Effect of pH and Different Substrates on the Electrokinetic Properties of (Na⁺, K⁺)-ATPase Vesicles

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Abstract. Some biophysical properties of a (Na⁺, K⁺)-ATPase preparation from guinea-pig kidney have been analysed. The recently developed technique of laser Doppler spectroscopy was applied to measure particle mobility under electrophoretic conditions. The following results were obtained:

1. magnesium ions at pH 7.3 decrease the mobility of the ATPase containing vesicles by binding to negatively charged surface groups. At pH 3.3 the competitive binding of protons causes a shift of the mobility vs. [Mg²⁺] curve to higher values of [Mg²⁺],

2. binding of ATP at pH 7.3 (K_d = 0.9 × 10⁻⁴ M for (mM 1 NaCl, 0.2 KCl, 0.1 MgCl₂, 0.1 Tris) was measured as an increase in particle mobility depending also on [Mg²⁺]. At pH 3.3 also unspecific ATP-binding occurred,

3. ITP and GTP had the same K_d value as ATP; ADP a slightly lower one (K_d = 1.2 × 10⁻⁴ M). Tris-H₃PO₄ (K_d = 2.6 × 10⁻⁴ M) was also able to increase particle mobility, but only at higher concentrations and not to the same extent as ATP; AMP induced only very small changes,

4. from the mobility-pH curve an isoelectric point of 4.1 is derived (buffer: 1 mM NaCl, 0.2 mM KCl, 0.1 mM MgCl₂, 0.1 mM Tris). In the presence of 0.9 mM ATP the isoelectric point is shifted to 3.2.

As the electrophoretic mobility is directly proportional to the net charge of the vesicles, the results may be interpreted as changes in surface charge density, originating from both a conformational change of the ATPase polypeptide and a decrease in vesicle size.

Key words: (Na⁺, K⁺)-ATPase – Laser Doppler electrophoresis – Surface charge – Conformational changes

Introduction

It is a well established fact that cytoplasmic membranes of intact cells have charged surfaces. The charge density may dominate physico-chemical properties
of the cell, like ion transport, ligand binding to receptors or vesicle adhesiveness and deformability.

Binding of a ligand to its membrane located receptor usually takes place by ionic, Van der Waals or dipole interactions. Binding by ionic forces would result in changes of the surface charge and is influenced by various ions and their concentrations.

It has been confirmed that the (Na\(^+\), K\(^+\))-ATPase is the enzyme of the active sodium and potassium transport across cell membranes (Dahl and Hokin 1974; Skou 1975; Schwartz et al. 1975). The enzyme transports cations against a concentration gradient by hydrolysing ATP to ADP and P\(_i\). For this process sodium, potassium and magnesium ions are necessary. The ATP, Mg\(^{2+}\), and Na\(^+\) binding sites are located on the inside surface of the cell, while potassium and cardiac glycosides are known to interact with the enzyme from the outside.

The preparation of (Na\(^+\), K\(^+\))-ATPase leads to spherical, closed vesicles which contain the ATPase polypeptide as protruding structures from the vesicle surface (Maunsbach et al. 1980; Schlieper et al. 1981). It is generally assumed that the vesicles are mainly inside out with the catalytic site of the enzyme accessible to substrates. Therefore ATPase containing vesicles seem to be suited to study ligand binding to surface active groups.

Surface charges may be analysed by various electrophoretic methods which have been applied to investigate liposomes, lysosomes, liver mitochondria and microsomes, synaptic vesicles and erythrocytes (Hauser et al. 1976; McDonald and Bangham 1972; Davenport 1964; Plummer 1965; Hannig 1969; Heidrich et al. 1970; Ryan et al. 1971; Blad-Holmberg 1979; Haydon and Seaman 1967). One of the most common technique which has been used is the slow and tedious procedure of microscopic observation of the bioparticles, moving in an uniform electric field.

Ware and Flygare (1971) and Uzgiris (1974) developed a much more rapid and accurate method for the measurement of particle mobilities, based on light scattering and the Doppler principle. This method has successfully been applied to characterize the surface groups of red blood cells (Uzgiris and Kaplan 1976), chromaffin granules (Siegel et al. 1978), synaptic vesicles (Siegel and Ware 1980), mast cells (Petty et al. 1980) and recently by us to study (Na\(^+\), K\(^+\))-ATPase vesicles (Schlieper et al. 1981).

In this report the influence of pH on substrate binding to the enzyme and the effect of magnesium are investigated measuring the electrophoretic mobility of ATPase vesicles by laser Doppler spectroscopy.

**Materials and Methods**

**Enzyme Preparations**

The extraction procedure for (Na\(^+\), K\(^+\))-ATPase from guinea-pig kidney was the same as described before (Schlieper et al. 1981).