SECONDARY STRUCTURE OF Na^+, K^+-DEPENDENT ADENOSINE TRIPHOSPHATASE

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1. Introduction

Na^+, K^+-dependent ATPase is a protein system built into the plasma membrane of eukaryotic cells. Its main function is the transfer of monovalent cations through the membrane against their electrochemical potentials using the energy of ATP hydrolysis [1,2]. According to modern concepts, the function of such systems is based on the structural features of their protein component. Active preparations of Na^+, K^+-ATPase are suspensions of rather large protein–lipid particles [3]; such a state of the active enzyme restricts the utilization of different physical methods for investigation of the protein structure.

This paper describes basic characteristics of secondary structure of the ATPase protein component as revealed by infrared spectroscopy in the amide band region. Studies were made of a highly active preparation of a protein–lipid complex of Na^+, K^+-ATPase; the protein component consisted of only two specific polypeptides (50 000 and 100 000 dalton) which comprised ~100% total protein. It is shown that ~20% of peptide groups form highly-ordered α-helical regions and about 25% are in pleated sheets (β-structure) with an anti-parallel packing of chains. Regions with regular structure are predominantly located in protein domains which are inaccessible to water. These regions seem to be located in the hydrophobic core. The major part of the protein structure (~55%) looks unordered and is easily accessible to water molecules.

2. Materials and methods

Na^+, K^+-ATPase preparations were obtained from the outer medulla of pig kidneys as in [4] which is a slight modification of Jørgensen's technique [5]. The final preparations varied from spec. act. 1600–1900 μmol P_i/mg protein/h. Proceeding from the sodium dodecyl sulfate gel electrophoresis data, the sum of the large and small polypeptides was estimated to be 96–98% total protein; their weight ratio was about 4 : 1 (fig.1). The preparation consisted of membrane fragments <1 μm diam. as judged from the ability of the particles to pass through membrane filters of fixed poresize. The same sizes of membrane fragments were also determined from electron microscopy.

Infrared spectra in solutions were registered with a Perkin-Elmer 180 spectrophotometer. The technique

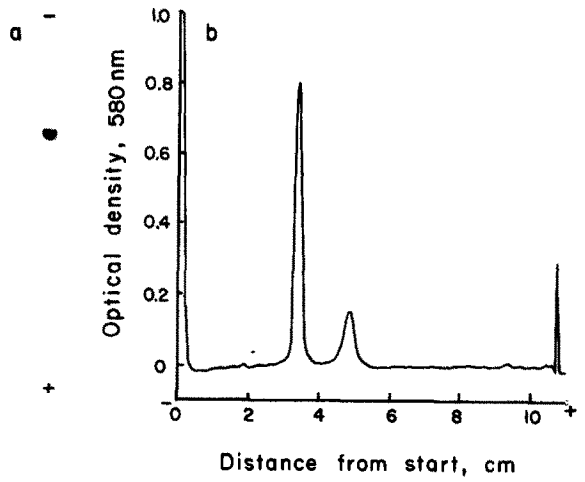

Fig.1. Analysis on the purity of the Na^+, K^+-ATPase preparation from pig kidneys. (a) Electrophoresis of the preparation in a polyacrylamide gel gradient (5–15%) in SDS under the conditions in [6]. (b) Scanning at 580 nm of the same gel dyed with Coomassie brilliant blue R-250. Loading: 8 μg preparation/gel; gel diam. 5 mm.

of obtaining quantitative infrared spectra of polypeptides in heavy water is given in [7,8] and the peptide spectrum was corrected for light-water absorption as described therein. Before the measurement, the Na^+, K^+-ATPase was brought into a complex with ouabain by incubation for 30 min at 37°C in a medium consisting of 25 mM Tris–HCl, pH 7.5, 100 mM NaCl, 2 mM $MgCl_2$, 2 mM ATP and 1 mM ouabain. Protein was transferred from solution in light water into heavy water using a column of Sephadex G-25 (fine) equilibrated in the same buffer in D_2O at 5°C. The initial protein concentration was from 0.7–2.0% and it was 0.6–1.6% after passing the protein through the column. Thermostatted cells 120 μm thick were used. The procedure from the beginning of the protein contact with heavy water to the start of the measurements took about 10 min. The protein concentration of the solutions was determined according [9].

3. Results

Figure 2 represents the infrared spectrum of the lipid complex of Na^+, K^+-ATPase in heavy water. It was obtained at 5°C and 15 min after contact with the heavy-water buffer. Rapid deuterium took place in practically all the side groups as well as in a part of peptide groups in the regions of easy accessibility to D_2O molecules. Intensive protein bands are seen in

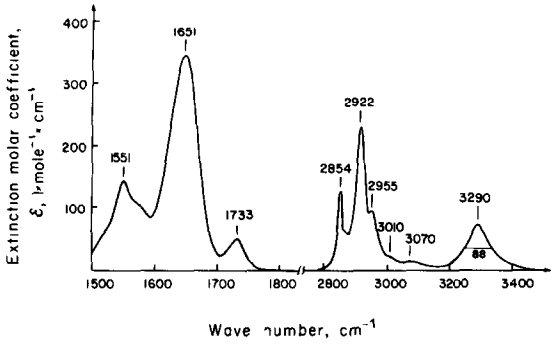

Fig.2. Infrared spectrum of Na^+, K^+-ATPase. Suspension in heavy water, 25 mM Tris–DCl, pD 7.5, 100 mM NaCl, 2 mM $MgCl_2$, 2 mM ATP and 1 mM ouabain. The spectrum was obtained at 5°C 15 min after transferring of the sample into heavy water. R is the calculated absorption of side groups on the basis of the method in [11].

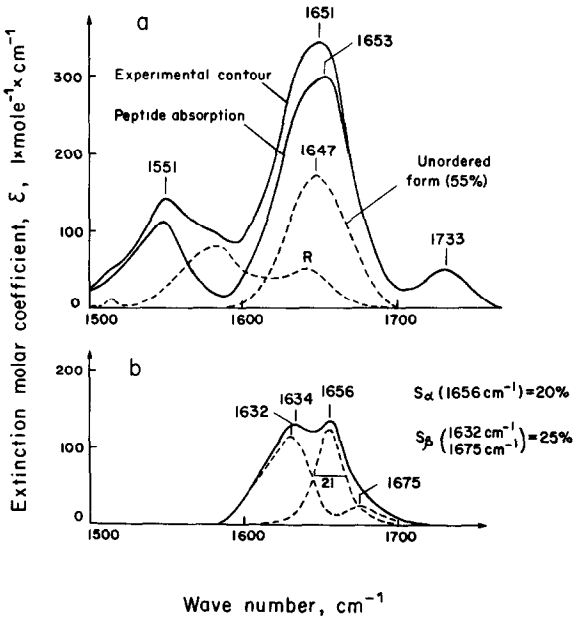

Fig.3. Graphic analysis of amide I band of Na^+, K^+-ATPase. (a) Determination of the contribution to amide I absorption band of the unordered form and side groups of amino acid residues (R). (b) Isolated spectrum of regular form.

the spectrum: amide A band at about 3290 cm^{-1}, amide I band with a ~ 1651 cm^{-1} max., and amide II band with a ~1551 cm^{-1} max. Lipid bands are also well displayed; carbonyl band with a ~1733 cm^{-1} max. and intensive CH_2 group bands at ~2854 cm^{-1} and ~2922 cm^{-1} (the contribution of protein CH groups in this region does not exceed 10% total intensity). The ratio of protein to lipids was estimated from integral intensities of the protein and lipid bands ~1651 cm^{-1} and ~1733 cm^{-1}. The weight fractions of these components in the complex were found to be approximately equal.

Amide A band (NH-stretching vibration) as well as amide II band (NH-bending vibration) are assigned to the non-deuterated peptide groups. Amide A band manifests itself as a narrow band with the half-width of about 88 cm^{-1} which is typical of only regular polypeptide structures [10]. Therefore we will assume that the spectrum in the region of amide A and amide II bands refers mainly to the regular part of protein. We used the integral intensity of amide A band for the evaluation of the fraction of the regular part in the protein structure. From the magnitude of this inten-

sity for the 100% regular form, 4.5×10^4 l. mol^{-1} . cm^{-2} [11], the amount of the regular form in the protein is estimated to be 45%. The spectrum obtained at 5°C 15 min after the sample contact with heavy water remains practically unchanged for 1 h. A subsequent exposure at 20°C for 2 h results in an additional deuterium exchange of only ~8% peptide groups.

Amide I band near 1650 cm^{-1} is the one most responsive to the type of secondary structure and we used it for secondary structure determination.

A graphic analysis of amide I band is given in fig.3. First we subtracted the absorption of side groups of amino acid residues (R) according to [12] in order to obtain the peptide absorption. Since the amino acid composition of Na^+, K^+-ATPase from pig kidneys is unknown, we used for this protein the data obtained for the preparation from dog kidneys [13]. This is reasonable because the differences in amino acid composition of this enzyme are negligible even for evolutionally widely-separated species [14]. Accurate data on the content of amide residues are also unavailable, therefore we took the number of glutamine and asparagine residues to be equal to the number of residues of the corresponding dicarboxylic acids. The corrected peptide contour of amide I band consists of the unexchanged regular part and the deuterated part of the non-ordered form. The parameters of this form were considered to be equal to those of hen-egg lysozyme denatured by heat [15], for histones in solutions of low ionic strength [16] and also for α_{S1}-casein from cow's milk as measured by us. For all these proteins in heavy-water solution the amide I band had the form of a wide band with similar parameters. The averaged contour of the non-ordered form had a maximum frequency of 1647 cm^{-1}, half-width of 50 cm^{-1} and integral intensity of 3.8×10^4 l. mol^{-1} . cm^{-2}.

As a result of subtracting the non-ordered part from the amide absorption in the region of amide I band (fig.3a), we obtained the contour to be assigned to the regular parts of the protein (fig.3b). The component with frequency 1656 cm^{-1} and half-width 21 cm^{-1} was assigned to the α-helical structure and could be easily isolated from the summary contour. The content of α-helices determined by the integral intensity of this band [8] is 20%. The narrow half-width of the α-helical component evidences a high degree of order of this structure [10]. Two components at 1632 cm^{-1} and 1675 cm^{-1} can be referred to the antiparallel β-form [17]. The content of this form estimated by the integral intensity [7] is about 25%. This structure is more distorted than the α-helix, since its components have greater half-widths. The splitting of the components which is equal to 43 cm^{-1} corresponds to 3 or 4 chains in the pleated sheet [17].

The greatest error in determining the amount of regular structures can result from inaccuracy in evaluating the protein concentration by Lowry's technique [9]; this is supposed to be about ± 10%. So the estimated amount of regular structures will be equal to 45 ± 4.5%. Naturally, to this should be added the error of the method of measuring the integral intensities and isolating the corresponding contours. This error is about ± 7%. As a result, the final estimate of regular structures is equal to 45 ± 8%. The error in determining each type of regular structures was estimated from the inaccuracy in determining the concentration and a possible error in the absorption of side groups. It was found that the amount of the α-helix is from 16–22%, and that of the β-structure from 18–34%.

It should be noted that this paper presents data on the structure of Na^+, K^+-ATPase which is associated with ouabain. However, we have found that both the shape of the main amide bands and the kinetics of deuterium exchange remain almost unchanged whether or not the enzyme is associated with ligand.

A structural investigation described above showed that about 55% of all the peptide hydrogen atoms are exchanged for deuterium at pD 7.5 and 5°C during 15 min. The high exchange rates in fact reflect the high degree of accessibility of a significant part of the protein structure to water molecules. Hydrogen exchange in the remaining regular part of the molecule is hindered. This part seems to be located in the hydrophobic core of the protein–lipid complex.

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