J. Antibiot. 61(6): 400–402, 2008

NOTE

THE JOURNAL OF ANTIBIOTICS

The Binding of Antibiotics to ERp57/GRP58

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Received: April 30, 2008 / Accepted: May 28, 2008

Abstract The effects of five antibiotics, previously described as ligands of protein disulfide isomerase PDI, have now been studied on the homologous protein ERp57. They bind to this protein with much higher affinity than to PDI, and some of them inhibit the reductase and the DNA-binding activities of ERp57. In view of the high affinity of vancomycin, erythromycin and streptomycin, some effects of their interaction with this protein might be expected *in vivo*.

Keywords ERp57/GRP58, aminoglycoside antibiotics, vancomycin, erythromycin

PDI and ERp57 (also called GRP58 or ERp60) are two members of the protein disulfide isomerase family of proteins, mainly present in the endoplasmic reticulum, where they are involved in the formation and reshuffling of the disulfide bonds of nascent proteins and in chaperoning their correct folding. However PDI and ERp57, and other members of the family as well, have been found also in other subcellular locations, where they are thought to be involved in a variety of processes [1]. ERp57 has been found on the cell surface of sperm, where it is involved in the process of gamete fusion [2] and on the cell surface of intestinal epithelial cells, where it binds the 1α,25-dihydroxyvitamin D_3 and appears to be responsible for the rapid response to this hormone [3]. ERp57 has been found also in the cytosol and the nucleus. The association of ERp57 with STAT3 in both compartments [4, 5] and its binding to specific sites of DNA in the nucleus *in vivo* [6] suggest that it is also involved in the regulation of gene expression. Furthermore, ERp57 has been shown to display a protease activity [7], whose biological importance is still unknown.

The discovery of specific ligands and inhibitors of these proteins might help to confirm the hypothesized functions of the two proteins, or possibly to disclose new roles for them. A variety of inhibitors of PDI has already been described, particularly among peptides and antibiotics [8, 9]. Nothing is known in this respect for ERp57. Taking into account the structural homology of PDI and ERp57, and acknowledging the capability of many antibiotics to bind to and inhibit the chaperone activity of PDI [9], we tested these compounds on ERp57, measuring their binding to the protein and their effects on its reductase and DNA-binding activities. In view of the importance of ERp57 suggested functions, a knowledge of possible inhibitory effect of antibiotics on this protein seemed highly desirable.

Experimental

The insulin, fluorescein-isothiocyanate and the various antibiotics were obtained from Sigma-Aldrich, and poly(dA)·poly(dT) from GE-Healthcare. Human recombinant ERp57 was obtained as described before [5].

The binding of the antibiotics was measured by adding increasing concentrations of each compound to a 34 nM solution of human recombinant ERp57 in Tris-HCl 0.01 M pH 8, and analyzing the fluorescence of the protein in a spectrofluorimeter at 25°C, with an excitation wavelength of 280 nm and an emission of 336 nm. When the binding of vancomycin was measured, the excitation wavelength was

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290 nm, in order to minimize the absorbance of the compound. The weak fluorescence of vancomycin was taken into account and was subtracted from that of the protein.

The effect of the ligands on the reductase activity of ERp57 was examined by measuring the reduction of insulin, labeled with fluorescein [10].

The inhibition of the ERp57-DNA interaction was tested by the use of a South-Western dot-blot method, as described before [11]. Briefly, ERp57 was spotted on nitrocellulose membranes, which were then overlayed with a solution of poly(dA)·poly(dT) labeled with digoxigenin, in the presence or absence of inhibitors. The polynucleotide bound to ERp57 was finally measured with an anti-digoxigenin antibody (Roche) and densitometry.

Results and Discussion

The addition of vancomycin, erythromycin and three aminoglycoside antibiotics to the protein induced in all cases a quenching of the intrinsic fluorescence emission of the protein, which can be attributed to a slight conformational change of the macromolecule. In all cases, the binding took place with hyperbolic saturation curves (Fig. 1) which allowed the determination of the K_Ds (Table 1). All five compounds displayed a significant affinity for the protein. The tightest binding was shown by vancomycin, with a K_D value of 6.7×10^{-6} M. Ampicillin did not bind, just as no binding was detected to PDI [9].

Next, the inhibition of the reductase activity of ERp57 on the disulfide bonds of insulin was measured. Only vancomycin and neomycin showed a significant, but modest, inhibitory activity, as shown in Table 1.

ERp57 has the specific property, not shared by PDI, of binding to double-stranded DNA with a preference towards the sequences rich in A/T [11]. The results of measurements of the binding of poly(dA)·poly(dT) to ERp57 in the presence of the antibiotics are shown in Fig. 2. Vancomycin, erythromycin and neomycin are good inhibitors of the DNA-ERp57 interaction. It should be noted that the K_D values as determined by fluorimetry do

Table 1 Dissociation constants of some antibiotics for ERp57 and PDI and their effects on reductase activities

Antibiotics	ERp57		PDI**	
	K_D (M)	Inhibition of reductase activity*	K_D (M)	Inhibition of reductase activity
Vancomycin	6.7×10^{-6}	22%	2.06×10^{-4}	—
Neomycin	2.0×10^{-5}	23%	8.72×10^{-4}	—
Ribostamycin	3.0×10^{-5}	—	3.19×10^{-4}	—
Streptomycin	5.0×10^{-5}	—	1.25×10^{-3}	—
Erythromycin	9.0×10^{-6}	—	4.32×10^{-2}	—
Ampicillin	—	—	—	—

* With a 0.1 mM concentration of antibiotic. ** Data from Horibe *et al.* [9]. — Not bound or not inhibited.

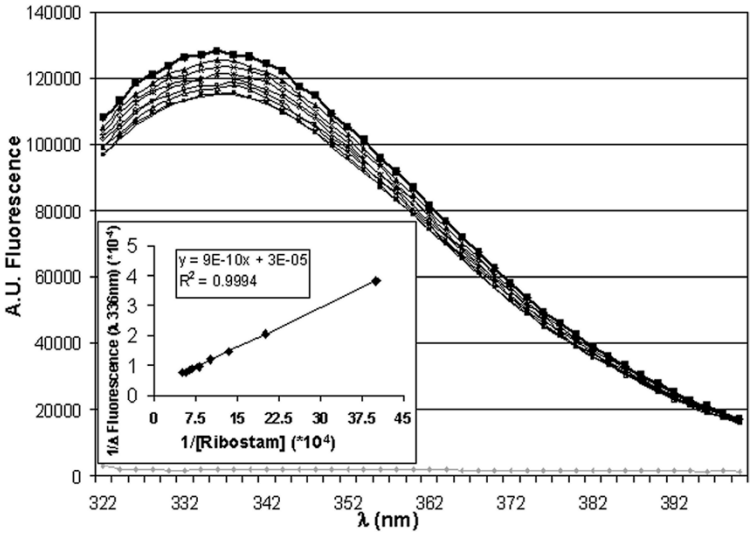

Fig. 1 Fluorescence emission spectra, with excitation at 280 nm, of ERp57 alone (top curve) and upon addition of increasing amount of ribostamycin (2.5~20 μM).

The insert shows the double reciprocal plot of the data (at λ 336 nm).

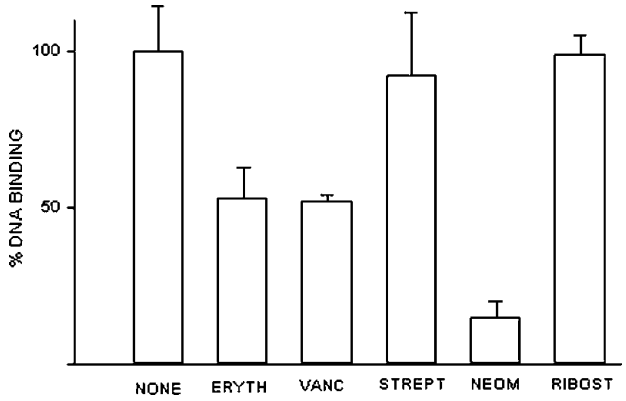

Fig. 2 Percent of residual binding of poly dA-poly dT to ERp57 in the presence of 0.1 mM antibiotics.

The values indicated are the averages of 3 or 4 measurements±s.d.

not necessarily reflect the respective inhibitory potencies. Thus, streptomycin and ribostamycin, which bind with K_Ds in the range of 10^{-5} M, have no significant inhibitory activity towards the ERp57-DNA interaction, while the other aminoglycoside antibiotic neomycin, with a similar K_D, is the most effective inhibitor. ERp57 has four domains, and it has been demonstrated that the fourth domain is responsible for the binding to DNA. This suggests that vancomycin, erythromycin and neomycin bind to this domain. This suggestion is strengthened by the fact that the fourth domain carries one of the two thioredoxin-like active site CGHC, consistent with inhibition of reductase activity of ERp57 by vancomycin and neomycin. The fact that streptomycin and ribostamycin do not inhibit the DNA binding might be explained by their binding to a different site of the protein or by their smaller size, so that, even if the five antibiotics have a common binding site, those which inhibit the DNA binding also occupy an extension of the site, thus being capable to exploit their additional inhibitory action.

When the behaviour of these five antibiotics towards PDI and ERp57 are compared, some striking differences are apparent (Table 1). They bind to ERp57 with higher affinities than to PDI [9], with affinity constants 1～3 orders of magnitude higher. Furthermore, vancomycin and neomycin display an inhibitory action towards the reductase activity of ERp57, while they are completely inactive in this respect upon binding to PDI [9]. Only ampicillin showed the same behaviour towards the two enzymes, being unable to bind and consequently unable to inhibit the reductase activity.

It should be noticed that the K_D values of vancomycin, erythromycin and streptomycin for ERp57 are of the same order of magnitude as the therapeutic concentrations of these antibiotics in the blood. Therefore some effects on this protein might be expected in the course of their therapeutic use.

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