Doctoral Thesis

Studien an Zuckertransportsystemen von Zellmembranen
chemische Modifikation am Na\(+\)-abhängigen D-
Glukosetransportsystem des Dünndarms

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STUDIEN AN ZUCKERTRANSPORTSYSTEMEN VON ZELLMEMBRANEN:

- REKONSTITUTION DES D-GLUKOSETRANSPORTSYSTEMS VON ERYTHROCYTEN

- CHEMISCHE MODIFIKATIONEN AM Na⁺-ABHÄNGIGEN D-GLUKOSE-TRANSPORTSYSTEM DES DÜNNARMS

ABHANDLUNG
zur Erlangung
des Titels eines Doktors der Naturwissenschaften
der
EIDGENÖSSISCHEN TECHNISCHEN HOCHSCHULE ZÜRICH

vorgelegt von
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SUMMARY

Reconstitution of the sugar transport system of human erythrocytes into artificial liposomes was achieved by freezing, thawing and sonicating preformed phospholipid vesicles in the presence of intact ghosts, protein depleted ghosts or detergent treated ghosts. D-glucose equilibrium exchange activities and affinity constants in the range of the reported erythrocyte values were reached in the best experiments. Whereas the extraction of peripheral membrane proteins did not depress the transport function crucially after reconstituting these protein depleted ghosts, the selective solubilization of integral membrane proteins by nonionic detergents, namely Triton N-101 and Triton X-100, resulted in an uncontrollable, continuously increasing inactivation of the carrier.

After chemical modification of rabbit small intestinal brush border membrane, $\text{Na}^+$-dependent D-glucose uptake activity was investigated. At least one carboxylate and three amino groups that can be blocked by DCCD, DIPC or fluorescamine, FDNB, imidoesters, formaldehyde/NaCNBH$_3$, isothiocyanates respectively are essential for a proper sugar transport or phlorizin binding function. Remarkably one of these amino groups is somehow influenced by the translocation (but most probably not by the binding) process, for the inactivation rate by several modifying reagents is reduced in the presence of D-glucose. Another critical amino group is embedded in a hydrophobic region, obviously in the vicinity of the bilayer, and a third one (with a $pK$ of 9 or higher) lies in a more hydrophilic environment.