Meeting Report

MEMBRANE PROTEINS: ANALYSIS OF MOLECULAR AND SUPRAMOLECULAR STRUCTURE

Workshop organized by the Sonderforschungsbereich 160 – Eigenschaften biologischer Membranen – October 5–7, 1980, Maria Laach, FRG

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The Sonderforschungsbereich 160 organized this workshop to discuss and evaluate recent activities and trends in the field of membrane protein structure. Some 20 scientists were invited to present their most recent investigations in an attempt to shed some light on various facets of the structural organization of this rather elusive class of proteins. Despite all efforts there is so far not a single example where the structural determination of a membrane protein has reached the degree of comprehensiveness, accuracy and definiteness which we are eventually seeking in order to begin inquiring sensibly into the way these molecules operate. It was the central aim of the workshop to discuss how the various rapidly advancing physical and chemical approaches presently available can be combined to achieve a 'holistic' picture of the structure of a membrane protein.

Ultimately it is only the atomic model which can provide us with the necessary structural information. Most advanced in this respect is bacteriorhodopsin. A model of this protein, obtained by piecing together information from various sources [1], was presented by R. Henderson (Cambridge). Low-dose electron microscopy combined with digital image reconstruction techniques provided the basic morphological information: The molecule is composed of seven helical rods traversing the membrane almost perpendicularly [2]. Analysis of a new two-dimensional crystal form obtained by recrystallisation [3] corroborated the boundaries of individual bacteriorhodopsin molecules in the lattice. The amino acid sequence [4] was then fitted tentatively into the morphological model. As important preliminaries the 7 helical segments and the link regions in the sequence were localized by applying the criteria of hydrophobicity and inaccessibility to proteolytic cleavage and assuming that the helices should be comparable in length. In the next step these helical segments had to be identified in the density map. To reduce the enormous number of possible assignments the following criteria were applied: (a) connectivity of non-helical link regions; (b) charge neutralization; (c) correlation of calculated electron scattering cross-sections for the individual helices with the densities determined experimentally. The model emerging as the most probable one exhibits a number of features favourable for a membrane-embedded proton pump. The buried charged amino acids are localized in the center of the molecule, possibly forming a hydrophilic channel through which protons could jump, whereas the hydrophobic, uncharged residues are directed outward towards the lipid environment. This 'inside-out' arrangement has meanwhile been confirmed by neutron diffraction experiments [5].

Further experimental verification of this model might be achieved either by electron optical means with two-dimensional crystals, pushing resolution further towards the instrumental limits, or by continuing efforts to prepare three-dimensional crystals suitable for X-ray analysis. Here considerable progress has been made recently, not only with bacteriorhodopsin [6], disproving the long prevailing opinion that crystallizing intrinsic membrane proteins is impossible.

J. P. Rosenbusch (Basel) reported about successful attempts to obtain three-dimensional crystals of porin, a pore-forming transmembrane protein from the outer membrane of *Escherichia coli* [7]. Several crystal

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forms were observed after precipitation of the protein, solubilised with mild non-ionic detergents. α-Octyl glucoside gave the largest crystals diffracting out to 0.38 nm resolution. This is close to what is needed to trace the β-pleated sheets which constitute the bulk of this protein. Porin is known to form a channel, able to assume an open and a closed conformation [8]. Therefore a high resolution determination of its structure, now under way, offers exciting prospects to relate structure and function on a molecular level.

W. Baumeister (Düsseldorf) introduced another pore-forming protein, the so-called HPI-layer protein from the radio-tolerant bacterium *Micrococcus radiodurans*. This protein (M_r 650 000) exists in an hexagonally ordered array intimately associated with the outer membrane of the bacterium and covering its entire surface [9]. A novel image processing method, 'correlation averaging' has been used to recover clear images of the unit cell structure of this protein complex at a resolution of ~1.5 nm [10]. This method of averaging, originally devised for single particles, avoids the loss of resolution encountered with the conventional Fourier-domain method when the specimen is not perfectly crystalline. A low resolution (~3 nm) three-dimensional model of the HPI-layer–protein complex was presented, obtained by combining information from unstained, negatively stained and shadowed HPI-layer sheets.

ATR (attenuated total reflection)–infrared spectroscopy is a particularly useful tool to study the fine structure of membrane proteins. It is sensitive enough to detect even rather subtle conformational changes and allows to mimic a wide range of environmental situations. U. P. Fringeli (Zürich) investigated the behaviour of acetylcholinesterase, adsorbed as a monolayer, under the influence of electric fields. Field-induced changes of protein charge and conformation due to a dissociation of the –COOH groups of A/sp and Glu residues were observed and interpreted by a new electrostatic regulation model for acetylcholinesterase activity in excitable membranes [11].

To derive quantitative data about protein secondary structure from infrared spectra a deconvolution of the overlapping components of the amide I and amide II region is required. B. Kleffel (Düsseldorf) outlined a general strategy for such quantitative band shape analyses and their calibration [12]. The method has been used to obtain estimates of the β-structure content of porin and the α-helix content of cytochrome *c* oxidase under a variety of experimental conditions.

A series of contributions centered around respiratory chain complexes III, IV and V of the inner mitochondrial membrane. Genetic information from the nucleus and the mitochondrium contributes to the assembly of ~10 subunits in each of these complexes.

H. Weiss (Heidelberg) presented a three-dimensional model of cytochrome bc_1 (complex III, EC 1.10.2.2) reconstructed from two-dimensional membranous crystals [13]. The data obtained from electron microscope tilt series show a transmembranous arrangement of the enzyme with 30% of its mass buried in the lipid phase [14]. Correspondingly some of the isolated subunits are hydrophilic, e.g., the 50 000, 45 000 and 12 000 M_r subunits, which are not involved in the redox reaction, others are amphiphilic, e.g., C_1, (31 000 M_r) with a hydrophilic 23 000 M_r segment which contains the catalytic center protruding from the cytoplasmic side of the membrane, and others hydrophobic such as the cytochrome *b* and FeS redox centers, which are solubilized only by ionic detergents [15]. Three-dimensional crystals have been obtained recently from the 50 000 and 45 000 M_r core proteins; thus X-ray crystallographic work with individual subunits will be an important way of further progress with the reductase.

Although a separation of functional subunits has so far not been achieved in the case of cytochrome *c* oxidase (EC 1.9.3.1) the sequence data presented by G. Buse (Aachen) allow for a similar characterization of the subunits of this enzyme [16]. Some cytoplasmically synthesized subunits, V (12 000 M_r) [17], and VII (10 000 M_r) [18] are clearly hydrophilic, while others are amphiphilic and possess a hydrophobic membrane penetrating sequence domain of ~20 residues length. Examples are polypeptide IV (17 000 M_r) [19], and the small polypeptides VIII a–c (5500 M_r) [20]. In these chains the hydrophobic domains are bordered by two charged residues, which probably bind to the charged head groups of the phospholipids. The mitochondrially synthesized subunits II (27 000 M_r) [21], a copper subunit, and III (30 000 M_r), proposed to be involved in H^+-pumping [22] may also be considered amphiphilic. Both have two hydrophobic membrane domains at their N-terminal sequence, which by folding back and forth through the membrane bring the enzymatic core to the cytoplasmic side of the inner membrane.

T. Frey (Philadelphia PA) showed that these subunits are located at this membrane side by reacting them with subunit-specific antibodies in the two-

dimensional crystalline vesicle-reconstituted enzyme [23]. With this method subunit IV (17 000 M_r) was traced at the matrix side of the membrane, while the large 36 000 M_r subunit I (the hydrophobic core of the oxidase) is not reacted. The picture emerging for the sidedness of the protein components is in agreement with the three-dimensional reconstruction of the enzyme obtained by R. Henderson (Cambridge) [24,25] and may be further specified by the method of photoaffinity labelling [26] with synthetic aryl-azidophospholipids reported by R. Bisson (Padua) [27,28]. Here 'deep' and 'shallow' probes provide tools for tracing the membrane domains of this [29] and other membrane proteins, among them cytochrome b_5 and the mitochondrial enzymes succinate ubiquinone reducatase, succinate cytochrome *c* reductase and ATP synthase.

W. Sebald (Braunschweig) presented another example of a membrane protein with two hydrophobic domains in the sequence: The 9000 M_r 'proteolipid' subunit of the F_0 fragment of the mitochondrial ATP synthase (EC 3.6.1.3) [30]. The author reported evidence for a hairpin-like orientation of two helical rods, in agreement with several amino acid sequences obtained from mitochondrial, chloroplast and bacterial sources [31]. In all these cases the second hydrophobic domain invariably contains a glutamic or aspartic acid residue which can be reacted specifically with dicyclohexylcarbodiimide (DCCD) [32]. Reacting only 1 out of 6 subunits present in the enzyme blocks the protonophoric function of the ATP synthase F_0 fragment as do mutations occuring at this position in the chain [33,34]. The vectorial proton translocation from the cytoplasmic to the matrix side driving the ATP synthesis at complex V corresponds to antidirectional proton movements across the inner membrane at three coupling sites including cytochrome *c* oxidase. First experiments reported by G. Steffens (Aachen) here again indicate a DCCD-reacting glutamic acid site (subunit III, pos. 90) sensitive to proton translocation [35].

Rhodopsin (M_r 38 000) is the predominant intrinsic protein of vertebrate photoreceptor disk membranes. Unlike bacteriorhodopsin, it has so far not been observed to form two-dimensional crystalline arrays. This precludes the electron optical approaches which have been so successful for bacteriorhodopsin and also for cytochrome reductase and oxidase. Nevertheless it is possible to prepare highly ordered rhodopsin samples using orientation of rod cell outer segments (ROS) by magnetic fields. Such preparations, in which all of the rhodopsin molecules are aligned perpendicularly to the observation axis, were used by M. Chabre (Grenoble) for the study of the orientation of the peptide backbone, the aromatic residues, and the retinal chromophore of the protein. Linear dichroism measurements in the infrared demonstrate that the hydrophobic core of the protein is mainly constituted by a bundle of α-helical segments oriented in the transmembrane direction [36]. In the hydrophilic peripheral regions of the protein the peptide backbone has no preferential orientation. Linear dichroism measurements in the ultraviolet revealed that a tryptophan residue in rhodopsin changes its orientation upon absorption of visible light by the chromophore [37], indicating conformational changes in the protein. The orientation of the chromophore and its variations at various stages of the photobleaching cycle are easily followed by linear dichroism measurements on oriented ROS suspensions [38].

H. B. Osborne (Grenoble) using hydrogen isotope exchange techniques, could demonstrate, that only ~50% of rhodopsin's peptide hydrogens are exchangeable under conditions where 80% would exchange in most proteins, demonstrating the hydrophobic environment for a large part of the polypeptide chain. A large number of sidechain hydrogens also exchanges extremely slowly as compared to soluble proteins. Solubilization of unbleached rhodopsin in mild detergents (non-ionic and zwitter-ionic) does not increase the accessibility of its hydrogens to exchange, indicating that the detergent micelles in these cases perfectly replace the lipid environment of the membrane without affecting the protein structure [39].

P. J. Bauer(Jülich) reported measurements, obtained by dielectric techniques, of the orientational dispersion of Triton X-100-solubilized rhodopsin in an electric field. The data indicate rhodopsin to be an elongated ellipsoid.

P. A. Hargrave (Carbondale IL) demonstrated the usefulness of chemical and enzymatic probes to characterize hydrophilic and hydrophobic domains of rhodopsin in the disk membrane. The N-terminus, bearing two carbohydrate side chains, is located at the internal surface, and the C-terminus at the external (cytoplasmic) disc surface [40]. About 35% of the amino acid sequence are established [40,41]. The hydrophobic nitrene precursor 1-azido-[^{3}H]pyrene (AP) was used to localize membrane-embedded sections of rhodopsin by photo-affinity labelling, particularly a segment containing the retinal binding site

[40]. In contrast, the water-soluble membrane-impermeable nitrene precursor *N*-4-azido-2-nitrophenyl-2-aminoethane [^{35}S]sulfonate (NAP-taurine) labels predominantly the C-terminal region of rhodopsin in intact disks.

Limited proteolysis at the cytoplasmic surface of the disk membrane releases two small peptides from rhodopsin's C-terminus [40]. The rate of cleavage is transiently increased upon illumination of rhodopsin, indicating a light-induced conformational change at rhodopsin's surface [42]. Upon more extensive digestion, rhodopsin is cleaved internally to produce two large membrane-bound fragments. Light-dependent binding of GTPase to rhodopsin was abolished by this internal cleavage [43].

Rhodopsin exerts its function as a light receptor protein by activating enzymes (GTPase, phosphodiesterase) peripherally bound to the surface of the disk membranes. H. Kühn (Jülich) reported on light-and GTP-dependent changes in the interaction of such peripheral proteins with the membrane. These changes probably reflect part of the light activation mechanism of the enzymes. At least three rod cell proteins undergo strong but reversible light-induced binding to the membranes: The enzymes GTPase and rhodopsin kinase as well as a 48 000 M_r protein of as yet unknown function [44–46]. The light-induced binding of GTPase is specifically reversed by GTP. Light-induced binding of GTP and its reversal occur in the 100 ms range [47]. These results suggest that photoexcited rhodopsin transiently becomes part of a multienzyme complex consisting of GTPase, phosphodiesterase, and probably other proteins. The phenomenon of light-induced protein binding offers convenient ways to purify several rod cell proteins with only a few centrifugation steps [46].

Integral membrane proteins mutually interact with their natural environment of lipids. M. P. Heyn (Basel) presented an analysis, by means of physical probes, of the influence of bacteriorhodopsin on the order and dynamics as well as on the thermotropic phase transition of large unilamellar vesicles of di-saturated phosphatidylcholines. In the liquid–crystalline state of the lipid bacteriorhodopsin is arranged as a monomer, transition to the gel state induced protein aggregation into the same hexagonal lattice which is also present in the native purple membrane [48]. Aggregation already occurs several degrees below the transition temperature of the lipid. Incorporation of bacteriorhodopsin into the vesicles induces an increased ordering of the lipids and a concomitant slowing of their rotational diffusion.

The rotational diffusion of bacteriorhodopsin in the lipid decreases with increasing fractional concentration of the protein, i.e., the protein experiences a higher microviscosity which demonstrates the pronounced influence of quantitative membrane composition on dynamic parameters [49]. Similar aspects were discussed by R. Cherry (Zürich). He presented comparative data on rotational motions of three integral membrane proteins, using the transient dichroism of probes and intrinsic chromophores. Bacteriorhodopsin rotates only around an axis normal to the plane of the membrane. All 'copies' are mobile at low protein concentration [48]. The same is true for cytochrome oxidase in reconstituted systems while in the native mitochondrial membrane only ~60% take part in the observed rotation [50]. In case of the (Ca^{2+} + Mg^{2+})-ATPase from sarcoplasmic reticulum a fast component of motion is tentatively assigned to segmental motions. The whole protein rotates more slowly, but still faster than cytochrome oxidase [51].

A somewhat different way to study the properties of a membrane protein in a lipid environment was discussed by E. Bamberg (Konstanz). Pieces of purple membrane from *Halobacterium halobium* were either adsorbed to or integrated into black lipid films. Photocurrents can be produced in these systems which exhibit time courses and decay times corresponding to the photochemical reactions produced by the exciting light. Photocurrents in the integrated system respond to external electric fields [52].

Bacteriorhodopsin acts as an isomerase for its chromophore, the 13-*cis* double bond of retinal. This was shown by K.-D. Kohl and W. Sperling (Jülich) using bacteriorhodopsin analogs formed by the reaction of the apoprotein with the retinal isomers 7-*cis* and 7,13-di-*cis* retinal, respectively. The isomerization occurs in the dark within <1 s.

Molecular models of membrane functions such as transport have to consider available structural data for the purported transport protein(s). From the oligomeric structure of a number of membrane-spanning proteins known or assumed to act as 'carriers', M. Klingenberg (München) derived a generalizing model for the operation of such transport systems. In this model an oligomeric, asymmetric arrangement of protein subunits either forms a single channel in its centre or provides separate, but interacting channels inside each subunit, exhibiting gating phenomena. Half-site

reactivity of the catalytic binding center, as observed in a number of carriers, is taken to result from a steric hindrance in the centre channel type, while indicating negative cooperativity in the parallel channel type [53].

A more specific transport model, based on protein structure, was presented by H. Passow (Frankfurt) for the anion (Cl^-/HCO_3^-) exchange system of the erythrocyte membrane, derived from molecular properties of the transport protein. This transport system is almost certainly located in the major intrinsic membrane protein, termed 'band 3', and undergoes conformation changes between a *cis*- and a *trans*-state, which can be induced by the presence of substrate and of inhibitors and activators of transport. This finding, combined with data on the covalent binding of competitive inhibitors, and results of net flux (conductance) measurements can be rationalized by the following model: The anion exchange system is formed by a channel with flexible regions acting as an adaptable (double) gate, closing itself around the substrate in the course of its binding. The subsequent *cis–trans* switch of the protein catalyzes the translocation by shifting the gates and thus forces the protein to act as a reciprocating, electrically silent exchange system. Ion slippage through the open wings of the gate, or uncomplexed isomerisations of the system account for the (low) anion conductance of the system observed experimentally [54,55].

Transport characteristics of band 3 protein incorporated, after isolation, into phospholipid vesicles were reported by W. Köhne and B. Deuticke (Aachen). The reconstituted system coincides with the native system in a number of characteristic properties but differs in other, less obvious, features, which clearly indicate alterations of the protein in the course of its manipulations [56]. The use of recombined protein–lipid systems for the elucidation of the molecular basis of transport functions, while attractive conceptually, thus still requires caution in its realisation.

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References

[1] Engelman, D. M., Henderson, R., McLachlan, A. D. and Wallace, B. A. (1980) Proc. Natl. Acad. Sci. USA 77, 2023–2027.
[2] Henderson, R. and Unwin, P. N. I. (1975) Nature 257, 28–32.
[3] Michel, H., Oesterhelt, F. and Henderson, R. (1980) Proc. Natl. Acad. Sci. USA 77, 338–342.
[4] Ovchinnikov, Y., Abdulaev, N., Feigina, M., Kiselev, A. and Lobanov, N. (1979) FEBS Lett. 100, 219–224.
[5] Engelman, D. M. and Zaccai, G. (1980) Proc. Natl. Acad. Sci. USA 77, 5894–5898.
[6] Michel, H. and Oesterhelt, D. (1980) Proc. Natl. Acad. Sci. USA 77, 1283–1287.
[7] Garavito, R. M. and Rosenbusch, J. P. (1980) J. Cell. Biol. 86, 327–329.
[8] Schindler, H. G. and Rosenbusch, J. P. (1981) Proc. Natl. Acad. Sci. USA 78, 2302–2306.
[9] Kübler, O., Engel, A., Zingsheim, H. P., Emde, B., Hahn, M., Heiße, W. and Baumeister, W. (1980) in: Electron Microscopy at Molecular Dimensions (Baumeister, W. and Vogell, W. eds) pp. 11–21, Springer-Verlag, Berlin, New York.
[10] Saxton, W. O. and Baumeister, W. (1981) Proc. Natl. Acad. Sci. USA 78, in press.
[11] Fringeli, U. P. and Hofer, P. (1980) Neurochem. Intl. 2, 185–192.
[12] Kleffel, B. and Baumeister, W. (1981) Biochim. Biophys. Acta in press.
[13] Wingfield, P., Arad, T., Leonard, K. and Weiss, H. (1979) Nature 280, 696–697.
[14] Leonard, K., Wingfield, P., Arad, T. and Weiss, H. (1981) J. Mol. Biol. in press.
[15] Weiss, H. (1981) in: Intl. Bari Symp. Vectorial Reactions in Electron and Ion Transport in Mitochondria and Bacteria (Palmieri, F. et al. eds) Elsevier/North-Holland, Amsterdam, New York, in press.
[16] Buse, G. (1980) in: 31. Mosbach Colloq. Biological Chemistry of Organelle Formation (Bücher, T. et al. eds) pp. 59–70, Springer-Verlag, Berlin, New York.
[17] Tanaka, M., Haniu, M., Yasunobu, K. T., Yu, C. A., Yu, L., Wei, Y. H. and King, T. E. (1977) Biochem. Biophys. Res. Commun. 76, 1014–1019.
[18] Steffens, G. C. M., Steffens, G. J. and Buse, G. (1978) Hoppe-Seyler's Z. Physiol. Chem. 360, 1641–1650.
[19] Sacher, R., Steffens, G. J. and Buse, G. (1979) Hoppe-Seyler's Z. Physiol. Chem. 360, 1385–1392.
[20] Buse, G. and Steffens, G. J. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359, 1005–1009.
[21] Steffens, G. J. and Buse, G. (1979) Hoppe-Seyler's Z. Physiol. Chem. 360, 613–619.
[22] Saraste, M., Penttilä, T. and Wikström, M. (1981) Eur. J. Biochem. 115, 261–268.

[23] Frey, T. G., Chan, S. H. P. and Schatz, G. (1978) J. Biol. Chem. 253, 4389–4395.
[24] Henderson, R., Capaldi, R. and Leigh, J. S. (1977) J. Mol. Biol. 112, 631–648.
[25] Fuller, S. D., Capaldi, R. A. and Henderson, R. (1979) J. Mol. Biol. 134, 305–327.
[26] Bercovici, T. and Gitler, C. (1978) Biochemistry 17, 1484–1489.
[27] Gupta, C. M., Radhakrishnan, R., Gerber, G. E., Olsen, W. L., Quay, S. C. and Khorana, H. G. (1979) Proc. Natl. Acad. Sci. USA 76, 2595–2599.
[28] Bisson, R. and Montecucco, C. (1981) Biochem. J. 193, 757–763.
[29] Bisson, R., Montecucco, C., Gutweniger, H. and Azzi, A. (1979) J. Biol. Chem. 254, 9962–9965.
[30] Kagawa, Y. (1978) Biochim. Biophys. Acta 505, 45–93.
[31] Sebald, W., Hoppe, J. and Wachter, E. (1979) in: Function and Molecular Aspects of Biomembrane Transport (Quagliariello, E. et al. eds) pp. 63–74, Elsevier/North-Holland, Amsterdam, New York.
[32] Sebald, W., Machleidt, W. and Wachter, E. (1980) Proc. Natl. Acad. Sci. USA 77, 785–789.
[33] Sebald, W., Graf, Th. and Lukins, H. B. (1979) Eur. J. Biochem. 93, 587–599.
[34] Hoppe, J., Schairer, H. U. and Sebald, W. (1980) FEBS Lett. 109, 107–111.
[35] Prochaska, L. J., Bisson, R., Capaldi, R. A., Steffens, G. C. M. and Buse, G. (1981) Biochim. Biophys. Acta in press.
[36] Michel-Villaz, M., Saibil, H. and Chabre, M. (1979) Proc. Natl. Acad. Sci. USA 76, 4405–4408.
[37] Chabre, M. and Breton, J. (1979) Photochem. Photobiol. 30, 295–299.
[38] Chabre, M. and Breton, J. (1979) Vision Res. 19, 1005–1018.
[39] Osborne, H. B. and Nabedryk-Viala, E. (1978) Eur. J. Biochem. 89, 81–88.
[40] Hargrave, P. A., Fong, S.-L., McDowell, J. H., Mas, M. T., Curtis, D. R., Wang, J. K., Juszczak, E. and Smith, D. P. (1980) Neurochem. Intl. 1, 241–244.
[41] Pellicone, C., Bouillon, P. and Virmaux, N. (1980) Comp. Rend. l'Acad. Sci. Paris 290, 567–569.
[42] Kühn, H., Mommertz, O. and Hargrave, P. A. (1981) unpublished.
[43] Kühn, H. and Hargrave, P. A. (1981) Biochemistry 20, 2410–2417.
[44] Kühn, H. (1978) Biochemistry 17, 4389–4395.
[45] Kühn, H. (1980) Nature 283, 587–589.
[46] Kühn, H. (1981) Curr. Top. Membr. Transp. 15, in press.
[47] Kühn, H., Bennett, N., Michel-Villaz, M. and Chabre, M. (1981) Hoppe-Seyler's Z. Physiol. Chem. 362, 217.
[48] Cherry, R. J., Müller, U., Henderson, R. and Heyn, M. P. (1978) J. Mol. Biol. 121, 283–298.
[49] Heyn, M. P., Cherry, R. J. and Dencher, N. A. (1981) Biochemistry 20, 840.
[50] Kawato, S., Sigel, E., Carafoli, E. and Cherry, R. J. (1980) J. Biol. Chem. 255, 5508–5510.
[51] Bürkli, A. and Cherry, R. J. (1981) Biochemistry 20, 138–145.
[52] Bamberg, E., Apell, H.-J., Dencher, N. A., Sperling, W., Stieve, H. and Läuger, P. (1979) Biophys. Struct. Mech. 5, 277–292.
[53] Klingenberg, M. (1981) Nature 290, 449–454.
[54] Passow, H., Fasold, H., Gärtner, E. M., Legrum, B., Ruffing, W. and Zaki, L. (1980) Ann. NY Acad. Sci. 341, 361–383.
[55] Passow, H., Kampmann, L., Fasold, H., Jennings, M. and Lepke, S. (1980) in: Alfred Benzon Symp. 14, Membrane Transport in Erythrocytes (Lassen, U. V. et al. eds) pp. 345–367, Munksgaard, Copenhagen.
[56] Köhne, W., Haest, C. W. M. and Deuticke, B. (1981) Biochim. Biophys. Acta 644, 108–120.