*Biochimica et Biophysica Acta*, 513 (1978) 78—88


BBA 78173

# MEMBRANE-BOUND COOPERATIVE ENZYMES

# STOKES' RADII, HILL PLOTS AND MEMBRANE FLUIDITY IN THE REGULATION OF ADENOSINETRIPHOSPHATASE FROM *ESCHERICHIA COLI* *

F. SIÑERIZ, H. MORENO and R.N. FARÍAS

*Instituto de Química Biológica, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Chacabuco 461, 4000 San Miguel de Tucumán, Tucumán (Argentina)*

(Received February 9th, 1978)

## Summary

The soluble $Ca^{2+}$-ATPase from *Escherichia coli* had a distinctive behavior with respect to inhibition by $Na^+$ measured at 36°C and 19°C. At the first temperature the Hill plots are linear and show a slope of 1.1 while at 19°C the plots are biphasic, with slopes of 1.8 and 0.8 before and after the break, respectively. The break occurs at about 50 mM NaCl. Gel chromatography was performed in jacketed Sepharose 4B columns kept at 2 temperatures in the presence of different concentrations of NaCl. It was found that the Stokes' radius of the enzyme was dependent on the temperature and on the salt concentration. Equilibrium sucrose gradients run at 19°C showed that the sedimentation constant of the enzyme remained constant irrespective of the NaCl concentration used. It is concluded that a "folding" of the enzyme takes place in the presence of NaCl, the process being complete at about 50 mM NaCl at 19°C and at about 20 mM at 36°C. The results are in excellent agreement with the kinetic data: the "folded" or "compact" configuration would show no cooperative response towards $Na^+$ while the "expanded" conformer would present strong cooperativity. This is also in agreement with the results obtained with the enzyme embedded in the membrane: when the membrane is fluid a high $n$ value (Hill coefficient) is found; when the membrane is more rigid the value of $n$ falls.

A model explaining all our results is proposed and discussed.

* Some results and interpretations present in this paper are included in a review by our group ((1975) Farías, R.N., Bloj, B., Morero, R.D., Siñeriz, F. and Trucco, R.E. Biochim. Biophys. Acta 415, 231—251).

## Introduction

The membrane-bound ATPase activity of *Escherichia coli* has been solubilized and purified by several workers [1—6]. The enzyme consists of 4 non-identical subunits, namely $\alpha$, $\beta$, $\gamma$ and $\epsilon$ [7—10]. A fifth polypeptide, $\delta$, is essential for the association of the enzyme with the membrane [8,11,12]. The stoichiometry of the polypeptide subunits is $\alpha_3\beta_3\gamma\epsilon$ [13]. The enzyme behaves, according to the model of Singer and Nicolson [14] as a peripheral protein since it can be easily removed from the membrane [2,4,6,8]. This paper deals with the conformational changes of the soluble ATPase in the presence of NaCl and a model which provides a molecular interpretation of our previous reports on the effect of the membrane on the cooperative regulation of the ATPase [15—18]. Part of the present work has been reported earlier in preliminary form [37].

## Materials and Methods

*E. coli* $K_{12}$ Hfr $M_1$ (*pho*; — alkaline phosphatase) and *E. coli* $K_{12}$ L010 [19] an auxotroph requiring unsaturated fatty acids were the source of the enzyme. Growth conditions and the isolation of the membranes have been described [15,18].

*Preparation of the soluble enzyme.* The soluble enzyme was obtained by washing the membranes with 0.002 M Tris-HCl buffer (pH 9.0) 2 ml of the suspension of the bacterial membranes (about 1.2 mg of protein per ml) were suspended in 12 ml of the buffer. The mixture was incubated for 30 min at 36°C and then spun at 37000 × *g* for 20 min. The pellet was resuspended in the same buffer and reincubated for another 20 min and centrifuged. The supernatants of the 2 washings were pooled and concentrated in a flash evaporator to a volume of 2 ml. The soluble enzyme was used immediately.

*Gel filtration.* Jacketed columns of Sepharose 4B (1.7 × 40 cm) were used for all experiments unless otherwise indicated. The columns were kept at the set temperatures by circulating water (Forma Sci. Co.).

*Stokes' Radii.* The values of the Stokes' radii of the enzyme under different conditions were obtained from the plot $(-\log K_d)^{1/2}$ vs. $R_s$ derived from the relationship of Laurent and Killander [20], calibrating the columns in the different conditions with markers of known Stokes' radii. The values of the radii of the marker proteins were taken from the work of Siegel and Monty [21].

*Sucrose density gradients.* The density gradients were prepared according to the method of Martin and Ames [22]. The particular conditions are described when necessary.

*Other assays.* ATPase activity was measured by the released phosphate as described before and expressed as $\mu$mol/h per mg of protein when needed [18]. Protein was determined by the method of Lowry et al. [23]. Catalase was measured as described by Beers and Sizer [24]. Thyroglobulin and bovine serum albumin were determined by their absorbance at 280 nm.

## Results

*Gel filtration at 19°C.* Fig. 1 shows the elution profile of the ATPase activity when the column was kept at 19°C. In the absence of any effector the activity

peak eluted in a position very close to that of thyroglobulin (mol. wt. 680000); when the column was equilibrated with 100 or 150 mM NaCl the activity peak eluted almost in the position of catalase (mol. wt. 230000). With intermediate NaCl concentrations a progressive shift towards the position of the lower mol. wt. markers was observed as the NaCl concentration increased. When the concentration of the enzyme was diluted to 1/4 similar results were found. If the activity peak which eluted at 100 mM NaCl was collected and poured into a column devoid of NaCl, the position of the peak was identical to that observed directly with the native enzyme (not shown), indicating a reversible behavior.

*Gel filtration at 36°C.* When the gel filtration of the soluble enzyme was performed at 36°C (Fig. 2) the position of the activity peak either with high NaCl

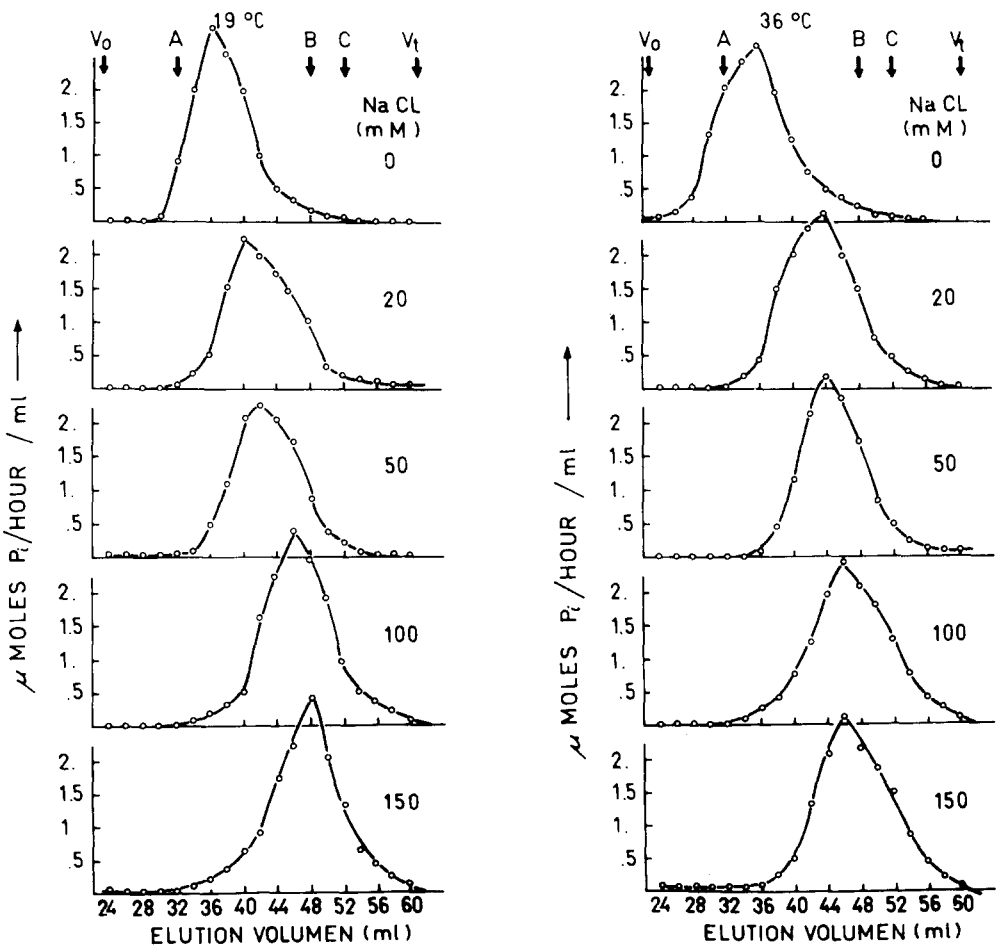


Fig. 1. Gel filtration of the ATPase through Sepharose 4B at 19°C; $V_o$ = void volume determined with Dextran Blue; $V_t$ = total volume determined with phosphates; A = thyroglobulin (680000); B = catalase (230000); C = Bovine serum albumin, dimer (130000). The column was equilibrated with 0.02 M Tris-HCl buffer, pH. 9.0 containing 1 mM $CaCl_2$, 1 mM cysteine and NaCl at (a) 0 mM; (b) 20 mM; (c) 50 mM; (d) 100 mM.

Fig. 2. Gel filtration of the ATPase through Sepharose 4B at 36°C. For details see legend to Fig. 1.

or without the salt in the equilibrating buffer was the same as with high or low NaCl concentration at 19°C, however, the response to NaCl was different: at a much lower level (20 mM) the shift towards the position of catalase was almost complete.

*Stokes' radii.* From the experiments with the Sepharose 4B columns, the Stokes' radius of the enzyme in the different conditions was determined and the results are shown in Fig. 3. The increase in the NaCl concentration brings about a diminution in the Stokes' radius of the enzyme. These results could be interpreted as a dissociation provoked by the rise in the salt concentration with an equilibrium of the type polymer ⇌ monomer; this type of equilibrium has been described as of considerable importance in enzyme regulation in vivo [25]. To test this hypothesis we studied the behavior of the enzyme in sucrose density gradient centrifugation. This approach has been succesfully applied by Datta and Epstein [26] to study the behavior of the homoserine dehydrogenase of *Rhodospirillum rubrum* in the presence and in the absence of threonine.

*Sucrose density gradients.* Sucrose density gradients were run in the same conditions used for gel filtration selecting 19°C as the working temperature because intermediate behavior could be found in the range 0—50 mM NaCl as suggested by Fig. 3. Besides, a temperature of 19°C was easier to control in the centrifuge chamber. This method has been used to study crude extracts [22]. ATPase activity appeared (Fig. 4) as a single peak with a sedimentation coefficient higher than catalase and in the same position at all NaCl concentrations tested. Exactly the same results were obtained with 50 and 150 mM NaCl (not shown); the position of the markers used (catalase or thyroglobulin) did not change in the presence of NaCl. Sedimentation coefficients for ATPase were in the range 14.6—16.0 S considering catalase as 11.3 S. If division into subunits of half the size had ocurred, as could also be inferred from the results from the columns, a noticeable change in the sedimentation constant should have ocurred too.

The value of the molecular weight determined by this method is about 400000 for 4 independent determinations using either marker, which is in good agreement with the value reported by Hanson and Kennedy [7]. The molecular weight of the sum of the polypeptide subunits $\alpha_3\beta_3\gamma\epsilon$ is 390000 [13].

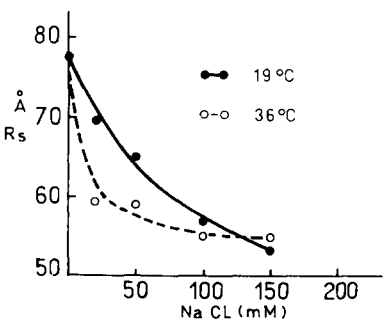


Fig. 3. Stokes' radii of the ATPase at 19°C (●- - - - -●) and 36°C (○- - - - -○) as a function of NaCl concentration.

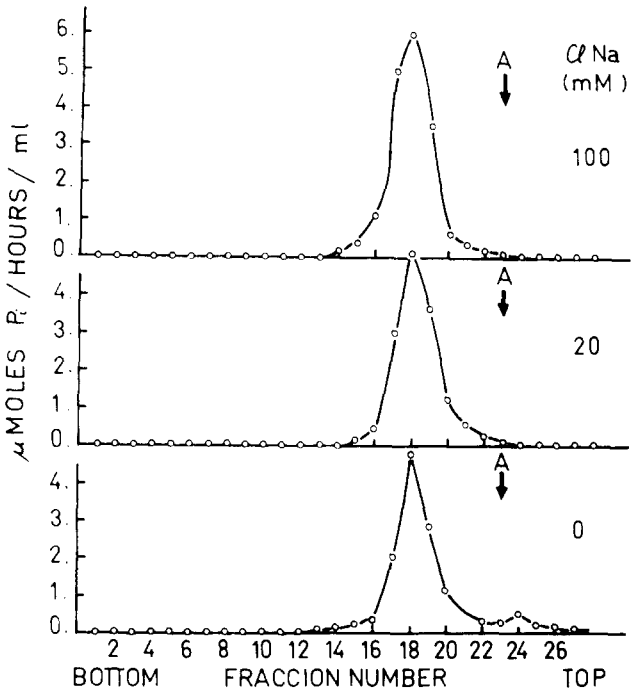


Fig. 4. Sucrose density gradient of the ATPase. The buffer was the same used for gel filtration. A = catalase. The gradient (10—40% w/v) was centrifuged for 7 h at 38000 r.p.m. and 34 fractions were collected.

*Hill coefficient and Stokes' radii of the enzyme.* Combining the data from the experiments of gel filtration and sucrose gradients we were able to conclude that a change in the conformation of the enzyme takes place as the NaCl concentration increases. Since the sedimentation coefficient relative to the markers remained constant, the conformation change could be a shift towards a more globular or "compact" molecule. The effect of the buffer concentration on the conformational change of the soluble ATPase was also studied and it was observed that the elution patterns changed when the buffer concentration (Tris-HCl, pH 9.0) was varied in the range 20—200 mM at 19°C. The Stokes' radius of the enzyme shifted from 77 to 65 Å in these conditions (not shown). A 200 mM Tris-HCl buffer of pH 9.0 has approximately the same ionic strength as a 20—30 mM NaCl solution (due to the extremely low dissociation constant of Tris) so the shift in Stokes' radius due to Tris-HCl agrees quite well with the one obtained by changing the ionic strength with NaCl to a comparable value (Fig. 3). As the increase in the Tris-HCl concentration caused no inhibition effect ($Na^+$ does), the Hill coefficient for the inhibition by $Na^+$ was determined at 19°C in the presence of different Tris-HCl concentrations (Fig. 5). A linear slope (1.1) was found with 300 mM Tris-HCl as buffer, whereas at 50 mM Tris-HCl a break in the slope is apparent with values of the Hill coefficient of 1.8 and 0.8 before and after the break respectively. So when the ionic strength of the solution is increased, the enzyme would become more compact and would lose cooperativity towards $Na^+$.

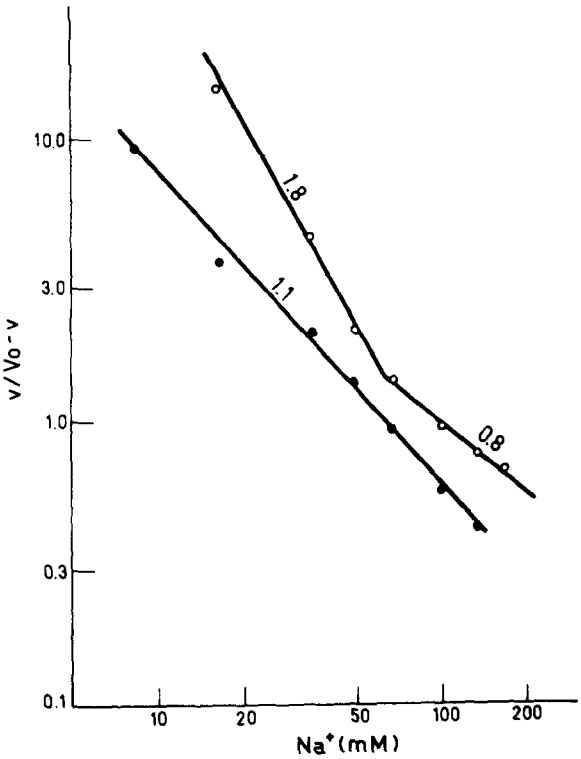


Fig. 5. Hill plots for the inhibition by $Na^+$ of the soluble ATPase at 19°C at different Tris-HCl concentrations: 50 mM (○- - - - -○) and 300 mM (●- - - - -●). 40 μg of protein per tube were used in the assay.

*Membrane fluidity and Hill coefficients at 19°C.* In a previous work we have shown that at 36°C the allosteric behavior of the membrane-bound ATPase from the unsaturated fatty acid auxotroph of *E. coli* was modulated by the fatty acid fluidity of the membrane [16]. The values of $n$ for inhibition by $Na^+$ increased (in the range 1.3—2.9) with the double bond index of the fatty acids of the membrane phospholipids and with the ratio double bond index/saturated fatty acids. These indexes were taken as indicative of the membrane fluidity.

When the inhibition by $Na^+$ was performed in the same membranes at 19°C the values of $n$ ranged from 1.0 in the case of the oleic acid-supplemented medium to 1.6 when linolenic acid was the supplement to the auxotroph. Significant differences are found with several groups (Table I). The plot of the values of $n$ against the double bond index/saturated fatty acids ratio shows a significant correlation ($y = 0.519 + 1.016\,x$; $r = 0.94$ $P < 0.02$). A correlation is also present when the other fluidity parameter, the double bond index, is used ($y = 0.628 + 1.29\,x$; $r = 0.94$ $P < 0.01$). So when the membrane fluidity is lowered it would appear that the protein (ATPase) is constrained to the globular or unexpanded conformation, hence the lower value of $n$.

TABLE I

*n* VALUES AT 19 C AND FLUIDITY PARAMETERS FROM *E. COLI* $K_{12}$ L010

The results are expressed as the mean ± S.E.M. Values followed by the same letters are significantly different ($P = 0.01$). The double bond index is the sum of the products of the mole fractions X the number of double bonds for each fatty acid. Double bond index (unsaturated) is the ratio between the double bond index and the sum of the mole fractions of the saturated fatty acid. (These values of double bond index (saturated) from data of Siñeriz et al. [16]). Values in brackets in the first column represent number of membrane preprations.

| Unsaturated fatty acid supplement | *n* | Double bond index | Double bond index (unsaturated) |
|---|---|---|---|
| Oleic (4) | 1.05 ± 0.03 [a,b] | 0.35 | 0.55 |
| Palmitoleic (5) | 1.16 ± 0.05 [c] | 0.36 | 0.57 |
| Linoleic (3) | 1.30 ± 0.07 [a,b] | 0.59 | 0.87 |
| Linoleic (4) | 1.60 ± 0.04 [c,b] | 0.70 | 0.99 |

## Discussion

### *Effect of NaCl on the soluble enzyme*

From chromatography on Sepharose 4B, the Stokes' radius changes by nearly 30% over a range of salt concentrations (Fig. 3). Yet, under identical conditions (Fig. 4), the sedimentation coefficient as determined by the method of Martin and Ames [22] is completely invariant with respect to salt concentrations. This last method is used for estimates of molecular weight among proteins with the same frictional factor, and the change in Stokes' radius detected with Sepharose 4B should in fact lead to a corresponding change in the diffusion constant even if there is no change in the molecular weight. The movement through the column is probably not exactly the same as the movement of the molecules through a liquid column under the influence of a gravitational field, but at this moment we have no better explanation for these controversial observations (Figs. 3 and 4). Nevertheless, the sedimentation experiment seems to rule out that NaCl provokes a dissociation of the enzyme, since the apparent *s* (relative to the marker) seems to be constant: a dimer ⇌ monomer equilibrium would noticeably alter *s*. Datta and Epstein [26] found that homoserine dehydrogenase from *Rhodospirillum rubrum* underwent an increase in the Stokes' radius with increasing ionic strength, with no change in the sedimentation constant. These authors suggest that the observations reflect a slight unfolding of the molecule rather than a polymerization. Our results would suggest that in the presence of different salt concentrations the molecular weight of the soluble ATPase from *E. coli* was constant and that a conformational rearrangement to a more compact configuration of the enzyme took place when the NaCl concentration increased. This would point to a double action of $Na^+$: it is an effector and at the same time provokes a conformational change by increasing the ionic strength. The influence of ions on the soluble $Ca^{2+}$-ATPase is well documented [1] and it is difficult to separate a specific ion action from an ionic strength effect since all the monovalent cations inhibit the enzyme. At the same time, no specific effect was found for several anions, among them $Cl^-$. Feinstein and Fisher [27] suggested the importance of ionic strength in the

inhibition of the *E. coli* ATPase by dicyclohexylcarbodiimide. Choline, generally used to keep constant ionic strength conditions also affects this inhibition [27].

Our results suggest that the same conformational changes that affect the movement of the molecules through the Sepharose column can be obtained with an increase of the ionic strength mediated by Tris-HCl. The ionic strength affects the conformation of the molecule though Tris-HCl does not inhibit the enzyme in our experimental conditions. In addition, this also showed that the high concentration of Tris-HCl prevents the appearance of cooperative effects for $Na^+$ because a more compact configuration of the enzyme is reached. The rearrangement to a more compact conformation is complete between 50 and 100 mM NaCl at 19°C and at about 20 mM NaCl at 36°C (Fig. 3). These results agree with previous cooperative kinetic data for $Na^+$ inhibition of the soluble enzyme [18]: at 36°C a linear slope ($n$ = 1.1) without any break was observed, but when the assay was done at 19°C a break in the Hill plot was present at a concentration of 70—80 mM NaCl, with slopes before and after the break of 1.8 and 0.8 respectively. So when a certain degree of compactness is achieved by NaCl, the cooperativity disappears with the ocurrence of a break in the Hill plot.

The reversible characteristics of the equilibrium "folded" ⇌ "unfolded" is proven by the fact that the enzyme recovered from a column equilibrated with NaCl will appear in the same position as the native enzyme when passed through a second column without NaCl.

*The enzyme in the structure (membrane)*

In a previous report [18] we showed that, in contrast to the soluble enzyme, the membrane-bound enzyme from wild type *E. coli* showed no cooperativity towards $Na^+$ at 19°C ($n$ = 1.1) while at 36°C values of $n$ = 1.8 and a break at 70—80 mM NaCl was present. It was clear from those experiments that the membrane acted as a "macroeffector" in inhibition by $Na^+$ at both 36°C and 19°C. At the first temperature the membrane enhanced the cooperativity shown by the soluble enzyme; at 19°C the opposite holds true. A strikingly similar kinetic behavior of the soluble enzyme at 19°C and of the membrane-bound enzyme at 36°C was observed: all the cooperative parameters (values of $n$ and position of the breaks) were the same [18]. These facts and the correlation between the breaks in the Hill plots and the Stokes' radii of the soluble enzyme strongly suggest that the membrane may affect the equilibrium "globular conformation" ⇌ "expanded conformation". At 36°C the packing of the lipid moiety is fluid; then the expanded conformation and the cooperative effects would occur. At 19°C the situation is the opposite since the cooperativity is greatly reduced when the enzyme is bound to the membrane; in this last case the lipids would be closely packed and the enzyme would be constrained to a more "folded" or "compact" conformation. The reversible cooperative alteration induced in the soluble enzyme when it binds $Na^+$ is lost at 19°C or recovered at 36°C in reconstituted membrane-like material [18]. All these considerations led us to propose a model compatible with our findings on the cooperative behavior of the soluble and membrane-bound ATPase from wild type *E. coli* for inhibition by $Na^+$ at 19°C and 36°C (Fig. 6).

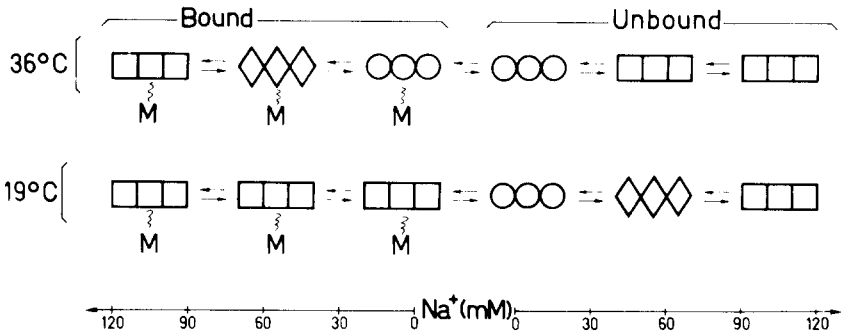


Fig. 6. Model for the action of temperature and NaCl concentration on the ATPase from *E. coli*. Circles and squares represent ATPase molecules with Stokes radii of 78 Å (circles) and 55—58 Å (squares). The romboid figure represents a molecule of intermediate Stokes: radius (60—70 Å). In the transition region from circles to squares, $n > 1.0$ is found for inhibition by $Na^+$ (before the break). After the break, the square is the only possible state and only $n = 1.0$ is found. The number of interacting protomers is 3, because the highest value of $n$ for inhibition by $Na^+$ of the $Ca^{2+}$-ATPase from *E. coli* actually obtained was 2.9 [16,31]. The polypeptide composition of the protomers is unknown. M = membrane.

From this model one could get the impression that at 19°C only a compact form of the enzyme can occur in the membrane: this is true for wild type *E. coli* grown at 37°C, where the 2 assay temperatures (36 and 19°C) determine the presence or absence of fluidity. However, when the auxotroph was grown in a medium supplemented with linolenic acid (Table I) or, when *E. coli* wild type was grown at 20°C instead of 37°C [28], conditions that increase the unsaturated fatty acid content, values of $n$ around 1.6 were obtained and a break was present.

The fluidity can be altered experimentally by (a) changing the temperature of assay from 19°C to 36°C in the membrane of the wild type [18] or of the mutant [4], leaving the composition constant, or by (b) changing the fatty acid composition of the membrane leaving the temperature constant, 36°C [16] or 19°C (Table I).

Also from the model in Fig. 6, a break should be present in the soluble enzyme at 36°C at low NaCl concentrations. They were not reported in earlier papers because the inhibition started to be measured at 20 mM NaCl since it was difficult to build a significant curve at lower concentrations because the extent of inhibition is very low. Experiments performed now in the range 0—30 mM NaCl showed the presence of the expected break.

*Hill plots as "probes" for membrane structure.*

Recently we reviewed the effect of the membrane lipid composition on the regulation of the allosteric membrane-bound enzymes in mammalian and bacterial membrane [29]. The convenience of the use of "allosteric probes" for the study of lipid-protein interactions in biological membranes has been suggested by our group [16,17,30] and the thermodynamical background was also reported [31]. In agreement with our theory it is interesting to note here that at 19°C the high $n$ values shown by the ATPase from membranes of the mutant strain supplemented with linolenic acid indicated that these membranes

were fluid while $n \approx 1.0$ at the same temperature for the wild type membranes as well as for the mutant when the supplement was oleic acid indicated a low fatty acid fluidity. X-ray diffraction techniques [32] showed the existence of different transition points for the lipids in intact membranes of the mutant grown with oleic (36—46°C) and linolenic acid (19—29°C) as supplements. Thus the evaluation of the Hill coefficient of a suitable allosteric enzyme would prove a sensitive method to detect changes in the membrane fluidity when the temperature is modified. In a different system, rat erythrocytes, the $n$ values for activation by $K^+$ of the membrane-bound *p*-nitrophenylphosphatase increased with the incubation temperature [33]. The increase in $n$ was observed between 25 and 37°C for the enzyme from rats fed a fat-supplemented diet (higher fluidity) and between 37 and 43°C for the enzyme from rats fed a fat-free diet (lower fluidity). These observations could be reinterpreted now as that the changes in the lipid fluidity surrounding the enzyme take place at different temperatures. This could be shown by a change in $n$ values. More recently, in isothermal conditions, we showed the action of some hormones on membrane fluidity in mammalian and bacterial systems by changes in the Hill coefficients of suitable membrane-bound enzymes [28,34—36].

## Acknowledgements

We acknowledge the support given to this work by the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentian) and the Secretaría de Ciencia Técnica de la Universidad Nacional de Tucumán. F.S. and R.N.F. are Career Investigators of the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina). This paper is taken in part from a thesis submitted by H.M. in partial fulfillment of the requirements for the Ph.D degree from the Facultad de Bioquimica, Quimica y Farmacia, Universidad Nacional de Tacumán.

## References

1 Evans, Jr., D.J. (1970) J. Bacteriol. 104, 1203—1212
2 Davies, P.L. and Bragg, P.D. (1972) Biochim. Biophys. Acta 266, 273—284
3 Kobayashi, H. and Anraku, J. (1972) J. Biochem. (Tokyo) 71, 387—399
4 Siñeriz, F., Farías, R.N. and Trucco, R.E. (1972) Abst. Annu. Meet. Am. Soc. Microbiol., p. 222
5 Carreira, J., Leal, A.J., Rojas, M. and Muñoz, E.M. (1973) Biochim. Biophys. Acta 307, 541—556
6 Roisin, M.P. and Kepes, A. (1973) Biochim. Biophys. Acta 305, 249—259
7 Hanson, R.L. and Kennedy, E.P. (1973) J. Bacteriol. 114, 772—781
8 Bragg, P.D., Davies, P.L. and Hou, C. (1973) Arch. Biochem. Biophys. 159, 663—670
9 Nelson, N., Kanner, B.J. and Gutnick, D.L. (1974) Proc. Natl. Acad. Sci. U.S. 71, 2720—2724
10 Kobayashi, H. and Anraku, J. (1974) J. Biochem. (Tokyo) 76, 1175—1182
11 Futai, M., Sternweis, P.C. and Heppel, L.A. (1974) Proc. Natl. Acad. Sci. U.S. 71, 2725—2729
12 Smith, J.B. and Sternweis, P.C. (1975) Biochim. Biophys. Res. Commun. 62, 764—767
13 Bragg, P.D. and Hou, C. (1975) Arch. Biochem. Biophys. 167, 311—312
14 Singer, S.J. and Nicolson, G.L. (1972) Science 175, 720—731
15 Farías, R.N., Londero, L. and Trucco, R.E. (1972) J. Bacteriol. 109, 471—473
16 Siñeriz, F., Bloj, B., Farías, R.N. and Trucco, R.E. (1973) J. Bacteriol 115, 723—726
17 Siñeriz, F., Farías, R.N. and Trucco, R.E. (1973) FEBS Lett. 32, 30—32
18 Moreno, H., Siñeriz, F. and Farías, R.N. (1974) J. Biol. Chem. 249, 7701—7706
19 Cronan, J.E. Jr., Birge, C.H. and Vagelos, R.P. (1969) J. Bacteriol. 104, 601—604
20 Laurent, T. and Killander, J. (1964) J. Chromatogr. 14, 317—330
21 Siegel, L.M. and Monty, K.J. (1966) Biochim. Biophys. Acta 112, 346—362
22 Martin, R.C. and Ames, B.N. (1961) J. Biol. Chem. 236, 1372—1379

23 Lowry, O.N., Rosebrough, N.H., Farr, A.L. and Randall, R.J. (1961) J. Biol. Chem. 193, 265–275
24 Beers, Jr., R.F. and Sizer, I.W. (1952) J. Biol. Chem. 195, 133–140
25 Frieden, C. (1971) Annu. Rev. Biochem. 40, 653–696
26 Datta, P. and Epstein, C.Ch. (1973) Biochemistry 12, 3888–3892
27 Feinstein, D.L. and Fischer (1977) Biochem. J. 167, 497–499
28 Moreno, H. and Farías, R.N. (1976) Biochem. Biophys. Res. Commun. 72, 74–80
29 Farías, R.N., Bloj, B., Morero, R.D., Siñeriz, F. and Trucco, R.E. (1975) Biochim. Biophys. Acta 415, 231–251
30 Bloj, B., Morero, R.D., Farías, R.N. and Trucco, R.E. (1973) Biochim. Biophys. Acta 311, 67–69
31 Siñeriz, F., Farías, R.N. and Trucco, R.E. (1975) J. Theor. Biol. 52, 113–120
32 Esfahani, M., Limbrick, A.R., Knutton, S., Oka, T. and Wakil, S.J. (1971) Proc. Natl. Acad. Sci. U.S. 68, 3180–3184
33 Goldemberg, A.L., Farías, R.N. and Trucco, R.E. (1972) J. Biol. Chem. 247, 4299–4303
34 Massa, E.M., Morero, R.D., Bloj, B. and Rarías, R.N. (1975) Biochem. Biophys. Res. Commun. 66, 115–122
35 de Mendoza, D., Moreno, H., Massa, E.M., Morero, R.D. and Rarías, R.N. (1977) FEBS Lett. 84, 199–202
36 de Mendoza, D. and Farías, R.N. (1978) J. Biol. Chem., in the press
37 Siñeriz, F., Moreno, H. and Farías, R.N. (1975) Abst. 75th Annu. Meet. Am. Soc. Microbiol., K 182