Polymyxin binding to charged phospholipid bilayer membranes: A cooperative lipid-protein-interaction

F. Sixl and H.-J. Galla

Universität Ulm, Abteilung für Biophysik, Oberer Eselsberg
D-7900 Ulm (Donau), Germany

We have investigated the interaction between negatively charged phospholipid bilayer membranes and the peptide antibiotic polymyxin-B. The molecular process of polymyxin binding was elucidated from the lipid phase transition curves. The outstanding result for phosphatidic acid membranes was the determination of a cooperative binding. The cooperativity of the binding was controlled by pH and ion concentration of the buffer solution. The formation of a lipid-peptide-domain was established as a consequence of the antibiotic molecular structure (1).

The strong binding of polymyxin to negatively charged lipid bilayer membranes is caused by both hydrophobic as well as electrostatic interaction. The packing density of the lipid membrane was found to control the binding process thus showing a Langmuir- or cooperative type of binding curve. In case of a cooperative binding we established a modell for the domain structure. From calorimetric data we could estimate the interaction energy within the polymyxin phosphatidic acid domain as well as the domain size.

Competitive binding experiments with Ca^{2+} were performed (2). Binding of polymyxin-B to mixed dipalmitoylphosphatidic acid/ distearoyl - phosphatidylcholine membranes led to a phase separation. Domains of polymyxin-bound phosphatidic acid were formed. Ca^{2+}-ions were found to be a strong competitor in displacing polymyxin from the complex in the mixed membrane system. Complete displacement was obtained at pH 9.0. With decreasing pH value Ca^{2+} became a less strong competitor and was ineffective at pH 5.0.

Binding of polymyxin was also observed to dipalmitoylphosphatidylclycerol membranes. Incorporation of polymyxin lowerd the lipid phase transition by 10° C. One polymyxin was found to bind five phosphatidylglycerol molecules. The binding curve was determined and in contrast to phosphatidic acid membranes, a non-cooperative binding could be established. Addition of Ca^{2+} decreased the amount of phosphatidylglycerol bound to polymyxin by about 20 %. No complete displacement was achieved even at a ten-fold excess of Ca^{2+}-ions with respect to phosphatidylglycerol.

1) F. Sixl and H.-J. Galla, Biochim. Biophys. Acta 557 (1979) 320-330
2) F. Sixl and H.-J. Galla, Biochim. Biophys. Acta 643 (1981) 626-635