Biochimica et Biophysica Acta, 732 (1983) 332–335
Elsevier

BBA Report

BBA 70106

COOPERATIVE LIPID ACTIVATION OF ($Na^+ + K^+$)-ATPASE AS A CONSEQUENCE OF NON-COOPERATIVE LIPID-PROTEIN INTERACTIONS

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(Received January 24 th, 1983)

Key words. ($Na^+ + K^+$)-ATPase; Lipid activation; Lipid-protein interaction; Cooperativity

Lipid activation data for ($Na^+ + K^+$)-ATPase (Ottolenghi, P. (1979) Eur. J. Biochem. 99, 113–131) have been subjected to a regression and fitting analysis based on a recent kinetic model (Sandermann, H. (1982) Eur. J. Biochem, 127, 123–128). The observed kinetic cooperativity could be generated from strictly non-cooperative binding events involving the known number of 30 boundary lipid-binding sites per ATPase monomer. Apparent lipid dissociation equilibrium constants of between 0.3 and 5 μM were obtained, enzyme activity being associated only with the fully lipid-substituted enzyme and enzyme-lipid complexes with less than six unoccupied lipid-binding sites. The enzyme appeared to operate close to a maximum of cooperativity.

A novel kinetic mechanism for the cooperativity of lipid-dependent membrane functions has recently been derived [1]. The model generates kinetic cooperativity from multiple non-cooperative lipid-protein binding steps. It has, however, remained open as to whether this mechanism is realized by lipid-dependent membrane enzymes.

The simplest rate equation of the kinetic model has in a recent independent study [2] been shown to describe the lipid activation of the mitochondrial β-hydroxybutyrate dehydrogenase. The basic Eqn. 3 of Ref. 2 was identical to Eqn. 4 of Ref. 1. Only two independent lipid-binding sites were assumed for the dehydrogenase [2]. More generally, however, integral membrane proteins possess about 20–100 lipid-binding sites [3,4]. The bound lipid has in many cases been shown to be required for function [5], but the kinetics of lipid activation have usually not been thoroughly studied. In the case of ($Na^+ + K^+$)-ATPase, detailed kinetic data and an elaborate kinetic model based on cooperativity in lipid binding have been published by Ottolenghi [6]. A completely different evaluation of these kinetic data is now presented. The kinetic model of Ref. 1 is applied, assuming strict non-cooperativity in lipid binding. Studies with electron spin labeled lipids have in fact yielded no evidence for cooperativity in lipid binding to ($Na^+ + K^+$)-ATPase [3,4,7,8]. These studies have indicated that a number ($n = 60$) of independent boundary lipid-binding sites are present per ($\alpha_2\beta_2$)-dimer of the enzyme, the dimer or the ($\alpha\beta$)-monomer being the basic unit of ATPase activity [9,10]. It will be shown that the cooperative lipid activation of the ATPase can be generated from non-cooperative lipid binding to the known number of sites.

The present analysis was carried out with the lipid activation data of Figs. 2 and 11 in Ref. 6. The original experimental data were kindly made available by Dr. P. Ottolenghi, Dept. of Physiology, University of Aarhus, Aarhus, Denmark. The

Abbreviations: DOPC, dioleoylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine.

analysis was based on Eqn. 10 of Ref. 1. Regression analysis was done with a SIMULA program, and non-linear curve fitting was performed using the SIMPLEX algorithm [11].

The experimental approach in Ref. 6 was to recombine a delipidated $(Na^+ + K^+)$-ATPase preparation from dogfish rectal glands with increasing concentrations of DOPC or DOPE in the presence of a non-activating detergent. The reappearance of K^+ phosphatase and $(Na^+ + K^+)$-ATPase activities was followed. The values for maximal velocity, V, as well as the lipid concentrations required to reach half-maximal velocity ($[L_{50}]$) are listed in Table I. Also listed are the cooperativity indices, $[L_{90}]/[L_{10}]$, which were determined graphically as the ratio of lipid concentrations required to reach 90% and 10%, respectively, of V (see Ref. 1).

The experimental datum points of Ref. 6 are shown in Fig. 1. When these data were fitted to a Hill-type equation (Eqn. 5 of Ref. 1) regression lines with correlation coefficients of at least 0.93 were obtained. The Hill coefficients, n_H, calculated as slope values of the regression lines, are listed in Table I. In agreement with the analysis by Ottolenghi [6], it is seen that $(Na^+ + K^+)$-ATPase activity appeared with significantly higher apparent cooperativity than K^+ phosphatase. As a first application of the kinetic model in Ref. 1, values for the index a were derived from the $[L_{90}]/[L_{10}]$ values of Table I by inspection of Fig. 2A of Ref. 1. The index a refers to the number of unoccupied lipid-binding sites which is still compatible with enzyme activity. Active lipoprotein species thus are EL_n, EL_{n-1}... and EL_{n-a} [1]. The estimated values for the index a of the reconstituted phosphatase and ATPase activities are listed in Table I.

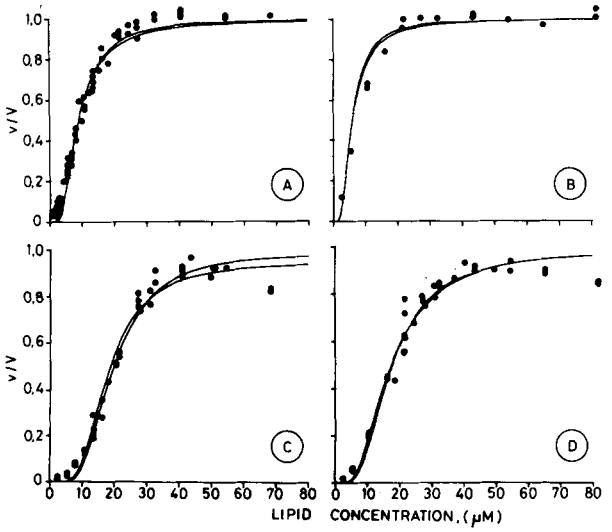

Fig. 1. Lipid activation of a purified delipidated $(Na^+ + K^+)$-ATPase preparation from dogfish rectal glands. Relative enzyme activity (v/V) is plotted against lipid concentration (μM). A, K^+ phosphatase regenerated with DOPC, B, K^+ phosphatase regenerated with DOPE, C, $(Na^+ + K^+)$-ATPase regenerated with DOPC, D, $(Na^+ + K^+)$-ATPase regenerated with DOPE. The experimental points shown are from [6]. The solid lines were calculated from Eqn. 10 of Ref. 1 using the optimal values for apparent K_1 and the index a of Table I. Values of $a = 2$ were used in C and D. The curves calculated for $n = 30$ as well as $n = 60$ are shown. Both curves largely superimpose. Where two lines are visible, the upper one was obtained for $n = 60$, except for the lower part of the curves in panel C.

Model calculations were carried out for membrane enzymes with 30 or 60 independent lipid-binding sites. It was first considered how the catalytic efficiency of lipid, expressed as the ratio, $[L_{50}]/K_1$, depends on the index a. K_1 is the microscopic lipid dissociation equilibrium constant [1]. The results are shown in Fig. 2A. There was a drastic decrease in $[L_{50}]/K_1$, equivalent to an increase in catalytic efficiency, in going from $a = 0$ to about $a = 5$. This may reflect the statistical difficulty in occupying the last unoccupied sites on

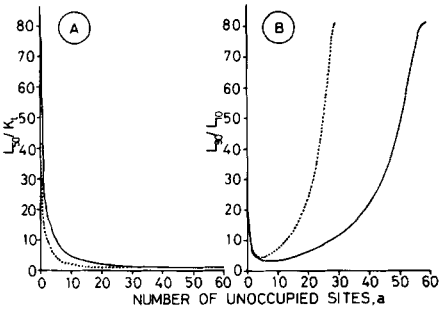

Fig. 2. Catalytic efficiency A and cooperativity B of membrane enzymes with $n = 30$ (· · · · · ·) and $n = 60$ (———) lipid-binding sites. The ratio, $[L_{50}]/K_1$, was used as a measure of catalytic efficiency, and cooperativity is expressed as the index, $[L_{90}]/[L_{10}]$. Both parameters are plotted against the index a. The latter indicates the maximum number of unoccupied lipid-binding sites which is still compatible with enzyme activity. The curves were calculated from Eqn. 10 of Ref. 1.

TABLE I

KINETIC CONSTANTS FOR THE RECONSTITUTED ENZYME ACTIVITIES

V is quoted in μmol/h per mg protein, taken from Ref. 6. $[L_{10}]$, $[L_{50}]$ and $[L_{90}]$ were determined graphically using the indicated values of V. The Hill coefficient, n_H, was determined from Eqn. 6 of Ref. 1. The correlation coefficient, r, is given in brackets. The approximate index, a was estimated by use of Fig. 2A in Ref. 1 using the indicated values of $[L_{90}]/[L_{10}]$ and $n = 60$. App. = apparent.

	Reconstituted with	V	$[L_{50}]$ (μM)	$[L_{90}]/[L_{10}]$	n_H (r)	Approx. index a	Calculated optimal values			
							$n = 60$		$n = 30$	
							App. K_1 (μM)	Index a	App. K_1 (μM)	Index a
$(Na + K^+)$-ATPase	PC	574	21	3.5	2.71 (0.98)	5	1.6	4.0	4.8	5.8
activity	PE	581	18	5.6	2.23 (0.95)	2	0.8	2.0	2.3	3.0
K^+ phosphatase	PC	260	10	6.7	1.90 (0.99)	2	0.31	1.3	0.6	1.4
activity	PE	190	8	8.0	1.56 (0.93)	1	0.27	1.5	0.6	1.6

a protein with multiple lipid-binding sites (cf. Ref. 1).

At higher values of a, there was a gradual approximation towards the value of $[L_{50}]/K_1 = 1$, which corresponds to hyperbolic activation [1]. When the cooperativity index, $[L_{90}]/[L_{10}]$, was calculated in dependence on the index a, there were well-defined minima of $[L_{90}]/L_{10}]$ which represented maxima of cooperativity (Fig. 2B). The value of $[L_{90}]/[L_{10}]$, 81, which is characteristic for hyperbolic activation [1], was reached only when the index a approximated n. The lowest cooperativity indices obtained were 4. 54 (for $n =$ 30 and $a = 5$) and 3.20 (for $n = 60$ and $a = 8$). These values correspond to Hill coefficients of 2.9 (for $n = 30$) and 3.8 (for $n = 60$). These limiting values were higher than the experimental Hill coefficients of Table I, so that the kinetic model of Ref. 1 could be used for analysis.

The values of V in Table I and fixed values of $n = 30$ or $n = 60$ were used to adjust the parameters of Eqn. 10 of Ref. 1 to the experimental points of Fig. 1. The optimal values for the apparent K_1 and for the index a are listed in Table 1. The optimal values for the index a were between 1.3 and 1.6 for the K^+ phosphatase and between 2.0 and 5.8 for the $(Na^+ + K^+)$-ATPase. These values were close to the preliminary values determined graphically (Table I, left part). The curves corresponding to the optimal values of apparent K_1 and the nearest integer of the index a are shown in Fig. 1. A close fit to the experimental points was generated for $n = 30$ as well as $n = 60$.

Inspection of Fig. 1 revealed several small but significant deviations of the calculated curves from the experimental data. No attempt was made to obtain a better fit by recalculation with altered assumptions. The present apparent K_1 values of between 0.27 and 4.8 μM were in the same range as the K_1 values of between 3.43 and 8.05 μM previously calculated on the basis of completely different kinetic assumptions [6].

The above results indicate that the kinetic cooperativity of the reconstituted $(Na^+ + K^+)$-ATPase and K^+ phosphatase activities can be generated from strictly non-cooperative lipid-binding events. The present analysis therefore reconciles the studies indicating cooperativity in lipid activation [6] with those indicating non-cooperativity in lipid binding [3,4,7,8]. This result could be obtained on the basis of the accepted number of lipid-binding sites of the ATPase (30 per monomer or 60 per dimer).

The present analysis led to different apparent K_1 values for the $(Na^+ + K^+)$-ATPase and the K^+ phosphatase activities. This result was difficult to understand, since both activities are functions of the same protein. Only experimental lipid-protein binding studies can clarify whether the differences in apparent K_1 values are real.

The optimal values of apparent K_1 were in the micromolar range, so that the catalytically active lipoprotein species of the ATPase possess a high free energy potential. This chemical potential could conceivably play a role in the conformational changes and the vectorial catalysis of the enzyme. The optimal values for the index a were in a range of particular regulatory importance. In this range of $a = 1$–6 the catalytic efficiency, expressed as $[L_{50}]/K_1$, was still strongly sensitive to changes in a. At the same time, the optimal values for the index a were close to the cooperativity maxima of Fig. 2B. This could enable the $(Na^+ + K^+)$-ATPase to act as a biochemical amplifier of structural changes in the lipid phase.

Special thanks go to Dr. P. Ottolenghi of Aarhus University, Aarhus, Denmark, for making his original experimental results available. This work has been supported by the Deutsche Forschungsgemeinschaft (SFB 46, C.7) and in part by the Fonds der Chemischen Industrie.

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