COMMUNICATIONS TO THE EDITOR

# Aminoglycoside Antibiotics Bind to Protein Disulfide Isomerase and Inhibit Its Chaperone Activity

Sir:

We have already reported that ribostamycin binds to protein disulfide isomerase (PDI) and inhibits its chaperone activity[1]. PDI is a multifunctional protein, which localizes most abundantly to the lumen of the endoplasmic reticulum (ER)[2]. It catalyses the formation, reduction, and isomerization of disulfide bonds in proteins and also assists protein folding[3]. In yeast, PDI is indispensable for viability[4]. Thus the inhibition of such an important protein activity might be expected to have a deleterious effect on the eukaryotic cell. As a step towards identifying the nature of these effects, we examined the binding of a variety of antibiotics, including ribostamycin, to PDI as well as the effects of these antibiotics on PDI chaperone and isomerase activities.

Interactions between PDI and a variety of antibiotics were examined using the BIACORE system 3000. Bovine PDI, purified according to the method of LAMBERT and FREEDMAN[5], was immobilized on the surface of CM5 sensor chip according to the manufacturer's instructions. As a control for nonspecific binding, sensor chips whose carboxymethyl groups had been blocked by ethanolamine were employed. As an analyte, several antibiotics were injected over the flow-cell at a flow rate of 20 $\mu$l/minute at 25°C. The sensorgrams obtained varied with the sample concentration of aminoglycoside antibiotics and vancomycin (data not shown). Using these sensorgrams, the dissociation constant ($K_D$) of PDI for aminoglycoside antibiotics, calculated using BIA evaluation ver.3.1 software, was found to range from $3.19\times10^{-4}$ M to $1.25\times10^{-3}$ M (Table 1). In the case of vancomycin, a peptide antibiotic, the $K_D$ was found to be $2.06\times10^{-4}$ M. Macrolide and $\beta$-lactam antibiotics or lincomycin did not bind to PDI, indicating that PDI binding to aminoglycoside antibiotics and vancomycin is specific.

Next, we examined the effect of antibiotics on the chaperone and isomerase activities of PDI. The isomerase activity of PDI was determined according to the method of LAMBERT and FREEDMAN[6]. The chaperone activity of PDI was determined using rhodanese[7] and citrate synthase[8].

Table 1. Dissociation constant of several antibiotics for bovine PDI and their effects on PDI activities.

| Antibiotics | Dissociation constant (M) | Inhibition of | |
|---|---|---|---|
| | | Isomerase activity | Chaperone activity |
| Polypeptide antibiotic | | | |
| Vancomycin | $2.06\times10^{-4}$ | − | + |
| Aminoglycoside antibiotics | | | |
| Ribostamycin | $3.19\times10^{-4}$ | − | + |
| Sisomicin | $3.92\times10^{-4}$ | − | + |
| Neomycin | $8.72\times10^{-4}$ | − | + |
| Gentamicin | $9.04\times10^{-4}$ | − | + |
| Kanamycin | $1.05\times10^{-3}$ | − | + |
| Streptomycin | $1.25\times10^{-3}$ | − | + |
| Macrolide antibiotics | | | |
| Erythromycin | $4.32\times10^{-2}$ | − | − |
| Novobiocin | $>10^{-2}$ | − | − |
| $\beta$-Lactam antibiotics | | | |
| Ampicillin | N.D. | − | − |
| Benzylpenicillin | N.D. | − | − |
| Cefoxitin | N.D. | − | − |
| Cefoperazone | N.D. | − | − |
| Lincomycin antibiotic | | | |
| Lincomycin | N.D. | − | − |

N.D.: Not detected

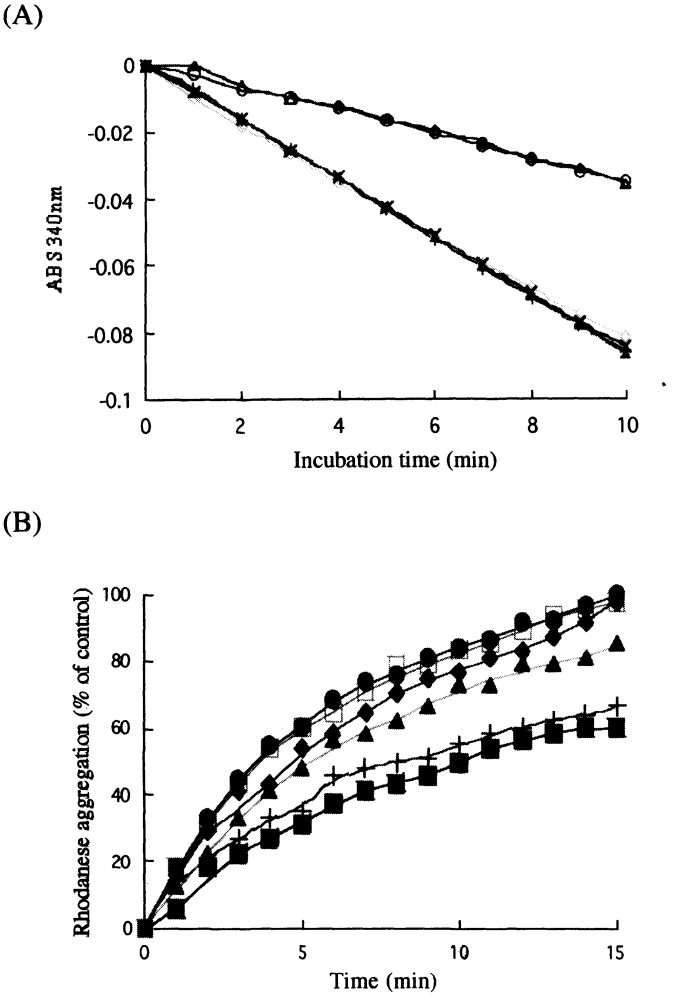
Inhibited: +
Not inhibited: −

The data analysis was performed using the BIA evaluation ver. 3.1 software.

The denaturation and reactivation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the presence of PDI were carried out according to the method of CAI *et al.*[9]. As shown in Fig. 1, sisomicin, which is an aminoglycoside antibiotic, inhibited the chaperone activity of PDI on denatured rhodanese (Fig. 1 (B)), but did not inhibit its isomerase activity (Fig. 1 (A)). The inhibition of PDI chaperone activity was also observed using denatured citrate synthase or GAPDH as a substrate (data not shown). A 100: 1 molar ratio of sisomicin to PDI was almost sufficient to completely inhibit the chaperone activity of PDI (Fig. 1 (B)). The molar ratio of each antibiotic to PDI required to inhibit chaperone activity was 100:1 for ribostamycin, sisomicin, and vancomycin, and 1000:1 for neomycin, gentamicin, kanamycin, and streptomycin (data not shown). Considering the $K_D$ values measured above, these ratios seem reasonable. As shown in Table 1, aminoglycoside antibiotics and vancomycin, which bound to PDI as measured by the BIACORE system, inhibited the PDI chaperone activity without affecting its isomerase activity. Other antibiotics such as macrolide and $\beta$-lactam antibiotics or lincomycin, which did not bind to PDI, failed to inhibit the isomerase or chaperone activity. The structures of aminoglycoside antibiotics and vancomycin show the presence of two or three aminosugars that differ from those of macrolide, $\beta$-lactam antibiotics, and lincomycin. Certain amino sugars (galactosamino-dimer) alone showed weak binding to PDI, whereas other sugars such as glucose, fructose, sucrose, and *N*-acetylglucosamine did not (data not shown). Thus it seems likely that the aminosugar moieties of certain antibiotics may function as key recognition motif for PDI binding, although further study will be required to establish this clearly. In addition, PDI contains two copies of the Cys-Gly-His-Cys (CGHC) motif (known as the thioredoxin box), which is located in the active site for isomerase activity[10]. The lack of an effect on PDI isomerase activity suggests that aminoglycoside antibiotics and vancomycin bind specifically to another region of PDI that is distinct from the active site for isomerase activity.

We are now trying to further elucidate in detail the molecular mechanism of inhibition of chaperone activity by aminoglycoside antibiotics and vancomycin.

Fig. 1. Effect of sisomicin on isomerase (A) and chaperone (B) activities of bovine PDI.




(A) The isomerase activity of PDI on insulin reduction was followed by changes in absorbance at 340 nm as a function of time of incubation at 25°C. The control with no PDI and no sisomicin (○) or with no PDI but with 100 $\mu$M sisomicin (△) showed the least decrease in absorbance. PDI alone at 1 $\mu$M (△) or in combination with sisomicin at 1 $\mu$M (×), 10 $\mu$M (+) or 100 $\mu$M (▲) showed comparable decrease in absorbance at 340 nm.

(B) The chaperone activity was measured by the suppression of rhodanese aggregation by PDI as followed by changes in absorbance at 320 nm. Bovine rhodanese was denatured and diluted (0.36 $\mu$M final concentration) in the absence of bovine PDI and sisomicin and the absorbance at 320 nm following incubation for 15 minutes at 25°C, and that value was set to 100% (control) (●) representing the maximum aggregation. Sisomicin at 100 $\mu$M with no PDI (□) showed no effect on aggregation. PDI at 1 $\mu$M with no sisomicin (■) showed the greatest suppression of rhodanese aggregation. PDI at 1 $\mu$M in the presence of sisomicin at 1 $\mu$M (+), 10 $\mu$M (▲), and 100 $\mu$M (◆) showed increasing inhibition of the chaperone activity of PDI to suppress rhodanese aggregation.

TOMOHISA HORIBE
HIROAKI NAGAI
HIROAKI MATSUI
YASUNARI HAGIWARA
MASAKAZU KIKUCHI*

Department of Bioscience & Technology,
Faculty of Science & Engineering,
Ritsumeikan University,
1-1-1 Noji-higashi, Kusatsu, Shiga 525-8577, Japan

(Received December 3, 2001)

## References

1) HORIBE, T.; H. NAGAI, K. SAKAKIBARA, Y. HAGIWARA & M. KIKUCHI: Ribostamycin inhibits the chaperone activity of protein disulfide isomerase. Biochem. Biophys. Res. Commun. 289: 967~972, 2001
2) NOIVA, R. & W. J. LENNARZ: Protein disulfide isomerase: a multifunctional protein resident in the lumen of the endoplasmic reticulum. J. Biol. Chem. 267: 3553~3556, 1992
3) FREEDMAN, R. B.; B. E. BROCKWAY & N. LAMBERT: Protein disulfide-isomerase and the formation of native disulfide bonds. Biochem. Soc. Transactions 12: 929~932, 1984
4) FARQUHAR, R.; N. HONEY, S. J. MURANT, P. BOSSIER, L. SCHULTZ, D. MONTGOMERY, R. W. ELLIS, R. B. FREEDMAN & M. F. TUITE: Protein disulfide isomerase is essential for viability in *Saccharomyces cerevisiae.* Gene 108: 81~89, 1991
5) LAMBERT, N. & R. B. FREEDMAN: Structural properties of homogeneous protein disulphide-isomerase from bovine liver purified by a rapid high-yielding procedure. Biochem. J. 213: 225~234, 1983
6) LAMBERT, N. & R. B. FREEDMAN: Kinetics and specificity of homogeneous protein disulphide-isomerase in protein disulphide isomerization and in thiol-protein-disulphide oxidoreduction. Biochem. J. 213: 235~243, 1983
7) MARTIN, J.; T. LANGER, R. BOTEVA, A. SCHRAMEL, A. L. HORWICH & F.-U. HARTL: Chaperonin-mediated protein folding at the surface of groEL through a 'moltenglobule'-like intermediate. Nature 352: 36~42, 1991
8) SHAO, F.; W. M. BADER, U. JAKOB & J. C. A. BARDWELL: DsbG, a protein disulfide isomerase with chaperone activity. J. Biol. Chem. 275: 13349~13352, 2000
9) CAI, H.; C.-C. WANG & C.-L. TSOU: Chaperone-like activity of protein disulfide isomerase in the refolding of a protein with no disulfide bonds. J. Biol. Chem. 269: 24550~24552, 1994
10) KANAI, S.; H. TOH, T. HAYANO & M. KIKUCHI: Molecular evolution of the domain structures of protein disulfide isomerases. J. Mol. Evol. 47: 200~210, 1998

* Corresponding author: kikuchi@se.ritsumei.ac.jp