Biochimica et Biophysica Acta, 433 (1976) 383–392

BBA 77273

NON-UNIFORM POPULATIONS OF g-STROPHANTHIN BINDING SITES OF $(Na^+ + K^+)$-ACTIVATED ATPase

APPARENT CONVERSION TO UNIFORMITY BY K^+

OTTO HANSEN

Institute of Physiology, University of Aarhus, DK-8000 Aarhus C (Denmark)

(Received July 7th, 1975)
(Revised manuscript received November 6th, 1975)

SUMMARY

(1) [^{3}H]g-strophanthin (ouabain) binding to $(Na^+ + K^+)$- activated ATPase from ox brain was studied as a function of the P_i concentration with the $(Mg^{2+} + P_i)$-facilitated pathway. Scatchard-type plots of bound versus bound/free g-strophanthin at equilibrium of binding resulted in curved lines. The non-linearity was most easily seen at low P_i concentration or after addition of the nucleotide analogue β,γ-methyleneadenosine triphosphate (ADPCP) or suramin at high P_i concentration.

(2) The curved lines obtained with $(Mg^{2+} + P_i)$-supported g-strophanthin binding are converted to straight lines at a certain, not very high K^+ concentration. This effect seems specific for K^+ and is apparently not due to a delay of the time of equilibrium.

(3) The experiments are explained by assuming the existence of two (or more) populations of enzymes with different affinities for substrates and ligands affecting g-strophanthin binding. The sites with apparently high affinity for g-strophanthin may be less dependent on P_i for g-strophanthin binding or they may have a higher affinity for P_i. On the other hand, they are more affected by K^+ such that all enzyme sites exhibit homogeneity with respect to g-strophanthin affinity after addition of K^+.

INTRODUCTION

g-Strophanthin (ouabain) binding to $(Na^+ + K^+)$- activated ATPase has been described as a second order reaction according to the equation $E + G \rightleftarrows EG$ (refs. 1–8), in which E denotes non-occupied g-strophanthin binding sites ($E = EG_{max} - EG$), G free g-strophanthin, and EG bound g-strophanthin. Enzyme-strophanthin interaction seems to follow this mass law reaction whether the binding is supported by $(Mg^{2+} + Na^+ + ATP)$ or by $(Mg^{2+} + P_i)$ [6, 7]. Although the binding studies have

Abbreviation: ADPCP, β,γ-methyleneadenosine triphosphate.

been performed on intact cells or on more or less crude membrane preparations from different tissues, the simple second order reaction should imply that binding always takes place to uniform populations of enzyme sites, i.e. homogeneity independent of source of material and preparation procedure. In the following, is described some conditions revealing that in microsomal enzyme preparation from ox brain, at least two populations of g-strophanthin binding sites with different characteristics towards substrate and K^+ exist. Part of this paper has been presented in a preliminary form elsewhere [9].

MATERIALS AND METHODS

Enzyme preparations of $(Na^+ + K^+)$-ATPase from ox brain were prepared as described by Skou and Hilberg [10] or according to Klodos, Ottolenghi and Boldyrev [11]. Both methods involve treatment of the homogenate with sodium deoxycholate (0.1 and 0.25 %, respectively) and differential centrifugation in sucrose-histidin. The latter method, which has a much higher yield, is combined with a step of NaSCN treatment and subsequent washes to get rid of ATPases which are not dependent on Na^+ and K^+.

Uniformly labelled g-strophanthin [^{3}H]-ouabain was obtained from New England Nuclear Corp. The purity of the label was determined by the extraction procedure by means of $(Na^+ + K^+)$-activated ATPase and taken into account in the calculations as described elsewhere [6]. Alternatively, pure [^{3}H]g-strophanthin was prepared by chromatography on NaK-ATPase by taking advantage of the stability of the enzyme-strophanthin complex at 0 °C. After incubation of the label with 3 mM Mg^{2+}, 3 mM P_i, 10 mM Tris (pH 7.25) and enzyme at 37 °C, cooling to 0 °C and centrifugation and wash in the cold, [^{3}H]g-strophanthin was released by incubation in 5 mM Tris (pH 7.25) at 55 °C and the enzyme subsequently sedimented by centrifugation. [^{3}H]g-strophanthin was diluted to the desired specific activities (25–200 Ci/mol) with unlabelled g-strophanthin (Merck).

[^{3}H]g-strophanthin binding was achieved by incubation at 37 °C of enzyme (0.1–0.2 mg protein/ml, g-strophanthin sensitive activity 170–240 μmol P_i/mg protein per h) with 3 mM Mg^{2+}, 0.2–3 mM P_i (buffered with propandiol), and 40–48 mM Tris/HCl (pH 7.25, 37 °C) to keep the ionic strength nearly constant. Where indicated K^+, suramin or β,γ-methyleneadenosine triphosphate (ADPCP) was added. The g-strophanthin binding level was determined at equilibrium of binding after 2–4 h of incubation depending on the conditions for binding, especially the presence of K^+. Bound and non-bound g-strophanthin was separated by filtration through Sartorius membrane filters (pore size 0.6 μm) with suction. Aliquots of the filtrates were taken to determine the non-bound ^{3}H radioactivity using a Triton X-114/xylene scintillation mixture and a Packard liquid spectrometer. The total ^{3}H radioactivity of the incubation medium was determined under similar conditions. The difference between ^{3}H radioactivity of the incubation medium and that of the filtrate was taken as bound [^{3}H]g-strophanthin. The results were corrected for quenching by external standardization.

Suramin® (Germanin®) was obtained from Bayer Leverkusen, Germany, and ADPCP was obtained from Miles Laboratories, Inc., Kanakee.

RESULTS

In previous reports [6, 12] it was concluded that the equilibrium data for g-strophanthin binding obtained with the (Mg^{2+}+P_i)-facilitated and (Na^+, K^+, Tris or Ca^{2+})-inhibited pathway for all ionic conditions seemed to be adequately described by the overall reaction $E+G \rightleftarrows EG$. Straight lines were obtained in Scatchard-type plots of bound g-strophanthin (EG) versus bound/free g-strophanthin (EG/G) at equilibrium for all ionic combinations and concentrations of the incubation medium. The straight lines were consistent with the equation deduced from the above-mentioned at equilibrium

$$EG = -\frac{k_{-1}}{k_1} \cdot \frac{EG}{G} + EG_{max}$$

k_1 and k_{-1} denoting the forward and backward velocity constants, respectively, for the overall reaction and k_{-1}/k_1 being equal to the apparent dissociation constant for the binding process. The apparent dissociation constant, i.e. the slope of the straight lines, was shown to be dependent on the ionic conditions.

According to the straight lines of the Scatchard-type plots and the identical ordinate intercept, equal to the total number of sites, EG_{max}, only one uniform population of g-strophanthin binding sites should exist. Reservation was only taken for the situation at low P_i concentrations. The binding values obtained at low P_i concentration were not well correlated to straight lines and the ordinate intercept was definitely decreasing with decreasing P_i concentrations. The explanation offered at that time was that the decreased ordinate intercept was paralleled by a decrease in (Na^++K^+)-activated ATPase activity when Mg^{2+} was present in high concentration relative to the P_i concentration. However, recent studies have shown that the g-strophanthin binding capacity of the enzyme is not always well correlated to the ATPase activity [13–16]. With an enzyme preparation similar to those used in the present study, Nørby and Jensen have shown in ATP- and ADP-binding experiments that homogeneity of the binding sites is less likely [17]. For these reasons, reexamination of g-strophanthin binding at varying P_i concentrations was decided.

Fig. 1 shows equilibrium binding data at varying P_i concentrations that are definitely better fitted to curved lines than to straight lines when g-strophanthin binding is studied in a wide range of g-strophanthin concentrations. The curvity is more pronounced at lower P_i concentrations. The observation has been confirmed with three different enzyme batches from ox brain prepared according to different schemes [10, 11].

P_i is probably bound to the substrate site, and competition of P_i and substrate analogues for binding to the substrate sites might be revealed by a similar effect on g-strophanthin binding of decreasing P_i concentration and addition of non-hydrolyzed substrate analogues at high concentration of P_i [18]. Fig. 2 shows that the addition of ADPCP at high P_i concentration decreases g-strophanthin binding and accentuates the curvity in the same way as lower P_i concentration.

The drug suramin which contains two naphthalene trisulphonate groups per molecule is a potent inhibitor of the (Na^++K^+)-activated ATPase among other hydrolytic enzymes. Fortes, Ellory and Keynes [19] have shown that suramin inhibits red cell membrane ATPase when present at the inside membrane surface, and

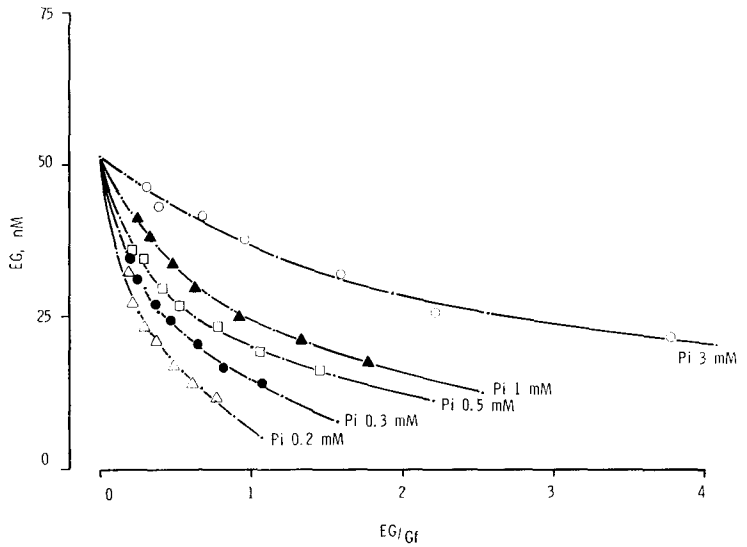

Fig. 1. Effect of the P_i concentration on ($Mg^{2+}+P_i$)-supported g-strophanthin binding to Na^+, K^+-ATPase. Equilibrium binding data of bound g-strophanthin (EG) in nM obtained after 2 h incubation at 37 °C are plotted against bound g-strophanthin/free g-strophanthin (EG/G_f). Incubation medium 3 mM Mg^{2+}, 0.2–3 mM P_i, 48–40 mM Tris (pH 7.25, 37 °C), $2.5 \cdot 10^{-5}$–$2 \cdot 10^{-4}$ mM [^{3}H]g-strophanthin, enzyme preparation 0.097 mg protein/ml, g-strophanthin sensitive activity 239 μmol P_i/mg protein per h. The curves were drawn through points (· —— ·) calculated from constants determined in the curve fitting procedure (see text).

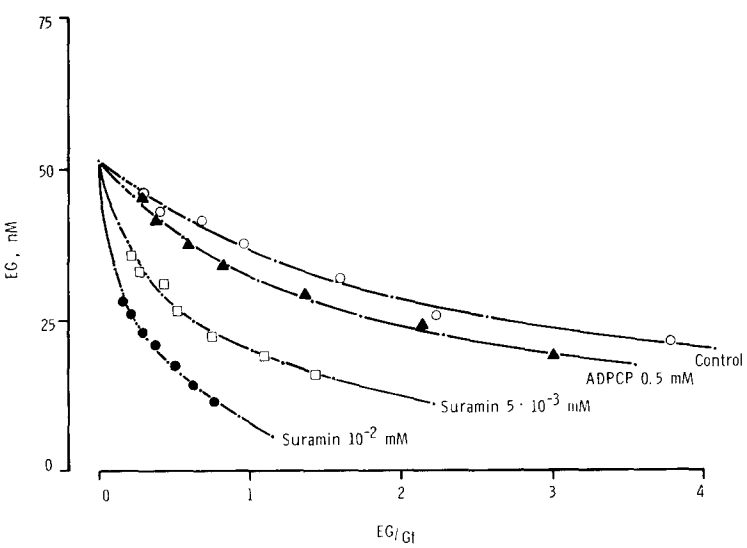

Fig. 2. Effect of ADPCP or suramin on g-strophanthin binding to Na^+, K^+-ATPase. Equilibrium binding data of bound g-strophanthin (EG) in nM obtained after 2 h incubation at 37 °C with 3 mM Mg^{2+}, 3 mM P_i, 40 mM Tris (pH 7.25, 37 °C), and $2.5 \cdot 10^{-5}$–$2 \cdot 10^{-4}$ mM [^{3}H]g-strophanthin and ADPCP or suramin as indicated are plotted against bound g/strophanthin-free g-strophanthin (EG/G_f). Enzyme preparation as in Fig. 1. The curves were drawn through points (· —— ·) calculated from constants determined in the curve fitting procedure (see text).

also g-strophanthin binding to red cell ghosts. They propose that the ATP-binding site is involved in the mechanism of inhibition, i.e. that suramin might be looked upon as a sort of ATP analogue. In that case suramin should behave as ADPCP or low P_i concentration towards g-strophanthin binding. Fig. 2 shows that the addition of suramin to the incubation medium at high P_i concentration results in curves identical to those obtained at low P_i concentration, which might mean that suramin competes with P_i for a common site. It is seen that suramin is effective at much lower concentration than ADPCP. The identical behaviour of lack of P_i or at least low P_i concentration and the addition of ADPCP or suramin at high P_i concentration favour the hypothesis that suramin may act as an ATP analogue and that suramin as well as ADPCP displace P_i.

A simple model compatible with non-linear Scatchard plots is one assuming the presence of two populations (component I and II) of independent binding sites with different affinity for the ligand (in the present case g-strophanthin) [20, 21]:

$$EG = \frac{[EG_t'] \cdot [G]}{[G]+K_{diss}'} + \frac{[EG_t''] \cdot [G]}{[G]+K_{diss}''}$$

where EG denotes the concentration of bound ligand, EG_t' and EG_t'' the concentration of total sites for component I and II, respectively, G the concentration of free ligand, and K_{diss}' and K_{diss}'' the apparent enzyme-ligand dissociation constant for component I and II, respectively.

In the present study this hypothesis was tested by means of an iterative curve-fitting procedure based on the method of least squares and carried out with a computer program* in the following way: It was assumed that EG_t' and EG_t'' were independent of the experimental conditions (for justification of this statement, see Discussion), whereas K_{diss}' and K_{diss}'' were allowed to vary. These assumptions allowed simultaneous consideration of all the data (represented by the symbols of Figs. 1 and 2) in the curve fitting procedure, which then resulted in the determination of one set of values of K_{diss}' and K_{diss}'' for each experimental condition as well as values of EG_t' and EG_t'' common to all the experiments.

The experimental data were compatible with two enzyme-strophanthin components of $EG_t' = 31.0$ nM and $EG_t'' = 20.6$ nM (at the enzyme concentration of the experiments). The apparent dissociation constants, K_{diss}' and K_{diss}'', were found to decrease with P_i concentration and increase with suramin concentration. When P_i varied from 0.2–3 mM K_{diss}' and K_{diss}'' decreased from 280–30 nM and from 16–1.5 nM, respectively.

The curves of Figs. 1 and 2 were drawn from calculated binding values (i.e. the dots) according to the model using the constants determined by the computer. It is seen that the experiments (the symbols) fit the calculated curves well and thus do not disprove the two component hypothesis of g-strophanthin sites.

Apart from lowering the P_i concentration, a decrease in the equilibrium binding level at a given g-strophanthin concentration can be achieved by lowering the Mg^{2+} concentration or by addition of Na^+, K^+, Tris or Ca^{2+} (ref. 6, 12), and curvity of the

---

* The program was developed for characterizing the two components of ATP binding in $(Na^+ + K^+)$-activated ATPase by Drs. Jørgen Jensen and Jens G. Nørby of this institute and kindly placed at my disposal.

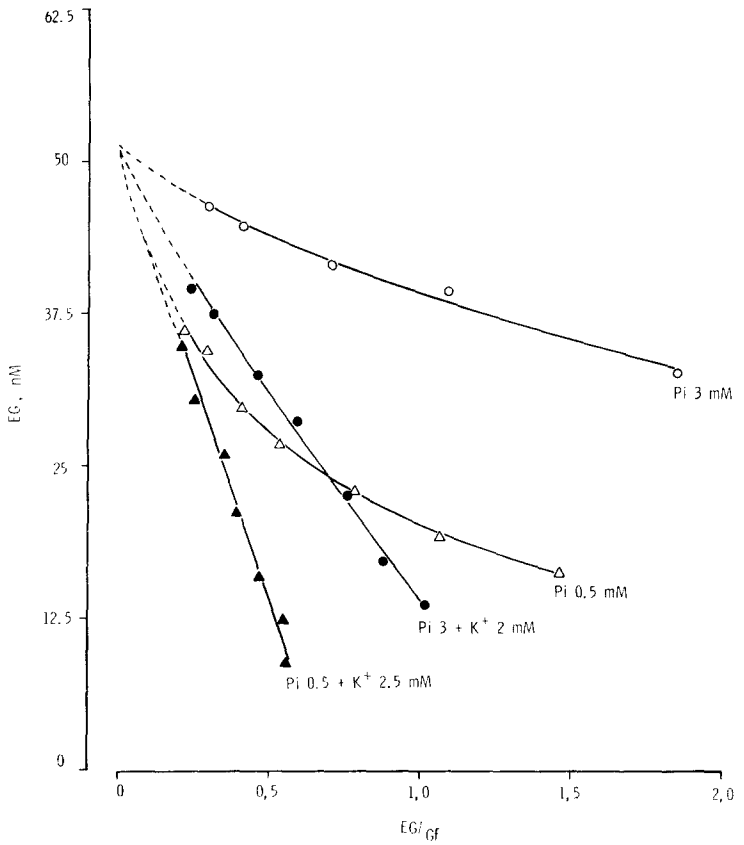

Fig. 3. Effect of K^+ on (Mg^{2+} + P_i)-supported g-strophanthin binding to Na^+, K^+-ATPase. Equilibrium binding data of bound g-strophanthin (EG) obtained after 2 h incubation at 37 °C without K^+ or 2–4 h with K^+ are plotted against bound g-strophanthin/free g-strophanthin (EG/G_f). Incubation medium 3 mM Mg^{2+}, 0.5 or 3 mM P_i, 46–40 mM Tris (pH 7.25, 37 °C), and $2.5 \cdot 10^{-5}$–$2 \cdot 10^{-4}$ mM [^{3}H]g-strophanthin. Enzyme preparation as in Fig. 1.

Scatchard plot might be expected with any of the changes mentioned. However, these changes, at a fixed high P_i concentration, do not bring about any curvity, apart perhaps from the weak curvity which may even be present before the changes. On the contrary, from Fig. 3 it is seen that the addition of 2–2.5 mM K^+ gives nearly straight lines whether added to an incubation medium of high or low P_i concentration and K^+ thus even seems to "straighten out" lines which from the outset were definitely curved. This effect seems to be specific for K^+. At lower K^+-concentrations intermediary forms of the lines are obtained, which are less curved than those without K^+. Addition of K^+ above a few millimolar concentration gives low g-strophanthin binding values and very steep Scatchard plots which are very difficult to handle, but we have no indication that the lines are qualitatively different from those obtained at 2–2.5 mM K^+.

K^+ inhibits and Mg^{2+} facilitates g-strophanthin binding so that they, according to the model, might be competitive [6]. That they are not really competitive is seen

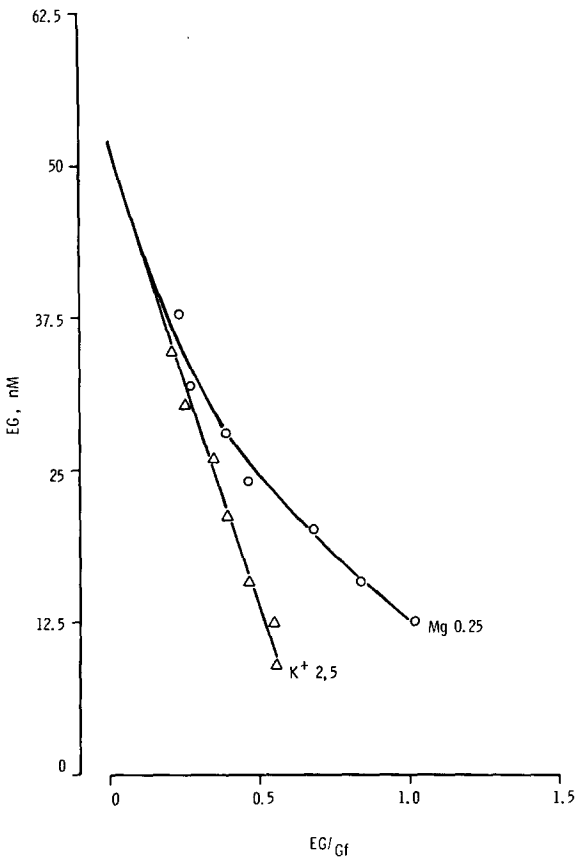

Fig. 4. Comparison of the effect of low Mg^{2+} concentration and K^+ on ($Mg^{2+}+P_i$)-supported g-strophanthin binding. Equilibrium binding data of bound g-strophanthin (EG) obtained after 4 h incubation at 37 °C are plotted against bound g-strophanthin/free g-strophanthin (EG/G_f). Incubation medium ○——○ 0.25 mM Mg^{2+}, 0.5 mM P_i, 53 mM Tris (pH 7.25, 37 °C), △——△ 3 mM Mg^{2+}, 0.5 mM P_i, 2.5 mM K^+, 40 mM Tris (pH 7.25, 37 °C), in each case $2.5 \cdot 10^{-5}$—$2 \cdot 10^{-4}$ mM [^{3}H]g-strophanthin. Enzyme preparation as in Fig. 1.

from the binding experiments with low P_i as well as low Mg^{2+} concentration in Fig. 4. A low Mg^{2+} concentration does not bring about straight lines in the Scatchard plot whereas the addition of K^+ does.

The rate of g-strophanthin binding is known to be delayed by K^+. That the equilibrium binding level is also lowered by K^+ is less accepted [6, 22, 23]. The nearly straight lines obtained with K^+ compared to the curved lines with low P_i concentration could be ascribed to a non-equilibrium situation and therefore a lower degree of binding than the final one, especially at the lower concentrations of g-strophanthin. This does not, however, seem to be the explanation of the straight lines with K^+ as seen from Fig. 5. Having reached an equilibrium level of binding without K^+ at low P_i concentration, the addition of K^+ always lowers the level of binding resulting in a big influence on the EG/G ratio and confirming the data of Fig. 3. Even the equilibrium binding obtained at low P_i as well as Mg^{2+} concentration (Fig. 4) is

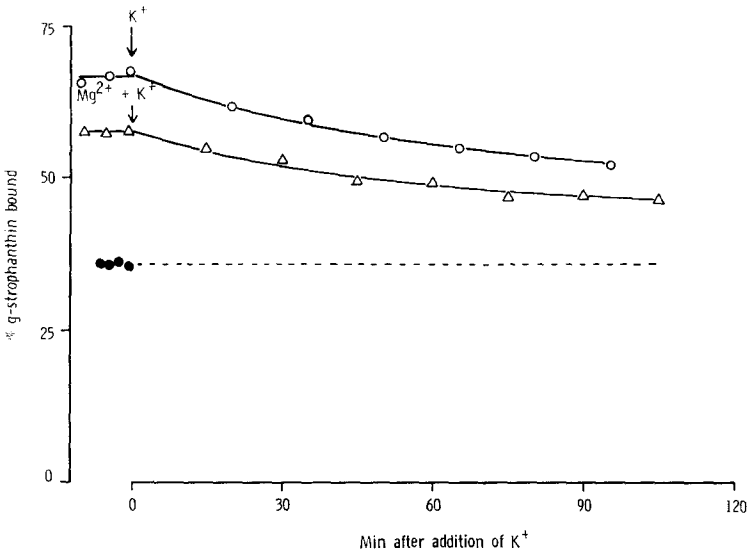

Fig. 5. Comparison of the effect of addition of K^+ or replenishing with Mg^{2+} on the equilibrium binding level of bound g-strophanthin. An equilibrium binding level was obtained after 2–4 h incubation at 37 °C with ●——● 3 mM Mg^{2+}, 0.5 mM P_i, 2.5 mM K^+, ○——○ 3 mM Mg^{2+}, 0.5 mM P_i or △——△ 0.25 mM Mg^{2+}, 0.5 mM P_i, in each case 40–46 mM Tris (pH 7.25, 37 °C), and $2.5 \cdot 10^{-5}$ mM [^{3}H]g-strophanthin. At the arrows, K^+ and Mg^{2+} was added, giving a final concentration of ○——○ 2 mM K^+ and △——△ 3 mM Mg^{2+} + 2.5 mM K^+.

lowered when K^+ and supplementary Mg^{2+} is added mimicking the conditions of the curves of Fig. 4.

DISCUSSION

This study supports other findings of non-homogeneity of $(Na^+ + K^+)$-activated ATPase preparations from ox brain. The curved lines in Scatchard-type plots of bound versus bound/free g-strophanthin is most easily explained by assuming the existence of non-uniform populations of g-strophanthin binding sites with varying apparent affinity to the drug in the $(Mg^{2+} + P_i)$-facilitated binding system. They are apparently converted to one uniform population of sites at a given K^+ concentration, judging from the straight lines obtained in this situation. Contrary to this, Nørby and Jensen [17] report linear plots of ATP binding data to NaK-ATPase in the absence of K^+, which are converted to non-linear plots in the presence of K^+. Their observations are explained by assuming the existence of two types of ATP-binding molecules differing in their sensitivity to K^+. The straight line obtained without K^+ might be due to identical ATP affinity of the two populations in this situation. Taniguchi and Iida [24] report two kinds of g-strophanthin binding sites under conditions with 14 mM K^+ where only one population of g-strophanthin binding sites was expected from the present study. The apparent g-strophanthin affinity is very low, and in our hands binding measurements are very unreliable under such circumstances. Erdmann and Schoner [25] have observed non-linearity of Scatchard-type plots when g-stro-

phanthin binding are measured with an incubation medium containing only Mg^{2+}. In the present study the non-linearity of g-strophanthin binding curves is generalized to all situations of ($Mg^{2+}+P_i$)-supported g-strophanthin binding without addition of K^+, although the phenomenon is most easily seen at low P_i concentration. This observation may most easily be explained by assuming that two or more populations of g-strophanthin sites exist, and this probably reveals a general property of non-homogeneity of the enzyme preparation, which is just not seen in other situations. Contrary to what several investigators [1–8] have claimed, at least ($Mg^{2+}+P_i$)-facilitated g-strophanthin binding seems only adequately described by the overall reaction scheme $E+G \rightleftarrows EG$ under special conditions, i.e. in the presence of a certain, not very high K^+ concentration and approximately described at high P_i concentration. Probably one of the reasons that it has been overlooked, is that impurities of [^{3}H]g-strophanthin (ouabain) were not taken into account. Just 5 % impurity heavily influences Scatchard plots, especially the values obtained at low concentrations of g-strophanthin. Assuming that two populations of g-strophanthin binding sites exist, the curved lines imply that one population cannot be converted to the other under conditions where non-linearity is seen, or at the most there would be a very slow rate of convertibility. If an equilibrium between a low affinity state (R_α) and a high affinity state (R_β) should exist and this equilibrium was influenced by ligands and substrates ($R_\alpha \rightleftarrows R_\beta$) [25], it can be shown that binding would only take place to the higher affinity state, which should finally be the only present and a straight line should be obtained in the Scatchard plot. So we can conclude that the most likely explanation for the curvity is that two (or more) populations with different affinities to P_i and thereby to g-strophanthin may exist. The results are compatible with the hypothesis that all the curves are composed of the same two populations, i.e. that the ratio between the concentration of sites of the two populations does not depend on the P_i concentration. The identical behaviour of lack of P_i, addition of ADPCP, which is definitely a nucleotide analogue [17], and the hypothetical analogue suramin, strongly suggests that P_i is directly involved in ($Mg^{2+}+P_i$)-supported g-strophanthin binding.

Although non-linear curves are obtained at low P_i concentration and nearly straight lines are obtained at high P_i concentration, only straight lines are seen at a certain K^+ concentration whether the P_i concentration is high or low. This means that at this K^+ concentration, all enzyme units behave identical towards g-strophanthin. Apparently only one population exists in this situation. This must mean that the population which is less dependent on P_i, since it binds g-strophanthin with high affinity, even at low P_i concentration (the tail in the Scatchard plots), is the population which is most affected by K^+, and in such a way that the two populations now behave identical towards g-strophanthin.

Whether the non-homogeneity of P_i and g-strophanthin binding sites is due to (1) different sources of origin, i.e different cells or organelles, (2) the preparation procedures or (3) an inborn property of the native enzyme, is an unsolved problem, but preliminary experiments suggest that not only ox brain enzyme exhibits diversity.

It is interesting that the presence of K^+ brings about linearity of otherwise curved Scatchard plots in the ($Mg^{2+}+P_i$)-supported g-strophanthin binding system, whereas in ATP binding experiments the presence of K^+ causes curvity of otherwise linear plots of ATP binding data [17]. The number of ATP and g-strophanthin binding

sites of $(Na^+ + K^+)$-activated ATPase usually differ very little [26]. ATP binding takes place to the inside of the membrane, whereas g-strophanthin binding takes place to the outside [27, 28]. The opposite effect of K^+ on ATP and g-strophanthin binding may reflect the same phenomenon just seen from two different sides, e.g. binding of K^+ from the outside in both cases. A mathematical resolution of the curved lines obtained in ATP and g-strophanthin binding experiments on the same enzyme preparation does not seem to give identical enzyme-ligand components in the two binding systems, however, we may be looking at different phenomena.

ACKNOWLEDGEMENTS

The skilled technical assistance of Mrs. Kirsten Pedersen is gratefully acknowledged. Thanks are due to T. Nørby for doing the calculations according to the computer program.

REFERENCES

1 Baker, P. F. and Willis, J. S. (1970) Nature, 226, 521–523
2 Hansen, O. (1971) Biochim. Biophys. Acta 233, 122–132
3 Barnett, R. E. (1970) Biochemistry 9, 4644–4648
4 Schönfeld, W., Schön, R., Menke, K.-H. and Repke, K. R. H. (1972) Acta Biol. Med. Germ. 28, 935–956
5 Erdmann, E. and Schoner, W. (1973) Biochim. Biophys. Acta 307, 386–398
6 Hansen, O. and Skou, J. C. (1973) Biochim. Biophys. Acta 311, 51–66
7 Whittam, R. and Chipperfield, A. R. (1973) Biochim. Biophys. Acta 307, 563–577
8 Clausen, T. and Hansen, O. (1974) Biochim. Biophys. Acta 345, 387–404
9 Hansen, O. (1974) Abstr. Commun. 9th Meet. Eur. Biochem. Soc. 1974, p. 238
10 Skou, J. C. and Hilberg, C. (1969) Biochim. Biophys. Acta 185, 198–219
11 Klodos, I., Ottolenghi, P. and Boldyrev, A. A. (1975) Anal. Biochem. 67, 397–403
12 Hansen, O. (1974) Ann. N. Y. Acad. Sci. 242, 635–645
13 Harris, W. E., Swanson, P. D. and Stahl, W. L. (1973) Biochim. Biophys. Acta 298, 680–689
14 Taniguchi, K. and Iida, S. (1973) Mol. Pharmacol. 9, 350–359
15 Erdmann, E. and Schoner, W. (1973) Biochim. Biophys. Acta 330, 316–324
16 Chipperfield, A. R. and Whittam, R. (1973) J. Physiol. 230, 467–476
17 Nørby, J. G. and Jensen, J. (1974) Ann. N. Y. Acad. Sci. 242, 158–167
18 Tobin, T., Akera, T., Lee, C. Y. and Brody, T. M. (1974) Biochim. Biophys. Acta 345, 102–117
19 Fortes, P. A. G., Ellory, J. C. and Lew, V. L. (1973) Biochim. Biophys. Acta 318, 262–272
20 Rosenthal, Hannah E. (1967) Anal. Biochem. 20, 525–532
21 Klotz, I. M. and Hunston, D. L. (1971) Biochemistry 10, 3065–3069
22 Akera, T. (1971) Biochim. Biophys. Acta 249, 53–62
23 Lindenmayer, G. E. and Schwartz, A. (1973) J. Biol. Chem. 248, 1291–1300
24 Taniguchi, K. and Iida, S. (1972) Biochim. Biophys. Acta 288, 98–102
25 Erdmann, E. and Schoner, W. (1973) Biochim. Biophys. Acta 330, 302–315
26 Hansen, O., Jensen, J. and Nørby, J. G. (1971) Nat. New Biol. 234, 122–124
27 Caldwell, P. C. and Keynes, R. D. (1959) J. Physiol. 148, 8P–9P
28 Perrone, J. R. and Blostein, R. (1973) Biochim. Biophys. Acta 291, 680–689