Biochimica et Biophysica Acta, 727 (1983) 101–107
Elsevier Biomedical Press

BBA 71486

EFFECT OF MAGNESIUM IONS ON THE HIGH-AFFINITY BINDING OF EOSIN TO THE $(Na^+ + K^+)$-ATPase

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(Received May 11th, 1982)

Key words: Mg^{2+}; Eosin binding; $(Na^+ + K^+)$-ATPase; Conformational transition; Oligomycin; Nucleotide binding

(1) The fluorescence of eosin Y in the presence of $(Na^+ + K^+)$-ATPase is enhanced by Mg^{2+}. The enhancement by Mg^{2+} is larger than that obtained with Na^+ (Skou, J.C. and Esmann, M. (1981) Biochim. Biophys. Acta 647, 232–240). Mg^{2+} shifts the excitation maximum from 518 to 524 nm, the emission maximum from 538 to 542 nm. Also a shoulder appears at about 490 nm on the excitation curve, as was also observed with Na^+. (2) The Mg^{2+}-dependent enhancement of fluorescence can be reversed by K^+ as well as by ATP. In the presence of $Mg^{2+} + P_i$ (i.e. under conditions of phosphorylation), the fluorescence enhancement can be reversed by ouabain. With Mg^{2+} and a low concentation of K^+ (i.e. conditions for vanadate binding), the enhancement of fluorescence can be reversed by vanadate. (3) There is a low-affinity binding of eosin which increases with the Mg^{2+} concentration. This is observed as a slight increase in the fluorescence when the excitation wavelength is above 520 nm. The low-affinity binding is K^+-, ATP-, ouabain- and vanadate-insensitive. (4) Scatchard analysis of the binding experiments suggests that there are two high-affinity eosin-binding sites per ^{32}P-labelling site in the presence of 5 mM Mg^{2+} both of which are ouabain-, vanadate- and ATP-sensitive. With 5 mM Mg^{2+} + 0.25 P_i, the K_d values are 0.14 μM and 1.3 μM, respectively. With 5 mM Mg^{2+}, 150 mM Na^+, the K_d values are 0.45 μM and 3.2 μM, respectively. With 5 mM Mg^{2+}, the addition of K^+ gives a pronounced decrease in affinity but does not decrease the number of binding sites (which remains at two per ^{32}P-labelling site). With 5 mM Mg^{2+} + 150 mM K^+, the affinities of the two binding sites become identical, at a K_d of 17 μM. (5) The rate of conformational transitions was measured using the stopped-flow method. The rate of the transition from the Mg^{2+}-form to the K^+-form is high. Oligomycin has only a small (if any) effect on the rate. Addition of Na^+ in the presence of Mg^{2+} does not appreciably change the rate of conversion to the K^+-form, giving a rate constant of about 110 s^{-1}. However, the addition of oligomycin in the presence of $Mg^{2+} + Na^+$ had a profound effect: the rate of conversion to the K^+-form was decreased by a factor of 2000 to about 0.063 s^{-1}. This suggests that the conformation with Mg^{2+} alone is different from the conformation with Na^+ alone. (6) The effects of K^+, ouabain, vanadate and ATP on the high-affinity binding of eosin suggest that the two eosin molecules bound per ^{32}P-labelling site are bound to ATP sites.

Introduction

The $(Na^+ + K^+)$-ATPase has one high-affinity ATP binding site per ^{32}P-labelling site in the presence of Na^+, whereas the affinity is decreased by K^+ [1,2]. However, when the system hydrolyses ATP (i.e. addition of Mg^{2+} together with Na^+, K^+ and ATP), there seem to be two effects of ATP. One which increases the rate of release of K^+ from the cytoplasmic side of the system [3,4]. This effect is observed as an increase in the Na^+

Abbreviations: P_i, inorganic phosphate; eosin, eosin Y (C.I. 45380); Ado*PP*[NH]*P*, 5′-adenylyl imidodiphosphate.

affinity relative to K^+ affinity for Na^+ activation of ATP hydrolysis [5]. This is an actual effect of ATP (i.e. not of MgATP) [5] and the apparent affinity is in the micromolar range [6,7]. The other effect is related to ATP hydrolysis and with an affinity of 0.1–0.6 mM (see, for example, Ref. 8). This effect may be related to the translocation of Na^+ from the inside of the membrane in exchange for K^+ from the outside.

The problem is whether this means two different ATP sites or a sequential reaction with two different ATP molecules on the same site, but with different affinities.

Eosin Y can be used as a probe to study ATP binding [6]. One molecule of eosin binds non-covalently per ^{32}P-labelling site to what seems to be the ATP site on the $(Na^+ + K^+)$-ATPase, and with an affinity which is comparable to the affinity for ATP (K_d 0.45 μM for eosin [6] and 0.12–0.2 μM for ATP [1,2]). K^+ eliminates the high-affinity binding or decreases the affinity to an undetectable level.

Under hydrolysing conditions (i.e., Mg^{2+} together with Na^+, K^+ and ATP), the addition of eosin has two effects [6]: It competes for ATP during hydrolysis and, on the molecules on which eosin has not displaced ATP from the catalytic site, eosin (like ATP) increases the Na^+ affinity relative to K^+ affinity for Na^+ activation of ATP hydrolysis. This means that eosin must be able to bind to both the catalytic site for ATP and to the site where ATP exerts its action on the Na^+ affinity relative to K^+ affinity.

Eosin thus shows an 'ATP effect' on the Na^+ affinity relative to K^+ affinity, it competes for ATP during hydrolysis, and it is not hydrolysed. We have therefore used eosin as a probe to elucidate whether one or two binding sites exist for ATP in the presence of Mg^{2+}.

Our results show that Mg^{2+} expose two high-affinity sites for eosin per ^{32}P-labelling site, and their sensitivity towards Na^+, K^+, ouabain, vanadate and ATP suggest that they are binding sites for ATP.

Methods

$(Na^+ + K^+)$-ATPase (EC 3.6.1.3) was prepared from the rectal glands of *Squalus acanthias* and the activity was tested as previously described [9,10]. The specific activity was 1276 ± 32 μmol P_i/mg protein per h and with 2.1 ± 0.1 ^{32}P-labelling sites/mg protein ($n = 4$).

The fluorescence probe, eosin Y (C.I. 45380), was obtained from Gurr (BDH Chemicals Ltd., Poole, U.K.). It was purified on a Sephadex G-25 column (2.5 × 30 cm) in 20 mM histidine (pH 7.0) at 4°C in the dark. The dye elutes very slowly from the column. The first fractions have an absorption maximum around 511 nm, whereas the major part of the eosin has the correct 517 nm absorption maximum of eosin Y. A minor fraction is irreversibly bound to the Sephadex. The 517 nm absorption maximum fraction is used in the present experiments. It should be noted, however, that essentially the same results are obtained without the purification step.

The fluorescence titrations and the binding of eosin to the enzyme were measured as previously described [6] and the rate of conversion between different enzyme forms was measured with a stopped-flow equipment as described earlier [7]. Data were collected with a Datalab DL 901 transient recorder interfaced to an HP 85 micro computer.

Results

Mg^{2+}-induced enhancement of eosin fluorescence

In the presence of enzyme, Mg^{2+} increases the intensity of fluorescence of eosin and shifts the excitation maximum from 518 to 524 nm and a shoulder appears around 490 nm on the excitation curve (Fig. 1A). The emission maximum is shifted from 538 to 542 nm (not shown). The effect of Mg^{2+} on the shifts is identical to the effect of Na^+ [6]. With optimum Mg^{2+} (3–5 mM, Fig. 2) the increase in fluorescence is higher than is seen with the optimum Na^+ concentration (20 mM, Fig. 1A). The $K_{0.5}$ for Mg^{2+} is 0.26 ± 0.03 mM ($n = 4$).

The Mg^{2+}-induced increase in fluorescence of eosin in the presence of enzyme is ATP- and K^+-sensitive (Fig. 1B and Fig. 2). In the presence of P_i, it is ouabain-sensitive and in the presence of a small amount of K^+, it is vanadate-sensitive (Fig. 3 A and B). However, there is a certain low fraction of the Mg^{2+}-induced increase in fluores-

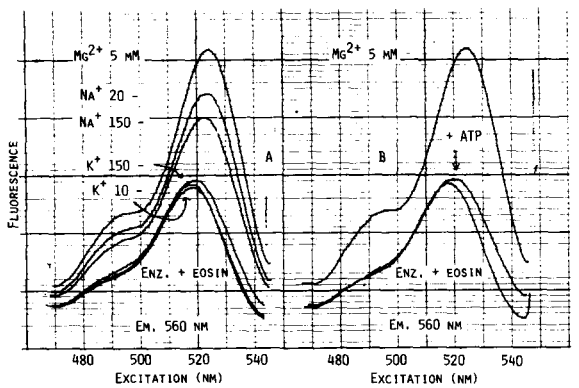

Fig. 1. The fluorescence of 0.1 μM eosin in the presence of 100 μg/ml ($Na^+ + K^+$)-ATPase in 30 mM histidine-HCl (pH 7.4) at 22°C. (A) Without and with 10 mM K^+, with 150 mM K^+, with 150 mM Na^+, with 20 mM Na^+ and with 5 mM Mg^{2+}, respectively. (B) Without and with 5 mM Mg^{2+} and with 5 mM Mg^{2+} + 0.2 mM ATP. Emission 560 nm, excitation wavelength varied. The slit was 10 nm both for excitation and emission and the time constant was 0.3 s.

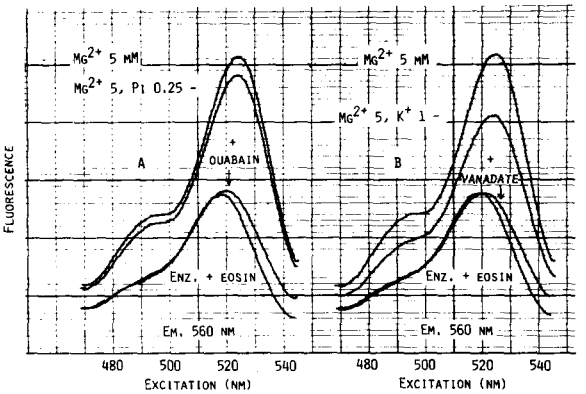

Fig. 3. The fluorescence of 0.1 μM eosin in the presence of 100 μg/ml ($Na^+ + K^+$)-ATPase in 30 mM histidine (pH 7.4) 22°C. (A) Without and with 5 mM Mg^{2+}, with 5 mM Mg^{2+} + 0.25 mM P_i, and with 5 mM Mg^{2+} + 0.25 mM P_i + 1 mM ouabain, respectively. (B) Without and with 5 mM Mg^{2+}, with 5 mM Mg^{2+} + 1 mM K^+ and with 5 mM Mg^{2+} + 1 mM $K^+ + 10^{-2}$ mM vanadate, respectively. The fluorescence was measured as in Fig. 1. The slit was 10 nm both for excitation and emission and the time constant was 0.3 s.

cence which is insensitive to all these agents and which is seen when the excitation wavelengths are higher than 520 nm (see Figs. 1B, 3 A and B). K^+ at a higher concentration (150 mM), increases the fluorescence to the level of the ATP-, ouabain- and vanadate-insensitive fluorescence seen in the presence of 5 mM Mg^{2+} (Fig. 1A, cf. Figs. 1B and 3 A and B). This fluorescence can also be seen as an ATP-insensitive, Na^+-induced fluorescence increase at high Na^+ concentrations (not shown). It is due to a low-affinity binding of eosin which increases with the concentration of K^+, of Na^+ and also of Mg^{2+} (see below).

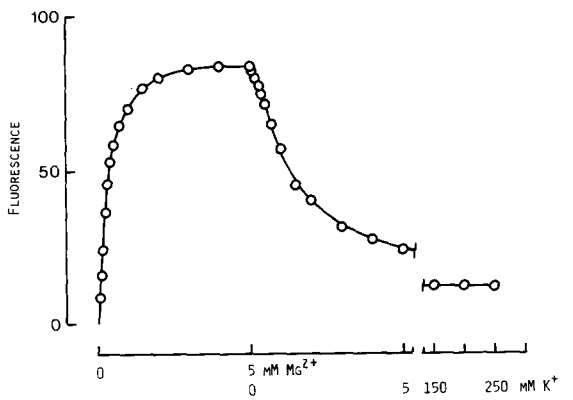

Fig. 2. The fluorescence of 0.1 μM eosin in the presence of 100 μg/ml ($Na^+ + K^+$)-ATPase as a function of the Mg^{2+} concentration and, with 5 mM Mg^{2+}, as a function of the K^+ concentration. Excitation at 530 nm, emission at 560 nm. The slit was 10 nm both for excitation and emission and the time constant was 0.3 s.

K^+ reverses the increase in fluorescence seen with Mg^{2+}. The titration curve is S-shaped and $K_{0.5}$ for K^+ is 1.4 ± 0.1 mM ($n = 3$) with 5 mM Mg^{2+} (Fig. 2). $K_{0.5}$ for K^+ increases with the Mg^{2+} concentration.

With 5 mM Mg^{2+}, the addition of 0.25 mM P_i (in order to support ouabain binding) gives a slight decrease in the fluorescence (Fig. 3A) whereas 1 mM K^+ added to increase the vanadate sensitivity (Fig. 3B) gives a more pronounced decrease.

Eosin binding

As mentioned above and shown in previous experiments [6] there is a low-affinity eosin binding to the ($Na^+ + K^+$)-ATPase in the presence of K^+ which increases with the K^+ concentration and with heat denaturation of the enzyme [6]. In the presence of Mg^{2+}, there is also a low-affinity binding of eosin to the enzyme which is ATP-, ouabain- and vanadate-insensitive. It increases with the Mg^{2+} concentration (not shown) and at a given Mg^{2+} concentration it decreases with an increase in the P_i concentration (Fig. 4).

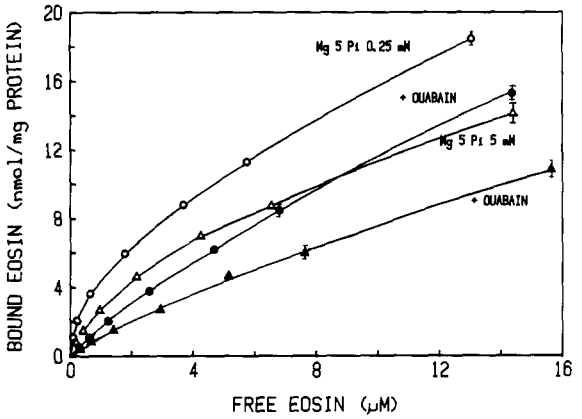

Fig. 4. Eosin bound (nmol/mg protein) to the $(Na^+ + K^+)$-ATPase as a function of the free eosin concentration in the presence of 5 mM Mg^{2+} + 0.25 mM P_i and of 5 mM Mg^{2+} + 5 mM P_i without and with 1 mM ouabain, respectively. The values given are the means of three determinations.

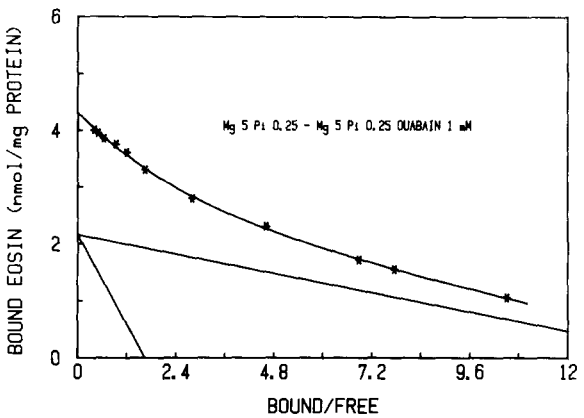

Fig. 5. A Scatchard plot of the ouabain-sensitive amount of eosin bound in the presence of 5 mM Mg^{2+} + 0.25 mM P_i. The values shown are obtained from the difference between the respective curves in Fig. 4.

Above this, there is in the presence of Mg^{2+} a high-affinity binding of eosin which is K^+-, ATP-, ouabain- and vanadate-sensitive. This gives the large increase in the fluorescence and the shift in excitation and emission maxima.

The nature of the high affinity binding sites has been investigated by Scatchard analysis. The experimental protocol was in each case as follows: the equilibrium binding at increasing eosin concentrations was determined for each set of ligands, and the high-affinity binding was found after subtraction of an appropriate control (see below).

Ouabain-sensitive eosin binding

In the presence of 5 mM Mg^{2+} + 0.25 mM P_i, the ouabain-sensitive eosin binding gives an upward curved line in the Scatchard plot (Fig. 5). The experimental points suggest an intercept at 4–5 nmol eosin bound per mg protein. With 2.1 nmol ^{32}P-labelling sites per mg protein, this means about two eosin-binding sites per ^{32}P-labelling site. Assuming that there are two equal populations of non-interacting sites, a computer-fitting of this model gives the two straight lines in Fig. 5, the sum of which is the curved line through the experimental points. The curved line intercepts at 4.2 nmol eosin bound per mg protein, i.e. two eosin molecules bound per ^{32}P-labelling site. The affinities are 0.14 and 1.3 μM, respectively. With a higher P_i concentration, the number of binding sites is the same but the affinity decreases, with 5 mM P_i it is 1.7 μM for both sites (not shown). From the point of view of binding sites for eosin, the conformation in the presence of Mg^{2+} (two sites per ^{32}P-site) is thus different from the conformation in the presence of Na^+ (1 site per ^{32}P-site, Ref. 6).

Vanadate-sensitive eosin binding

K^+ decreases the affinity of the high-affinity

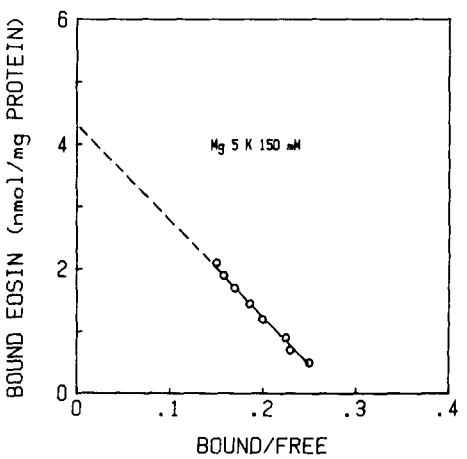

Fig. 6. A Scatchard plot of the vanadate-sensitive amount of eosin bound to the $(Na^+ + K^+)$-ATPase in the presence of 5 mM Mg^{2+} + 150 mM K^+. The values are obtained from the difference between the respective curves, based on the mean of three determinations.

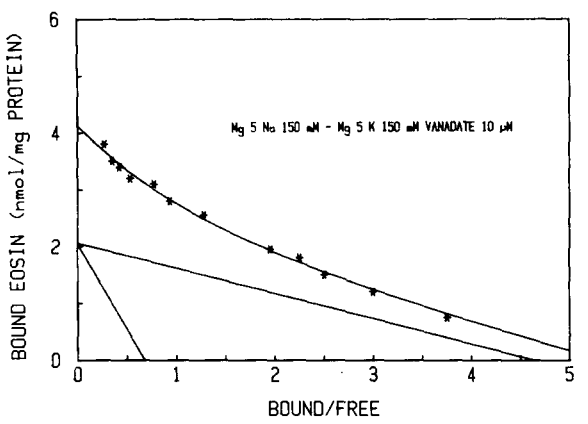

Fig. 7. A Scatchard plot of the difference in amount of eosin bound to the $(Na^+ + K^+)$-ATPase in the presence of 5 mM Mg^{2+} + 150 mM Na^+ and 5 mM Mg^{2+} + 150 mM K^+ + 10^{-2} mM vanadate. The values are obtained from the difference between the respective curves, based on the mean of three determinations.

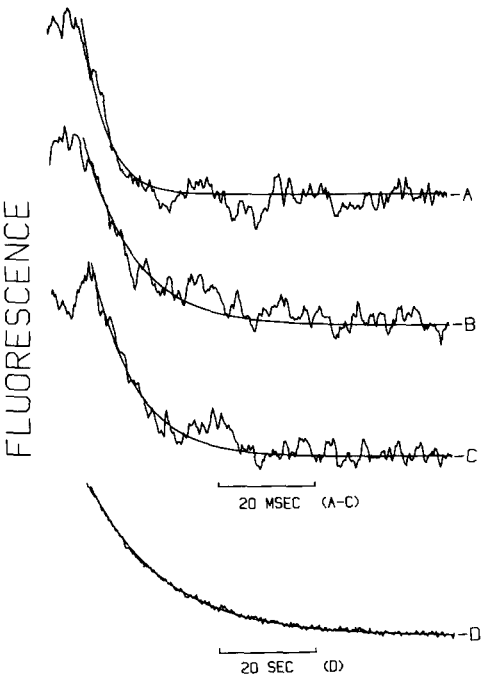

Fig. 8. Stopped-flow measurements of the conformational changes. The conditions were: (A) Enzyme + 4 mM Mg^{2+} mixed with 150 mM K^+, the data are fitted by the monoexponential decay curve with a rate constant of 210 s^{-1}. (B) Enzyme + 4 mM Mg^{2+} + 20 μg/ml oligomycin mixed with 150 mM K^+, rate constant 95 s^{-1}. (C) Enzyme + 4 mM Mg^{2+} + 20 mM Na^+ mixed with 150 mM K^+, rate constant 110 s^{-1}. (D) Enzyme + 4 mM Mg^{2+} + 20 mM Na^+ + 20 μg/ml oligomycin mixed with 150 mM K^+, rate constant 0.063 s^{-1}. The instrumental time constant was 1.5 ms for tracings A–C and 15 ms for tracing D, the exitation was at 530 nm, the emission was at 560 nm, and both slits were 10 nm.

eosin binding in the presence of Mg^{2+}. With 5 mM Mg^{2+} and 150 mM K^+, the vanadate-sensitive eosin binding gives a straight line in the Scatchard plot with an affinity of 17 μM (Fig. 6). The straight line extrapolates (if it is permissible) to 4.2 nmol sites per mg protein, i.e. two eosin-binding sites per ^{32}-labelling site.

$Na^+ + Mg^{2+}$ high-affinity binding

150 mM Na^+ in the presence of 5 mM Mg^{2+} renders the high-affinity eosin binding ouabain- and vanadate-insensitive. Using the vanadate-insensitive low-affinity eosin binding in the presence of 5 mM Mg^{2+} + 150 mM K^+ as reference, the high-affinity eosin binding in the presence of 5 mM Mg^{2+} + 150 mM Na^+ gives an upward curved line in a Scatchard plot (Fig. 7). Assuming two equal populations of sites, the curve extrapolates to two high-affinity binding sites per ^{32}P-labelling site with K_d values of 0.5 and 2 μM, respectively. 150 mM Na^+ + 5 mM Mg^{2+} thus give a slightly lower affinity on both sites than was observed in the presence of 5 mM Mg^{2+} + 0.25 mM P_i.

Rate of conversion from Mg^{2+}-form to K^+-form of the enzyme

The rate by which the Mg^{2+}-form is transformed into the K^+-form is very high at both 22°C and 4°C. When enzyme in the presence of 4 mM Mg^{2+} at 4°C (pH 7.4) is mixed with 150 mM K^+ the half-time, $t_{1/2}$, for the transformation from the Mg^{2+}-form to the K^+-form is about 3 ms (Fig. 8A). This value must be taken as an upper limit, since the instrumental set up does not allow measurement of rates much faster than this. Oligomycin, which decreases the rate of the transformation from the Na^+-form to the K^+-form [7], has only a small effect on the rate of the transformation from the Mg^{2+}-form to the K^+-form; $t_{1/2}$ increases to about 7 ms (Fig. 8B).

Addition of 20 mM Na^+ in the presence of 4 mM Mg^{2+} also has a small effect on the rate of the transformation to the K^+-form; $t_{1/2}$ is about 6 ms (Fig. 8C). However, in the presence of Mg^{2+}

and Na^+, the rate of the transformation becomes very sensitive to oligomycin. Addition of 20 μg oligomycin/ml increases $t_{1/2}$ for the transformation at 4°C (pH 7.4) from about 6 ms to 10–11 s (Fig. 8D) (note the different time scales).

The enzyme in the presence of Mg^{2+} and of $Mg^{2+} + Na^+$ thus differs from enzyme in the presence of Na^+ alone by having two sites for eosin per ^{32}P-labelling site instead of only one. In the presence of $Mg^{2+} + Na^+$, the enzyme differs from enzyme in the presence of Mg^{2+} by the oligomycin sensitivity of the conversion to the K^+-form of the enzyme. This indicates that E · Mg, E · Mg · Na and E · Na are different conformations of the enzyme.

Discussion

As the low-affinity binding of eosin to the enzyme increases with the concentration of K^+, it was suggested that this was due to the increase in ionic strength [6]. However, the present experiments do not support this view. With 5 mM Mg^{2+} + 0.25 mM P_i, which give a lower ionic strength effect than 150 mM K^+, there is a ouabain-insensitive low-affinity binding which is comparable to the one obtained with 150 mM K^+ (cf. Fig. 4A and Fig. 8 in Ref. 6). Furthermore an increase in the concentration of P_i from 0.25 to 5 mM, which gives an increase in ionic strength, decreases the ouabain-insensitive, low affinity binding of eosin to the enzyme. The low-affinity binding is thus not dependent only on the ionic strength. It is not possible to investigate the low-affinity binding in detail using the present methods. In the concentration range used, the low-affinity binding seems to be ouabain-, vanadate- and ATP-insensitive and may represent a non-specific background binding.

Mg^{2+} exposes more high-affinity binding sites than the one per ^{32}P-labelling site seen in the presence of 150 mM Na^+. In the Scatchard plot, the experimental observations can be fitted to a curve which is a sum of two high-affinity binding sites per ^{32}P-labelling site. With 5 mM Mg^{2+} + 0.25 mM P_i, the affinity for eosin of one site is higher (K_d 0.14 μM) than the affinity for eosin in the presence of 150 mM Na^+ without Mg^{2+} (K_d 0.45 μM [6]), while the other site has a lower affinity (K_d 1.3 μM). As an increase in the P_i concentration from 0.25 to 5 mM gives a decrease in affinity, the affinity of the two sites may be even higher in the presence of Mg^{2+} without 0.25 mM P_i. Due to ATP hydrolysis during the binding experiments and also due to the effect on the low-affinity binding of eosin of the complexing of Mg^{2+} with ATP, the ATP-insensitive binding cannot be used as a control for binding in the presence of Mg^{2+}.

The K^+-effect on the fluorescence in the presence of Mg^{2+} is due to an effect of K^+ on the affinity of eosin and not to an effect on the number of binding sites. There are also two vanadate-sensitive binding sites for eosin in the presence of 150 mM K^+.

With 5 mM Mg^{2+}, the addition of 150 mM Na^+ does not expose an additional high-affinity binding site for eosin, suggesting that one of the two sites seen with Mg^{2+} is the site also seen with Na^+ alone. However, the effect of Na^+ on the oligomycin sensitivity of the transformation from the Mg^{2+}-form to the K^+-form shows that binding of Na^+ to the enzyme induces a conformation of the system which is different from the conformation in the presence of Mg^{2+} without Na^+. The 'Na^+ effect' in the presence of Mg^{2+} is not seen as a change in the number of high-affinity eosin-binding sites but as a slight decrease in affinity for eosin.

In the previous paper, it was argued that the high-affinity binding site seen in the presence of 150 mM Na^+ was an ATP-binding site. What is the nature of the other site which is exposed in the presence of Mg^{2+} and is it a ouabain-binding site?

The high-affinity eosin binding is ATP-sensitive not only in the presence of Mg^{2+} but also in the presence of $Mg^{2+} + Na^+$, which are conditions under which ouabain is bound [11]. Vanadate (which supports ouabain binding [12]) eliminates the high-affinity eosin binding. Both arguments suggest that it is not a ouabain-binding site. K^+ decreases the affinity for eosin just as it decreases the affinity for ATP [1,2]; furthermore, ATP eliminates the eosin binding. This suggests that both high-affinity binding sites for eosin are at ATP binding sites.

The presence of two ATP binding sites does not, however, agree with the measurements of ATP binding which show one high-affinity ATP-

binding site per ^{32}P-labelling site [1,2]. This is done in the absence of Mg^{2+} and, as seen from the present experiments, Mg^{2+} is necessary in order to fully reveal both binding sites for eosin. However, preliminary binding experiments with a filtration technique, by which the enzyme can be separated from the supernatant before appreciable hydrolysis has occurred, have never shown more than one ATP binding site in the presence of Mg^{2+}.

There seem to be at least two possible explanations. One is that a second ATP-binding site has too low an affinity for ATP (but not for eosin) to be measured using these methods. This is supported by equilibrium binding of the ATP analog, Ado*PP*[NH]*P*, where a second site with low affinity has been reported in the presence of Mg^{2+} [13]. A number of experiments where the effect of ATP on the inactivation of the enzyme has been investigated also suggests that more than one ATP site exists on the enzyme (for a review, see Ref. 14). The other explanation is that bound ATP on the first site eliminates ATP binding to the second site. This is, however, not the case for eosin. For example, the two binding sites could have one part in common; with one bound ATP molecule there is not room for another ATP to bind. Eosin may only occupy that part of the two sites which is not in common. This would mean that the reactions with the two ATP molecules in the turnover situation are consecutive.

Acknowledgements

The authors wish to thank Ms. Hanne Ouertani for excellent technical assistance and 'Ingeborg and Leo Dannin's Foundation for Scientific Research' and 'The Danish Medical Research Council' for financial support.

References

1 Nørby, J.G. and Jensen, J. (1971) Biochim. Biophys. Acta 233, 104–116
2 Hegyvary, C. and Post, R.L. (1971) J. Biol. Chem. 246, 5234–5240
3 Karlish, S.J.D., Yates, D.W. and Glynn, I.M. (1978) Biochim. Biophys. Acta 525, 252–264
4 Karlish, S.J.D. and Yates, D.W. (1978) Biochim. Biophys. Acta 527, 115–130
5 Skou, J.C. (1974) Biochim. Biophys. Acta 339, 246–257
6 Skou, J.C. and Esmann, M. (1981) Biochim. Biophys. Acta 647, 232–240
7 Skou, J.C. (1982) Biochim. Biophys. Acta 688, 369–380
8 Robinson, J.D. (1967) Biochemistry 6, 3250–3258
9 Skou, J.C. and Esmann, M. (1979) Biochim. Biophys. Acta 567, 436–444
10 Skou, J.C. (1979) Biochim. Biophys. Acta 567, 421–435
11 Schwartz, A., Matsui, H. and Laughter, A.H. (1968) Science 160, 323–325
12 Hansen, O. (1979) Biochim. Biophys. Acta 568, 265–269
13 Schuurmans Stekhoven, F.M.A.H., Swarts, H.G.P., De Pont, J.J.H.H.M. and Bonting, S.L. (1981) Biochim. Biophys. Acta 649, 533–540
14 Nørby, J. (1983) Curr. Top. Membranes Transport, in the press