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Dinitrophenyl glutathione efflux from human erythrocytes is primary active ATP-dependent transport

Edward F. LABELLE, Shivendra V. SINGH, Satish K. SRIVASTAVA and Yogesh C. AWASTHI*
Department of Human Biological Chemistry and Genetics, The University of Texas Medical Branch, Galveston, TX 77550, U.S.A.

Dinitrophenyl *S*-glutathione is accumulated by inside-out vesicles made from human erythrocytes in a process totally dependent on ATP and Mg^{2+}. The vesicles were shown to accumulate dinitrophenyl *S*-glutathione against a concentration gradient. The vesicles were able to concentrate this glutathione derivative even in the absence of membrane potential. This indicated that the ATP-dependent uptake of dinitrophenyl *S*-glutathione by inside-out vesicles represented an active transport process. Neither extravesicular EGTA nor intravesicular ouabain inhibited the transport process, indicating that neither the Ca^{2+}-ATPase nor the Na^+,K^+-ATPase were involved. These results indicated that dinitrophenyl *S*-glutathione uptake by inside-out vesicles probably represented primary active transport. The uptake of dinitrophenyl *S*-glutathione was a linear function of time (up to 5 h) and vesicle protein. The rate of uptake was optimal between pH 7.0 and 8.0 and at 37 °C. The K_m values determined for dinitrophenyl *S*-glutathione and ATP were 0.29 mM and 1 mM, respectively. The transport process was completely inhibited by vanadate and by *p*-hydroxymercuribenzene sulphonate and inhibited to a lesser extent by *N*-ethylmaleimide. GTP could efficiently substitute for ATP as an energy source for the transport process, but CTP and UTP were comparatively much less effective.

INTRODUCTION

The reduced form of the tripeptide glutathione (GSH) exists in rather high amounts (2–10 mM) in most mammalian cells (Beutler, 1983). It has been implicated in the protection of cells from oxidative damage (Cohen & Hochstein, 1963; Srivastava *et al.*, 1980). Many cells, such as hepatocytes and erythrocytes contain glutathione *S*-transferases that permit GSH to conjugate covalently with a variety of xenobiotics such as 1-chloro-2,4-dinitrobenzene (Booth *et al.*, 1961; Boyland & Chasseaud, 1969; Marcus *et al.*, 1978). This conjugation process renders such toxic compounds water-soluble and facilitates their removal from the organism. Transport systems have been demonstrated in the plasma membrane of erythrocytes, hepatocytes and cardiac cells that facilitate the efflux of glutathione–xenobiotic conjugates from these cells (Board, 1981; Akerboom *et al.*, 1982; Awasthi *et al.*, 1983; Ishikawa & Sies, 1984; Ishikawa *et al.*, 1986). Evidence exists that such a system may be similar to the system that is responsible for the active ATP-dependent efflux of oxidized glutathione (GSSG) from rabbit lens and human erythrocyte (Srivastava & Beutler, 1968*a*,*b*, 1969*a*,*b*; Kondo *et al.*, 1980, 1981, 1982). The glutathione *S*-conjugate Dnp-SG can be transported into inside-out vesicles from erythrocytes in an ATP-dependent manner (Kondo *et al.*, 1982). The purpose of the current study was to characterize further the transport of Dnp-SG into erythrocyte inside-out vesicles with respect to molecular mechanism. The present studies demonstrate that the ATP-dependent uptake of Dnp-SG into inside-out vesicles from erythrocytes represents primary active transport that is not dependent on any ion gradients produced by either the Na^+,K^+-ATPase or the Ca^{2+}-ATPase.

EXPERIMENTAL

Materials

Dextran T70 was obtained from Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A. [^{3}H]Glutathione was obtained from New England Nuclear, Boston, MA, U.S.A. Silica gel K5 t.l.c. plates were obtained from Whatman, Clifton, NJ, U.S.A., and 1-chloro-2,4-dinitrobenzene, GSH, GSSG, Dowex 1 X8-100, ATP, Sephadex G-50 (fine), Triton X-100, EGTA, GTP, CTP and UTP were all purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Preparation of dinitrophenyl *S*-[^{3}H]glutathione ([^{3}H]Dnp-SG)

[^{3}H]Dnp-SG was synthesized enzymically according to our previously described method (Awasthi *et al.*, 1981) except that, in this case, [^{3}H]glutathione and a homogenous preparation of the anionic glutathione *S*-transferase from human erythrocytes (Awasthi & Singh, 1984) were used.

Preparation of inside-out vesicles from human erythrocytes

Inside-out vesicles from human erythrocytes were prepared by a slight modification of the method of Steck & Kant (1974). Dextran T-70 was substituted for Dextran T-110 and the centrifugation over the Dextran barrier solution was performed for 3 h at 28000 rev./min in a Spinco SW 28 rotor. The protein concentration of the inside-out vesicles was determined by the method of Bradford (1976). The accessibility of the inside-out vesicles to neuraminidase was determined by the method of Steck & Kant (1974). The amount of *N*-acetylneuraminic acid released from the inside-out

Abbreviation used: Dnp-SG, 2,4-dinitrophenyl *S*-glutathione.

* To whom reprint requests should be addressed.

vesicles was increased by 170% upon pretreatment with detergent, indicating that the vesicle preparation contained about 62% sealed inside-out vesicles (Steck & Kant, 1974).

[^{3}H]Dnp-SG transport into vesicles

The uptake of [^{3}H]Dnp-SG into the inside-out vesicles from erythrocytes was measured by the Sephadex centrifugation procedure (Penefsky, 1977). A 1 ml syringe barrel (5 mm internal diam.) was plugged with glass wool and filled with Sephadex G-50 (40–80 mesh) previously swollen in a solution of NaCl (150 mM), $MgCl_2$ (2 mM), and Tris/HCl (10 mM, pH 7.4). The syringe barrel was placed in a test tube and centrifuged for 2 min at 800 *g*. An aliquot (100–200 μl) of the reaction mixture containing inside-out vesicles, [^{3}H]Dnp-SG and other compounds was applied to the top of the Sephadex in the syringe barrel and the centrifugation was repeated as above. The ^{3}H content and the protein content of the effluent were determined after brief sonication. In a typical experiment about 50% of the membrane vesicles applied to each Sephadex column were eluted during the centrifugation, and over 99.9% of the extravesicular [^{3}H]Dnp-SG was retained by the column.

The uptake of [^{3}H]Dnp-SG into the inside-out vesicles was also determined by the Dowex column chromatography and centrifugation procedures described previously (LaBelle & Valentine, 1980).

3-*O*-Methyl[^{3}H]glucose uptake into vesicles

The uptake of 3-*O*-methyl[^{3}H]glucose into inside-out vesicles from erythrocytes was measured by the Sephadex centrifugation procedure. The vesicles (0.45 mg of protein) were incubated at 25 °C with 3-*O*-methyl[^{3}H]-glucose (5 μCi/0.16 mM) and sodium phosphate buffer (0.5 mM, pH 7.0) in a total volume of 0.72 ml for increasing periods of time (up to 60 min) and then 0.1 ml aliquots of the vesicles were applied to 1 ml Sephadex columns and centrifuged as described above. The radioactivity in the eluted vesicles was determined. The centrifugation was performed at 0 °C to inhibit any diffusion of 3-*O*-methylglucose out of the vesicles.

RESULTS

Dnp-SG transport requires Mg^{2+} and ATP

In the absence of ATP or Mg^{2+}, very little radioactivity accumulated within the inside-out vesicles prepared from human erythrocytes incubated in a medium containing [^{3}H]Dnp-SG. This process was stimulated approx. 4-fold by the addition of ATP and Mg^{2+} to the medium (Table 1). The uptake of Dnp-SG into the vesicles was not stimulated by the addition of Mg^{2+} alone to the medium and was slightly increased by the addition of ATP alone. This increase could be due to the presence of some Mg^{2+} that was reversibly bound by the vesicles (Table 1). The rate of [^{3}H]Dnp-SG uptake into eight separate membrane vesicle preparations in the presence of ATP was determined to equal 121 ± 31 pmol/min per mg.

The uptake of Dnp-SG shown in Table 1 was measured by the Sephadex centrifugation procedure of Penefsky (1977). Similar results were obtained when Dnp-SG transport was measured by means of Dowex column chromatography or by means of high-speed centrifugation (results not shown).

Table 1. Stimulation of Dnp-SG uptake into inside-out vesicles from erythrocytes by ATP

Erythrocyte inside-out vesicles (0.27 mg of protein) were incubated for 60 min at 37 °C with [^{3}H]Dnp-SG (0.1 μCi, 0.36 mM), sodium phosphate buffer (0.25 mM) and the compounds below in a total volume of 0.11 ml at pH 7.0. The incubation mixtures were applied to syringe barrels containing Sephadex G-50 and centrifuged as described in the Experimental section. The radioactivity in the eluted vesicles was determined as described. Values are means ± S.D. ($n = 4$).

Additions	Dnp-SG uptake (pmol/min per mg of protein)
None	29.2 ± 6
$MgCl_2$ (2.7 mM)	20.5 ± 10
ATP (6.4 mM)	45.4 ± 9
ATP + $MgCl_2$	133.1 ± 38

The uptake of Dnp-SG into the vesicles was shown to require transmembrane movement rather than non-specific binding to the membrane exterior by the following controls. The ATP-dependent uptake of Dnp-SG into the vesicles was shown to represent transport into a restricted space when Dnp-SG accumulated by vesicles in the presence of ATP could be removed from vesicles by the detergent Triton X-100. When vesicles were incubated with [^{3}H]Dnp-SG and ATP plus $MgCl_2$ as described in Table 1, and then treated briefly with Triton X-100 (0.2%) before application to Sephadex columns, the amount of [^{3}H]Dnp-SG taken up by the vesicles was shown to equal 17 ± 4 pmol/min per mg ($n = 4$), a value not significantly different from the amount of [^{3}H]Dnp-SG accumulated by vesicles in the absence of ATP. Further evidence that the ATP-dependent accumulation of Dnp-SG by vesicles represented transport rather than binding was provided when the extent of the accumulation was decreased to nearly zero by increasing the osmolarity of the extravesicular medium with the sucrose (Fig. 1). The increase in osmolarity would be expected to lower the intravesicular volume without inhibiting the non-specific binding of Dnp-SG to the vesicle surface.

The possibility of Dnp-SG binding to the vesicular exterior was further ruled out by freeze–thaw experiments similar to those used by Kondo *et al.* (1982). When vesicles in which [^{3}H]Dnp-SG was accumulated in the presence of ATP and Mg^{2+} were subjected to a cycle of freeze–thaw most of the radiolabelled substrate associated with vesicles could be released.

Uptake of Dnp-SG by inside-out vesicles as a function of time and protein content

The background uptake of Dnp-SG into the vesicles observed in the absence of ATP appeared to be maximal (1.2 nmol/mg of protein) at the shortest times measured (15 min), whereas the ATP-dependent Dnp-SG uptake calculated by subtracting the background uptake increased with time and remained linear for at least 2 h (Fig. 2). The uptake of Dnp-SG into the vesicles was a linear function of the vesicular protein concentration,

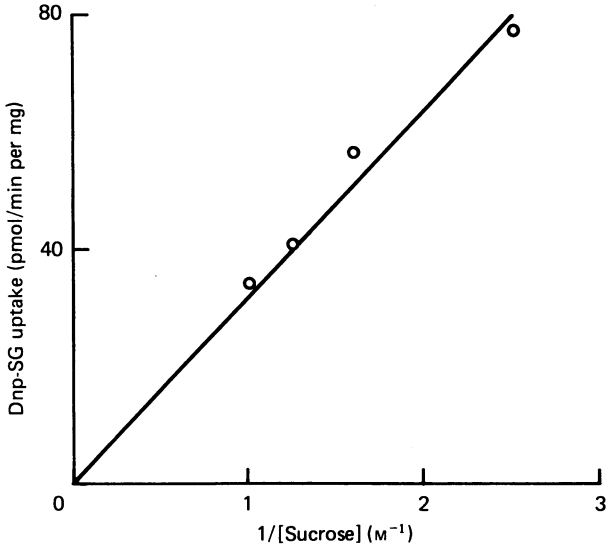

Fig. 1. Effect of increasing osmolarity on Dnp-SG uptake into inside-out vesicles

Samples of inside-out vesicles (0.22 mg of protein) were incubated for 30 min at 37 °C with [^{3}H]Dnp-SG (0.1 μCi, 0.2 mM), sodium phosphate buffer and increasing amounts of sucrose in a total volume of 0.2 ml at pH 7.0. ATP (7 mM) and $MgCl_2$ (2 mM) were then added to the incubation mixtures, the incubation continued for 60 min longer, and the uptake of [^{3}H]Dnp-SG into the vesicles was measured as described in the Experimental section.

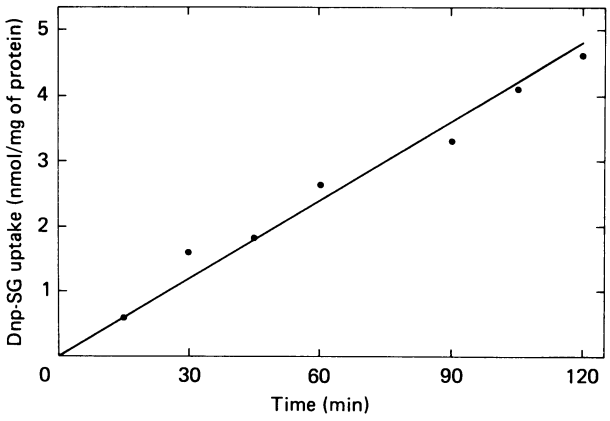

Fig. 2. Effect of incubation time on Dnp-SG uptake into inside-out vesicles from erythrocytes

Samples (0.27 mg of protein) of inside-out vesicles were incubated for the times indicated at 37 °C with [^{3}H]Dnp-SG (0.1 μCi, 0.36 mM), sodium phosphate buffer (0.25 mM), either with or without ATP (6.4 mM), plus $MgCl_2$ (2.7 mM) in a total volume of 0.11 ml at pH 7.0. The incubation mixtures were applied to syringe barrels containing Sephadex G-50 and centrifuged as described in the Experimental section. The radioactivity in the eluted vesicles was determined as described. The ATP-dependent Dnp-SG uptake is shown.

both in the presence and the absence of ATP and Mg^{2+} (results not shown).

Dnp-SG transport is sensitive to temperature and pH

The transport process was very sensitive to the temperature of the incubation mixture (Table 2). At 20 °C, ATP plus Mg^{2+} stimulated the Dnp-SG uptake into vesicles by about 50% while at 37 °C they stimulated the transport by over 500%. The background uptake of Dnp-SG observed in the absence of ATP was not sensitive to temperature. ATP-dependent Dnp-SG uptake increased as the pH increased from 6.0 to 6.8 and appeared to have reached a plateau between pH 6.8 and 8.0 (Fig. 3).

Kinetics of Dnp-SG transport

When [^{3}H]Dnp-SG uptake into vesicles was determined as a function of the concentration of Dnp-SG, a linear double-reciprocal plot was obtained (Fig. 4*a*). The K_m value determined for Dnp-SG was 0.29 mM and the $V_{max.}$ was 217 pmol/min per mg. When Dnp-SG uptake was measured as a function of ATP concentration, a K_m of 0.93 ± 0.06 mM ($n = 5$) was observed (Fig. 4*b*). In order to determine the K_m value for ATP accurately, it was necessary to measure transport activity in the presence of EGTA, so that the very low amounts of Ca^{2+} (10 μM) that might contaminate the vesicles or the Dnp-SG would not stimulate the Ca^{2+}-ATPase in the vesicles. The activity of this Ca^{2+}-ATPase in the vesicles is at least 20 nmol/min per mg of protein (results not shown). If the concentration of Ca^{2+} is about 10 μM or more, the Ca^{2+}-ATPase will degrade most of the added ATP during the 60 min incubation period and decrease the accuracy of the transport measurements. The uptake of Dnp-SG into the vesicles was shown to equal 124 ± 39 nmol/min per mg ($n = 4$) in the presence of ATP (12 mM) plus $MgCl_2$ (5 mM), while Dnp-SG uptake equalled 121 ± 37 nmol/min per mg ($n = 4$) in the presence of ATP (12 mM), $MgCl_2$ (5 mM) and EGTA (1 mM). EGTA did not interfere with ATP-dependent Dnp-SG uptake into the vesicles.

DNP-SG is concentrated in vesicles by active transport

In order to determine if the ATP-dependent transport of Dnp-SG into the inside-out vesicles represented an active transport process, we determined the intravesicular Dnp-SG concentration by measuring the amount of Dnp-SG taken up by the vesicles in the absence of any membrane potential and dividing this number by the intravesicular volume. The amount of 3-*O*-methyl[^{3}H]-glucose taken up by the vesicles at equilibrium was determined and from this value the intravesicular volume of 3.9 μl/mg of protein was calculated (results not

Table 2. Effect of temperature on Dnp-SG uptake into vesicles

The uptake of [^{3}H]Dnp-SG into inside-out vesicles from erythrocytes was measured as described in Table 1. The incubations were performed at either 20 °C or 37 °C. Values are means ± S.D. ($n = 4$).

Temperature	Addition	Dnp-SG uptake (pmol/min per mg of protein)
20 °C	None	36.8 ± 8
	ATP + $MgCl_2$	50.5 ± 5
37 °C	None	30.7 ± 4
	ATP + $MgCl_2$	154 ± 27

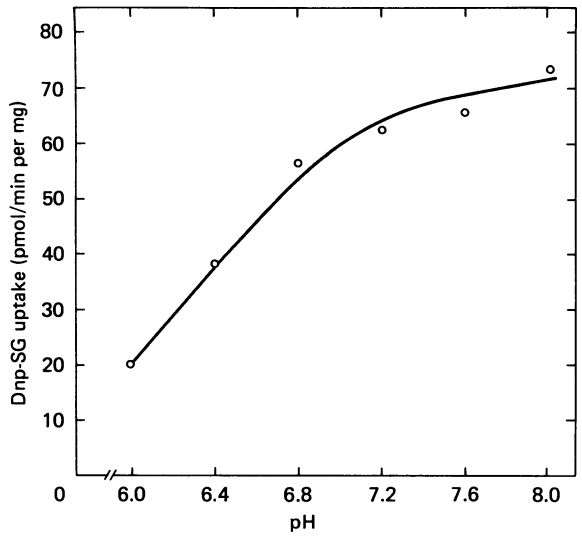

Fig. 3. Effect of pH on Dnp-SG uptake into vesicles

The uptake of [^{3}H]Dnp-SG into inside-out vesicles was measured as described in the legend of Table 1 either with or without ATP (6 mM), plus $MgCl_2$ (2.6 mM) in the presence of Tris/Mes/sodium phosphate buffer (40 mM) set at the indicated pH values. The ATP-dependent Dnp-SG uptake is shown.

shown). The ATP-dependent uptake of DNP-SG into the vesicles was then measured as a function of time in the presence of valinomycin and KCl in order to eliminate any membrane potential (Fig. 5). After 5 h incubation, the amount of Dnp-SG taken up by the vesicles in the presence of ATP was 11.3 nmol/mg of protein and when this value was divided by the intravesicular volume, the final concentration of Dnp-SG inside the vesicles was determined to be 2.9 mM, or about 8 times greater than the extravesicular concentration (0.35 mM).

Dnp-SG uptake is primary active transport

It was important to demonstrate that the active uptake of Dnp-SG into the vesicles represented primary rather than secondary active transport. Since the ATP-dependent uptake of Dnp-SG into the vesicles was insensitive to added EGTA, it was concluded that the Ca^{2+}-ATPase was not mediating the effects of ATP on Dnp-SG transport by producing a Ca^{2+} gradient that would drive Dnp-SG uptake through an exchange mechanism.

The ATP-dependent uptake of Dnp-SG into inside-out vesicles from erythrocytes was also insensitive to inhibition by Ruthenium Red, an inhibitor of the Ca^{2+}-ATPase (Schatzmann, 1983). The ATP-dependent uptake of Dnp-SG in the presence of Ruthenium Red was shown to equal $87 \pm 12\%$ ($n = 3$) of uptake observed in the absence of Ruthenium Red. This also indicated that the Ca^{2+}-ATPase was not required to mediate ATP-dependent Dnp-SG uptake into the vesicles. The uptake of Dnp-SG by the vesicles was also found to be independent of the Na^+,K^+-ATPase. Again the possibility existed initially that ATP hydrolysed by the Na^+,K^+-ATPase produced either a Na^+ or a K^+ gradient that

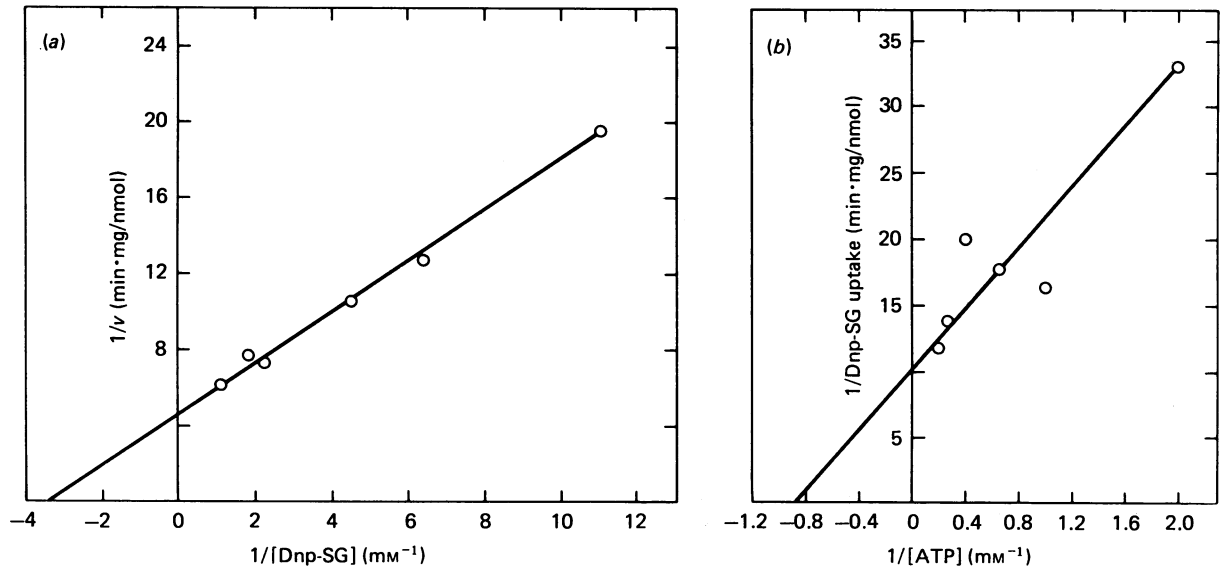

Fig. 4. Effect of concentration of Dnp-SG and ATP on [^{3}H]Dnp-SG uptake into inside-out vesicles

(*a*) Inside-out vesicles (0.22 mg of protein) were incubated for 120 min at 37 °C with ATP (12.5 mM), $MgCl_2$ (5 mM), sodium phosphate buffer (0.4 mM, pH 7.0), [^{3}H]Dnp-SG (0.1 μCi) and increasing amounts of non-radioactive Dnp-SG in a total volume of 0.2 ml. The uptake of [^{3}H]Dnp-SG into the vesicles was measured as described in Table 1 both in the presence and absence of ATP and the ATP-dependent Dnp-SG uptake was determined. A double-reciprocal plot of the data is shown. (*b*) Inside-out vesicles (0.6 mg of protein) were incubated for 60 min at 37 °C with [^{3}H]Dnp-SG (0.35 μCi, 0.18 mM), sodium phosphate buffer (2.5 mM, pH 7.0), EGTA (1 mM), and increasing amounts of ATP as indicated in a total volume of 0.19 ml. $MgCl_2$ was also included at a final concentration equal to 45% of the ATP concentration. The uptake of [^{3}H]Dnp-SG into the vesicles was also measured in the absence of ATP and this background uptake was subtracted from uptake measured in the presence of ATP. A double-reciprocal plot of the data is shown.

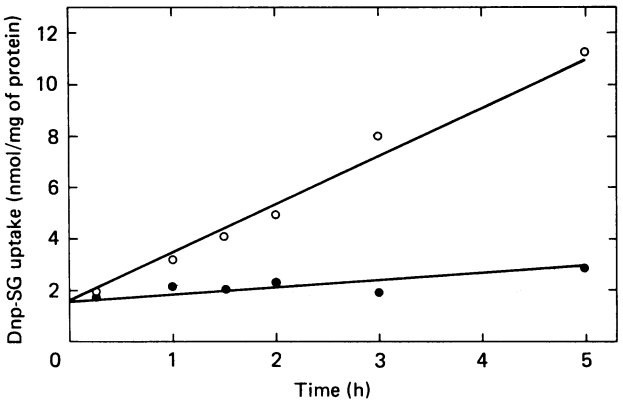

Fig. 5. Active accumulation of Dnp-SG by vesicles

Samples of inside-out vesicles (0.09 mg of protein) were preincubated for 16 h at 0 °C with valinomycin (1.7 μM) and KCl (9 mM) in a total volume of 0.03 ml, and then [^{3}H]Dnp-SG (0.15 μCi, 0.35 mM) was added to the vesicles together with KCl (9 mM) either with (○) or without (●) ATP (22 mM), plus $MgCl_2$ (9 mM) in a final volume of 0.1 ml and the incubations continued at 37 °C for the times indicated. The uptake of Dnp-SG was determined as described in the Experimental section.

could drive Dnp-SG uptake either by the co-transport of Dnp-SG and K^+ or by a counter-transport of Dnp-SG plus two monovalent cations for Na^+. The inside-out vesicles were prepared from erythrocytes by an extensive centrifugation procedure in the absence of K^+ and the final K^+ concentration in the vesicles would be expected to be below 0.1 μM if there was no K^+ bound to vesicle proteins during the preparation process. Therefore, it seemed reasonable to assume that the final K^+ concentration in the incubation mixture would not be high enough to activate the Na^+,K^+-ATPase, which has a K_m for K^+ of 1 mM (Skou, 1962). However, in order to rule out the possible contamination of the incubation mixture by K^+ that might have bound to membrane proteins, inside-out vesicles were prepared in the presence of 0.1 mM-ouabain. Preparations of inside-out vesicles were formed in the presence of [^{3}H]ouabain, purified by centrifugation, and shown to contain [^{3}H]oubain in the intravesicular space that was sensitive to detergent lysis. The rate of ATP-dependent Dnp-SG uptake into vesicles loaded with non-radioactive ouabain was 205 ± 61 pmol/min per mg while the rate of ATP-dependent Dnp-SG uptake into control vesicles formed in the absence of ouabain was 206 ± 38 pmol/min per mg ($n = 2$). Essentially similar results were obtained in three other experiments. These results indicate that the ATP-dependent Dnp-SG transport process was totally independent of the ouabain-sensitive Na^+,K^+-ATPase. Additional experiments were performed in order to determine if there was any co-transport of Dnp-SG with K^+ or exchange of Dnp-SG with Na^+. No such K^+–Dnp-SG co-transport or Na^+–Dnp-SG counter-transport was detected (results not shown). This further ruled out the involvement of the $Na^+ + K^+$-ATPase in the transport of Dnp-SG.

The uptake of Dnp-SG into the vesicles remained unaffected when $MgSO_4$ was substituted for the $MgCl_2$ in the incubation system (results not shown). Since Dnp-SG transport was shown to be ATP-dependent, active, and independent of the known ion-transporting ATPases, it seemed reasonable to assume that this transporter was capable of the primary, active transport of Dnp-SG. The energy for this process may be provided by the direct hydrolysis of ATP by the transporter.

Effect of vanadate and SH reagents on Dnp-SG uptake

Once it was established that Dnp-SG transport required energy in the form of ATP, it was assumed that some form of ATPase, possibly a Dnp-SG ATPase, was required. Since vanadate, besides being an excellent inhibitor of the Na^+,K^+-ATPase and the Ca^{2+}-ATPase, inhibits many ATPases in general, the effect of this compound on Dnp-SG transport was studied (Cantley *et al.*, 1978; O'Neal *et al.*, 1979; Nechay, 1984). The ATP-dependent Dnp-SG transport process was sensitive to inhibition by vanadate. The uptake of Dnp-SG by the vesicles in the presence of ATP, $MgCl_2$ and Na_3VO_4 (0.6 mM) was only 26.5 ± 10 pmol/min per mg ($n = 4$), which was statistically indistinguishable from Dnp-SG uptake observed in either the presence of vanadate alone (26.7 ± 4 pmol/min per mg) or in the absence of both ATP and vanadate (24.2 ± 2 pmol/min per mg). The ATP-dependent uptake of Dnp-SG into the vesicles was inhibited by 50% in the presence of 50 μM-vanadate (results not shown). The ATP-dependent uptake of Dnp-SG into the vesicles was also inhibited by the SH reagents *p*-hydroxymercuribenzene sulphonate and *N*-ethylmaleimide by 97% and 27%. The SH reagent 5,5′-dithiobis-(2-nitrobenzoic acid) did not inhibit transport significantly.

Nucleotide specificity of Dnp-SG uptake

The transport of the Dnp-SG into the vesicles was found to be almost equally (5.6-fold) stimulated by either ATP or GTP (Table 3). Pyrimidine nucleotides (CTP and UTP) were much less effective in stimulating this transport process (Table 3).

Sensitivity of Dnp-SG uptake to GSSG

Since active ATP-dependent GSSG transport out of erythrocytes has been demonstrated (Srivastava & Beutler, 1969*a*), it was of interest to determine if GSSG could inhibit the ATP-dependent uptake of Dnp-SG into inside-out vesicles from erythrocytes. For these studies the uptake of Dnp-SG by the vesicles was determined in the presence of different concentrations of GSSG in the medium. GSSG was included in the medium and the ATP + Mg^{2+}-dependent uptake of Dnp-SG by the vesicles was studied as described in the Experimental section. When the concentration of Dnp-SG in the media was kept fixed at 0.16 mM and GSSG equivalent to 0.9, 1.87, 2.89 and 10.2 mM was included in different incubations the Dnp-SG uptake by the vesicles was found to be equal to 90 ± 14%, 101 ± 24%, 82 ± 10% and 111 ± 15%, respectively, of the uptake measured in the absence of GSSG ($n = 4$). This indicates that GSSG has no significant effect on the uptake of Dnp-SG by the vesicles.

DISCUSSION

We have demonstrated that Dnp-SG is concentrated in inside-out vesicles, even when the membrane potential is eliminated by inclusion of valinomycin and K^+. This

Table 3. Nucleotide specificity of Dnp-SG uptake into vesicles

The uptake of [^{3}H]Dnp-SG into inside-out vesicles from erythrocytes was measured at 37 °C as described in Table 1 in the presence of the nucleotides. Values are means ± S.D. ($n = 4$).

Addition	Dnp-SG uptake (pmol/min per mg of protein)
None	20 ± 5
ATP (3 mM) + $MgCl_2$ (1.4 mM)	115 ± 12
GTP (3 mM) + $MgCl_2$ (1.4 mM)	116 ± 19
CTP (3 mM) + $MgCl_2$ (1.4 mM)	47 ± 1
UTP (3 mM) + $MgCl_2$ (1.4 mM)	60 ± 4

proves that active transport is required for Dnp-SG uptake into the vesicles. The transport of Dnp-SG is a primary active process rather than a secondary active process because a complete inhibition of the Na^+,K^+-ATPase by ouabain or complete inhibition of the Ca^{2+}-ATPase by EGTA, and Ruthenium Red, had no effect on this transport system. This primary active transport process is highly sensitive to vanadate, which is a well-known general inhibitor of transport ATPases (Nechay, 1984). Similar to other transport ATPases the Dnp-SG transport ATPase is temperature-sensitive (Kielley & Meyerhof, 1948) and appears to be highly specific for purine nucleotides. The active, energy-dependent efflux of GSSG from the erythrocyte was first demonstrated by Srivastava & Beutler (1969*a*). This ATP-dependent process has also been shown to occur in resealed erythrocyte ghosts and in inside-out vesicles prepared from erythrocytes (Prchal *et al.*, 1975; Kondo *et al.*, 1980, 1981). The ATP-dependent efflux of Dnp-SG from erythrocytes has been shown by Board (1981) and by Awasthi *et al.* (1983). Kondo *et al.* (1982) demonstrated the ATP-dependent uptake of Dnp-SG into inside-out vesicles from erythrocytes. It has been proposed by several investigators that GSSG as well as Dnp-SG are transported by the same system (Board, 1981; Awasthi *et al.*, 1983; Kondo *et al.*, 1982). Kondo *et al.* (1982) have suggested that the high-K_m (7.3 mM) system of GSSG transport is inhibited by Dnp-SG. In vesicles prepared from bilary canaliculi, Inoue *et al.* (1984*a*) have demonstrated that GSSG inhibits Dnp-SG transport. In the present studies we do not observe the expected inhibition of Dnp-SG transport by GSSG at concentrations up to 10 mM.

The transport of GSSG and Dnp-SG is not unique to the erythrocytes. GSSG transport has been demonstrated in the ocular lens (Srivastava & Beutler, 1968*a*) and liver (Akerboom *et al.*, 1982) and Dnp-SG transport has been demonstrated in the lens (Srivastava *et al.*, 1984), in hepatocytes (Akerboom *et al.*, 1982; Inoue *et al.*, 1984*b*) and in heart (Ishikawa & Sies, 1984). The transport system in the liver seems to be more complex than the transport system in the erythrocytes.

The uptake of GSSG and DNP-SG into plasma membrane vesicles from liver does not prefer GSSG over GSH or Dnp-SG as does the erythrocyte system (Akerboom *et al.*, 1984; Inoue *et al.*, 1984*a*,*b*). Furthermore, the liver system does not appear to require ATP, although a GSSG-stimulated ATPase and a similar Dnp-SG-stimulated ATPase have recently been reported in plasma membranes from liver (Nicotera *et al.*, 1985*a*,*b*).

These studies indicate that a membrane ATPase responsible for the transport of molecules other than ions is present in the erythrocyte membrane and the transport system may play a significant role in the defence of tissues against toxic electrophilic xenobiotics.

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