Biochimica et Biophysica Acta, 985 (1989) 248–254
Elsevier

BBAMEM 74619

Thallium binding to native and radiation-inactivated Na^+/K^+-ATPase

Jørgen Jensen and Jens G. Nørby

Institute of Physiology and Institute of Biophysics, University of Århus, Århus C (Denmark)

(Received 2 June 1989)

Key words: ATPase, Na^+/K^+-; Radiation inactivation; Thallium binding; Potassium site

The number of high-affinity K^+-binding sites on purified Na^+/K^+-ATPase from pig kidney outer medulla has been assessed by measurement of equilibrium binding of thallous thallium, Tl^+, under conditions (low ionic strength, absence of Na^+ and Tris$^+$) where the enzyme is in the E_2-form. Na^+/K^+-ATPase has two identical Tl^+ sites per ADP site, and the dissociation constant varies between 2 and 9 μM. These values are identical to those for Tl^+ occlusion found previously by us, indicating that all high-affinity binding leads to occlusion. The specific binding was obtained after subtraction of a separately characterized unspecific adsorption of Tl^+ to the enzyme preparations. Radiation inactivation leads to formation of modified peptides having two Tl^+-binding sites with positive cooperativity, the second site-dissociation constant approximating that for the native sites. The radiation inactivation size (RIS) for total, specific Tl^+ binding is 71 kDa, and the RIS for Tl^+ binding with original affinity is approx. 190 kDa, equal to that of Na^+/K^+-ATPase activity and to that for Tl^+ occlusion with native affinity. This latter RIS value confirms our recent theory that in situ the two catalytic peptides of Na^+/K^+-ATPase are closely associated. The 71 kDa value obtained for total Tl^+ sites is equal to that for total binding of ATP and ADP and it is clearly smaller than the molecular mass of one catalytic subunit (112 kDa). The Tl^+-binding experiments reported thus supports the notion that radiation inactivation of Na^+/K^+-ATPase is a stepwise rather than an all or none process.

Introduction

The stoichiometry of the Na^+/K^+-pump is generally considered to be 3Na:2K:1ATP (for a recent review, see De Weer et al. [1]), and this is reflected in most, if not all, models for transport and reaction mechanism, where a similar stoichiometry of binding sites for these ligands is assumed. In the present study we use equilibrium binding of Tl^+ to characterize the high-affinity binding sites for K^+. The direct estimation of the binding capacity by equilibrium binding of K^+ to Na^+/K^+-ATPase is difficult, as evidenced from reports in the recent literature [2–6], where one finds a maximum of close to 2 ions can be bound per α-subunit, but also 3 [7] and 4–5 [8] can be found. Several studies have shown that Tl^+ binds to Na^+/K^+-ATPase and activates the system as a congener of K^+. Gehring and Hammond [9] have reported the activation of rat erythrocyte ATPase by Tl^+ in the presence of Na^+ and have compared this to similar activation obtained with K^+. Earlier work [10] of these authors demonstrated metabolically dependent accumulation of Tl^+ by rabbit erythrocytes that was inhibited by ouabain and K^+. Moreover, it has been shown [11] that Tl^+ is handled like K^+ by rat sartorius muscle and also that Tl^+ behaves like K^+ during electrical excitation of that tissue. Tl^+ has been found to replace K^+ in activation of the Na^+/K^+-ATPase of rabbit kidney. In comparison to other known substitutes for K^+ in activating Na^+/K^+-ATPase Tl^+ is unique in that it has an affinity approx. 10-times greater than K^+ for the K^+-activating site [12] and the same when *p*-nitrophenylphosphatase is considered [13]. The findings of Cavieres and Ellory [14] that Tl^+ has an effect on ouabain-sensitive Na^+ efflux and K^+ influx, are by them interpreted in terms of a high-affinity substitution for K^+ at the external potassium sites of the sodium pump. It has likewise been demonstrated that Tl^+ has a specific, K^+-like role in activating the dephosphorylation of the phosphointermediates of Na^+/K^+-ATPase [15].

Abbreviations: Na^+/K^+-ATPase, Na^+/K^+-transporting ATPase (EC 3.6.1.37); K-pNPPase, K^+-activated *p*-nitrophenylphosphatase activity; SDS, sodium dodecyl sulphate.

Correspondence: J. Jensen, Institute of Physiology, University of Århus, DK-8000 Århus C, Denmark.

Here we have investigated the properties of equilibrium binding of Tl^+ while trying to optimize the conditions for binding of K^+ and its congeners. We have used a centrifugation method with high enzyme concentration, low ionic strength and low monovalent cation concentration to obtain accurate isotherms for unspecific and specific binding. We find two identical high-affinity binding sites per ADP site and that equilibrium binding equals occlusion [16]. Likewise it is demonstrated that the radiation inactivation size is equal to that for Na^+/K^+-ATPase activity (about 190 kDa = $1.8 \cdot m(\alpha)$) when unmodified binding is concerned and significantly smaller than $m(\alpha)$ for the total Tl^+-binding capacity. This is in line with our recently published model for the stepwise radiation inactivation of a dimeric α-peptide arrangement in the membrane [16].

Experimental procedure

Na^+/K^+-ATPase. Membrane-bound Na^+/K^+-ATPase was isolated from pig kidney outer medulla by the method of Jørgensen [17], i.e., selective extraction of plasma membranes with SDS in the presence of ATP, followed by isopycnic zonal centrifugation (zonal enzyme). Also enzyme prepared according to the procedure described by Jensen et al. [18], with additional washes to avoid K^+, was used (SDS-enzyme). The enzymes were stored at $-20\,°C$ in 250 mM sucrose, 12.9 mM imidazole and 0.625 mM EDTA at pH (38 °C) = 7.15. The specific, ouabain-inhibitable Na^+/K^+-ATPase activities for the two types of preparation were: 20–30 and 10–15 units $\cdot$ (mg protein)$^{-1}$, and the ADP-binding capacity was about 2.5 and 1.3 nmol $\cdot$ (mg protein)$^{-1}$, respectively. These last numbers were used in calculating the enzyme concentration in the different assays.

Reagents. Glucose-6-phosphate dehydrogenase from *L. mesenteroides*, SDS, Tris and imidazole were from Sigma, EDTA and sodium *p*-nitrophenylphosphate from Merck, sucrose from British Drug House. Tl_2SO_4 was from Analar and the sodium salt of ATP from Boehringer, Mannheim. [^{14}C]ADP and $^{204}Tl_2SO_4$ was obtained from Amersham International, Amersham, U.K. The ammonium salt of [^{14}C]ADP was converted to its Tris salt by chromatography on DEAE-Sephadex [19].

Enzyme assays and protein determination. Na^+/K^+-ATPase was determined at 37 °C by the coupled assay [20] with 3 mM ATP, 4 mM $MgCl_2$, 120 mM NaCl and 20 mM KCl, 1 mM phospho*enol*pyruvate, 0.2 mM NADH, 10 units $\cdot$ ml^{-1} pyruvate kinase, and 30 units $\cdot$ ml^{-1} lactate dehydrogenase. K-pNPPase activity was measured at 37 °C with 10 mM *p*-nitrophenyl phosphate, 20 mM $MgCl_2$ and 150 mM KCl [21].

Glucose-6-phosphate dehydrogenase was assayed at 37 °C in 50 mM Tris-HCl, pH 7.8 at 25 °C, 3 mM $MgCl_2$, 3 mM NAD, and 3.3 mM glucose 6-phosphate [22].

Protein concentrations were measured with the method of Lowry et al. [23] with bovine serum albumin as standard.

Binding assays. [^{14}C]ADP binding was measured by a centrifugation assay [24]. The medium contained 200 mM sucrose, 10 mM EDTA, 30 mM Tris and 50 mM NaCl. The nucleotide-binding isotherms with the non-irradiated Na^+/K^+-ATPase samples gave straight lines in the Scatchard plot (Ref. 18 and Fig. 3) so that the binding capacity and dissociation constant were easily obtained.

Equilibrium binding of Tl^+. Determination of isotherms for the binding of Tl^+ was carried out by incubating enzyme (2.3–3.3 μM) for 1 h at 25 °C with varying concentrations (1.5–100 μM Tl^+) of Tl_2SO_4 with $^{204}Tl^+$, in 10 mM imidazole, 200 mM sucrose and 1 mM EDTA titrated to pH 7.1 at 25 °C with H_2SO_4 (TlCl is much less soluble than Tl_2SO_4). Under these low ionic strength conditions, the enzyme has a high affinity for Rb^+ [7] and is in the E_2-form (Skou, J.C., personal communication). The concentration of bound Tl^+ (from 9% of total Tl^+ at the highest Tl^+ concentration to 60% at the lowest) was determined as the difference between total Tl^+ concentration in the assay mixture and the Tl^+ concentration in the supernatant of a sample centrifuged at $100\,000 \times g$ for 45 min at room temperature. Tl^+ binding was found to be independent of preincubation time from 0.5 to 3 h, also for irradiated samples. Unspecific binding of Tl^+ to native and heat-denatured enzyme preparations was determined by binding measurements at Tl^+ concentrations from 2 up to 28 mM. The subtraction of unspecific binding from the total binding to the native preparations gave a specific binding to a single population of sites (see Results).

Irradiation procedure. The enzyme samples to be irradiated were kept in perspex tubes under N_2 and were transported to and from the Accelerator Department, Risø National Laboratory, Roskilde, Denmark, on solid CO_2. The irradiation was carried out at $-15\,°C$. The radiation source was a linear accelerator producing 10-MeV electrons, and successive passes through the electron beam, each lasting about 3 min, and exposing the samples to about 2 Mrad, were performed until the appropriate dose was reached. The dose was measured for each pass by calorimetry with an accuracy within $\pm$ 5% [25]. Glucose-6-phosphate dehydrogenase was added to several samples to serve as a standard for dose correction as described earlier [16,28] and the K-pNPPase and the Na^+/K^+-ATPase activity served as internal standards. In this paper results from two different irradiation series are reported.

It should be noted that the irradiated samples were kept in the frozen state until the activities were measured, except for a very brief thawing followed by rapid freezing in connection to withdrawal of a sample for

measuring Tl^+ binding. Na^+/K^+-ATPase and especially glucose-6-phosphate dehydrogenase activity were found to be sensitive to repeated thawing and freezing. Likewise it was important to keep the samples under N_2 throughout the manipulations prior to the activity measurements.

To present the radiation inactivation data, the logarithm of biological activity (A) was plotted versus radiation dose. When this relation is linear, i.e., $A = A_0 \cdot e^{-\gamma D}$, the inactivation can be described by the decay constants, γ ($Mrad^{-1}$), which is proportional to the radiation inactivation size, RIS (kDa).

Results

Unspecific binding of Tl^+

Unspecific binding of Tl^+ constitutes a considerable part of the total binding of Tl^+ to both the zonal enzyme and the SDS-enzyme used in this study (see Fig. 2), and to characterize the specific binding it is therefore necessary to obtain accurate estimates of the unspecific binding. Fig. 1 shows the binding of Tl^+ to SDS-enzyme, the concentration of free Tl^+ being between 2 and 28 mM. Under these conditions bound Tl^+ varies from 300 to about 2000 μM, and as the specific binding is only of the order of 6 μM (corresponding to 2 Tl^+ sites per ADP site, Figs. 2 and 3), the experiments in Fig. 1 are well-suited to characterize unspecific binding. The values are pooled from native enzyme and enzyme denatured at 65 °C for 0.5 h, since the ratio (Tl^+ bound to native)/(Tl^+ bound to denatured) was

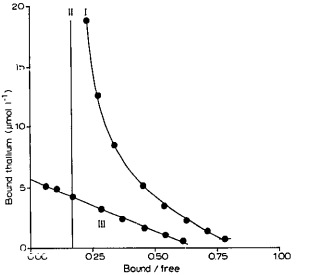

Fig. 2. Scatchard-type plot of Tl^+ binding to Na^+/K^+-ATPase. The concentration of free Tl^+ varies between 0.9 and 82 μM, and total binding is represented by curve I, whereas curve II represents the unspecific binding found in Fig. 1. The specific binding of Tl^+ (curve III = I − II) is a straight line. The S.E.M. was smaller than the size of the points, except in one case. It appears that the ratio between specific and unspecific binding varies between 3 (at high bound/free) and 1/3 (at low bound/free). The specific binding capacity is the intercept with the ordinate, 5.69 μM, and the dissociation constant is the slope of the straight line, 8.56 μM. The protein concentration was 2.06 $g \cdot l^{-1}$.

equal to 1 over the whole range of Tl^+ concentrations: 1.050 ± 0.024 in one study ($n = 8$) and 0.996 ± 0.044 in another ($n = 4$). The data were fitted to a simple binding equation:

$$\frac{B_u}{[Tl^+]_{free}} = \frac{B_u(max)}{K_{d,u} + [Tl^+]_{free}}$$

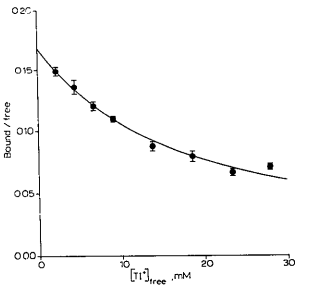

Fig. 1. Tl^+ binding to SDS-treated Na^+/K^+-ATPase under conditions (2–28 mM Tl^+) where unspecific binding is dominating. Data from native and heat-denatured (65 °C for 0.5 h) enzyme are pooled since they were not significantly different. The number of experiments at each concentration is 4–8, and the S.E.M., which was independent of Tl^+, is shown. The curve was fitted to a simple binding equation: $B_u/F = B_u(max)/(K_{d,u} + F)$. $B_u(max) = 2.80$ mM; $K_{d,u} = 16.6$ mM, as described in the text. F is $[Tl^+]_{free}$.

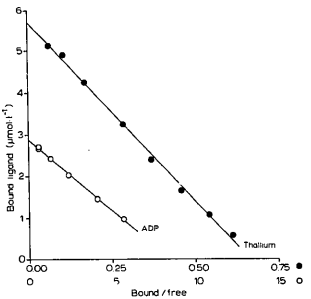

Fig. 3. Scatchard plot of specific Tl^+ binding and of ADP binding to the same enzyme as in Fig. 2. The ratio between Tl^+-binding and ADP-binding capacity is 1.98 ± 0.03.

which gives the binding capacity, $B_u(\max) = 2.80$ mM, and the dissociation constant, $K_{d,u} = 16.6$ mM. This means that in the typical experiment shown in Fig. 2, where the concentration of free Tl^+ varies between 0.9 and 82 μM, i.e., $[Tl^+]_{free} \ll K_{d,u}$, $B_u/[Tl^+]_{free}$ varies only from 0.1686 to 0.1675. Henceforth, the unspecific binding may be illustrated by a vertical binding isotherm like (II) in Fig. 2, and B_u can be expressed as the product $K \cdot [Tl^+]_{free}$ as outlined in the next section.

Specific binding of Tl^+ to native and radiation inactivated Na^+/K^+-ATPase

Fig. 2 shows a binding isotherm (*I*) for total Tl^+ binding to native Na^+/K^+-ATPase. The curve is upward concave indicative of inhomogeneity as regards binding affinity. Subtraction of the unspecific binding (II) from total binding (I) gives the specific binding (III). This curve is linear pointing to uniform sites, and by linear regression it can be shown that the binding has a capacity of 5.69 ± 0.07 μM and a dissociation constant of 8.56 ± 0.19 μM ($r = 0.999$). As outlined above, the unspecific binding can be expressed as $K \cdot [Tl^+]_{free}$, and generally the binding curves for total Tl^+ binding to native enzyme were therefore fitted to:

$$B = \frac{a \cdot [Tl^+]_{free}}{b + [Tl^+]_{free}} + K \cdot [Tl^+]_{free}$$

where a is the binding capacity for specific sites, and b is the dissociation constant.

The specific Tl^+ binding is in Fig. 3 compared to an isotherm for ADP binding to the same enzyme. The number of Tl^+ sites per ADP site is 1.98 ± 0.03. Table I shows determinations of the specific Tl^+-binding capacity relative to the ADP-binding capacity from five different native enzyme preparations, three SDS-treated and two zonal. The ratio is not significantly different from 2.

Binding isotherms for the specific Tl^+ binding to irradiated enzymes are given in Fig. 4, but only a part of this is Tl^+ binding with original affinity. In accordance with the results on Tl^+ binding to native enzyme (described above) and our previous observations on Tl^+ occlusion [16], we now assume that total binding to radiation inactivated enzyme is composed of binding to sites with original affinity, binding to sites with changed affinity (and possible interaction) due to radiation damage, and unspecific binding:

$$B = \frac{a \cdot F}{b + F} + \frac{c(F^2 + d \cdot F)}{F^2 + 2d \cdot F + e \cdot d} + K \cdot F$$

where a and c are the binding capacity for sites with original and changed affinity, respectively; b is the native dissociation constant; and d and e are the second and first site-dissociation constants of the modified sites. F is the concentration of free Tl^+. Fig. 4 illustrates the picture when unspecific binding ($K \cdot F$) has been subtracted. When we furthermore substract a binding isotherm with original affinity for sites (A in Fig. 5) from the specific isotherm (C) we get a curve (B) that is upward convex, i.e., indicating positive cooperativity [26,27]. Besides the site concentrations, a and c (Fig. 6, and next section), the following parameters were obtained by fitting the model to the data in Fig. 4: $b = 6.07$ μM, $e = 30.8$ μM, and $d = 5.49$ μM. It is interesting, that with this method of resolution, the second site-dissociation constant of the modified molecules, d, is equal to the native dissociation constant, b.

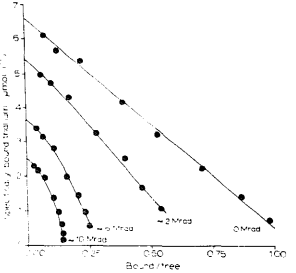

Fig. 4. Scatchard plot of specific Tl^+ binding (obtained as described in Fig. 2 and the text) to native and radiation-inactivated SDS-treated Na^+/K^+-ATPase. Each point represents the average of three to four determinations. The protein concentration was 2.72 g·l^{-1}. The fitted curves are computed as described in the text with the assumption that the irradiated samples contain two different and independent classes of Tl^+-binding peptides, one of which is identical to that in the native enzyme. The results of this analysis is described in the text, and the computed binding capacities for native and modified sites are used to determine RIS (Fig. 6, Table II).

TABLE I

The ratio between specific Tl^+-binding capacity and ADP-binding capacity for five Na^+/K^+-ATPase preparations (three SDS-treated and two zonal enzymes)

Enzyme preparation	(Tl^+/ADP)-binding capacity
SDS-treated	1.98
	2.05
	2.07
Zonal	2.05
	2.09
Average	2.05 ± 0.02

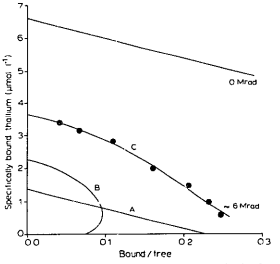

Fig. 5. Scatchard plot illustrating the resolution of the isotherm for specific Tl^+ binding to radiation-inactivated enzyme (5.96 Mrad, see Fig. 4). It appears that total, specific binding (C) is the sum of the component with original affinity (A) and a component with positive cooperativity (B). Curve C has been fitted to the data as referred in the text.

Radiation inactivation size of Tl^+ binding, K-pNPPase and Na^+/K^+-ATPase activity

Two series of radiation experiments were performed. To provide a standard, glucose-6-phosphate dehydrogenase was added to the enzyme preparation, and its activity was measured as a function of dose together

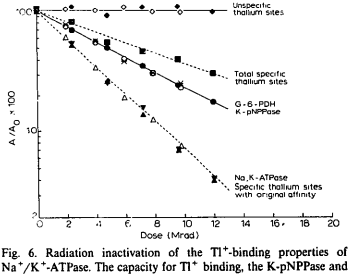

Fig. 6. Radiation inactivation of the Tl^+-binding properties of Na^+/K^+-ATPase. The capacity for Tl^+ binding, the K-pNPPase and Na^+/K^+-ATPase activity as function of radiation dose is shown for zonal enzyme (closed symbols) and SDS-enzyme (open symbols, dotted lines). Unspecific, specific-native, and total-specific capacity for Tl^+ were obtained as described in Figs. 4 and 5 and in the text. The decay constants ($Mrad^{-1}$)±S.E.M. for the different properties are: K-pNPPase (●, ○), 0.146±0.002 and 0.150±0.002; Na^+/K^+-ATPase (▲, △), 0.273±0.004 and 0.266±0.005; Total Tl^+ binding (■, - - -), 0.100±0.007 and 0.099; Native Tl^+ binding (▼, - - -), 0.268±0.011 and 0.262; Unspecific Tl^+ binding (◆, ◇), 0.001±0.004. For comparison, the decay curve for the molecular mass standard (molecular mass 104 kDa) glucose-6-phosphate dehydrogenase (•) is shown: $\gamma = 0.146 \pm 0.007\ Mrad^{-1}$.

TABLE II

Radiation inactivation sizes (RIS) for some biochemical activities of Na^+/K^+-ATPase

The decay constants were obtained as described in the text and the legends to figures. Using glucose-6-phosphate dehydrogenase as standard (molecular mass 104 kDa) the following average RIS values could be calculated.

	RIS (kDa)	
	SDS-treated	zonal enzyme
K-pNPPase	104	107
Na^+/K^+-ATPase	189	194
Tl^+ binding with original affinity	187	191
Total Tl^+-binding capacity	71	71

with the K^+-dependent *p*-nitrophenylphosphatase and the Na^+/K^+-ATPase activity. The decay constants for these activities in the second series of experiments relative to those obtained in the first series, were used to normalize the experiments, the correction factor being 1.18. The resulting decay curves are shown in Fig. 6 together with those for the Tl^+-binding capacity. The unspecific Tl^+-binding sites are apparently not affected by radiation, whereas the total Tl^+-binding sites and the binding sites with original affinity, both decrease linearly with radiation dose in this semilogarithmic plot. The decay constant for Tl^+ sites with original affinity is 0.262 and 0.268 $Mrad^{-1}$ in two experiments (SDS-treated and zonal enzyme). This is very much like the decay constant for Na^+/K^+-ATPase (0.266 and 0.273 $Mrad^{-1}$). The decay constant for total specific Tl^+-binding capacity (original + changed affinity) is 0.099 and 0.100 $Mrad^{-1}$. The values for K^+-dependent phosphatase activity are 0.150 and 0.146 $Mrad^{-1}$, while that for glucose-6-phosphate dehydrogenase is 0.146. Using 104 kDa as the molecular mass of the functional dimer of glucose-6-phosphate dehydrogenase (see Ref. 28), we obtain the radiation inactivation sizes (RIS, kDa) shown in Table II by multiplying the decay constants with 104/0.146.

The RIS values for K-pNPPase and Na^+/K^+-ATPase activity are identical to those obtained previously [16,28] and Tl^+ binding has the same RIS as Tl^+ occlusion [16] when the molecules with original affinity are considered. The RIS for total Tl^+ binding is here found to be higher than that reported for total occlusion (71 versus 40 kDa). These results will be discussed in the following.

Discussion

The stoichiometry of specific Tl^+ binding to native preparations is 2 per ADP site and the two sites are independent under our conditions. The dissociation constant varies between 2 and 9 μM in different experi-

ments, and this is equal to that for occlusion, which means that the bound Tl^+ is also occluded [16].

Since the unspecific binding constitutes a significant proportion of total binding, characterization of the specific Tl^+ binding is entirely dependent upon accurate measurement of the unspecific binding (e.g., Figs. 1 and 2). Here this is done either by binding measurements with high Tl^+ concentrations, where specific binding is negligible, or indirectly by assuming that total binding is the expression of two classes of sites, specific and unspecific. The two methods gave the same result, and the direct method furthermore showed that heating the enzyme to 65°C for 30 min. (which abolishes all biological activities) leaves the unspecific binding unaltered. When we earlier [7] have found about three rubidium sites per ADP site (and an upward curved Scatchard isotherm), it was probably due to the fact that we subtracted the binding of Rb^+ at low concentrations to heat-denatured preparations to get the specific binding, and this unspecific binding is too low. The stoichiometry of 2 Tl^+ sites per ADP site found in this work is supported by the finding of the same stoichiometry in occlusion experiments, where specific occlusion is by far the largest part of the measured occlusion [16].

The nature of the unspecific binding is not known, but it is tempting to suggest that it represents binding of Tl^+ to phospholipids: the binding capacity is 10^3-times the ADP site concentration (compare Figs. 1 and 2) which is of the right order of magnitude for the molar ratio between lipid and Na^+/K^+-ATPase molecules [29], and the radiation inactivation size is very small (Fig. 6).

As regards our target size measurements, we confirm our previous finding [28] that RIS for K-pNPPase activity is close to the mass of one α-subunit, 112 kDa. The RIS for K-pNPPase is equal to that of nucleotide-, vanadate- and ouabain-binding capacity with unchanged affinity, which again is equal to the target size of an α-peptide [16,28]. The total, specific binding capacity for Tl^+ has a lower RIS, 71 kDa, which is equal to the RIS for total binding capacity for ADP, ATP and ouabain [16,28]. Because RIS values equal to 70 kDa are obtained, we have concluded [16] that ionization by absorbed radiation is not indiscriminately transmitted along the peptide backbone, and that fragments of the catalytic peptide may retain biochemical activity, albeit with modified properties.

The RIS value = 71 kDa for total, specific binding found here is higher than the 47 ± 7 kDa and the 37–40 kDa estimated for Rb^+ occlusion by Richards et al. [30] and for Tl^+ occlusion by us [16]. We have at the present no explanation for this discrepancy. It is certain that these values are significantly lower than $m(\alpha)$. The decrease in Tl^+ affinity combined with the positive cooperativity in Tl^+ binding to the partly damaged molecules show that important control functions have been disconnected or annihilated in the fragments that arise during irradiation.

The RIS for Tl^+ binding with unchanged affinity, about 190 kDa, is equal to RIS for full Na^+/K^+-ATPase activity and to RIS for native Tl^+ occlusion (Fig. 6 and Ref. 16). Firstly this supports the notion that Tl^+ binding measured here is equal to Tl^+ occlusion. Secondly, it might indicate that there is a close connection between full Na^+/K^+-ATPase activity and the ability to perform Na^+/K^+-transport, and that these properties are connected with a structural unit larger than the catalytic subunit. Thirdly, it also suggests that the K^+-ATP antagonism prominent in nucleotide-binding experiments with membrane-bound Na^+/K^+-ATPase but much less expressed in solubilized enzyme [31], reflects normal K^+ binding (occlusion) by the α-peptides in an α_2-dimer. On the basis of the many RIS data (smaller than, equal to, or larger than 112 kDa) for the biological activities of Na^+/K^+-ATPase, we have recently [16] proposed a model for the dimeric organization and the stepwise radiation inactivation of the α-peptide. Based on this and on the present study we conclude that Na^+/K^+-ATPase activity and Tl^+ binding with original affinity is an expression of a single, intact α-peptide in a dimeric α_2-arrangement (112/112), and in the irradiated samples it is manifested by both α-peptides in the original (112,112)-dimer and by the single α-peptide in (112,70). This, of course, leads to a RIS value smaller than $2 \times 112 = 224$ kDa. The theoretical value is 112 + 70 = 182 kDa, where we find about 190 kDa. Thus the 112 kDa-peptide as such has Na^+/K^+-ATPase activity and original Tl^+ binding as long as it is 'stabilized' by a sufficiently large peptide domain from another α-peptide. This assumption is in accordance with the studies of Ottolenghi and Jensen [32,33], who found that the only possible explanation for the heterotropic, negative cooperativity between K^+ and ATP in nucleotide-binding studies was an α–α interaction. When the effect of ouabain binding on Na^+/K^+-ATPase activity was probed, it was just as evident that each of the two α-subunits could turn over independently of the other.

Acknowledgements

We wish to thank Toke Nørby for his invaluable help in performing the computations, Ms. Edith Bjørn Møller for excellent technical assistance, and Ms. Inga Edney for expert secretarial assistance. This work was supported by the Biomembrane Research Center, University of Århus.

References

1 De Weer, P., Gadsby, D.C. and Rakowski, R.F. (1988) in The Na^+/K^+-Pump, Part A: Molecular Aspects (Skou, J.C., Nørby, J.G., Maunsbach, A.B. and Esmann, M., eds.), pp. 421–434, Alan R. Liss, New York.

2 Matsui, H. and Homareda, H. (1982) J. Biochem. (Tokyo) 92, 193–217.
3 Cantley, L.C., Cantley, L.G. and Josephson, L. (1978) J. Biol. Chem. 253, 7361–7368.
4 Yamaguchi, M. and Tonomura, Y. (1979) J. Biochem. (Tokyo) 86, 509–523.
5 Jørgensen, P.L. and Petersen, J. (1982) Biochim. Biophys. Acta 705, 38–47.
6 Matsui, H., Hayashi, Y., Homareda, H. and Taguchi, M. (1983) Curr. Top. Membr. Trans. 19, 145–148.
7 Jensen, J. and Ottolenghi, P. (1983) Curr. Membr. Trans. 19, 223–227.
8 Hastings, D. and Skou, J.C. (1980) Biochim. Biophys. Acta 601, 380–385.
9 Gehring, P.J. and Hammond, P.B. (1964) J. Pharmacol. Exp. Ther. 145, 215–221.
10 Gehring, P.J. and Hammond, P.B. (1967) J. Pharmacol. Exp. Ther. 155, 187–201.
11 Mullins, L.J. and Moore, R.D. (1960) J. Gen. Physiol. 43, 759–773.
12 Britten, J.S. and Blank, M. (1968) Biochim. Biophys. Acta 159, 160–166.
13 Inturrisi, C.E. (1969) Biochim. Biophys. Acta 173, 567–569.
14 Cavieres, J.D. and Ellory, J.C. (1974) J. Physiol. 242, 243–266.
15 Post, R.L., Hegyvary, C. and Kume, S. (1972) J. Biol. Chem. 6530–6540.
16 Nørby, J.G. and Jensen, J. (1989) J. Biol. Chem., in press.
17 Jørgensen, P.L. (1974) Biochim. Biophys. Acta 356, 36–52.
18 Jensen, J., Nørby, J.G. and Ottolenghi, P. (1984) J. Physiol. 346, 219–241.
19 Nørby, J.G. and Jensen, J. (1971) Biochim. Biophys. Acta 233, 104–116.
20 Nørby, J.G. (1971) Acta Chem. Scand. 25, 2717–2726.
21 Skou, J.C. (1974) Biochim. Biophys. Acta 339, 258–273.
22 Olive, C. and Levy, H.R. (1975) Methods Enzymol. 41B, 196–201.
23 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
24 Nørby, J.G. and Jensen, J. (1988) Methods Enzymol. 156, 191–201.
25 Miller, A. (1985) Nucl. Instr. Methods Phys. Res. B10/11, 994–997.
26 Fletcher, J.E., Spector, A.A. and Ashbrook, J.D. (1970) Biochemistry 9, 4580–4587.
27 Klotz, I.M. and Hunston, D.L. (1975) J. Biol. Chem. 250, 3001–3009.
28 Jensen, J. and Nørby, J.G. (1988) J. Biol. Chem. 263, 18063–18070.
29 Peters, W.H.M., Fleuren-Jakobs, A.M.M., De Pont, J.J. H.H.M. and Bonting, S.L. (1981) Biochim. Biophys. Acta 649, 541–549.
30 Richards, D.E., Ellory, J.C. and Glynn, I.M. (1981) Biochim. Biophys. Acta 648, 284–286.
31 Jensen, J. and Ottolenghi, P. (1983) Biochim. Biophys. Acta 731, 282–289.
32 Ottolenghi, P. and Jensen, J. (1983) Biochim. Biophys. Acta 727, 89–100.
33 Ottolenghi, P. and Jensen, J. (1985) In The Sodium Pump (Glynn, I.M. and Ellory, J.C., eds.). pp. 219–227. The Company of Biologists Ltd., Cambridge.