

Cooperative Lipid-Protein-Interaction: The Binding of Polymyxin to charged Phospholipid Bilayer Membranes.

F. Sixl and H. J. Galla

Department of Biophysics, University of Ulm, Oberer Eselsberg, D-7900 Ulm, F.R.G.

The binding of polymyxin-B to vesicles of dipalmitoylphosphatidic acid (DPPA) has been investigated by calorimetry and fluorescence depolarisation of diphenylhexatriene, incorporated into the membrane.

Recently it has been shown, that the binding curves of Polymyxin to DPPA are strongly influenced by the fluidity of the membrane, which may be triggered by changing the external pH and/or the ionic strength(1).

At pH 9.0 a cooperative binding process is observed. The cooperativity decreases by decreasing the ionic strength and pH and is totally lost at pH 3.0.

The size of the lipid-peptide-complex could be estimated by analysing the peaks of the calorimetric scans at pH 9.0. For the inner core a diameter of about 100 Å is obtained, and it consists of about 40 peptide molecules.

A further evidence for the cooperativity of the lipid-protein-interaction is the kinetics of the incorporation of polymyxin into the outer layer of DPPA vesicles, which has been studied by the fluorescence polarisation technique, using a stopped flow apparatus. A lag time in the order of 0.1 second is observed, which suggests the formation of condensation nuclei. This process is followed by the clustering of the polymyxin-DPPA-complexes within 1-2 seconds. The incorporation time is independent of polymyxin concentration and of temperature in the range between 35 and 60 °C. A diffusion controlled process has to be considered.