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Changes in Na^+,K^+-ATPase structure induced by cation binding

Approach by Raman spectroscopy

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Raman analysis of Na^+,K^+-ATPase structural changes induced by cation binding reveals a slight decrease (<10%) of the α-helical content upon E_1–E_2 transition. Pronounced conformational changes of the enzyme are unlikely as the character of the environment of tyrosine residues remains unaltered. However, local changes can take place as evidenced by changes in tryptophan vibration at about 880 cm^{-1}.

Na^+,K^+-ATPase; Secondary structure; Structural change; Raman spectroscopy

1. INTRODUCTION

Although a considerable body of information accrued since Skou's discovery of Na^+,K^+-ATPase (the enzyme responsible for the asymmetric distribution of univalent cations between animal cells and their medium [1]), discussion on the conformational changes associated with its function is still open. The ATPase can reversibly alternate between two major conformations: the E_1 form, which is the high-affinity 'sodium form', and the E_2 form, which is the low-affinity 'potassium form' [2,3].

The E_1–E_2 transition may cause changes in the representation of various secondary structure types, as well as changes in the position of α-helices or β-sheets with or without changing their relative proportion. So far, circular dichroism [4,5] and vibrational spectroscopies (IR [6] and Raman [7]) have been used to monitor the changes of the secondary structure: The results gave, however, an unclear picture as the E_1–E_2 (Na^+-to-K^+) transition was reported to be accompanied by a 7% decrease of α-helical content (by CD [4]), or by a negligible change of the secondary structure (by CD [5] and IR [6]), or, by a 10% increase of the relative amount of α-helices (Raman [7]).

The careful Raman analysis carried out here reveals a slight decrease of the α-helical content upon the E_1–E_2 transition. Greater conformational changes of the enzyme are, however, unlikely as the environment of the ATPase tyrosine residues does not significantly change.

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2. EXPERIMENTAL

The Na^+,K^+-ATPase was purified from the outer medulla of frozen lamb kidneys by the method of Lane et al. [8] and stored at 4°C until used. After polyacrylamide gel electrophoresis, the protein showed only two protein bands corresponding to molecular weights of the α (~100 kDa) and β (~50 kDa) subunits. The initial specific activity when purified was between 1,000 and 1,200 μmol of P_i/mg/h. Activity measurements were made as described by Abbott et al. [9].

For Raman experiments, the samples with ATPase concentrations of approximately 10–15 mg/ml were used (Tris-HCl buffer, pH 7.4). The final concentration of K^+ ions used to convert the E_1 form (as isolated) to the E_2 form was 20 mM (by addition of KCl). The samples retained at least 90% of their activity after several hours of illumination.

Raman spectra were taken using a 514.5 nm excitation from an argon ion laser with, typically, 100 m*W* light power unfocussed at the sample to a beam with a diameter of about 0.5 mm. The samples were held in a quartz thermostated microcell (5 μl) stabilized at 4°C. A computer-assisted modular Raman system [10], consisting of a Jobin-Yvon THR 1500 monochromator with a Raman holographic edge filter (POC), placed at the entrance slit and a cooled RCA 31034-A02 photomultiplier coupled with an ORTEC photon counting system was used. Spectral bandpass was set to 7 cm^{-1}. Data points were taken at every 1 cm^{-1} with 1.5 s/point integration time for one scan. Routinely, up to 64 scans in 250 or 150 cm^{-1} ranges were taken. The spectra were not subjected to smoothing procedures. The difference spectrum was obtained by direct subtraction of the accumulated spectrum of the E_1 form from the E_2 one.

3. RESULTS AND DISCUSSION

Experimental data for the Amide I region of Raman spectra of Na^+,K^+-ATPase in both the E_1 and E_2 forms and the respective (E_2–E_1) difference spectrum are shown in Fig. 1A. The spectra exhibit a characteristic shape for ATP-dependent ion pumps, which are integral membrane proteins [11,12]. Raman spectra of these pro-

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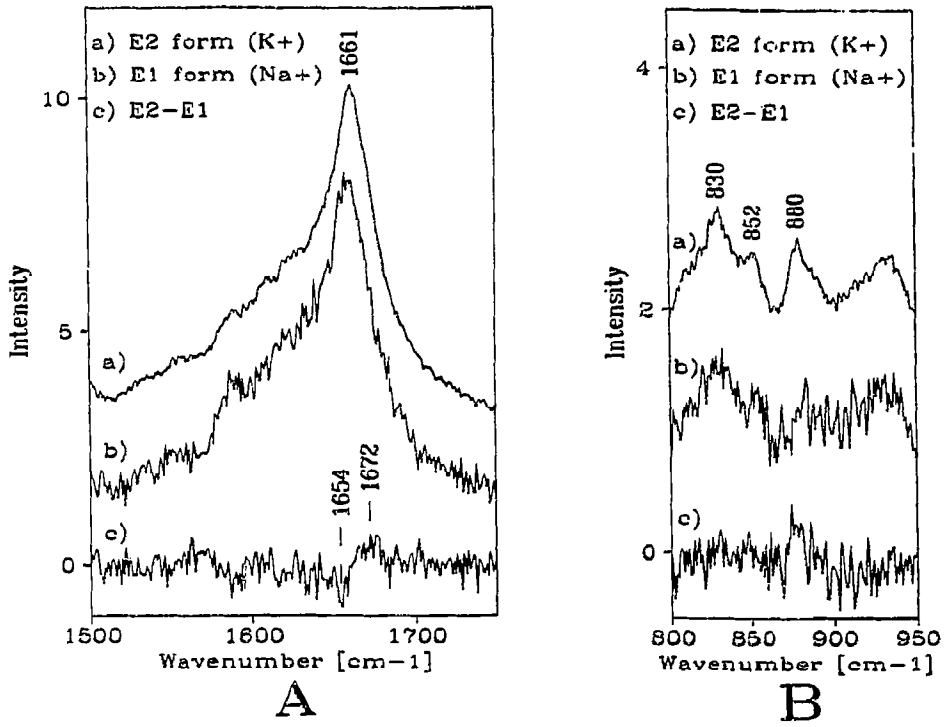

Fig. 1. Raman spectra of sheep Na^+,K^+-ATPase. (A) The Amide I region. Traces a and b correspond to the E_2 and E_1 forms of the Na^+,K^+-ATPase, respectively; trace c, E_2–E_1 difference spectrum. (B) The 800–950 cm^{-1} region. The traces are the same as for A. For the experimental details, see section 2.

teins are known to contain a contribution from the lipid molecules bound to the protein, together with a signal from the aqueous solvent. Hence, for studies of the relative amounts of the various secondary structures in each particular state (E_1 and E_2), a subtraction of the lipid and solvent spectra was commonly done [7,11,12]. Then, after subtraction, an estimate of secondary structure in both states, E_1 and E_2, was performed and the structural changes were then characterized as large (a 10% increase in α-structure [7]). As the subtraction of solvent and lipid contribution from the spectra may significantly influence the experimental data, we compared the spectra of the enzyme in the E_1 and E_2 states without any subtraction. The result (Fig. 1A) shows a characteristic differential feature with a minimum at 1,654 cm^{-1} and a maximum at about 1,672 cm^{-1}, which can be interpreted as a decrease in α-helical content together with a corresponding increase of β-structure during the E_1–E_2 change. The extent of this change can only be assessed as being less than 10%.

This result is in agreement with CD data of Gresalfi and Wallace [4] who found a 7% decrease in α-helicity. Another CD study performed by Hastings et al. [5], as well as an IR study of Chetverin and Brazhnikov [6] did not, however, confirm changes in the secondary structure. On the other hand, the Raman study mentioned above [7] (with data processing including double subtraction) indicated pronounced changes of the secondary structure but in an opposite direction to those found here and by Gresalfi and Wallace [4]: a 10% increase in α-helicity in the E_2 form (in the presence of K^+). This disagreement is difficult to explain. We feel that there may be two reasons. (i) Treatment of samples may lead to changes in their optical properties and structure. We experienced this when examining three types of samples which differed in the method of preparation and in the way of preserving the purified enzyme. Although both highly purified samples gave perfectly reproducible Raman spectra, the extent of structural changes and the spectral quality was higher with the sample which was kept all the time at about 5°C. The data presented here are those obtained with this sample. Hence, it may be possible that the degree of purification, the influence of lipid molecules attached to the isolated enzyme and possible conformational changes caused by changing the temperature, may influence the final result. (ii) The subtraction method may be another source of error, as already mentioned in the original paper [11]. Hence, the subtraction of the lipid and solvent spectra was avoided here to obtain the difference based solely on the effect of the E_1–E_2 state transition.

Another marker of possible conformational changes is the relative exposure of aromatic, mainly tyrosine residues as monitored by the intensity changes of a tyrosine doublet at 850/830 cm^{-1} [13]. The intensity ratio, I_{850}/I_{830}, is known to be sensitive to the states of

hydrogen bonding at the phenolic OH group; however, an E_1–E_2 state transition apparently did not cause significant change of the ratio. The value of the I_{850}/I_{830} intensity ratio ~0.5 can be interpreted as reflecting a low relative accessibility of the tyrosine residues (i.e. tyrosine residues are buried inside the enzyme) and a high one (above 1) for the more exposed ones which can form H-bonds with the solvent, water. The value of about 0.6 found for the Na^+,K^+-ATPase sample in this study (Fig. 1B) indicates that the 24 tyrosines [14] in this enzyme are relatively buried. On the other hand, the presence of certain local conformational changes influencing the exposure of tryptophan residues [15] is indicated by the presence of a positive peak at about 880 cm^{-1} in the difference spectrum (Fig. 1B).

Hence, it can be stated that Raman spectroscopy, which is a powerful tool and complementary to CD spectroscopy and other methods used for studying protein structure [16–18], revealed only subtle changes of the secondary structure of the sheep kidney Na^+,K^+-ATPase: a decrease in the α-structure and a concomitant increase of the β-structure. However, gross conformational changes are unlikely as the average exposure of the tyrosine residues does not change.

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