Addition of subunit γ, K⁺ ions, and lipid restores the thermal stability of solubilized Na,K-ATPase

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Differential scanning calorimetry (DSC) was applied to ascertain the effect caused by K⁺, Na⁺, ATP, detergent, DPPC, DPPE, and subunit γ on the thermostability of Na,K-ATPase. The enthalpy variation (∆H) for the thermal denaturation of the membrane-bound is twice the ∆H value obtained for solubilized Na,K-ATPase. Denaturation occurs in five steps for membrane-bound against three steps for the solubilized enzyme, therefore a multi-step unfolding process. In the presence of Na⁺, the melting temperature is 61.6 °C and the ∆H is lower as compared with the ∆H obtained in the presence or in the absence of K⁺. Addition of ATP does not alter the transition temperatures significantly, but the shape of the curve is modified. Subunit γ probably stabilizes Na,K-ATPase in the beginning of thermal unfolding, and different amounts of detergents in the solubilized sample change the protein stability. Reconstitution of Na,K-ATPase into a liposome shows that lipids exert a protector effect. These results reveal differences on the thermostability depending on the conformation of Na,K-ATPase. They are relevant because it allows a comparison with future studies, e.g. how the composition of the membrane interferes on the stability of Na,K-ATPase, elucidating the importance of the lipid type contained in cell membrane.

Introduction

The discovery of Na,K-ATPase by Skou [1] was a critical step in the study of the cell as a basic unit of animal life [2]. The Na,K-pump moves three Na⁺ ions out of and two K⁺ ions into the cell for each hydrolyzed ATP molecule. The sodium pump is ubiquitous because it is necessary for regulation of the volume of animal cells. Moreover, the Na⁺ gradient is the energy source for facilitated transport of other ions and metabolites. The generation and maintenance of membrane potentials are necessary for nerve transmission and muscle contraction and excitability [3–5]. A number of studies have suggested that Na,K-ATPase interacts with neighboring membrane proteins in caveolae and organizes cytosolic cascades of signaling proteins, so that messages can be sent to intracellular organelles in different tissues [6]. The Na,K-pump acts as receptor for the cardiac glycosides that are effective in heart failure through inhibition of the enzymatic activity [7]. Na,K-ATPase is essential for cell viability, and it has been indirectly implicated in the etiology of diseases such as hypertension and diabetes [8]. The functional Na,K-ATPase is a heterodimer, and subunits α and β are crucial to the catalytic and transport functions. In some tissues, like the kidneys, Na,K-ATPase contains a third subunit, γ, which displays a regulatory function [9]. Subunit α has a transmembrane domain formed by 10 segments: α M1–α M10. Subunit β contains a large glycosylated extracellular portion and is anchored to the membrane by a single helical segment [5]. Subunit γ or protein FX4D2 displays a single trans-membrane segment with an extracellular N terminus and a cytoplasmic C terminus [10]. New information on the structure and function of Na,K-ATPase has been obtained from crystal structures. The structure of the protein from the shark was determined at a resolution of 2.4 Å. It is similar to that of the pig protein, previously determined at 3.5 Å. It completes the structure of the ectodomain of β- subunit and identifies cholesterol at a proposed lipid-binding site. The crystal form of the shark Na,K-ATPase was also soaked with cardioactive steroid ouabain, which provided insights into a low-affinity cardioactive steroid-binding site in Na,K-ATPase among helices αM1, αM2 and αM4–M6 [5,11–13]. Although the crystal structure of Na,K-ATPase has been established, many studies are still required for deeper understanding of the biological pump operation [8].

Calorimetric techniques have played a pivotal role in the development of our understanding of the energetic and thermodynamic mechanisms underlying protein folding–unfolding transitions [14]. Differential Scanning Calorimetry (DSC) studies on the thermal denaturation of proteins have had a central part in the development of the current views about the factors that determine protein stability [15].

Our group has investigated the structure–function and stability of Na,K-ATPase by means of biophysical techniques such as Circu-
lar Dichroism (CD), Infrared Absorption (IR), Spectroscopy Fluorescence Emission, Surface Tension, Dilatational Elasticity, and Small-Angle X-ray Scattering (SAXS). CD showed that the surfactant/enzyme ratio affects the aggregation of the solubilized protein with C12E8 [16–21]. At high detergent concentrations (greater than 2.7 mg/mL), the activity is almost completely lost, and the form (α) of the enzyme ratio affects the aggregation of the solubilized protein with C12E8 [16–21]. At high detergent concentrations (greater than 2.7 mg/mL), the activity is almost completely lost, and the form (α) of the protein was maintained in solubilization buffer (20 mM imidazole buffer, pH 7.0, containing 250 mM sucrose, 6 mM EDTA, and 6 mM Tris). After solubilization, the purified protein was maintained in solubilization buffer (5 mM Tris–HCl buffer, pH 7.0, containing 15 mM KCl, 6 mM EDTA and 0.005 mg/mL C12E8).

**Preparation of Na,K-ATPase**

Membrane-bound and solubilized/purified Na,K-ATPase were obtained from the dark red medulla of the rabbit kidney as previously described in [16]. The membrane-bound enzyme was maintained in homogenization buffer (20 mM imidazole buffer, pH 6.8, containing 250 mM sucrose, 6 mM EDTA, and 6 mM Tris). After solubilization, the purified protein was maintained in solubilization buffer (5 mM Tris–HCl buffer, pH 7.0, containing 15 mM KCl, 6 mM EDTA and 0.005 mg/mL C12E8).

**Extraction of subunit γ**

Extraction of subunit γ was carried out as described in [22] with some modifications. Membrane-bound Na,K-ATPase (1.0 mL, 1.0 mg) was diluted 16-fold with 46% (v/v) methanol, 46% (v/v) chloroform, and 8% (v/v) 750 mM NH4HCO3 mixture. The sample was centrifuged for 5 min at 600g, and the supernatant (subunit γ) was dried under N2 flux. The dry material was suspended in 500 μL of the homogenization buffer.

**Preparation of the proteoliposome**

A DPPC:DPPE (1:1 w/w) proteoliposome was prepared by the co-solubilization method using a lipid:protein ratio 1:3 (w/w), as previously described in [17,20].

**Proteolysis with trypsin**

Membrane fractions containing Na,K-ATPase were treated with trypsin at a protein:trypsin ratio 3:1 (w/w). After 30 min at 37 °C, the smaller polypeptide units and trypsin were eliminated by ultracentrifugation for 1 h, at 100,000g and 4 °C. The pellet was suspended in homogenization buffer.

**Analysis of the protein**

Concentration of the protein was estimated in the presence of 2% (w/w) SDS as described in [16]. Determination of the protein in the proteoliposome was performed according to the methodology described in [23]. Bovine serum albumin was used as standard.

**Enzymatic activity**

Activity of the enzyme ATPase was discontinuously assayed at 37 °C in a final volume of 1.0 mL by quantification of phosphate release as described in [24]. The reaction was initiated by addition of the enzyme, and it was stopped with 0.5 mL of cold 30% TCA solution.

**Differential scanning calorimetry (DSC)**

Melting temperatures (Tm) and variation of the thermal denaturation enthalpy (ΔH) of the membrane-bound Na,K-ATPase, the solubilized enzyme and the proteoliposome were measured by DSC. The samples and reference (buffer) were placed in the calorimeter and analyzed on the apparatus Nano-DSC II from Calorimetry Sciences Corporation, CSC (Lindon, Utah, USA). All the samples were degassed under vacuum (140 mbar) for 30 min before use. Scans were recorded from 20 to 90 °C at an average heating rate of 0.5 °C/min, under pressure of 3 atm. The baseline was determined by filling the sample and the reference cells with buffer solution. Data was analyzed with the fitting program CpCalc provided by CSC. The plot and deconvolution were carried out by using Origin version 8.0 (Gaussian deconvolutions with R² > 0.993).

**Results and discussion**

To study the effects of factors such as the presence of detergent, lipids, K+ and Na+ ions, substrate and, subunit γ on the stability of Na,K-ATPase, we conducted DSC experiments using Na,K-ATPase from the rabbit kidney in different conditions.

The heat capacity profile of a macromolecular system undergoing a temperature-induced transition; e.g. protein unfolding is characterized by the presence of one or more peaks in the transition region. The change in enthalpy associated with the unfolding, ΔH, is the area under the curve [14]. A recent review described DSC as a tool for protein folding and stability [25].

**Membrane-bound Na,K-ATPase**

Fig. 1-A shows the heat capacity profile of the membrane-bound Na,K-ATPase from the rabbit kidney. Deconvolution of the endotherm indicates a five-step thermal denaturation, with transition temperatures (Tt) of 47.9, 52.9, 57.7, 62.9 and 69.0 °C (Tm ≈ 58 °C, Table 1). Grinberg et al. [26] described transition temperatures of 47.5, 54.3 and 58.4 °C (Tm ≈ 53 °C) for the membrane-bound Na,K-ATPase extracted from the pig kidney. Fodor et al. [27] found transition temperatures of 33.2, 41.5, 45.1 and 50.2 °C (Tm ≈ 44 °C) for membranous Na,K-ATPase from the shark and 49.0, 52.7, 54.8, 62.7 and 70.1 °C (Tm ≈ 54 °C) for membranous Na,K-ATPase from pig kidney. Rescan of the sample reveals that
Na,K-ATPase comprises domains that are exposed to the aqueous medium and domains that are in contact with the lipid components of the membrane [18]. It is known that the thermal denaturation of membrane and soluble proteins are different, because the two classes of protein have very distinct environments [28–30]. The total enthalpy variation (ΔH) values of ca. 500 kcal/mol obtained here are smaller than those achieved for the unfolding enthalpies of small globular proteins. This indicates that transmembrane helices probably retain most of their secondary structure [26,31], and that only part of the protein unfolds, most likely the cooperative domains situated outside the membrane. The ΔH value found for the thermal denaturation of membrane-bound Na,K-ATPase (513 kcal/mol, Table 2) is relatively close to values reported by other groups (∼420 kcal/mol [26] and ∼690 kcal/mol [27]), and the difference is probably due to the use of different sources, preparation methods, and protein conditions in the medium.

To assign the transitions, we carried out digestion of membrane fractions with trypsin. The exposed regions of subunit α of Na,K-ATPase are attacked, whereas subunit β and the intramembranous moiety remain intact [26,32]. Comparison of the endotherm of the intact membrane-bound Na,K-ATPase (Fig. 1-A) with that of its digested counterpart (Fig. 1-B) reveals that only the central part of the curve is maintained, with a melting temperature of 57 °C, and the first (T1) and last (T5) transitions completely disappear (Fig. 1-B). Moreover, the ΔH values are reduced to more than half the initial value (Table 2). Grinberg et al. [26] identified an intermediate transition, assigned to subunit β. Here, the central part of the peak is maintained after proteolysis, suggesting that the intermediate transition may also be related to subunit β. Thus, the beginning and the end of the unfolding process can be ascribed to extramembranous parts of subunit α.

Unfortunately, we were not able to identify which domain is denaturing and the kind of conformational change that is happening induced by the increase in temperature.

Detergent-solubilized Na,K-ATPase: effect of solubilization and subunit γ

Most studies have been accomplished on membrane-bound Na,K-ATPase. However, solubilization of the protein is required for some biochemical and biophysical techniques. Therefore, it is very important to maintain the protein in a functional and folded state by solubilization with detergent molecules for studies in vitro [33]. Na,K-ATPase was removed from the natural membrane with the detergent C12E8, which was followed by purification in one chromatographic step, as described in [16]. The active enzyme was maintained mainly in its dimeric form (2αβ) in a solubilization buffer. The thermal denaturation profile recorded for the detergent-solubilized sample in the presence of K+ ions is displayed in Fig. 2. The thermal unfolding of this sample takes place at higher temperature as compared with the membrane-bound Na,K-ATPase. However, deconvolution evidences three peaks only, at 55.0, 62.7 and 69.5 °C (Table 1), and ΔH is lower (245 kcal/mol, Table 2).

The thermal denaturation of domain 1 (T1 = 47.9 °C) and 2 (T2 = 52.9 °C) is no longer seen after solubilization (Table 1). This is probably because the solubilization process causes the unfolding of these structures, although the solubilized and purified enzyme retains catalytic activity (∼60%). This indicates that these structures are not primordial for Na,K-ATPase function.

The environment around the Na,K-ATPase should promote this different thermal denaturation. The replacement of the endogenous lipid membrane with detergent molecules and loss of subunit γ during solubilization can determine the change on the shape of the endotherm.

No band corresponding to subunit γ can be seen in the electrophoresis gel of solubilized Na,K-ATPase (data not shown), because this subunit is probably lost during the solubilization/purification process. Subunit γ had been previously extracted from the membrane fractions and incubated with a sample of solubilized Na,K-ATPase, which led to an increase in enzymatic activity (15%). The heat capacity profile of this sample is exhibited in Fig. 3, and the endotherm can be separated into four components. Interestingly, one peak appears below 55 °C, more specifically at 48.4 °C, and...
the other three peaks observed for the solubilized protein in the absence of subunit γ are maintained.

The subunit γ or FXYD2 belongs to the family of proteins FXYD. It is not essential for the function of Na,K-ATPase, but it modulates the kinetic properties of this enzyme. There are seven small proteins in this family, and they are expressed and associated with Na,K-ATPase in a tissue-specific manner [10]. FXYD2, or subunit γ has two sliced variants (FXYD2a and FXYD2b), which are mainly expressed in the medullary thick ascending limb of the kidney [34,35]. Interaction with FXYD2a and FXYD2b affects the apparent affinity of ATPase for extracellular K⁺ and Na⁺ weakly, and this interaction diminishes the apparent affinity of the enzyme for intracellular Na⁺ [35]. It is known that FXYD2 enhances the thermal stability of Na,K-ATPase [10,36].

Comparison of the thermograms of the membrane-bound enzyme (Fig. 1-A), the solubilized protein in the absence of subunit γ (Fig. 2), and containing subunit γ (Fig. 3) evidences that FXYD2 is probably involved in the initial step of thermal denaturation. After heating of the Na,K-ATPase at 55 °C, Donnet et al. [29] showed that three transmembrane spans (αM8–αM10) and the C terminus of subunit α were extruded, whilst the rest of subunit α retained its normal topology with respect to the lipid bilayer.

Bearing in mind that the crystal structure revealed that subunit γ is in contact with αM9 [12], we expected that the presence of γ would influence the beginning of thermal denaturation; i.e., up to 55 °C. Our DSC experiment shows that denaturation initiates around 48 °C, and that this transition is affected by the presence of subunit γ. The part of the structure of the enzyme (domain 1) that would be stabilized by γ is probably unfolded in its absence. Therefore, the first step of thermal denaturation can be related to the structure portion next to αM9. The present result shows that the structure of domain 1 is probably restored by presence of subunit γ, which has a pivotal role in the whole structure of Na,K-ATPase in addition to its regulatory function.

**Solubilized protein in different concentrations of detergent**

The study of membrane proteins is hindered by their inherent hydrophobic nature, which makes their expression and purification in sufficient quantities difficult. This kind of proteins is embedded in a mosaic lipid bilayer, which is a complex, heterogeneous, and dynamic environment even in the simplest organism. These proteins have a strong tendency to aggregation when they are removed from their native environment. The use of detergents is thus essential for maintenance of the membrane protein in a functional, folded state in the absence of its natural environment. Although it is a simpler system, the interaction between membrane proteins with detergent or lipid vesicles (liposome) mimics their interaction with the natural membrane. It is necessary to use this system first if one is to gain enough information before moving onto a more complex and realistic system [30,33,37,38].

C₁₂E₈ has been successfully used for Na,K-ATPase solubilization and this detergent was also used for protein crystallization [11].

We conducted a calorimetric study, for verification of the influence of the detergent/protein (D/P) molar ratio on the thermal stability of Na,K-ATPase.

The protein can be kept in its solubilized form at a concentration of C₁₂E₈ of 0.005 mg/mL, which is below its critical micelle concentration (CMC = 0.053 mg/mL, determined in working buf-
The thermal profile of Na,K-ATPase determined by DSC at the CMC, below the CMC and above this concentration can be viewed in Fig. 4. The same three transitions are observed for all the D/P ratios until D/P = 344. At higher concentration of C12E8, the shape of the curve is altered, showing that the amount of detergent molecules has direct influence on the thermal unfolding of Na,K-ATPase. A high concentration of detergent causes extinction of the transition at 55 °C and diminishes the enzymatic activity (Figs. 4F, G and 5). We also heated a pure sample of detergent, for control. One peak appeared around 80 °C, which is out of the range of the denaturation temperature (data not shown).

The mainly oligomeric form obtained by means of our solubilization/purification method is a dimer of the (αβ)2 type. Having this in mind, we studied the effect of the concentration of the surfactant on the oligomerization of Na,K-ATPase. Using small-angle X-ray scattering, Barbosa et al. [21] described that a high concentration of C12E8 leads to the inactivation via a chemical denaturation that would occur thermally at 55 °C. This result is in accordance with those found by Miles et al. [41] that described that the active site region may be amongst the earliest parts of the protein structure to unfold. Here, the first transition observed for solubilized Na,K-ATPase at 55 °C must be related to the unfolding of the active site part.

**Detergent-solubilized Na,K-ATPase: effect of Na⁺ ions and ATP**

The reaction cycle of Na,K-ATPase involves transition between two main conformations. The presence of Na⁺ or K⁺ ions induces conformation E1 and E2, respectively. Biochemical and biophysical evidence support the idea that the structure of the enzyme in E2 (K2) is rather different from that in E1 [42]. Up to now, only one state: E2-2 K⁺–MgF2− analogs of E2-2 K⁺–Pi in the presence of gamma subunit (and with the specific inhibitor ouabain: E2-2 K⁺–MgF2−–ouabain) was resolved by crystallography and the structure of E1(P) remains currently unavailable [43].
The effect of these ions was examined by microcalorimetry. We carried out solubilization of the membrane in the presence of K+ ions. It is known that these ions stabilize Na,K-ATPase [29,44].

The DSC scan was recorded in the same solubilization buffer, but without K+ ions, and the heat capacity profile of the protein in this medium is depicted in Fig. 6-A. In the absence of K+, the unfolding temperatures are below those recorded in the presence of the same ion. This decrease reaches a value of 5 °C for the second peak, confirming the protector effect of K+ ions. Addition of Na+ ions to the sample in the absence of K+ changes the shape of the thermal denaturation curve (Fig. 6-B), and increases the Tm, but the values are slightly minor as compared with the solubilized enzyme in the presence of K+ ions. The total change in enthalpy is also smaller and this indicates a higher thermal lability of E1 as compared with E2 conformation. The Tm values (Table 1) shows that binding of physiological ions enhances thermal stability.

Recently, Kaufman and collaborators [42] found opposing effects for Na+ and K+ on the thermal stability of Na,K-ATPase by using fluorescence and CD. These authors showed that K+ stabilizes the enzyme, whereas Na+ decreases its stability. Here, we can conclude that conformations acquired in the presence of Na+ eK+ are different enough to change the thermostability of Na,K-ATPase.

When ATP is added to solubilized Na,K-ATPase in the presence of K+, neither the unfolding temperatures nor ΔHt are significantly altered, but the shape of the curve clearly changes (Fig. 7). The peaks become more defined, suggesting that domains unfold with little dependence on each other. One review [45] explained the structural and allosteric effect of ATP. The addition of ATP to the form E2(K+)2 of the enzyme induces separation of the cytoplasmic domains, with consequent releasing of K+ ions. The decreased interaction between the cytoplasmic domains could culminate in the lower cooperativity observed during thermal denaturation, indicating that the difference between the closed and open states is enough to modify the thermal unfolding profile.

**Proteoliposome**

Membrane proteins exist in a heterogeneous environment, interacting with lipid chains, head groups, and aqueous phases. These proteins are also influenced by their lipid surroundings, which take an active role in the topology of the protein and its function [30]. The question is if the protein-lipid interaction has effect on the stability of the membrane protein indirectly via, e.g., fluidity or more specific, as a closer interaction by binding certain lipids to the protein. This question can be addressed using reconstitution into liposomes of defined lipid composition [23].

### Table 3

Denaturation temperature (T) and denaturation enthalpy (ΔHi) of the i-domains (i = 3–5) of solubilized Na,K-ATPase at different Detergent/Protein (D/P) molar ratios.

<table>
<thead>
<tr>
<th>D/P molar ratio</th>
<th>[C12E8] (mg/mL)</th>
<th>T3 (°C)</th>
<th>T4 (°C)</th>
<th>T5 (°C)</th>
<th>ΔH3 (kcal/mol)</th>
<th>ΔH4 (kcal/mol)</th>
<th>ΔH5 (kcal/mol)</th>
<th>ΔHt (kcal/mol)</th>
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<tr>
<td>1.72</td>
<td>0.005</td>
<td>55.0</td>
<td>62.7</td>
<td>69.5</td>
<td>18</td>
<td>176</td>
<td>51</td>
<td>245</td>
</tr>
<tr>
<td>17.2</td>
<td>0.05</td>
<td>55.2</td>
<td>61.8</td>
<td>68.5</td>
<td>37</td>
<td>167</td>
<td>51</td>
<td>255</td>
</tr>
<tr>
<td>34.4</td>
<td>0.10</td>
<td>54.4</td>
<td>61.0</td>
<td>67.7</td>
<td>39</td>
<td>167</td>
<td>79</td>
<td>285</td>
</tr>
<tr>
<td>172</td>
<td>0.50</td>
<td>54.1</td>
<td>62.0</td>
<td>67.9</td>
<td>33</td>
<td>215</td>
<td>28</td>
<td>266</td>
</tr>
<tr>
<td>344</td>
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<td>54.4</td>
<td>61.0</td>
<td>67.7</td>
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</tr>
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Fig. 6. Excess heat capacity of solubilized Na,K-ATPase after baseline subtraction. Dashed lines represent the best peak deconvolution. Thermograms were obtained in solubilization buffer in the (A) absence of KCl and NaCl; (B) presence of 15 mM NaCl.

Fig. 7. Excess heat capacity of solubilized Na,K-ATPase after baseline subtraction. Dashed lines represent the best peak deconvolution. The thermogram was obtained in solubilization buffer containing 3 mM ATP.
We incorporated the solubilized enzyme into DPPC:DPPE vesicles by the co-solubilization method standardized in our laboratory [17]. In contrast to solubilized protein, the detergent molecules are replaced with lipids in the proteoliposome and Na,K-ATPase retains about 70% of its activity after incorporation. The heat capacity profile can also be divided into three peaks, as seen in Fig. 8. We also examined a DPPC:DPPE (1:1) liposome in the calorimeter, for control and a peak of much smaller area appeared around 45 °C (results not shown) and the area was subtracted from the area of protein denaturation.

The first transition temperature (T3) is maintained at 55 °C, while T4 and T5 are 2 °C above the temperatures observed for the solubilized enzyme (Table 1). The total enthalpy variation (ΔHt) is increased almost twofold in the presence of lipids. The elevation in T4 and T5 could indicate protection by lipids mainly on the second and third domain. However, the ΔH3 value increased 10-fold.

The effect of lipid bilayer on the stability was also reported for many membrane proteins, e.g., cytochrome c oxidase [46], Ca2+-ATPase [47], and rhodopsin [48].

Lipids may impact protein stability through both direct protein-lipid interactions and through the bulk properties of the lipid bilayer. Indeed, a specific phospholipid acyl chain length and the presence of cholesterol are essential to support optimal hydrolytic activity of Na,K-ATPase [49]. The work of Cohen, et al. [50] revealed a dependence on activity of Na,K-ATPase expressed in Pichia pastoris without lipid and with specific phospholipids like dioleoylphosphatidylcholine and dioleoylphosphatidylserine. Beside this, Esmann and Marsh [51] reviewed the lipid interactions with Na,K-ATPase by means of electron spin resonance spectroscopy. The authors showed the stoichiometry and selectivity of some spin-labeled lipids with Na,K-ATPase. A special lipid environment, e.g., a high concentration of cholesterol may be needed to stabilize Na,K-ATPase for structure studies. The high cholesterol content is likely to impact fluidity of the membrane and hence the crystal structure obtained suggested a specific site for cholesterol in Na,K-ATPase structure. This cholesterol molecule, carried through from native tissue, occupies the position in which a phospholipid head group was previously located [11,12,43].

In conclusion, a quantitative description of how the investigated factors influence the stability of Na,K-ATPase was done in this work. Fig. 9 shows the ΔHt values found for thermal denaturation of Na,K-ATPase in different conditions. Observation of Tables 1 and 2 shows that each factor studied has influenced differently on the thermal unfolding of the domains. This suggests that the factors interact and have effect on different parts of the Na,K-ATPase structure. The results are relevant because it allows a comparison with future studies with Na,K-ATPase, e.g. how the interaction with other proteins or molecules interferes on the stability, or even how the composition of the lipid bilayer can affect its stability and function, revealing the importance of the lipid type contained in cell membrane.

Fig. 9. Comparative values of Total variation enthalpy of thermal unfolding in different conditions, as described previously: (MF) Na,K-ATPase membrane fraction; (SP + K) solubilized protein in the presence of K+ ions; (SP + K) solubilized protein in the absence K+ ions; (SP + Na) solubilized protein in the presence of Na+ ions; (SP + K + γ) solubilized protein in the presence of K+ ions and subunit γ; (SP + K + ATP) solubilized protein in the presence of K+ ions and ATP and (SP + K + lipid) proteoliposome sample in the presence of K+ ions.

Conclusion

In conclusion, a quantitative description of how the investigated factors influence the stability of Na,K-ATPase was done in this work. Fig. 9 shows the ΔHt values found for thermal denaturation of Na,K-ATPase in different conditions. Observation of Tables 1 and 2 shows that each factor studied has influenced differently on the thermal unfolding of the domains. This suggests that the factors interact and have effect on different parts of the Na,K-ATPase structure. The results are relevant because it allows a comparison with future studies with Na,K-ATPase, e.g. how the interaction with other proteins or molecules interferes on the stability, or even how the composition of the lipid bilayer can affect its stability and function, revealing the importance of the lipid type contained in cell membrane.

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