The effect of the pore forming antibiotic, alamethicin, on the hepatic microsomal glucose-6-phosphatase system.

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Hepatic microsomal glucose-6-phosphatase is a multicomponent system comprising a catalytic subunit which is in the lumen of the endoplasmic reticulum and transport proteins which allow substrates, including glucose-6-phosphate and inorganic pyrophosphate, access to the active site of the enzyme, and let the products of hydrolysis leave the endoplasmic reticulum [1]. The transport of substrate to the active site of the system is rate limiting thus microsomal glucose-6-phosphatase displays a 'latency' which can be abolished by the complete disruption of microsomes. The complete disruption of microsomes results in an increase in the Vmax of the system and a decrease in the Km [2]; effectively the activity of the catalytic subunit alone. The apparent hydrolytic activity of intact microsomes is actually the combined transport capacities of the system together with the activity of the catalytic subunit.

Alamethicin, a helical oligopeptide, is thought to insert into membranes as a hexamer or nonamer in a voltage dependent manner, forming a pore and removing the lipid barrier to the passage of solutes.[3,4]. The voltage dependency of alamethicin may be due to the need for a trans membrane voltage to aggregate the individual alamethicin molecules at the site of membrane insertion [4]. The predicted action of alamethicin on microsomal glucose-6-phosphatase would be similar to that of complete disruption of the microsomes, the removal of the rate limitations of substrate transport.

The effects of alamethicin on microsomal glucose-6-phosphatase were assayed using rat liver microsomes prepared as in [5] and the assay method described in [6].

The results below show that, as expected, the addition of alamethicin, increases the Vmax of microsomal glucose-6-phosphatase in intact microsomes (the combined rates of transport and hydrolysis) and has no effect on the activity of the catalytic subunit of the system in disrupted microsomes (hydrolysis alone). Alamethicin is therefore affecting the transport of substrate across the microsomal membrane and not the hydrolytic activity of the catalytic subunit. It fails however, to reduce the Km of the system to that of disrupted microsomes. It also fails to abolish the effects of 6mM NaCl which the results suggest activates the inorganic pyrophosphate transporter by lowering the Km but not affecting the Vmax. These results indicate that alamethicin is not acting as a simple pore, activating the microsomal glucose-6-phosphatase system by disrupting the microsomal membrane but is activating substrate transport.

![EFFECTS OF ALAMETHICIN and NaCl on Vmax and Km](image)

Disrupted data obtained using microsomes fully disrupted with 0.2% Lubrol PX.

PP=Inorganic pyrophosphate
G-6-P=Glucose-6-phosphate.

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