bilayer results in the generation of a vesicle-associated hydrophobic peptide and the release of a mixture of hydrophilic peptides. The former peptide probably constitutes the hydrophobic domain which serves to anchor the native γGT to the renal brush border membrane. The hydrophilic peptides may be derived from the sequence of amino acids which connects the separate domains of this amphipathic protein.

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STRUCTURE OF THE H^+-ATPASE ($F_1 \cdot F_0$) FROM *ESCHERICHIA COLI* AND ITS DICYCLOHEXYLCARBODIIMIDE-PROTEIN IN SOLUTION BY X-RAY SCATTERING METHODS

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The H^+-ATPase ($F_1 \cdot F_0$) synthetase of energy-transducing membranes couples the vertical movement of H^+ through the membrane to synthesize ATP (1). The F_1 portion, having a molecular weight of 365,000 (2) can be released from membranes at low ionic strength. The F_0 part, which is the integral membrane portion, renders dicyclohexylcarbodiimide (DCCD) sensitivity (3). Heavy atom labeling of the F_1-portion with tetrakis (acetoxymercuri) methane (4) (TAMM) or mercuriated *N*-pyrrolo-isomaleinimide (5) (NEM-Hg), which carries four Hg^{2+}, was applied on F_1 and $F_1 \cdot F_0$. The localization of the Hg label was determined from the distance distribution function, D_{PL}, by measuring the scattering curves of $F_1 \cdot F_0$ and F_1 with and without the label, and measuring the scattering intensities of the unbound Hg^{2+} label ($I_L[h]$). $I_L(h)$ was automatically subtracted from the difference of the scattering curves of labeled and unlabeled $F_1 \cdot F_0$. The distribution of distances between the label and any position in the $F_1 \cdot F_0$ structure is given through D_{PL} and enables us to locate the heavy atom level.

RESULTS AND DISCUSSION

The Hg_4^{2+}-label peaks at R=18 Å only, whereas the maximum chord length was determined to be 174 ± 10 Å for $F_1 \cdot F_0$; for $F_1 \cdot F_0$-Hg_4^{2+} it was 180 ± 10 Å; and for F_1 it was 125 ± 10 Å. The morphological parameters of the labeled and unlabeled complexes are listed in Table I. No severe changes in volume or radii of gyration are detected, indicating that size and shape heterogeneities are absent. Furthermore, the biological activity of $F_1 \cdot F_0$ as well as of F_1 is not affected by the heavy atom label, indicating that the SH group, which is attainable from the outside of $F_1 \cdot F_0$ is involved neither in the ATP hydrolysis, nor in the catalysis of the $^{32}P_i$-ATP exchange rate.

When the F_1 portion was labeled with TAMM or NEM-Hg, the distance distribution function peaked at 31 ± 5 Å, without any change in D_{max}=125 ± 10 Å. Chemical analysis of the location of the Hg_4^{2+} cluster revealed that Hg_4^{2+} is located on the β-subunit of F_1; however, for the $F_1 \cdot F_0$ complex the label is situated on the α-subunit. Possibly, a molecular rearrangement of the five subunits of F_1 occurred when F_0 assembled to the DCCD-sensitive H^+-ATPase. A second Hg_4^{2+} cluster can be inserted into the F_1 complex, located at D_{PL} = 50 Å which is not possible with the $F_1 \cdot F_0$ complex. Methyl-mercury nitrate (CH_3HgNO_3) can be bound to the tail end of the distribution function of $F_1 \cdot F_0$, revealing a profile of $D_{PL}(R)$ of $F_1 \cdot F_0$ along its major axis of a = 85 Å. The distance between the CH_3-$HgNO_3$ label, situated in the F_0 portion, and the Hg_4^{2+} cluster was evaluated at 140 ± 10 Å.

The DCCD-protein involved in the H^+-transmembrane channeling process undergoes a reversible water-induced transformation from α-helical to β-structure. The funda-

TABLE I

STRUCTURAL PARAMETERS OF THE H^+-ATPASE ($F_1 \cdot F_0$) AND THE DCCD-PROTEIN FROM *E. COLI* (3°C).

Parameter	$F_1 \cdot F_0$	$F \cdot F_0 \cdot Hg_4^{2+}$	F_1	$F_1 \cdot Hg_4^{2+}$	DCCD-protein	
					DMSO	$CHCl_3$:CH_3OH:H_2O
$m_i \times 10^5$e*	2.40 ± 0.12	2.48 ± 0.1	1.89 ± 0.05	1.95 ± 0.02	0.0476	0.0905
$M_r \times 10^5$						
anal. UZ‡	4.75 ± 0.3	—	3.60	3.61	—	—
from x-ray§	4.60 ± 0.22	4.62 ± 0.20	3.60	3.68	0.08 ± 0.005	0.171 ± 0.02
laser light scattering‖	4.80 ± 0.3	4.80 ± 0.20	3.62	3.51	~0.10	0.161 ± 0.03
R_g, Å	58.1 ± 0.2	58.0 ± 0.3	42.3 ± 0.04	42.2 ± 0.03	15.0 ± 0.3	17.7 ± 0.5
$V \times 10^5$, Å^3	1.18	1.15	7.15	7.05	0.17	0.28
v_2, ml · g^{-1}*	0.8012	0.8013	0.7420	0.7431	0.7610	0.7645
R, Å	75.3	75.3	55.0	54.8	19.48	22.90
R_v, Å	65.1	65.2	57.4	57.5	—	18.84
w, ml · g^{-1}	0.75	0.70	0.62	0.58	—	0.25
D_{max}, Å	174 ± 10.0	180 ± 10.0	125 ± 10	125 ± 10	48.5	61.5 ± 1.5
$D^0_{20,w} \times 10^7$ cm^2 · s^{-1}¶	2.60 ± 0.03	2.61 ± 0.04	3.47 ± 0.02	3.49 ± 0.03	—	2.01 ± 0.03

*Determined as described in (6);

‡Determined by sedimentation equilibrium at 20,000 rpm (8);

§According to $M_r = 1.89\ m_i$;

‖Wavelength $\lambda = 546.5$ nm; $(\partial n/\partial c)_{\mu\text{-const.}} = 0.1710 \pm 0.002$ ml · g^{-1} as described in (8);

¶Determined by inelastic light scattering (9); R, R_v are the radii of a sphere whose radius of gyration is R_g and volume V; the difference in these values is an indication of the departure from spherical.

mental unit of association of the DCCD-protein in organic solutions is the dimer ($CHCl_3$:CH_3:H_2O=5:1:1), giving evidence that this hydrophobic protein can aggregate in low-polarity media. The maximum chord length of the dimer was found to be 61.5 ± 1.5 Å; however, at a concentration of 25% (vol/vol) of DMSO the maximum chord length was determined to be 48.5 Å. The weight-average mol wt was found to be 9,000, apparently the monomer of the DCCD-protein, suggesting that the conformation changes upon addition of DMSO. Small-angle x-ray scattering intensity curves obtained from the DCCD-protein in water show a discrete interference maximum indicative of a considerable degree of regularity in the two dimensional arrangement in the plane perpendicular to the DCCD-protein-axis. A well-orientated diffraction pattern of the DCCD-protein was obtained from which cylindrically average intensity distribution curves were calculated, revealing a π-left-handed helix rather than α-helix.

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THE EFFECTS OF LIPID ENVIRONMENT, ION-BINDING AND CHEMICAL MODIFICATIONS ON THE STRUCTURE OF THE GRAMICIDIN TRANSMEMBRANE CHANNEL

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Gramicidin A is a linear polypeptide antibiotic which forms ion channels in membranes. It is capable of adopting a number of different conformations, depending on its environment. Nuclear Magnetic Resonance (NMR) studies have indicated that its conformation in phospholipid vesicles is likely to be an N-terminal to N-terminal dimer of single helices (1). Circular dichroism (CD) and infrared spectroscopic studies of gramicidin in methanol and ethanol solutions have suggested that the molecule forms a family of intertwined double helical dimers in these hydrophilic organic solvents (2). The present CD study of gramicidin demonstrates that neither the structures which the molecule assumes in a variety of organic solvents, including such amphipathic solvents as decanol and decanoic acid, nor likely those formed in crystals prepared from hydrophilic solvents, are equivalent to its structure in phospholipid vesicles. Our data support the single helical channel as being the structure formed in membranes.

RESULTS AND DISCUSSION

Circular dichroism spectroscopy has also been used to ascertain those features necessary for maintenance of the active channel conformation of gramicidin A, and to examine this antibiotic's mechanism of action, alterations in its structure accompanying cation binding, chemical modification, and variations in lipid structure. X-ray diffraction of crystals formed from methanol and ethanol solutions have suggested that the cation binding mechanism might involve a widening of the channel and, consequently, foreshortening of the helix (3). Our CD study of gramicidin in membranes demonstrates that the helical