Biochemical Coupling between the DrrA and DrrB Proteins of the Doxorubicin Efflux Pump of *Streptomyces peucetius* *

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The *drrAB* operon of *Streptomyces peucetius* encodes for resistance to the antibiotics doxorubicin and daunorubicin. Subcloning of the *drrAB* genes in *Escherichia coli* has previously been shown to result in expression of DrrA and DrrB proteins and resistance to doxorubicin in a sensitive strain of *E. coli*. DrrA, a peripheral membrane protein, binds ATP in a UV-catalyzed reaction in a doxorubicin-dependent manner; DrrB, a hydrophobic protein, is localized to the inner membrane of *E. coli* (Kaur, P. (1997) *J. Bacteriol.* 179, 569–575). The present study provides evidence that DrrA, the membrane component of the complex, is stably maintained in the cell only if DrrA is present. Furthermore, it was found that the catalytic component DrrA is in an active conformation only when it is in a complex with DrrB. In a subclone containing the *drrB* gene by itself, no DrrB protein could be detected, although a translational fusion of the first 15 amino acids of DrrB to β-galactosidase indicated that DrrB is translated in the absence of DrrA. Upon co-transformation with a plasmid containing the *drrA* gene in trans, DrrB could again be detected in these cells. UV cross-linking studies with [γ-32P]ATP showed that only the membrane-bound form of DrrA in cells containing both DrrA and DrrB was in a conformation competent to bind ATP. Chemical cross-linking studies also provided direct evidence for interaction between the two proteins. Based on these analyses, a model for interaction between DrrA and DrrB proteins is presented.

Doxorubicin and daunorubicin, two antibiotics produced by *Streptomyces peucetius*, are widely used in chemotherapy. *S. peucetius* is itself resistant to the inhibitory action of these drugs. Resistance in *S. peucetius* is encoded by the *drr* operon that contains two open reading frames, *drrA* and *drrB* (1). DrrA and DrrB proteins have been hypothesized to form an efflux pump that confers resistance by transport of the drugs out of the cell (1, 2). DrrA protein consists of one nucleotide binding domain with homology to the ABC family of proteins to which P-glycoprotein (Pgp); Ref. 3) belongs. The product of the *drrB* gene is a membrane protein (1, 2) with homology to membrane components of the bacterial ABC transporters (4). Pgp is expressed in human cancer cell lines, where it confers resistance to a variety of structurally unrelated chemotherapeutic drugs, including doxorubicin and daunorubicin (3). This phenomenon is referred to as multidrug resistance. Pgp contains a drug-stimulated ATPase activity (5); however, its mode of action and the mechanism by which it can transport structurally unrelated substrates is not understood. Since DrrA and Pgp are homologous proteins and both confer resistance to doxorubicin and daunorubicin, an understanding of the structure and function of DrrA pump is expected to contribute to a better understanding of the function and evolution of Pgp.

The *drr* operon has previously been subcloned in an *Escherichia coli* expression vector, and both proteins have been identified using antisera raised in rabbits (2). Expression of the proteins in an *acr* strain of *E. coli* resulted in doxorubicin resistance in these cells, indicating that the proteins expressed in *E. coli* retain proper conformation and function. Isolation of active alkaline phosphatase fusions in DrrB showed that DrrB is an integral membrane protein (2). DrrA, a peripheral membrane protein was shown to bind ATP or GTP in the presence of Mg2+. The nucleotide binding to DrrA was found to be doxorubicin-dependent (2), indicating that the activity of DrrA is regulated by the substrate of the pump.

Most ABC transporters, including Pgp, consist of two nucleotide binding domains and two membrane-spanning domains of six α-helices each. In Pgp, the four domains form one large protein of about 170 kDa (3). The Drr system, instead, consists of two subunits, DrrA and DrrB, that carry individual domains. Together, a monomer of DrrA and a monomer of DrrB form a molecule about half the size of Pgp; however, the stoichiometry of the DrrA and DrrB proteins in the Drr pump is not known. The present study focuses on an interaction between DrrA and DrrB; the data indicate a strong dependence of the proteins on each other for their expression and function such that the two protein subunits behave as domains of a larger protein. The DrrB protein was detected in the cell membrane of these cells only if there was simultaneous expression of DrrA. In addition, DrrA was in an active conformation to bind and/or hydrolyze ATP only if it was in a complex with DrrB in the membrane. Chemical cross-linking studies also suggest a direct interaction between DrrA and DrrB with the suggested stoichiometry of the complex being DrrA2B2. These results bear important implications for the evolution of multidomain proteins, including Pgp, and for understanding the interaction between their domains.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Plasmids—**The *E. coli* strains and plasmids used in this study are described in Table I.

**Media and Growth Conditions—**The cells were grown in LB medium (6) at 37 °C. Chloramphenicol was added to 20 μg/ml, and ampicillin was added to 100 μg/ml.

**DNA Manipulations—**The conditions for plasmid isolation, DNA endonuclease restriction analysis, ligation, and sequencing were described elsewhere (7).

**Subcloning of the *drrA* Gene—**The 1.0-kilobase pair *NdeI–*HindIII fragment from pDX102 was subcloned behind the *lac* promoter in

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† The abbreviations used are: Pgp, P glycoprotein; PAGIE, polyacrylamide gel electrophoresis; IPTG, isopropyl-1-thio-β-D-galactopyranoside; DSP, dithiobis(succinimidyl propionate); DTSSP, dithiobis(sulfosuccinimidyl propionate).
pUC18 vector by substitution of the araS gene in pUC18arsA. This clone has been designated pDX108. The drrA gene in this construct utilizes the translational start signals of the araS gene.

Creation of Translational Fusions of DrrB to β-Galactosidase—A translational fusion of the first 15 amino acids of DrrB to the 8th amino acid of β-galactosidase was created. Using pDX101 or pDX103 as the template (Fig. 1 and Table I), the region between the lac promoter and the 45th nucleotide of the drrB gene was polymerase chain reaction-amplified. Polymerase chain reaction primers had the following nucleotide sequence, and each primer contained the following HindIII restriction site: 1, 5'-GCGCCACAGTCCTCCTCCCGCGCGGGTTGG-3'; II, 5'-GCCGCGCCAGCTGCTCATACGCGGGTTGGG-3'. The amplified product was ligated to the translational fusion vector pMC1069 that had been digested with HindIII. Amplification and subcloning of the fragment from pDX101 and pDX103 resulted in plasmids pDX109 and pDX110, respectively. pDX109 contains plc and the ribosome binding site of araS, followed by the drrA gene translationally coupled to drrB-lacZ fusion, as is seen in the original operon. The drrA gene, however, is missing from pDX110.

Analysis of Translational Fusions by β-Galactosidase Assay—CC118 cells containing pDX109 or pDX110 were grown to mid-log phase and induced with 1 mM IPTG for 3 h. The cells were harvested and lysed by a single passage through a French pressure cell at 20,000 p.s.i. The lysed cells were centrifuged at 10,000 g for 15 min to remove unbroken cells. 100 µg of protein was then added to a tube containing 0.5 ml of Z buffer containing 60 mM NaHPO4, 40 mM NaH2PO4, 10 mM KCl, 1 mM MgSO4, 50 mM β-mercaptoethanol, 200 µl of α-nicotinyl β-n-galactosidase, and the reaction was continued for 5 min. The reaction was stopped with 0.5 ml of 1 M NaCO3. Analysis by Western blotting using anti-DrrB serum suggested that the lac promoter and the translational start signals of the araS gene. pDX101 also contains a putative ribosome binding site (RBS) for drrB, which is contained within the coding sequence of drrA. In addition, the region between drrA and drrB (shown as ATGA in Fig. 1A) shows translational coupling such that the stop codon of drrA overlaps with the start codon of drrB. Translational coupling is absent in pDX103. drrB in pDX103 lacks its own putative RBS, and it utilizes the RBS of araS. E. coli TG1 cells containing the indicated plasmids were induced with IPTG, and samples were collected after 3 h of induction. The cells were fractionated into cytosolic and total membrane fractions. The fractions were analyzed by Western blotting using anti-DrrA or anti-DrrB serum (Fig. 1B), and the intensities of the bands were determined by densitometric analysis. Western blot analysis using anti-DrrA serum suggested that DrrA is expressed both in pDX101- and pDX102-containing cells; however, the level of expression in pDX102 (Fig. 1B, a, lanes 1 and 2) was lower than in pDX101 (Fig. 1B, a, lanes 1 and 2). Densitometric analysis suggested that the total level of DrrA in pDX102 was about 70% that of in pDX101. However, in both situations, i.e. in the presence or the absence of DrrB, roughly 80% DrrA was membrane-associated. A band of faster mobility than DrrA that reacts with anti-DrrA antibody (Fig. 1B, a) is unrelated to DrrA, since this band is also observed in the control samples that do not contain either DrrA or DrrB (2).

Analysis of Western blotting using anti-DrrB serum sug-

### Table I

| Strain or plasmid | Genotype or description | Reference or source |
|-------------------|-------------------------|---------------------|
| TGL               | K12 Δlac-pro, supE, thi, hsd D51 F' traS6, pro A- B- lacIq, lacZAM15 | 7, 11 |
| CC118             | F' araD139 Δara-lev17697 ΔlacX74 phoAΔ20 galE18 galK16 thi rpsE rpoB argE(Am) recA1 | |
| pUC18arsA        | araS, Amp'             | 12               |
| pMC1069          | Vector, Amp'           | 6                |
| pDX101           | drrAB in pSU2718arsA, Cm' | 2               |
| pDX102           | drrA in pSU2718arsA, Cm' | 2               |
| pDX103           | drrB in pSU2718arsA, Cm' | 2               |
| pDX110           | drrA and 45 nt from the 5' -end of drrB fused to 24 nt of lacZ in pMC1069 | This study |
| pDX110           | 45 nt from the 5' -end of drrB fused to 24 nt of lacZ in pMC1069 | This study |

* nt, nucleotide(s).
gested that DrrB is present only in pDX101-containing cells (Fig. 1B, b, lanes 3 and 4). Little if any DrrB could be detected in the membrane fraction prepared from cells containing pDX103 (Fig. 1B, b, lanes 1 and 2). To determine if the expression of DrrB in pDX101 is the result of translational coupling with DrrA and the absence of DrrB in pDX103 is a result of the absence thereof, a translational fusion of the first 15 amino acids of DrrB with β-galactosidase was created. Using pDX101 or pDX103 as a template, the region starting at the promoter plac and ending with the 45th nucleotide of drrB was amplified by polymerase chain reaction, and the amplified fragment was fused to the eighth codon of β-galactosidase. Two clones resulting from this (Fig. 2), one (pDX109) containing the drrA gene in cis and the other without (pDX110), were tested for β-galactosidase activity. The data in Fig. 2 indicate that drrB in pDX110 can be translated, although the β-galactosidase activity in pDX109, where drrA is present in cis, was about 2-fold higher as compared with pDX110.

Since DrrB is translated in the absence of coupled translation with DrrA, but not detected in these cells by Western blot analysis (Fig. 1), we wanted to determine if maintenance of DrrB in the cell depends on simultaneous expression of DrrA. E. coli TG1 cells containing pDX103 were co-transformed with a compatible plasmid pDX108 containing the drrA gene. pDX108 contains the drrA gene in pUC18 and, as in pDX101--pDX103, uses the translational start signals of arsA (Table I and Fig. 1). After induction of the drrA and drrB genes with IPTG, total cell lysates were analyzed for the presence of DrrA and DrrB proteins. Data in Fig. 3 show that the expression of DrrB in pDX103 is restored in cells containing DrrA in trans. The amount of DrrB protein detected was roughly the same whether the drrA gene was present in cis (pDX101-containing cells; lane 4) or in trans (pDX103 and pDX108; lane 5), indicating that the presence of the DrrA protein is required for maintenance of DrrB in the cell. As is also seen in Fig. 1, the level of DrrB in pDX102-containing cells was lower (Fig. 3, lane 2) as compared with pDX101-containing cells (Fig. 3, lane 4). However, expression of DrrA in pDX108 (lane 1) results in the same level of DrrA protein as in pDX101 (Fig. 3, lane 4). This may be due to the higher copy number of plasmid pUC18 in pDX108 compared with the plasmid pSU2718 in pDX102. The band at around 29 kDa is the protein reacting nonspecifically with the anti-DrrA antibody, and it is present in all of the samples.

The chemical nature of the association of DrrA or DrrB to the cell membrane was evaluated by extraction with urea (Fig. 4). About 90% DrrA was still membrane-associated after treatment with 1.5 M urea in both DrrA- and DrrAB-containing membranes (Fig. 4, lanes 2 and 5), whereas it could be completely extracted by 6 M urea in both cases (lanes 3 and 6). DrrB was not affected at all by 6 M urea treatment (Fig. 4), confirming the earlier observation that DrrB is an integral membrane protein, whereas DrrA is peripherally associated with the membrane in both DrrA- or DrrAB-containing cells.

**ATP Binding to DrrA**—It has previously been shown that DrrA can form a UV-activated adduct with ATP or GTP in a doxorubicin-dependent manner in total cell lysates containing both DrrA and DrrB (2). To determine if DrrB affects ATP binding to DrrA, ATP binding was characterized in the cytosolic and membrane fractions prepared from E. coli cells containing pDX101 or pDX102. [γ-32P]ATP binding in a UV-catalyzed cross-linking experiment showed that DrrA cross-links to ATP only in membranes prepared from cells containing both DrrA and DrrB (Fig. 5A, lanes 3 and 4). No ATP cross-linking was seen to DrrA in membranes containing DrrA alone (Fig. 5A, lanes 1 and 2). Western blot analysis of a similar gel using antibodies to DrrA or DrrB showed that DrrA was present in all samples (Fig. 5B, lanes 1–4), whereas DrrB was present only in lanes 3 and 4 (Fig. 5C). Further, it was tested whether the soluble fraction of DrrA in cells containing both DrrA and DrrB can be UV-cross-linked to ATP. Data in Fig. 5A, lanes 5 and 7, show that the soluble fraction of DrrA in DrrAB-con-
taining cells was also not in a state competent to cross-link to ATP. Only the membrane bound form of DrrA in DrrAB-containing cells was competent (Fig. 5A, lanes 6 and 8). Again, Western blot analysis showed that DrrA was present in both the cytosolic and the membrane fractions (Fig. 5B, lanes 5–8), whereas DrrB was present only in the membrane fractions (Fig. 5C, lanes 6 and 8). Two other species in the membrane-containing samples, that move with slower mobility compared with DrrA, were also seen to form a UV-activated adduct with ATP (Fig. 5A, lanes 1–4, 6, and 8). Both of these species are unrelated to DrrA and bind ATP irrespective of the presence or absence of DrrB.

![Chemical extraction of the cell membrane with urea.](image)

**Fig. 3. Effect of co-expression of DrrA and DrrB.** E. coli cells, co-transformed with pDX103 and pDX108, were induced with IPTG and lysed as described under “Experimental Procedures.” The total lysates were analyzed by SDS-PAGE followed by Western blot analysis using anti-DrrA or anti-DrrB serum. The same amount of total protein was loaded on the gel in each lane. After transfer of the proteins to the membrane, the top part of the blot was probed with anti-DrrA, and the bottom part was probed with anti-DrrB serum. Lane 1, pDX108: lane 2, pDX102; lane 3, pDX103; lane 4, pDX101; lane 5, pDX103 and pDX108. The positions of molecular mass markers are shown on the left, and the cell genotypes are shown at the bottom of the gel.

![Chemical extraction of the cell membrane with urea.](image)

**Fig. 4. Chemical extraction of the cell membrane with urea.** The cell membrane prepared from cells containing pDX101 or pDX102 was extracted with different concentrations of urea as described under “Experimental Procedures.” The extracted membrane was analyzed by SDS-PAGE followed by Western blot analysis using anti-DrrA or anti-DrrB serum. Lanes 1 and 4, control; lanes 2 and 5, 1.5 M urea; lanes 3 and 6, 6 M urea.

![Chemical extraction of the cell membrane with urea.](image)

**Fig. 5. [α-32P]ATP binding to DrrA protein.** UV-catalyzed adduct formation between DrrA protein and [α-32P]ATP was performed in the cytosol and membrane fractions as described under “Experimental Procedures.” The reactions contained 35 μM doxorubicin (Dox) where indicated. The proteins were resolved by SDS-PAGE on 12% gels, followed by autoradiography. The proteins from a similar gel were electrophoretically transferred to nitrocellulose membrane, which was followed by sequential Western blot analysis of the membrane with anti-DrrA and anti-DrrB serum. A, autoradiogram showing [α-32P]ATP binding to DrrA protein. A1, experiment 1 (lanes 1–4). Membrane fraction prepared from cells expressing DrrA (lanes 1 and 2) or both DrrA and DrrB proteins (lanes 3 and 4) was used. Each lane contained 25 μg of protein. A2, experiment 2 (lanes 5–8). Cytosol or membrane fraction prepared from cells expressing both DrrA and DrrB was used, as indicated at the bottom of the gel. The same volume of the indicated fraction was loaded in lanes 5 and 6 and in lanes 7 and 8. B, Western blot probed with anti-DrrA serum. C, Western blot in 8 stripped of the DrrA antibody and reprobed with anti-DrrB serum. Lanes 1–8 contained similar samples in A, B, and C. Lanes 1–4, 6, and 8, membrane fraction; lanes 5 and 7, cytosol. C, cytosol; M, cell membrane.
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Fig. 6. Chemical cross-linking between DrrA and DrrB. The cell membrane fraction containing both DrrA and DrrB proteins was subjected to chemical cross-linking as described under “Experimental Procedures.” The proteins were resolved by SDS-PAGE, followed by Western blot analysis using anti-DrrA or anti-DrrB antibodies. A, anti-DrrB; B, anti-DrrA. Lanes 1 and 8, uncross-linked control. Lanes 2–7, chemical cross-linking using DTSSP. Lanes 9–13, chemically cross-linked using DSP. Lanes 2 and 9, 0.05 mM. Lanes 3 and 10, 0.1 mM. Lanes 4 and 11, 0.25 mM. Lanes 5 and 12, 0.5 mM. Lanes 6 and 13, 1 mM. Lane 7, 5 mM. The positions of the molecular mass markers are shown on the left, and the chemically cross-linked species are indicated on the right with single, double, and triple asterisks.

Fig. 7. Two-dimensional analysis of the chemically cross-linked species. Lane 11 from the gel in Fig. 5 was excised, and the cross-linked species were detected by incubation with β-mercaptoethanol. The excised lane was placed on top of a 10% polyacrylamide-SDS gel and subjected to electrophoresis, followed by sequential Western blot analysis with anti-DrrB and anti-DrrA antibodies. A and C, unreduced controls. B and D, after reduction with β-mercaptoethanol. The position of the cross-linked species is marked with a single, double, and triple asterisk on the unreduced gels in A and C.

In the present study, expression and function of the DrrA and DrrB proteins was found to be dependent on each other, resulting in a strong biochemical coupling. In cells containing the plasmid pDX101, which contains drrA and drrB genes in cis, both DrrA and DrrB proteins were detected. Upon subcloning of the drrA gene separately in pDX102, DrrA was detected but at levels lower than in pDX101, indicating that DrrB might affect the expression or stability of DrrA. Effect of DrrA on DrrB expression or stability, however, was much more dramatic. Subcloning of the drrB gene alone in pDX103 showed the absence of DrrB protein (Fig. 1). Since pDX101, -102, and -103 all contain the same promoter, the regulation of expression of drrB appears to be at the level of translation or post-translational. DrrA and drrB in the original operon are translationally coupled so that the stop codon of drrA overlaps with the start codon of drrB (Fig. 1). To determine if coupling is essential for expression of drrB, a translational fusion containing first 15 amino acids of DrrB fused to the eighth codon of β-galactosidase was created. Analysis of the fusion indicated that DrrB is translated in the absence of DrrA in pDX110 although at levels lower than in pDX109 that contains DrrA in cis (Fig. 2). Hence, translational coupling does not seem to be essential for translation of DrrB, although it appears to have some role in increasing efficiency of translation of DrrB in pDX109. Further experiments showed that the simultaneous expression of DrrA, whether from a gene in cis or in trans, was crucial for stable maintenance of DrrB in the cell. When drrA and drrB genes were co-transformed into the same cell on compatible plasmids, DrrB protein, translated from pDX103, was again detected at the same levels as in pDX101 (Fig. 3), implying that the regulation is mostly post-translational. To understand the contribution of translational coupling in pDX101 and pDX109 toward increased translation of DrrB, it is important to understand the role of the putative RBS of drrB. This is presently under investigation; however, it is clear that drrB is translated in pDX103, but the protein is not detected in these cells by Western blot analysis. When the drrA gene is co-transformed into these cells, DrrB is again detected at the
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FIG. 8. A model for interaction between DrrA and DrrB. DrrB is shown as an integral membrane protein (2) containing seven transmembrane domains with the N terminus inside the cytoplasm and the C terminus in the periplasm (P. Kaur and J. Levine, unpublished results) and DrrA as a protein peripherally associated with the membrane (2). In the absence of DrrA, DrrB is degraded either soon after it is translated or after it inserts into the membrane. Hence, DrrB is not present at detectable levels in the membrane if DrrA is not simultaneously expressed. DrrA goes through different conformations (Confo.) in the presence or absence of DrrB and the antibiotic ligand doxorubicin. The uncomplexed form of DrrA (either the soluble fraction of DrrA or the membrane-associated form of DrrA in the absence of DrrB) is in conformation I, which is inactive as determined by the lack of UV-mediated ATP cross-linking in the absence or the presence of the ligand doxorubicin. In the presence of both DrrA and DrrB, DrrB is inserted and/or stably maintained in the membrane due to an interaction with DrrA. In this complexed state, DrrA acquires conformation II, which seems to be essential for achieving the active conformation (conformation III) upon the addition of doxorubicin. In conformation III, DrrA is able to form an adduct with ATP on UV cross-linking (Fig. 5). Based on the chemical cross-linking data, the complex of DrrA and DrrB is shown as consisting of two subunits of DrrA and two subunits of DrrB, where DrrA only contacts DrrB. Dash, not applicable.

Since we found strong evidence for a very specific interaction between DrrA and DrrB, chemical cross-linking studies were carried out to characterize the interaction further and to determine the stoichiometry of the complex in the membrane. Studies carried out with both a membrane-permeable and a membrane-impermeable cleavable cross-linker showed the presence of two cross-linked species: one species of 60 kDa and another species of 120 kDa. Both species were shown by two-dimensional analysis, after reduction, to consist of both DrrA and DrrB. DrrA migrates as a 36-kDa protein, and DrrB, a 30.6-kDa protein, migrates at roughly 25 kDa; hence, the 60-kDa cross-linked species should correspond to a complex consisting of one subunit of DrrA and one subunit of DrrB, whereas the 120-kDa species probably consists of a complex of two subunits of DrrA and two subunits of DrrB. A cross-linked species with a mass greater than 200 kDa, possibly representing a nonspecific aggregate containing DrrA, DrrB, and other membrane proteins, was also observed; the amount of this species was seen to increase at higher concentrations of the cross-linker until it became the major species at 1 mM. Hence, the formation of the 60-kDa chemically cross-linked species suggests a strong interaction between DrrA and DrrB. Based on the available data, it can also be speculated that the DrrAB complex consists of two subunits of DrrA and two of DrrB. The failure to detect species corresponding to (A)2 or (B)2 might imply that in the Drr complex, DrrA only contacts DrrB and not another subunit of DrrA and that the complex has the arrangement of A\(^2\)B\(^1\)A\(^1\)B\(^2\) (Fig. 8), where the superscripts 1 or 2 indicate subunit 1 or subunit 2 within the complex. This would make the DrrAB pump equivalent to Pgp in terms of the number of domains and the overall structure, both containing two nucleotide-binding ABC cassettes and two membrane-spanning domains. The 60-kDa cross-linked species indicates that the closest interaction is between A\(^1\) and B\(^1\) or between A\(^2\) and B\(^2\). Within the arrangement A\(^1\)B\(^1\)A\(^2\)B\(^2\), the species A\(^1\)B\(^2\) and A\(^2\)B\(^1\) would also be expected upon cross-linking, but these species were not detected, perhaps because a cross-link between B\(^1\) and A\(^2\) results in the 120-kDa species A\(^1\)B\(^1\)A\(^2\)B\(^2\) instead of A\(^1\)B\(^1\)A\(^2\) or B\(^1\)A\(^2\)B\(^2\) due to the close proximity of A\(^1\) and B\(^1\) or A\(^2\) and B\(^2\).

Interaction between subunits of transport complexes has been reported (10); however, such a tight regulation of expression and function as seen in the DrrAB system has not been observed before. It has generally been observed that the prokaryotic transport systems exist as complexes consisting of subunits that are encoded by genes within an operon, whereas the eukaryotic transporters occur as single large proteins that contain multiple domains. DrrAB, a typical prokaryotic transporter, consists of two separately expressed subunits; however, the subunits are tightly coupled and appear to behave like the domains of a single protein. In addition to DrrA regulating the stability or membrane insertion of DrrB, the function of DrrA itself was found to be dependent upon its interaction with DrrB. DrrA has previously been shown to be an ATP/ADP-binding protein (2). In the present study, we have shown that no ATP cross-linking to DrrA was seen in the cytoplasmic or membrane-bound form of DrrA in cells containing only DrrA.
Further, ATP cross-linking to DrrA was seen only in the membrane-bound fraction of DrrA in DrrAB-containing cells (Fig. 5). These data suggest that it is the specific interaction between DrrA and DrrB in the membrane that introduces a conformational change in the ATP binding site of DrrA. Hence, although DrrA is tightly associated with the membrane in the absence of DrrB (Fig. 4), it is not in an active conformation. Hence, the ability of DrrB to be stably maintained in the cell and/or inserted into the membrane depends on DrrA, and the ability of DrrA to bind and/or hydrolyze ATP depends on the presence of DrrB in the membrane. In this respect, the DrrAB complex is a novel ABC transporter, where two components assist each other in a stepwise manner. Based on the data presented in this paper, we propose that DrrA is an allosteric protein that goes through different conformations depending on the presence or absence of DrrB and the ligand doxorubicin (Fig. 8). The DrrB protein is shown as consisting of seven transmembrane α-helices based on preliminary analysis of gene fusions with alkaline phosphatase and β-galactosidase. In its uncomplexed state, DrrA is in conformation I, which is inactive in terms of its ability to UV-cross-link with ATP. Interaction with DrrB results in maintenance of DrrB in the cell membrane, and this interaction introduces a conformational change in the active site of DrrA, which is shown as conformation II. Active conformation of DrrA, depicted as conformation III, can be achieved via conformation II upon the addition of doxorubicin. It is in this conformation that DrrA is able to form the UV-activated cross-link with ATP (Figs. 2 and 5).

A better understanding of how DrrA and DrrB interact would undoubtedly lead to a better understanding of how domains within Pgp might interact and stabilize each other and how energy might be transduced between the catalytic ABC domain and the membrane-spanning domain of Pgp. This has implications for understanding the evolution of multidomain proteins found primarily in eukaryotic cells.

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REFERENCES

1. Guilfoile, P. G., and Hutchinson, R. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8553–8557
2. Kaur, P. (1997) J. Bacteriol. 179, 569–575
3. Gottesman, M. M., and Pastan, I. (1993) Annu. Rev. Biochem. 62, 385–427
4. Reizer, J., Reizer, A., and Saier, M. H. (1992) Protein Sci. 1, 1326–1332
5. Senior, A. E., Al-Shawi, M. K., and Urbatsch, I. L. (1995) J. Bioenerg. Biomembr. 27, 31–36
6. Miller, J. (1992) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
7. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
8. Yue, V. T., and Schimmel, P. R. (1977) Biochemistry 16, 4678–4684
9. Laemmli, U. K. (1970) Nature 227, 680–685
10. Hwang, J., Zhong, X., and Tai, P. C. (1997) J. Bacteriol. 179, 6264–6270
11. Manoil, C., and Beckwith, J. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 8129–8133
12. Kaur, P., and Rosen, B. P. (1993) J. Bacteriol. 175, 351–357

2 P. Kaur and J. Levine, unpublished data.