Methionine Transport in *Halobacterium halobium* Vesicles: Noncompetitive, Asymmetric Inhibition by L-Cysteine†

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ABSTRACT: Methionine transport in *Halobacterium halobium* cell envelope vesicles containing bacteriorhodopsin can be driven either by light, primarily in response to the electrochemical gradient of Na^+ which arises, or by a high chemical gradient of Na^+ (outside > inside) in the dark. L-Cysteine inhibits methionine transport in either case without dissipating the driving force, and the inhibition can be completely reversed by washing. The K_m for light-energized methionine transport is unchanged in the presence of L-cysteine, indicating noncompetitive inhibition. The K_i for L-cysteine inhibition of both light- and Na^+-gradient-energized transport is 60 μM. D-Cysteine and other analogues of L-cysteine do not inhibit methionine transport or block the inhibition by L-cysteine. The inhibition by L-cysteine is apparently highly specific but its mechanism has not been defined; various agents known to interact with thiol groups do not cause similar inhibition. The exchange of methionine in both directions across the vesicle membrane has been studied under nonenergized (dark) and energized (light) conditions. It is concluded that (a) the efflux of methionine is dependent on Na^+ as is the uptake process; hence both fluxes utilize transport carrier(s); (b) the inhibition is asymmetric in that L-cysteine inhibits only the inward transport process and only when present outside the vesicle; and (c) the protonmotive force effects the functional symmetry of the transport system and this effect is removed by L-cysteine.

The question of symmetry in membrane transport carriers has been approached with kinetic studies of the effects of specific inhibitors. *N*-Ethylmaleimide and *p*-chloromercuribenzoate inhibit the active transport of galactosides in *E. coli* membrane vesicles (Barnes & Kaback, 1971) and whole cells (Kepes, 1960). The galactoside carrier protein is directly affected since the presence of a nonmetabolizable galactoside protects the carrier from reaction with *N*-ethylmaleimide (Fox & Kennedy, 1965). The effect is symmetrical: both the influx and efflux of galactosides are inhibited when *p*-chloromercuribenzoate is added externally to the vesicles (Kaback & Barnes, 1971). Facilitated diffusion of D-glucose in human erythrocytes is also sensitive to *N*-ethylmaleimide and *p*-chloromercuribenzenesulfonate; this inhibition is noncompetitive and asymmetrical since it affects efflux much more than influx (Batt & Schachter, 1973). Recently, it was shown using impermeant maleimides that the sulfhydryl group involved in the inhibition is accessible from the external medium and that D-glucose protects the group from alkylation (Batt et al., 1976). Thus, the inhibition of both galactoside transport in *E. coli* and of D-glucose transport in human erythrocytes occurs via the action of reagents known to react with sulfhydryl groups, but, while the inhibition of galactoside transport is symmetric, the inhibition of D-glucose transport appears to be asymmetric.

MacDonald et al. (1977) have shown that the transport of some amino acids but not others in *H. halobium* cell envelope vesicles is inhibited by cysteine. Cysteine itself is not transported into the vesicles. This report further defines the specific nature of the inhibition by examining the effect of cysteine on methionine transport and presents evidence for asymmetric inhibition with respect to influx and efflux.

Materials and Methods

Halobacterium halobium cell envelope vesicles were prepared as described previously (Lanyi & MacDonald, 1978). The inside salt composition was adjusted by osmotic shock (MacDonald & Lanyi, 1975) and the diluted vesicle suspension (1.6 mg of protein/mL) stored 1 to 2 days in the cold before further use. When L-cysteine was present inside the vesicles, the osmotic shock mixture included 1 mM L-cysteine, 2 mM DTE,[1] and 20 mM Hepes buffer (pH 6.5). Light- and Na^+-gradient-energized methionine uptake were assayed at 24 °C by filtration as described previously (Lanyi & MacDonald, 1978). For measurement of the exchange of accumulated methionine during and after light-energized uptake, the vesicles were loaded with labeled methionine in the light for 5–6 min, the indicated additions made, and samples taken as above. When L-cysteine was present inside the vesicles, the experiment was initiated by diluting vesicles 125-fold into the assay mixture. Unless otherwise indicated, the assay mixture (0.6–2.0 mL) consisted of 3 M salt containing 20 mM Hepes (pH 6.7), 0.3–0.9 mg of protein/mL, and 5.7×10^{-7} M [^{3}H]methionine (1.0 μCi/mL, 1.75 Ci/mmol).

For the nonenergized and Na^+-driven efflux experiments, the vesicles were loaded with, and stored 1 to 2 days in, the desired salt composition. The vesicles were then resuspended in the same salt mixture at 8.0 mg of protein/75 μL. The suspension was made 14 μM in methionine (20 μCi/mL, 1.33 Ci/mmol), and stored for 2 days in the cold in order to equilibrate the methionine across the membrane. Efflux was initiated by diluting the vesicles 125-fold into the assay mixture containing 14 μM methionine (cold). Subsequent sampling to assay the amount of labeled methionine retained in the vesicles was identified with the procedure used in the methionine uptake experiments, except that the samples were washed with a chilled salt solution of the same composition as the external salt mixture used in the experiment.

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[1] Abbreviations used: Cys, L-cysteine; Met, L-methionine; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; DTE, dithioerythritol; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

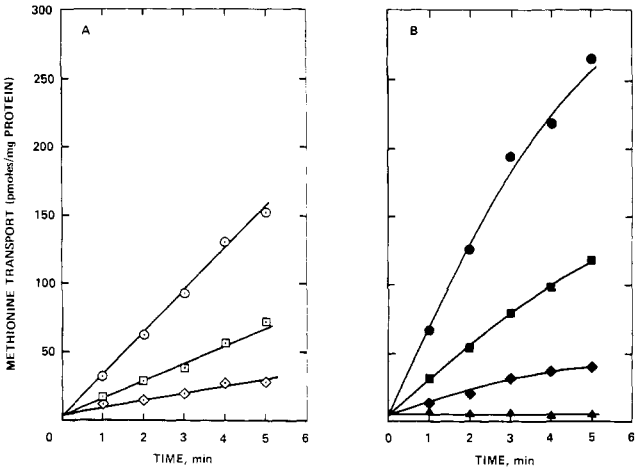

FIGURE 1: Inhibition by L-cysteine of light- and Na^+-gradient-energized methionine uptake in *H. halobium* vesicles. (A) Transport was initiated by illuminating the sample starting at time zero. The salt composition inside and outside the vesicles was 2 M KCl + 1 M NaCl, and the assay mixture contained 0.38 mg of protein/mL and 8.1×10^{-7} M [^{3}H]methionine (1.43 μCi/mL, 1.75 Ci/mmol). (B) Transport was initiated at time zero in the dark by diluting vesicles, loaded, and suspended in 3 M KCl, 3.5-fold into the assay mixture. The final outside concentrations were 2.1 M NaCl + 0.9 M KCl, 0.42 mg of protein/mL, and 8.1×10^{-7} M [^{3}H]methionine. Uptake was assayed in the absence (⊙ and ●) and presence of 71 μM (⊡ and ■) and 286 μM (◇ and ◆) L-cysteine. Also shown in B is the lack of uptake when the salt composition is 3 M KCl inside and outside the vesicles (▲).

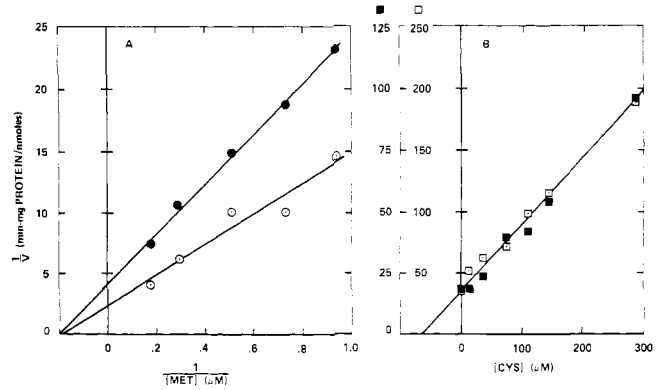

FIGURE 2: Effect of L-cysteine on the light- and Na^+-gradient-energized methionine uptake. (A) Double-reciprocal plot of the initial light-energized transport rate and methionine concentration in the absence (⊙) and presence (●) of 30 μM L-cysteine. Conditions were as in Figure 1A except that 0.88 mg of protein/mL and 2×10^{-4} M phosphate buffer (pH 6.4) were present. (B) Plot of the reciprocal initial transport rate and L-cysteine concentration for light- (⊡) and Na^+-gradient- (■) energized uptake. Conditions were as in Figure 1.

For the nonenergized influx experiments, the vesicles were loaded with 3 M NaCl, resuspended to 4.4 mg of protein/200 μL, made 19.6 μM in unlabeled methionine, and stored for 2 days. Influx was initiated by adding labeled methionine (3 μCi, 14.6 Ci/mmol) to a final concentration of 1.9 μM; the final assay mixture (100 μL) consisted of 3 M NaCl, 19.6 μM methionine, 30 mM Hepes (pH 6.6), and 0.53 mg of protein. Samples were taken with a 10-μL Carlsberg pipet and filtered as in the methionine uptake experiments.

The half-times ($t_{1/2}$) reported for and the curves drawn to fit the efflux experiments were determined by fitting the data points to a single exponential curve using a least-squares fit program. Correlation coefficients were typically 0.90 to 0.99.

[^{3}H]Methionine (L isomer, 14.6 Ci/mmol) was obtained from New England Nuclear; [^{14}C]cysteine (L isomer, 52 mCi/mmol) was from The Radiochemical Center, Amersham, England; β-mercaptoethanol, L-cystine, dithioerythritol, β-mercaptoethylamine, D-cysteine, β-mercaptopropionic acid, and phenylmercuric acetate were from Sigma; iodoacetamide was from Calbiochem; and glutathione was from Boehringer/Mannheim.

Results

Kinetics of the Inhibition of Methionine Transport by Cysteine. Addition of L-cysteine to *H. halobium* vesicles causes inhibition of methionine transport, whether energized by the light-induced transmembrane electrical potential or a prearranged chemical gradient of Na^+ (Figure 1). The proposed mechanism of amino acid transport in *H. halobium* vesicles by these two processes has been recently reviewed (Lanyi, 1978; MacDonald & Lanyi, 1977). The time required for L-cysteine to inhibit transport is less than 1 min. Both methionine (Figure 2A) and histidine (MacDonald et al., 1977) transport are noncompetitively inhibited by L-cysteine. The K_m for methionine transport is 5.5 μM and remains essentially unchanged at 5.0 μM in the presence of 30 μM L-cysteine. As shown in Figure 2B increasing concentrations of L-cysteine cause increased inhibition of both light- and Na^+-gradient-energized methionine transport; the K_i for the inhibition is 60 μM. Cysteine inhibition is reversible; washing with 3 M salt solution restores completely the transport of methionine.

L-Cysteine has been shown to inhibit the energized transport of lysine, histidine, alanine-glycine-serine-threonine,[2] valine-leucine-isoleucine-methionine,[2] and proline but not asparagine, tryptophan, and phenylalanine in *H. halobium* vesicles (MacDonald et al., 1977). Thus, the inhibition of transport is specific for certain amino acids and is not the result of dissipating the transmembrane driving force.

Specificity of L-Cysteine Inhibition. Analogues of L-cysteine (D-cysteine, L-cysteic acid, glutathione, β-mercaptoethylamine, β-mercaptopropionic acid, L-cystine) when tested at a concentration of 1 mM caused less than 10% inhibition of methionine transport (not shown). The binding and inhibition are stereospecific for L-cysteine since D-cysteine does not inhibit methionine transport or prevent the inhibition by L-cysteine. The structure necessary for binding at the inhibitor site is apparently lost with chemical modification of the inhibitor, since L-cystine, L-cysteic acid, and β-mercaptoethylamine at high concentrations (1 mM) do not affect the inhibition of transport by a much lower concentration (67 μM) of L-cysteine. The thiols β-mercaptoethanol and dithioerythritol are ineffective as inhibitors. These results imply L-cysteine binds to a highly specific site in the membrane to affect the inhibition of methionine transport.

Since the thiol group of L-cysteine could act as a reducing agent to break a disulfide bond or an oxidizing agent to form a mixed disulfide, various agents known to interact with sulfhydryl groups were tested for their effect on transport. The results of experiments designed to define the chemical mechanism by which L-cysteine inhibits transport were inconclusive: (a) treatment with the reducing agent dithionite or aerating the reaction mixture prior to assaying transport had no effect on the extent of cysteine inhibition; (b) the mercurials phenylmercuric acetate and mersalyl caused only slight inhibition (10–15%); (c) the thiol alkylating agent iodoacetamide,

[2] These amino acids share common carrier(s) within each group (MacDonald et al., 1977).

TABLE I: Half-times for Exchange of Accumulated Methionine during and after Light-Driven Transport in Energized Vesicles.

Salt composition[a]		Efflux conditions		$t_{1/2}$
Internal	External	$h\nu$	Addition (M)[b]	(min)[c]
NaCl	NaCl	(−)	None	5 (6.5)
		(−)	Met	6.5 (9.5)
		(−)	Cys	5
		(−)	Met + Cys	7
		(+)	Met (5×10^{-5})	16 (15.5)
		(+)	Cys	8
		(+)	Met + Cys	7.5 (6.5)
		(+)	Met + gramicidin (2×10^{-6})	8.5
		(+)	Met + monensin (10^{-5})	10.5
		(+)	Met + gramicidin + monensin	8
		(+)	Met + FCCP (10^{-5})	11.5
NaCl + Cys	NaCl	(−)	Met	8.5
		(−)	Met + Cys	5.5
		(+)	Met (5×10^{-5})	15.5
		(+)	Met + Cys	6
KCl	NaCl + KCl	(+)	Met (5×10^{-5})	d
		(+)	Met + Cys	77

[a] See Figure 3 for experimental conditions. [b] The concentrations were 1.2×10^{-5} M methionine and 10^{-3} M L-cysteine unless otherwise indicated. [c] Labeled methionine was accumulated in the light for 5–6 min to ~35 pmol/mg of protein before efflux was measured. The results given in parentheses were obtained after 20 min of illumination, so that the accumulation had reached the steady-state value of ~90 pmol/mg of protein; in this case 3×10^{-5} M methionine was added where indicated. [d] Labeled methionine was accumulated in the light for 6 min, ~300 pmol/mg of protein; no influx or efflux was observed in 60 min after the addition of unlabeled methionine.

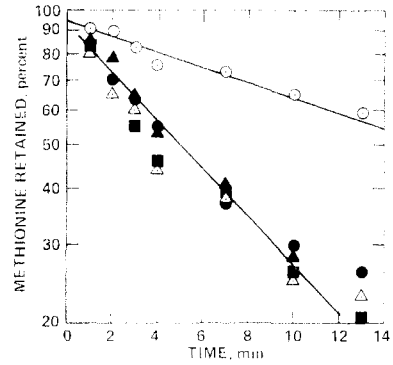

FIGURE 3: Efflux of labeled methionine during and after light-energized uptake in NaCl-loaded vesicles. The assay mixture contained 3 M NaCl, 20 mM Hepes (pH 6.5), 0.8 mg of protein/mL, and 5.7×10^{-7} M [^{3}H]-methionine. Transport was initiated by illumination at −5 min. L-Cysteine (1 mM), 2 mM DTE, and 20 mM Hepes (pH 6.5) were present inside the vesicles in two cases (△ and ▲). At time zero efflux was measured under the following conditions: (+) $h\nu$ + 5×10^{-5} M Met (⊙); (+) $h\nu$ + 1.2×10^{-5} M Met + 10^{-3} M Cys (△); (−) $h\nu$ (■); (−) $h\nu$ + 1.2×10^{-5} M Met (●); (−) $h\nu$ + 1.2×10^{-5} M Met + 10^{-3} M Cys (▲).

alone or with L-cysteine, failed to inhibit transport; (d) sulfite, which reacts with thiols and disulfides in the presence of oxidants, mercurials, cysteine, or β-mercaptoethylamine to form *S*-sulfonates (Means & Feeney, 1971), stimulated transport ~40% when present alone or with Cu^{2+} as an oxidant but had no additional effect in the presence of cysteine or mercurials; (e) $CdCl_2$ and arsenite, which are known to form tight complexes with dithiols but not monothiols (Hockster & Quastel, 1963), caused only slight inhibition of transport (15%); and (f) cyanide, azide, and sulfide, which at high concentrations can reduce disulfide bonds in proteins (Dixon & Webb, 1958), caused 30–40% inhibition at 1 mM but did not cause further inhibition at higher concentrations. These treatments may have failed due to the inability of the agents to reach the site of the postulated groups. Considering the highly specific nature of the inhibition by L-cysteine this rationale would be an obvious explanation. However, mercurials (Lanyi, 1972; Barnes & Kaback, 1971; Batt & Schachter, 1973), iodoacetamide (Webb, 1966), $CdCl_2$ (Jacobs et al., 1956), and cyanide and azide are known to be effective in modifying the activity of membrane-bound proteins. Any role for sulfhydryl groups in methionine transport and in the mechanism of inhibition by L-cysteine appears unlikely but remains unresolved.

Exchange of Accumulated Methionine in Energized Vesicles. While the chemical mechanism by which L-cysteine inhibits methionine transport is not known, the site of action can be further defined. In the experiments described above, L-cysteine was added to the external medium and the inhibition of the initial rate of uptake measured. These experiments show that the energized uptake of methionine can be inhibited by externally added L-cysteine. The experiments described below test for asymmetry in the inhibition of energized and nonenergized exchange of methionine when L-cysteine is present on the cis and/or trans side of the membrane relative to the direction of transport.

When either L-cysteine or an excess of unlabeled methionine is added to vesicles energized by light and actively transporting methionine, loss of the accumulated labeled methionine occurs. The results can be summarized as follows (Table I, Figure 3).

(a) When a 100-fold excess of unlabeled methionine is added to a suspension containing vesicles loaded with 3 M KCl and suspended in 2 M NaCl + 1 M KCl, no further net flux of labeled methionine occurs. Since the external concentration of unlabeled methionine is now ten times the K_m for transport, any recapture phenomenon would be blocked (Robbie & Wilson, 1969). Thus, there is very little exchange of methionine from KCl-loaded vesicles during light energized transport. Under these conditions the internal concentration of Na^+ is very low since the vesicles are actively pumping Na^+ out (Lanyi & MacDonald, 1976). When the vesicles are loaded with and suspended in 3 M NaCl, so that the interior cannot be depleted of Na^+, methionine exchange during the light-driven transport occurs with a half-time ($t_{1/2}$) of 16 min. Thus Na^+ inside the vesicles stimulates the rate of methionine exchange during light-driven active transport.

(b) When NaCl-loaded vesicles are allowed to accumulate labeled methionine and the light is turned off, the loss of label is more rapid, with a half-time of 5 min. The addition of unlabeled methionine at a concentration equal to that of the labeled methionine inside the vesicles has little further effect on the loss of the labeled amino acid in the dark ($t_{1/2}$ = 6.5 min). The exchange of methionine is thus three times slower in the light-energized than in the deenergized NaCl-loaded system. These properties of methionine efflux can be contrasted to those of glutamate, which does not exit from *H. halobium* vesicles during or after light-driven transport, even in the presence of internal Na^+ (Lanyi et al., 1976), and of leucine, which shows exchange and efflux kinetics during and after transport relatively unaffected by illumination or by the presence of internal Na^+ (Lanyi & MacDonald, 1975).

(c) When 1 mM L-cysteine is added externally, loss of ac-

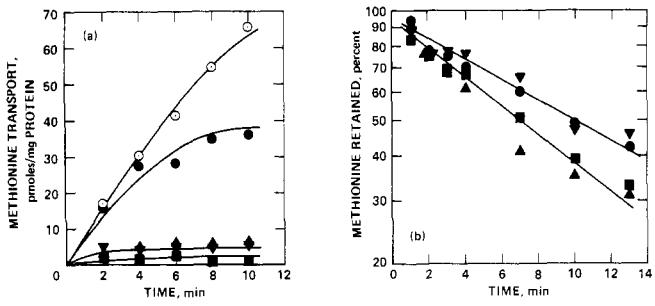

FIGURE 4: Effect of L-cysteine and various ionophores on the efflux of labeled methionine during light-driven transport. The assay conditions were as given in Figure 3. (A) Transport was initiated by illumination at time zero. (B) Transport was initiated by illumination at −5 min and at time zero 1.2×10^{-5} was added. In both A and B the following additions were made at time zero: control (⊙); 10^{-5} M nonensin (●); 10^{-5} M FCCP (▼); 2×10^{-6} M gramicidin (■); 10^{-3} M Cys (▲).

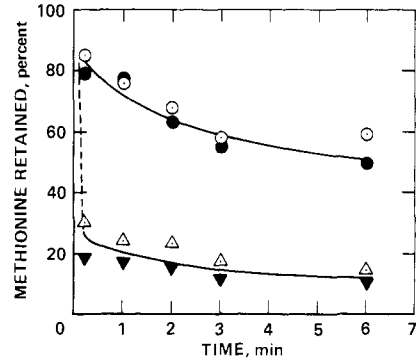

FIGURE 5: Effect of L-cysteine on Na^+-gradient-driven efflux. The vesicles were loaded with 14 μM [^{3}H]methionine. Efflux was initiated by diluting the vesicles 125-fold into the outside assay mixture given below. The initial inside/outside conditions were: 2.7 M KCl + 0.3 M NaCl/2.7 M KCl + 0.3 M NaCl (⊙); 2.7 M KCl + 0.3 M NaCl + 1 mM L-cysteine/2.7 M KCl + 0.3 M NaCl (●); 2.7 M KCl + 0.3 M NaCl/2.9 M KCl + 0.1 M NaCl (△); 2.7 mM KCl + 0.3 M NaCl + 1 mM L-cysteine/2.9 M KCl + 0.1 M NaCl + 1 mM L-cysteine (▼).

cumulated methionine occurs from NaCl-loaded energized vesicles. The rate is the same in the presence or absence of unlabeled methionine ($t_{1/2}$ = 7.5–8 min, Table I) and is close to that for the exchange in deenergized vesicles ($t_{1/2}$ = 5–6.5 min). This concentration of L-cysteine is sufficient to inhibit the uptake process by ~85% (Figure 4A). The half-time for exchange in KCl-loaded vesicles is much longer (77 min).

(d) The presence of 1 mM L-cysteine inside the vesicles has little effect on the rate or extent of methionine uptake (not shown). That the vesicles retain L-cysteine under these conditions is demonstrated by the fact that vesicles loaded with [^{14}C]-L-cysteine and diluted 100-fold into 3 M NaCl lose only about 10% of the cysteine during 3 h of incubation (not shown). Similarly, internal L-cysteine has no effect on the exchange of methionine in energized or deenergized vesicles.

(e) As shown in Figure 4, ionophores which inhibit the energized membrane state also cause loss of accumulated labeled methionine from illuminated vesicles. Gramicidin and FCCP cause loss with half-times of 8.5 and 11.5 min when added at concentrations sufficient to inhibit the initial rate of methionine uptake by 95% and 85%, respectively. Monensin, which does not affect the initial rate of uptake but does inhibit the amount of methionine accumulated at longer times, causes loss with a half-time of 10.5 min. Under these conditions gramicidin would be expected to collapse the membrane potential but leave the pH gradient intact, while FCCP would diminish both gradients. As with turning off the light to deenergize the system, dissipation of the proton-motive force allows faster loss of methionine than the rate of exchange observed in the energized system. The effect of monensin on influx suggests that a sodium gradient may be slowly forming even in an "all" NaCl system due to the presence of a small amount of contaminating KCl which can act as a counterion. It is unlikely, however, that the effect of light on exchange is related to the presence of a Na^+ gradient which would be very small after only 5–6 min of illumination. Longer illumination times (Table I) have no further effect on the rate of exchange.

Thus, in light-energized vesicles which are actively transporting methionine, L-cysteine acts to inhibit the inward translocation of methionine and to uncouple the outward flux of the amino acid from the effect of the protonmotive force. These effects are observed only when L-cysteine is present on the outside of the membrane. The inhibitor has no further effect on the exchange process.

Passive Exchange of Methionine in Nonenergized Vesicles. The nonenergized, passive exchange of methionine in vesicles was measured as described in Materials and Methods, with

TABLE II: Half-times for Methionine Exchange in Preloaded, Nonenergized Vesicles.[a]

Salt composition	L-Cysteine (1 mM) Internal	L-Cysteine (1 mM) External	$t_{1/2}$ (min)
KCl	(−)	(−)	79
NaCl	(−)	(−)	9
	(−)	(+)	11
	(+)	(−)	8
	(+)	(+)	9

[a] Exchange measured by loss of labeled methionine. See Materials and Methods for experimental conditions.

equal concentrations of labeled and unlabeled methionine outside and inside the vesicles, respectively. Uptake of labeled methionine takes place only in the presence of NaCl under these conditions. The passive exchange of methionine, measured in the inward direction, was found to have an initial rate of 3.3 pmol per mg of protein per min at a methionine concentration of 19.6 μM and was inhibited approximately 90% by 1 mM L-cysteine. The effect of L-cysteine on the passive exchange of methionine was studied similarly in the opposite direction, by loading nonenergized vesicles with labeled methionine and suspending in a medium containing an equal concentration of unlabeled methionine (Table II): (a) the kinetics of the loss of labeled methionine from vesicles loaded and suspended in 3 M KCl ($t_{1/2}$ = 79 min) or in 3 M NaCl ($t_{1/2}$ = 9 min) indicate that exchange proceeds at the same rate as in the deenergized or cysteine-inhibited system (Table I); (b) the presence of L-cysteine inside and/or outside NaCl-loaded vesicles has no further effect on methionine exchange when measured in the outward direction.

The Effect of L-Cysteine on Na^+-Gradient-Driven Release of Methionine. Both the inward (MacDonald & Lanyi, 1977) and outward (see above) translocations of methionine are dependent on Na^+. It is of interest to determine if net loss of methionine can be induced by a reverse Na^+ gradient (inside > outside). Vesicles loaded with 2.7 M KCl + 0.3 M NaCl and labeled methionine were rapidly diluted 125-fold into 2.7 M KCl + 0.3 M NaCl + unlabeled methionine. Under these conditions 15% of the labeled methionine was lost within 0.2 min, followed by slow efflux (Figure 5). This initial release of methionine is presently unexplained and seems to be due to

having both K^+ and Na^+ present. Vesicles loaded with 2.7 M KCl + 0.3 M NaCl were then suspended in 2.9 M KCl + 0.1 M NaCl as above, so that a threefold Na^+ gradient was imposed across the membrane. As shown in Figure 5, 75% of the labeled methionine was released in 0.2 min, thus causing an approximately fivefold increase in the amount of methionine released from the vesicles. This Na^+-gradient-driven efflux was not affected by 1 mM L-cysteine present both inside and outside the vesicles (Figure 5).

Discussion

The results show that the rates of both the inward and outward fluxes of methionine are dependent on the presence of Na^+ and can be driven by a Na^+ gradient arranged outside > inside or inside > outside, respectively. These results suggest that Na^+-dependent methionine carrier(s) exist, which can facilitate fluxes in both directions across the membrane, for both passive and active transport. Separate carriers for influx and efflux are not excluded and would explain the different sensitivities of inward and outward fluxes of methionine to L-cysteine. Since the exchange loss of methionine from energized vesicles is slower than from deenergized or nonenergized vesicles, the kinetic properties of efflux appear to be regulated by the transmembrane protonmotive force. Energy-dependent asymmetries in the properties of membrane bound transport carriers have been reported in *E. coli* (Winkler & Wilson, 1966; Rudnick et al., 1976; Winkler, 1973) and *Klebsiella aerogenes* (Deshusses & Reber, 1977). Kaback (1976) has proposed that the functional orientation of the transport carrier within the membrane is regulated by the interaction between the proton-motive force and a negatively charged site on the carrier.

The inhibition by L-cysteine is asymmetric in both energized and nonenergized vesicles, since the inward translocation of methionine is affected only when L-cysteine is present outside the vesicles. Similarly, only L-cysteine added externally causes loss of accumulated methionine from energized vesicles. The results show that L-cysteine (a) binds at an externally accessible inhibitory site in a stereospecific manner, (b) affects the inward translocation process directly since the influx of methionine during passive exchange is also inhibited, and (c) uncouples the transport system from the regulatory effect of the protonmotive force since the rate of the outward flux during exchange in the energized system is increased by L-cysteine to equal the rate in the deenergized system.

The mechanism of the inhibition is at present unknown; however, the action of L-cysteine to uncouple the transport carrier(s) from the transmembrane driving force is an interesting observation. The binding of L-cysteine may cause or prevent conformational changes in the carrier protein, or act in a more localized fashion to chemically modify essential groups (e.g., sulfhydryl) which are necessary for energetic coupling. Considering the highly specific binding and the lack of inhibition by a variety of sulfhydryl reagents, it may be necessary to isolate the carrier and binding site protein(s) in order to define the mechanism. The strict vectorial nature of inhibition by L-cysteine does indicate that there must be very few misoriented carriers in the membrane, resulting from the vesicle preparation procedure and suggests that the L-cysteine binding site does not become internally accessible during the functioning of the methionine carrier system. It is not clear what, if any, physiological significance the inhibition of transport by cysteine, present externally to the cells, might have. Cysteine inhibits both the L and A neutral amino acid transport systems in S37 ascites tumor cells, but acts as both cis and trans inhibitor of these two systems (R. Matthews, personal communication).

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