of proteinase inhibitors and amines on hexose transport in membrane vesicles

We previously reported that pretreatment of whole cells with NH_4Cl, methylamine and proteinase inhibitors resulted in an inhibiton of antibody-mediated activation of dGlc uptake (Lo & Duronio, 1984*b*). This inhibition was not effective if these reagents were applied after the cells had been exposed to antibody. To determine whether a similar effect on the antibody-mediated activation of hexose transport occurs in membrane vesicles, vesicles were pretreated with these reagents before exposure to antibody. Table 2 summarizes these results. It can be seen that dGlc-transport activation by antibody is completely abolished by pretreatment with Tos-Lys-CH_2Cl and leupeptin. PMSF, NH_4Cl and methylamine had a lesser, but significant, inhibitory effect on the activation process. It should be noted that these reagents alone had no effect on hexose transport in whole cells (Lo & Duronio, 1984*b*) or in plasma-membrane vesicles (results not shown) and that proteolytic activity could not be detected in the antibody preparations (Lo & Duronio, 1984*a*). These results suggest that activation of hexose transport may be brought about by an endogenous membrane-associated protease.

Effect of trypsin on hexose transport in membrane vesicles

In order to test the hypothesis that limited proteolysis may be involved in the activation of hexose transport, vesicles were treated for 35 min with various amounts of trypsin before the transport assay. Fig. 4 shows that

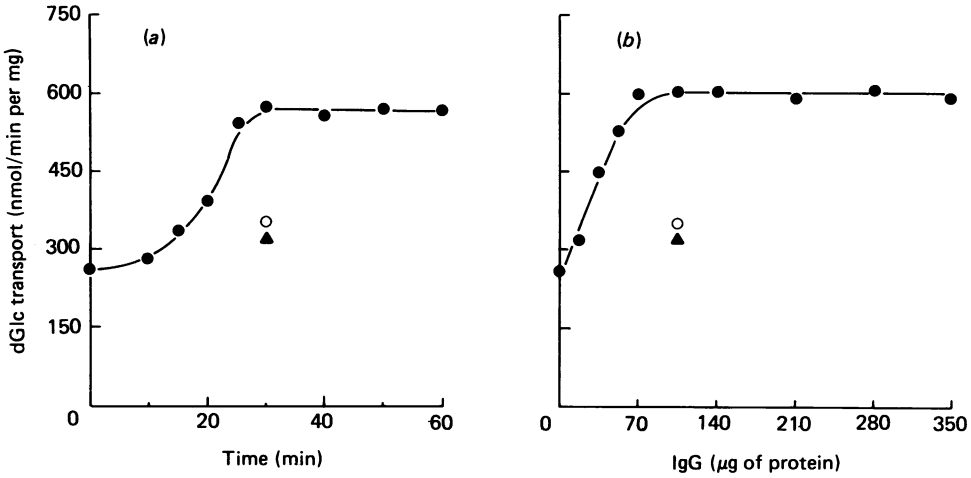

Fig. 3. Effect of antibody on dGlc transport in plasma-membrane vesicles

(*a*) Vesicles (14 μg of protein) were treated with sheep anti-L6 antibody (70 μg of protein) for various lengths of time before the transport assays. (*b*) Vesicles (14 μg of protein) were treated with various amounts of antibody for 35 min before the transport assays. In both cases, ● represents the transport activity by vesicles incubated with anti-L6 antibody, ▲ represents the level of transport by vesicles incubated with buffer alone, and ○ represents the level of transport by vesicles incubated with IgG from a pre-immunized animal. The concentration of [^{3}H]dGlc used was 500 μM (specific radioactivity 16 mCi/mmol). Transport rates were determined as described in the Materials and methods section.

Table 2. Effect of proteinase inhibitors and amines on the antibody-mediated activation of dGlc transport in membrane vesicles

The concentration of [^{3}H]dGlc used was 0.5 mM with a specific radioactivity of 16 mCi/mmol. Membrane vesicles (14 μg of protein) were first incubated with or without inhibitors for 15 min followed by sheep anti-L6 antibody (70 μg of protein) for 35 min before addition to the top chamber. The concentrations of inhibitors used were: PMSF, 0.003%; Tos-Lys-CH_2Cl, 0.1 mM; leupeptin, 0.5 mg/ml; NH_4Cl and methylamine, 30 mM. The control consisted of membrane vesicles mixed with IgG and added to the top chamber immediately. These reagents alone were observed to have no effect on whole cell hexose transport (Lo & Duronio, 1984*b*). Transport rates were determined as described in the Materials and methods section. The results are averages for at least four trials.

Treatment	*v* (nmol/min per mg of protein)	*v* as % of control
Control	263	100
IgG	502	191
PMSF + IgG	373	142
Tos-Lys-CH_2Cl + IgG	250	95
Leupeptin + IgG	248	94
NH_4Cl (+ IgG)	398	151
Methylamine + IgG	362	138

exposure of vesicles to 0.1–0.5 μg of trypsin/ml resulted in a 2-fold increase in transport activity. Higher trypsin concentrations resulted in a decrease in transport activity, probably the result of extensive proteolysis of the transporters. These results are very similar to those obtained with whole cells (Lo & Duronio, 1984*b*).

DISCUSSION

Activation of hexose transport has been observed in a number of mammalian cells treated with various surface-active reagents, such as specific antibodies (Jacobs *et al.*, 1978; Pillion & Czech, 1978; Lo & Duronio, 1984*a*,*b*), lectins (Czech *et al.*, 1974), proteinases (Kono & Barham, 1971), tumour promoters (Nordenberg *et al.*, 1983) and peptide hormones (Carpenter & Cohen, 1979; Czech, 1981). This activation of hexose transport usually occurs within 15–20 min after the addition of the surface-reactive agents and in the absence of protein synthesis. About 2–3-fold increases in transport capacity are usually observed, and changes in transport affinity cannot be detected. At the moment, not much is known about the activation mechanism.

Treatment of glucose-grown whole cells with either sheep or rabbit anti-L6 antibody or glucose starvation was observed to cause a 2-fold stimulation in dGlc and MeGlc uptake with no change in affinities (Table 1). The concentration ranges selected were such that dGlc uptake reflected the high-affinity system and MeGlc uptake reflected the low-affinity system (D'Amore & Lo, 1986*a*). Therefore antibody treatment or glucose starvation was found to activate the high- and low-affinity transport systems. Further increases of hexose uptake in these glucose-starved or antibody-treated cells by reciprocal treatment with antibody or glucose starvation could not

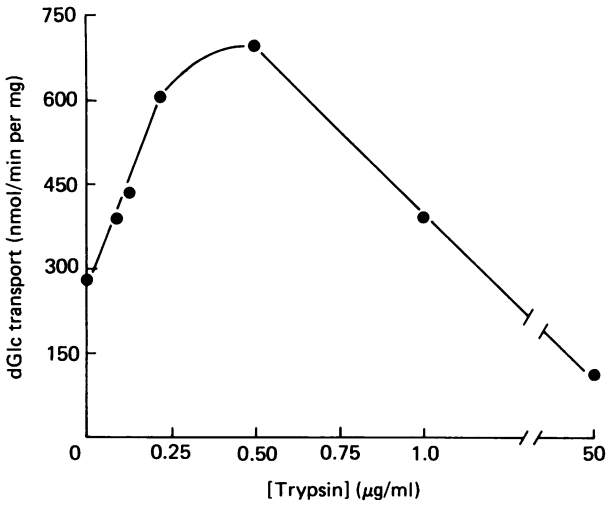

Fig. 4. Effect of trypsin on dGlc transport in vesicles

Vesicles (20 μg of protein) were treated with various amounts of trypsin for 35 min before the transport assays. The concentration of [^{3}H]dGlc used was 500 μM (specific radioactivity 16 mCi/mmol). Transport rates were determined as described in the Materials and methods section.

be observed. Thus, once stimulation of hexose uptake has occurred, further stimulation cannot be produced. This indicates that maximum activation of hexose uptake is achieved by antibody treatment or by glucose starvation.

Although antibody treatment and glucose starvation have the same end result of stimulating hexose transport, the mechanism of activation does not appear to be similar. For example, antibody-mediated activation occurred within 15 min after exposure to antibody, whereas cells had to be starved for glucose at least 8 h to observe a similar increase. Furthermore, antibody-mediated activation is independent of protein synthesis (Lo & Duronio, 1984*a*,*b*), whereas stimulation of transport by glucose starvation is dependent on protein synthesis (Christopher *et al.*, 1976*a*,*b*; T. D'Amore & C. Y. Lo, unpublished work). Although the mechanism of glucose-transport regulation has not been elucidated, the stimulation of hexose uptake by glucose starvation appears largely due to decreased carrier inactivation in the face of continued carrier synthesis (Yamada *et al.*, 1983; Tillotson *et al.*, 1984).

On the other hand, we have recently isolated a mutant in which the hexose-transport system cannot be stimulated by either antibody treatment or glucose starvation. In addition, the kinetic properties of both the high- and low-affinity transport systems in this mutant are not altered (T. D'Amore & T. C. Y. Lo, unpublished work). Preliminary results indicate that this mutant has much decreased amounts of a 112 kDa membrane-associated protein. Thus this mutant may be of value in establishing the mechanism of antibody- and glucose-starvation-mediated stimulation of hexose transport.

Our whole-cell studies suggest that the mechanism of antibody-mediated stimulation involves activation of some membrane-associated proteinase(s). This proteinase may act by conversion of an inactive form of the carrier into the active form (Lo & Duronio, 1984*b*). Whole-cell studies cannot provide definitive information on the mechanism of this activation process. This is because the

interpretation of the results is complicated by various metabolic events, such as the possible involvement of chemical mediators, recruitment of cytosolic transport components, protein synthesis and phosphorylation, internalization of antibody–receptor complex, among others. We therefore used plasma-membrane vesicles from L6 cells to study the antibody-mediated activation of hexose transport. These vesicles are devoid of ATP, hexokinase, marker enzymes for the cytosol, mitochondria and microsomes. These sealed right-side-out vesicles exhibit hexose-transport properties similar to those of whole cells (Cheung & Lo, 1984).

Treatment of purified plasma-membrane vesicles from glucose-grown L6 cells with anti-L6 antibody results in a 2-fold activation of dGlc transport in a time- and dosage-dependent fashion (Fig. 3). Transport kinetics reveal that the K_m and $V_{max.}$ values of dGlc transport in untreated membrane vesicles are 0.6 mM and 333 nmol/min per mg of protein respectively. Treatment of these vesicles with anti-L6 antibody results in K_m and $V_{max.}$ values of 0.6 mM and 830 nmol/min per mg of protein respectively (M. O. Cheung & T. C. Y. Lo, unpublished work). Thus antibody treatment of membrane vesicles increases the $V_{max.}$ of the high-affinity transport system, with no change in K_m, as in whole cells. At the time, the effect of antibody on the low-affinity transport system in vesicles has not been tested, although it would be expected to be activated as in whole cells. As with whole cells, vesicles prepared from glucose-starved cells also exhibited higher rates of hexose transport (results not shown). It should be noted that treatment of whole cells (Lo & Duronio, 1984*a*) or plasma-membrane vesicles (Fig. 3) with pre-immune IgG has no effect on hexose transport. This demonstrates that activation results from interaction with specific antibody. Furthermore, these results demonstrate that vesicles could be used to study the mechanism of antibody-mediated activation. In addition, these results indicate that only a plasma-membrane component(s) is involved in the activation process and precludes the possible involvement of recruitment or internalization in this activation process.

Studies with various proteinase inhibitors suggest that proteolytic cleavage of a plasma membrane component(s) leads to activation of hexose transport (Table 2). In support of this, it was observed that treatment of vesicles with low concentrations of trypsin also leads to activation of hexose transport (Fig. 4). These results are in excellent agreement with those obtained in the whole-cell studies (Lo & Duronio, 1984*a*,*b*). It is unlikely, for several reasons, that the anti-L6 antibody fraction contains a contaminating proteinase. First of all, no activation of hexose transport was observed in cells or vesicles treated with IgG obtained from a pre-immunized animal. Secondly, and more importantly, proteinase activity could not be detected in the anti-L6 antibody preparation (Lo & Duronio, 1984*b*).

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In conclusion, the present results indicate that hexose transport in L6 cells can be activated by treatment with specific antibody or by glucose starvation. Our results indicate that antibody-mediated activation occurs by specific interaction of the antibody with a membrane-associated component(s). This interaction may result in a proteolytic event that causes the conversion of an inactive form of the carrier into the active form.

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