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What we can learn from the effects of thiol reagents on transport proteins

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I. Summary

Many secondary membrane transport systems contain reactive sulfhydryl groups. In this review the applications of SH reagents for analyzing the role of sulfhydryl groups in membrane transport systems will be discussed. First an overview will be given of the more important reagents, that have been used to study SH-groups in membrane transport systems, and examples will be given of transport proteins in which the role of cysteines have been analyzed. An important application of SH-reagents to label transport proteins using various SH-reagents modified with fluorescent or spin-label moieties will be discussed. Two general models are shown which have been proposed to explain the role of sulfhydryl groups in some membrane transport systems.
II. Introduction

By examining the reactivity toward membrane permeable and impermeable SH reagents, information about the localization of reactive SH-groups can be obtained. From studies on the effect of different SH reagents on the functional properties of transport proteins, information about the substrate binding site or the role of the SH-groups in the transport mechanism can be obtained, in certain cases. It is important in these kind of experiments to establish the effect of the SH-reagent on other proteins in the membrane. For instance, in studies of secondary transport systems particularly the effect of SH-reagents on protonmotive force (Δp) generation should be analyzed. When there is an effect on Δp generation, a good alternative is to examine the effect on those reactions which do not use the Δp (efflux, exchange, counterflow) or to use artificially generated driving forces (Δp and/or ΔpH).

III. Reaction of SH-groups with sulphydryl reagents

III-A. Introduction

In most reactions, thiol (SH-) groups take part as mercaptide (or thiolate) ions, RS-. The nucleophilic strength of this ion depends upon basicity and polarizability. Thus, to understand the reactivity of the SH-groups, it is important to have information about the pKₐ values of SH-groups in proteins. Some information can be obtained by comparing the values for cysteine and other small thiols [1]. However, the pKₐ values of SH-groups in proteins can vary within wide limits under the influence of a variety of factors. One important factor is the location of the SH-group in the tertiary structure of the protein. SH-groups, which are ‘buried’ or ‘masked’ inside the protein in a hydrophobic environment and have no contact with the solvent, have higher pKₐ values than SH-groups on the outer surface of the protein. Another important factor is the electrostatic influence of neighboring charged groups. On the basis of the behavior of low molecular weight thiols, it is concluded that a positively charged group in the immediate vicinity of an SH-group lowers its pKₐ, whereas a negatively charged group rises its pKₐ [2].

Sulphydryl groups of low molecular weight thiols and proteins can react with numerous sulphydryl reagents. Table I summarizes some of these different reactions. It should be noted that SH-groups of proteins have usually high reactivities which differ from those of small thiols. Of the reagents listed in Table I only the disulfides are absolutely specific. They do not react

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Reactions of SH-groups with various sulphydryl reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of reaction</strong></td>
<td><strong>Reagent</strong></td>
</tr>
<tr>
<td>Aerylating agents</td>
<td>ketene; acetyl chloride; acetic and succinic anhydrides; ethylthiofluoroacetate (formation of thioesters)</td>
</tr>
<tr>
<td>Cyanate</td>
<td>HNCO (cyanate)</td>
</tr>
<tr>
<td>Carbonyl compounds</td>
<td>aldehydes; ketones (formation of hemimercaptoaldehydes and hemimercaptocarboxylates)</td>
</tr>
<tr>
<td>Addition to activated</td>
<td>N-ethylmaleimide; N-prenylmaleimide; N-pyrrolsosomaleimide; N,N'-phenylenebismaleimides; N(44-dimethylamino-3,5-dinitrophenyl)maleimide; N,N'-hexamethylenebismaleimide; 4-chloroacetylphenylmaleimide; 4-azidophenylmaleimide; acrylonitrile; acryamide; malonic acid</td>
</tr>
<tr>
<td>Quinone compounds</td>
<td>2-methyl-(1,4)napthoquinone (plumbagin); 2,3,5,6-tetramethylbenzo-1,4-quinone (formation of thioethers and/or oxidation to disulfides)</td>
</tr>
<tr>
<td>Alkylating compounds</td>
<td>isoacacetate; isoacacetamide; bromoacetate; chloroacetate; bromopyruvate; ethylene oxide and -imine; methyl iodide; dimethylsulfate; 1,3-propane sulfone</td>
</tr>
<tr>
<td>Arylation</td>
<td>fluoroacetobrombenzene (FDNB); chlorodinitrobenzene (CDNB); 2,4,6-trinitrobenzene-1-sulfonic acid (TNBS); arsenic oxides</td>
</tr>
<tr>
<td>Metal ions</td>
<td>Ag⁺, Cu⁺, Au⁺, Hg²⁺, Pb²⁺, Cu²⁺, Cd²⁺, Zn²⁺, Sb³⁺-compounds (formation of mercurates)</td>
</tr>
<tr>
<td>Organic mercury compounds</td>
<td>p-chloromercuribenzoate; p-chloromercuriphenylsulfonate; phenylmercuriacetate; methylmercury iodide; methylmercury bromide; O-(3-hydroxymercuri-2-methylpropyl)carbamylphenoxyacetate (mersalylic); chloromercurinatrioethenes</td>
</tr>
<tr>
<td>Arsene compounds</td>
<td>S-mercury N-dansyl cysteine (formation of mercaptides)</td>
</tr>
<tr>
<td>Oxidation</td>
<td>iodine; ferricyanide; tetrathionate; Na₂S₂O₃; porphyrindia; -iodoiodobenzene; H₂O₂; sulfonates; benzofuroxan; dansylchloride; thiocyanogen (SCN⁻); hypochromeinate ion, OSCN⁻; tetraniitromethane (formation of inter- or intramolecular disulfide bonds and, depending upon the strength of the oxidizing agent, sulfenic, sulfine- or sulfonic acid; photoxidation; autoxidation by atmospheric oxygen)</td>
</tr>
<tr>
<td>Thiol-disulfide exchange</td>
<td>5,5'-dithio-bis(2-nitrobenzoic acid); 2,2'-dithiopyridines; n-propyl-2-pyridyl disulfide; N-succinimimidyl-3,4-pyridyldiethyldithioapatropionate; 2,2'-dithiodi-3-indolecarboxylic acid; β-hydroxyethyl-2,4-dinitrophenyl disulfide; oxidized glutathione; cystamine; cystamine monosulfide; di-N,N-phosphonic acid; cystamine-5,5'-disulfide</td>
</tr>
<tr>
<td>Conversion into thiocyano-groups (cyanation)</td>
<td>2-nitro-5-thiocyanobenzoic acid; 1-cyano-4-dimethylamino pyridinium salts; 2-thiocyanopyridine</td>
</tr>
<tr>
<td>Sulfenyl halides</td>
<td>2-nitro-, 4-nitro and 2,4-dinitrophenyl sulfenyl chlorides; azobenzen 2-sulfenyl bromide</td>
</tr>
</tbody>
</table>
with any other groups than cysteines in proteins. Organic mercury compounds, arsenite and arsenoxides have high specificity while other thiol reagents have only relative specificities for SH-groups. They often also react with other functional groups in proteins. However, by choosing appropriate reaction conditions selective modification of SH-groups can often be achieved.

**III-B. Addition to activated double bonds**

SH-groups form irreversible adducts to polarized or easily polarizable double bonds in compounds as acrylonitrile, acrylamide, N-ethylmaleimide (NEM), etc. [3,4]. NEM has been used extensively to study SH-groups in transport proteins. Fig. 1 shows the reaction of an SH-group with NEM. The rate of reaction increases sharply with increasing pH, indicating the involvement of the mercaptide ion [5,6]. NEM reacts not only with SH-groups, but can also react with the imidazole group of histidine and the α-amino group of amino acids [7]. These reactions occur only at relatively high concentrations and/or high pH. Under conditions normally used for reacting NEM with SH-groups (< 1 mM, pH 6–7) reaction with histidine or α-amino groups usually does not occur or only to an extremely small extent [8]. However, an increased reactivity as a result of its particular environment in the protein can lead to a reaction of some amino groups with NEM even at low concentrations.

When a reactive SH-group is located at or near the substrate binding site and reaction with NEM can be protected by substrate, radioactively labeled NEM can be used to label specifically transport proteins [9,10], allowing the detection of the protein during its purification, for example. Alternatively, colored derivatives of maleimide are available for labeling protein SH-groups [11,12]. Some nonfluorescent N-substituted maleimides react with thiols to form adducts which are highly fluorescent, making them suitable for the use of reporter groups [13,14]. N-Pyrene maleimide was used successfully to study coupling between transport and changes in the conformation of lac permease [16] with a fluorescence anisotropy decay technique. Upon passing through the lipid phase transition the transport rate decreases several orders of magnitude, whereas binding activity remains unaltered. This change in transport activity was accompanied by a reduced relaxation rate of the fluorescence of bound N-pyrene maleimide, suggesting a correlation between these fluctuations and transport.

**III-C. Reaction with quinones**

Reaction of thiols with certain quinones result in the formation of a thioether of a hydroquinone or a quinone. Fig. 2 shows the reaction of a SH-group with 2-methyl-1,4-naphthoquinone (plumbagin). The hydroquinone formed is reoxidized by atmospheric oxygen to the original quinone and H₂O₂ [17]. When the substituent at position 2 or 3 of the quinone ring is CH₃ (as in 2,3,5,6-tetramethylbenzo-1,4-quinone) or OH, the reaction does not proceed [17]. Quinones not only alkylate SH-groups, but are also able to oxidize them to disulfides [2]. Because of the possibility of two kinds of reactions, one should be very careful with the interpretation of observed inhibition by compounds such as plumbagin.
III-D. Reaction with metal ions and organic mercury compounds

Reaction of SH-groups with metal ions results in the formation of mercaptides:

\[
R-SH + M^\text{III} \rightarrow R-SM^- + \text{H}^+
\]

This reaction is reversible, but the equilibrium is shifted towards the formation of the weakly dissociating mercaptides. Depending on the spatial arrangement of the SH-groups in the protein, Hg\(^{2+}\) can produce either a monomer containing one mercury atom or a dimer of two protein molecules joined by a mercury atom [18,19]. Organomercury compounds of the type R-Hg-X (pCMB, pCMBS and others) react only with one SH-group. When mercurials completely inhibit transport proteins they can be used successfully in rapid sampling techniques [20,21]. The inhibitor must be efficient, rapid and block the transport protein without unwanted side-effects. In this respect, some, but not all, organomercurials can cause an unspecific flux through the protein, as was observed with the mitochondrial aspartate/glutamate carrier [22]. However, this unspecific leak is not observed in other systems such as lac permease of E. coli [75] or the arginine/ornithine exchanger of Lactococcus lactis [21].

Labeling of proteins with chloromercuriphenols and S-mercuric N-dansylcysteine can be used in principle for the detection of conformational changes in the protein, because the spectroscopic properties of these compounds are sensitive to their environment [23,24].

III-E. Arsenic compounds

Inhibition of enzyme activity by arsenite and arsenoxides supplies strong evidence for the presence of vicinal SH-groups [25–27]. Arsenic compounds form very stable five-membered rings with 1,2-dithiols (Fig. 3). Enzymes are completely or partially reactivated by the addition of DTT or 2,3-dimercaptoopropanol.

III-F. Oxidation of SH-groups

Oxidation of SH-groups under mild conditions by compounds such as iodine, ferricyanide, tetrathionate, \(\alpha\)-iodosobenzoate or hydrogen peroxide [28] results in the formation of inter- or intramolecular disulfide bonds:

\[
2R-SH \rightarrow R-S-S-R + \text{2H}^+ + \text{2e}^-
\]

The disadvantage of many of these oxidizing agents is that they can also oxidize other groups, such as tyrosine, tryptophan and methionine residues, dependent on the particular system. Another possibility is that oxidation of SH-groups can occur to a state of sulfur more oxidized than that of a disulfide, by formation of sulfinites or sulfonates [29–31]. These difficulties can be avoided by using more specific reagents such as benzofuoroxan (Fig. 4) [32]. The rate and type of oxidation depends on the oxidation-reduction potentials of the SH-groups and the oxidizing agent, the concentration of reagents, pH, temperature and on the spatial arrangement of the SH-groups in the protein. When proteins contain only one SH-group or when contact between SH-groups is not possible because of the tertiary structure intramolecular disulfides cannot be formed. These proteins, with single or sterically restricted SH-groups, can be oxidized to sulfenic, sulfonic (cysteic) acids, depending upon the strength of the oxidizing agent:

\[
\text{RSH} \rightarrow \text{RSOH} \rightarrow \text{RSO}_2\text{H} \rightarrow \text{RSO}_3\text{H}
\]

Furthermore, SH-groups can undergo photooxidation in the presence of ultraviolet light to form a disulfide or cysteic acid. SH-groups can also be oxidized "spontaneously" by atmospheric oxygen ("autooxidation"). This reaction is facilitated by the presence of catalytic amounts of metal ions, especially iron and copper [33–36]. Mercaptoethanol and dithiothreitol are used for the protection of SH-groups from oxidation. However, these thiols can also undergo oxidation in air to form \(\text{H}_2\text{O}_2\) [2], which can in turn oxidize SH-groups. Addition of catalase, EDTA or an increased amount of DTT can further stabilize the enzyme preparation.

III-G. Thiol-disulfide exchange

Oxidation of SH-groups by disulfides is absolutely specific. The reaction consists of two steps of nucleo-
Philic substitution with the formation of a mixed disulfide as an intermediate:

\[
R'S^- + R''S^- \rightarrow R'S.SR'' + R''S
\]

\[
R'S - R''S \rightarrow R'S-SR'' - R'S
\]

In a protein containing a single or sterically restricted SH-group the reaction may stop with the formation of a mixed disulfide.

Ellman's reagent, 5,5'-dithiobis(2-nitrobenzoate), is used for the quantitative determination of SH-groups [37,38]. Reaction of Ellman's reagent with SH-groups results in the formation of mixed disulfides, leaving stoichiometrically a nitrothiobenzoate anion, which has an intense absorbance at 412 nm.

Several disulfides are available which can be used to label proteins at their SH-groups. Examples of fluorescent probes are 1,4-N-2-aminophenyl-2-(pyridyl disulfide)-7-nitrobenzo-2-oxa-1,3-diazole [39], di(1-dimethylamino)naphthalene-5-sulfonate-(dansyl)-L-cystine and difluorescein isothiocarbamido-cystamine [40,41]. The advantage of these disulfides is their absolute specificity. However, the disadvantage of these probes for obtaining structural information about the protein is their relatively large size. A good alternative is methanethiosulfonate spin-label, which is much smaller. By combining site-directed mutagenesis with EPR (electroparamagnetic resonance) spectroscopy of purified spin-labeled single cysteine mutants, tertiary structural information on bacteriorhodopsin [42] and colicin E [43] has been obtained.

Another application of mixed disulfides is covalent chromatography of proteins using thiol-Sepharose 4B or 6B. Proteins containing a reactive SH-group can be bound specifically to a column containing a Sepharose derivative and are subsequently eluted by washing the column with buffer containing an excess of thiol compound (see for example Ref. 44).

**IV. The role of cysteine residues in membrane transport systems**

**IV-A. Localization of reactive SH-groups**

In Table II a number of membrane transport systems is listed in which the role of cysteinyl residues has been studied. Different membrane transport systems exhibit different reactivities toward SH reagents. Some systems are inhibited by permeable (apolar) and impermeable (polar) reagents while others are inhibited by permeable reagents only. In this way different classes of essential sulfhydryl groups can be identified. Examples of membrane permeable SH-reagents which have been used frequently are NEM, p-chloromercuribenzoate (pCMB) and plumbagin. Many used membrane impermeable SH-reagents are eosin-5-maleimide, ferricyanide, glutathione maleimide, 4-(2-arsenophenyl)azo-3-hydroxy-2,7-naphthalene disulfonic acid (thorin), 5,5'-dithiobis(2-nitrobenzoic acid) and mersalyl.

By using eosin-5-maleimide and NEM, it was determined that the ADP/ATP carrier of mitochondria has two distinct classes of sulfhydryl groups at and near the matrix surface of the mitochondria, respectively [50]. On the other hand the phosphate transporter, which contains six cysteines, has a single reactive class at the cytoplasmic surface of the mitochondria [50,51]. The high reactivity of this class (i.e., Cys-42) has been attributed to electrostatic effects of two neighboring basic groups, Lys-41 and Arg-43 [72]. These basic groups would facilitate the dissociation of the sulfhydryl-proton, and therefore increase the reactivity with NEM. The other cysteine residues exist either as disulfides or are sterically inaccessible to NEM. Similar studies on the glucose facilitator of human erythrocytes

<table>
<thead>
<tr>
<th>Transport system</th>
<th>Location and role of SH-groups</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proline permease (E. coli)</td>
<td>one class at outer and one class at inner surface</td>
<td>[47]</td>
</tr>
<tr>
<td>Lactose permease (E. coli)</td>
<td>more reactive classes; Cys-148 at or near substrate binding site</td>
<td>[15,46]</td>
</tr>
<tr>
<td>Mannitol permease (E. coli)</td>
<td>phosphorylation site</td>
<td>[69]</td>
</tr>
<tr>
<td>Arginine-ornithine antiporter (L. lactis)</td>
<td>one or more classes at outer surface; probably located at or near binding site</td>
<td>[66]</td>
</tr>
<tr>
<td>Bilirubin translocase (rat liver)</td>
<td>two classes with different reactivity</td>
<td>[72]</td>
</tr>
<tr>
<td>Uncoupling protein (brown adipose tissue mitochondria)</td>
<td>three different classes; one class essential for H⁺, but not for CI⁻ transport</td>
<td>[73]</td>
</tr>
<tr>
<td>Phosphate transport system (rat heart and -liver mitochondria)</td>
<td>single class near external surface; not involved in substrate binding</td>
<td>[50,51]</td>
</tr>
<tr>
<td>ADP/ATP antiporter (rat liver mitochondria)</td>
<td>two classes at or near internal surface</td>
<td>[50]</td>
</tr>
<tr>
<td>Glucose facilitator (human erythrocytes)</td>
<td>three different classes involved in translocation process</td>
<td>[73,74]</td>
</tr>
</tbody>
</table>
led to the identification of three different classes of reactive SH-groups [73]. One class (type III), is directly involved in the translocation process and is required for the dissociation of glucose bound at the transport sites.

IV-B. Lac-permease of Escherichia coli

In the lac permease of F coli the role of the eight sulphydryl groups has been analyzed in detail. As demonstrated initially by Fox and Kennedy [52,53], the lac permease is irreversibly inactivated by NEM and protection is offered by substrates such as β-D-galactopyranosyl-β-D-thiogalactopyranoside (TDG). On the basis of these findings, it was postulated [52] that a cysteinyl residue is at or near the substrate-binding site of lac permease. Beyreuther et al. [54] later showed that the substrate-protectable residue is Cys-148. In addition, the permease is reversibly inactivated by other sulphydryl reagents like pCMBS or by sulphydryl oxidants such as diamide [55] or plumbagin [46], while TDG prevents inactivation by these reagents. These observations, together with other studies [45,47,48], led to the model shown in Fig. 5.

In 1971, Kaback and Barnes [49] proposed a model in which dithiol-disulfide interchange plays an important role in the mechanism of lactose transport. In this model the transport system was depicted as an electron transfer intermediate having one redox-center which undergoes reversible oxidation-reduction (Fig. 5). In the oxidized state, the system has a high affinity for its substrate which it binds on the outside. The critical disulfide in the transport system can be reduced by accepting electrons which results in a conformational change. This conformational change leads to a markedly decreased affinity of the system for its substrate and ligand is released at the outside. The reduced form of the transport system is also able to catalyze low affinity, permease-mediated, non-energy-dependent transport of ligand across the membrane.

Another model was proposed by Konings and Robillard [45–47] in 1982, in which dithiol-disulfide interchange plays an important role in many membrane-related processes such as transport, energy transduction and hormone-receptor interactions. These authors proposed that the affinity of the substrate binding site is regulated by a dithiol and a disulfide located at different depths in the membrane. Furthermore, the oxidation states of these two redox centers are coupled by dithiol-disulfide interchange such that, when one is oxidized, the other is reduced (Fig. 6). Furthermore, when the reactive thiols of the transport system at the outside are in the reduced state the system has a high affinity for its substrate. When it is in the oxidized state the system has a low affinity for its substrate. It can be derived that the redox states of the two centers will vary in response to ΔΨ or ΔpH (see Ref. 48 for a detailed treatment). According to this model Δp drives active transport (symport or antiport) and other energy transducing processes by inducing changes in affinity.

Both models emerged from studies demonstrating that lac permease is inactivated by NEM in a substrate-protectable manner [52] and is reversibly inhibited by several SH reagents which oxidize SH-groups [46,47]. Although there are eight cysteine residues in the lac permease (Fig. 7) their precise role is not known. Therefore, a question arises as to whether or not dithiol-disulfide interconversion plays a role in the mechanism and/or in the regulation of lactose transport.

Given the importance attributed to sulphydryl groups in lac permease, particularly Cys-148, site-directed mutagenesis was used to study the role of the cysteine residues in lac permease. Out of a total of eight cysteinyl residues in the permease, only Cys-154 appears to be important for transport and even this residue is not absolutely essential [56–65]. Although the results from site-directed mutagenesis provide strong evidence that cysteinyl residues in the permease do not play a direct role in the mechanism, alkylation of Cys-148 or replacement of Cys-154 with Gly leads to inactivation. Furthermore, Cys-148 and Cys-154 are both located in putative transmembrane helix V (Fig. 5).
adduct with either Cys-148 or Cys-154 and not from disulfide formation [64]. Modification of either of these cysteinyl residues strongly decreases the activity of the permease. However, regulation of the permease activity by sulfhydryl-disulfide interconversion seems to be unlikely. A similar conclusion was reached in studies of Page and West [77]. Plumbagin can react with sulfhydryl groups by adduct formation or by the catalysis of the formation of a disulfide bridge [17]. On the other hand the similar compound 2,3,5,6-tetramethylbenzo-1,4-quinone can react with sulfhydryl groups only by the formation of a disulfide bridge [17]. Lactose transport in whole cells was inhibited by plumbagin, but not by 2,3,5,6-tetramethylbenzo-1,4-quinone [77], thereby providing indirect evidence that the formation of a disulfide by plumbagin in lac permease is unlikely. Subsequently, using oligonucleotide-directed, site-specific mutagenesis a lac permease molecule was constructed in which all eight cysteinyl residues were simultaneously mutagenized (Cys-less permease). Cys-154 was replaced with valine, and Cys-117, -148, -176, -234, -333, -353, and -355 were replaced with serine [65]. Remarkably, Cys-less permease catalyzes lactose accumulation in the presence of a Δp (interior negative and alkaline). As anticipated, moreover, active lactose transport via Cys-less permease is completely resistant to inactivation by NEM [65] and by plumbagin (P.R. van Iwaarden, unpublished results). These results pro-
vide definitive evidence that sulfhydryl groups do not play an essential role in the mechanism of lactose/H⁺ symport.

The construction of a functional permease devoid of cysteiny1 residues provides the basis for an approach to the analysis of static and dynamic aspects of permease structure-function relationships. By using the lacY gene encoding C-less permease, for instance, it is now possible to design mutants in which an individual amino acid residue in putative hydrophilic or hydrophobic domains is replaced with a cysteiny1 residue which can then be reacted specifically with either permeant or impermeant sulfhydryl reagents in right-side-out or inside-out membrane vesicles, followed by solubilization and immunoprecipitation. In addition, single Cys mutants can be tagged with appropriately reactive EPR or fluorescent probes after solubilization and purification and studied spectroscopically after reconstitution. Finally, it should be possible to study proximity relationships between transmembrane domains by placing single cysteiny1 residues in pairs of helical domains predicted to lie close to each other within the membrane.

IV-C. Other transport systems

In the proline carrier of E. coli an active cysteine was postulated to be involved in substrate and cation recognition [78], on the basis of substrate protection against NEM inactivation. Using site-directed mutagenesis, Cys-281 and 344 were identified as the residues that are modified by NEM, and shown not to be essential for proline transport [79]. Interestingly, Cys-344 and Cys-349 have similar configurational localization as Cys-148 and Cys-154 of the lac permease of E. coli in the secondary structure models. Also in the raffinose permease of E. coli [80] and the lactose permease of Klebsiella pneumoniae [81] Cys-148 and Cys-154 are conserved. The role of the residues has not been analyzed in these permeases.

In other membrane transport systems the reactive SH-group is not protected by substrates towards SH-reagents, as in the phosphate transporter of rat heart and liver mitochondria [50,51]. An interesting observation in the case of the arginine-ornithine antiporter of L. lactis [66] and the mitochondrial carriers for dicarboxylates, 2-oxoglutarate and neutral amino acids [67], is that the reactive SH-groups are relatively insensitive to NEM, while organomercurials inhibit activity at low concentrations. It is known that these reagents not necessarily react with the same SH-groups [3,27]. Reaction of SH-groups with NEM is strongly dependent on the polarity of the environment. NEM (like other alkylating agents and disulfides) usually blocks only the more accessible or reactive SH-groups, leaving the more buried residues unmodified. Organomercurials, on the other hand, have the ability to penetrate into partially masked regions of the protein, where they can react with less accessible residues [2]. Furthermore, organomercurials have higher specificity towards SH-groups than maleimides [2].

A different critical role for a cysteiny1 residue, the transport mechanism is found in the mannitol-specific carrier of the E. coli phosphoenolpyruvate-dependent phosphotransferase system [68]. In this system the activity-linked cysteine (Cys-384) was identified as one of two phosphorylation sites, located at the cytoplasmic side of the protein [69]. The other phosphorylation site is His-554. This protein is only active when Cys-384 can be phosphorylated, for which Cys-384 has to be in its reduced form [70]. The phosphorylated protein is protected against oxidation, because Cys-384 is not available for disulfide formation [71].

V. Concluding remarks

SH-reagents can be very useful for identifying structurally and functionally important cysteine residues in transport proteins. However, as might be evident from the different examples care should be taken with respect to the hypothesis that reactive SH-groups play a crucial dynamic role in the mechanisms of substrate transport. Apparently SH-groups can play different roles in different transport proteins. Modification of reactive SH-groups located in functionally important regions of the protein can have drastic effects on the functioning of the protein, without playing direct roles in the transport mechanism. To characterize the SH-groups of a transport protein it is important to examine the interactions with SH reagents of various types. In any case, a general role for SH-groups in the mechanism of membrane transport has not been shown thusfar.

The high reactivity of the SH-group can be very useful for labelling many transport proteins specifically with different reporter groups based on SH reagents. Subsequently, many structural and functional aspects of the transport system can be analyzed. The specificity of the labelling is usually strongly dependent on the transport protein and the reagent. Furthermore, when SH reagents completely inhibit the transport system they can be very useful in rapid sampling techniques and in different control experiments.

Acknowledgement

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References