Chapter 5

Conclusions

The different aspects of the interaction of phloretin with lipid layers as already discussed in the chapters 2, 3 and 4 are mainly based on a biophysical point of view. However, the effects of phloretin on the permeability of membranes have been observed first on human red blood cells (LeFevre and Marshall, 1959), i.e., on an intact biological system. It is not self-evident that the results gained with model systems apply to that gained with biomembranes and vice versa. Thus we also have to focus our interest on the physiological effects and its possible implications to the biophysical ones.

5.1 Phloretin binding to proteins

Jennings and Solomon (1976) have investigated the binding of phloretin to red blood cells. They found in their study that the binding to ghosts is a saturable process exhibiting two different binding affinities. Phloretin binds with a high affinity ($k = 1.5\ \mu$M) to about 2.5×10^6 sites per cell; it also binds with lower affinity ($k = 54\ \mu$M) to a second (5.5×10^7) set of sites. However, in sonicated total lipid extracts of red cell ghosts, phloretin binding consists of a single, saturable component. Its affinity and total number of sites are not significantly different from those of the low affinity binding process in ghosts. No high affinity binding of phloretin is exhibited by the red cell lipid extracts. Jennings and Solomon concluded that the high affinity phloretin binding sites are related to membrane proteins, and the low affinity sites result from phloretin binding (adsorption) to lipid[1].

Forman et al. (1982) found that phloretin bind to the anion transport protein, a major integral protein of the red cell membrane. Similar results are reported for the glucose carrier (Krupka, 1985) and the urea transport system (Toon and Solomon, 1987). There is no doubt that the binding (adsorption) of phloretin to lipid and the binding to proteins are fundamentally different and independent of each other, which is, e.g., confirmed by the fact that it is actually the *charged* form of phloretin binding to the

[1]Our study of the adsorption of phloretin to unilamellar lipid vesicles (see paragraph 4.4.3) exhibited a dissociation constant of $k = 49\ \mu$M, which agrees well with the value found by Jennings and Solomon (1976) for the low affinity sites. This confirms that the low affinity can be attributed to lipid adsorption.

anion transport protein at red blood cells (Forman et al., 1982). However, at least in the case of the urea transport system in the red cell membrane it has been shown that the inhibition of urea transport is modulated by both the phloretin adsorption to lipid and the phloretin binding to the urea transport protein (Toon and Solomon, 1987). Moreover, the experiments of Toon and Solomon (1987) have shown that lipid/protein interactions, influenced by phloretin, can alter the conformational state of the urea transport protein. Toon and Solomon (1987) have not shown of what kind these interactions are, but their results suggested that the effects of phloretin to the lipid layer may also affect membrane protein functions.

5.2 Indirect effects of phloretin on cell membranes

What mechanisms are conceivable for possible physiological effects of the phloretin-induced dipole potential reduction on membranes and membrane proteins? A physiological effect has been reported by De Jonge et al. (1983): phloretin acts as uncoupler and inhibitor of mitochondrial oxidative phosphorylation. It is clear that the primary effect of this finding cannot be explained by mechanisms similar to that of ionophores since the mode of action of phloretin at the membrane is fundamentally different. De Jonge et al. (1983) provided no information about the mechanisms by which phloretin exerts its influence. However, they found a reduction in transmembrane potential difference in isolated mitochondria indicating that the phloretin effect is likely an electrostatic one. Strichartz et al. (1980) found that phloretin decreases the potassium conductance of the giant axon membrane of the squid, *Loligo peali*. Lowering the pH, which favors the presence of neutral phloretin, potentiates the action of internally perfused phloretin. These results are of particular interest since phloretin usually *increases* the membrane conductance for cations. In fact, high phloretin concentrations applied for brief periods increased the resting potassium conductance of the axon soma membrane of *Aplysia* (Owen, 1974). These contradictory results can be explained by two different mechanisms. The ionic conductances in excitable membranes are controlled by the membrane potential through the motion of specific "gating" molecules, which change their position of configuration in response to changes of the electric field (Hodgkin and Huxley, 1952). It is conceivable that even small concentrations of phloretin adsorbed to the membrane lead to a dipole potential change sufficient to trigger these voltage sensitive molecules[2]. Due to the regulatory activity of these molecules the potassium conductance decreases. On the other hand, this regulation may be covered by the potential increasing effect at higher phloretin concentrations, which can explain the result gained by Owen (1974).

Taking into account that physiological functions of proteins are usually combined with their charge states, which also determine the folding and conformational states, it is clear that membrane potentials and surface charges in close proximity to membrane

[2]The phloretin concentrations used at the experiments of Strichartz et al. (1980) were 10^{-5} M, which produces a dipole potential change of nearly -100 mV at PC membranes (see paragraph 2.5.2).

proteins can influence their functions. Therefore it is very likely that the membrane dipole potential and dipole potential changes contribute to the complex interactions between lipids, membrane proteins and the signal transduction pathways within the cell. Surface active molecules, especially those affecting the dipole potential of lipid layers, are certainly designated by nature to regulatively interfere with membrane functions.