BBA 75585

THE FUNCTIONAL PROPERTIES OF ATPases BOUND TO AND SOLUBILIZED FROM THE MEMBRANE COMPLEX OF THE HEN'S EGG

E. ETHEREDGE, J. E. HAALAND AND M. D. ROSENBERG

Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minn. 55101 (U.S.A.)

(Received August 24th, 1970)

SUMMARY

Similarities and differences between "soluble" and 'membrane-bound' ATPase (ATP phosphodyhrolase, EC 3.6.1.3) have been found for the membrane complex of the hen's egg. Under conditions of varying ionic strength at pH 9.0, both "soluble" and "membrane-bound" ATPase activities are enhanced by Na^+ and are suppressed by Ca^{2+}. At both pH 6.0 and 9.0 the "membrane-bound" and "soluble" ATPases exhibit opposite patterns of K^+ enhancement or suppression of activity. The "soluble" and "membrane-bound" fractions exhibit similar cold lability and biphasic pH dependencies. The presence of similar pH differentials *in vivo* suggests a functional correlation between activation of "membrane-bound" ATPase and changes in the ionic environment. The ATPases appear to represent a family of enzymes with marker characteristics of enzymes associated both with "transport" and "contractility" depending on variations in the species, absolute concentrations and ratios of ions, as well as variations in pH and ionic strength of the reaction.

INTRODUCTION

ATPase (ATP phosphohydrolase, EC 3.6.1.3) is implicated in such diverse functions as transport of inorganic ions[1] and oxidative phosphorylation[2], and has been shown to differ in its activation and enhancement responses to cations, depending on the membrane system studied[3]. Differences between the soluble and particulate state, called "allotopy" by RACKER[4] have been described for mitochondrial ATPase; and recently, allotopic behavior in the solubilized ATPase of *Micrococcus lysodeikticus* was described[5]. This fascinating phenomenon poses many critical questions concerning the nature and influence of membrane–enzyme interaction. In another paper[6] we reported on the solubilization of an ATPase from the plasma vitelline–membrane complex of the chicken egg. In an attempt to elucidate the nature of membrane-ATPase interaction, we have partially characterized this ATPase in its membrane-bound form and compared these functional aspects to those of an ATPase solubilized from the same membrane source.

MATERIALS AND METHODS

Membranes (plasma–vitelline–membrane complex) and the soluble fraction (S100/30) were obtained by the procedures detailed in a preceding paper[6]; likewise,

ATPase and protein assays were performed as previously described, except as indicated[6]. Following the imidazole wash, membranes were cut into pieces approx. 80–100 mm^2, using a dissecting microscope. For studies of ATPase salt dependency* with ionic strength varying with the concentration of the added salt species, the reaction volume was kept constant and the salts, when used, were added in the following final concentrations: Mg^{2+} alone, 7.35 mM; Ca^{2+} alone, 7.35 mM; Mg^{2+} *plus* Ca^{2+}, 7.35 mM and 7.35 mM; Mg^{2+} *plus* Na^+, 7.35 mM and 90.0 mM; Mg^{2+} *plus* Na^+ *plus* K^+, 7.35 mM, 90.0 mM, and 20.0 mM, respectively. For studies of ATPase salt dependency with constant ionic strength ($I = 0.387$), choline chloride was added in appropriate concentrations to the various test samples and blanks, the reaction volume being held constant at 0.85 ml. In this text the ATPase activity will be qualified by the salt species present, *i.e.* "Mg^{2+}-ATPase" will refer to the ATPase activity occurring in the presence of Mg^{2+} in the reaction mixture. The change in activity of a specified divalent-cation-ATPase by an added monovalent cation will be called, for example, "Na^+ enhancement" or "K^+ suppression". Specific activity is defined as the μmoles of P_i released per h per mg of protein.

Determination of the temperature dependency of the soluble fraction was made by incubating the enzyme sample for 30 min in the standard reaction mixture (0.2 M Tris–histidine, pH 9.0, *plus* magnesium, sodium, and potassium salts), in water baths of the appropriate temperature. Determination of the temperature dependency of membrane pieces was made by incubating them for 30 min, in a reaction mixture containing 0.2 M imidazole–HCl, pH 7.0, *plus* magnesium, sodium and potassium salts, at the required temperature. Following the requisite incubation, the reaction was stopped with cold trichloroacetic acid, and the remainder of the assay completed as described in the preceding paper[6].

For studies of the relationship of specific activity to reaction pH, 0.2 M Tris–histidine buffers were adjusted to the approximate pH range and the final reaction mixture pH values were measured. Final reaction pH of the unknown and the enzyme, substrate and buffer blanks were maintained constant using buffers of slightly different pH, since constancy of the final pH is critical for the correct color development. For the broad range of pH required, a 0.2 M Tris–histidine–HCl buffer was found to be most satisfactory in its ability to hold our reaction pH at the desired pH point.

RESULTS

Influence of salt conditions on ATPase acitvity

(*a*) *Influence of cations at constant ionic strength*

Table I summarizes the specific activity of the membrane-bound and "soluble" fraction ATPase specific activity as functions of ionic strength, cation species, and pH. The first four columns illustrate the effects of divalent cations at low ionic strength, the second group of these columns illustrate the effects of monovalent cations at high ionic strength.

(*1*) *Membrane-bound ATPase activity.* Using the first column as a reference specific activity (in the presence of 10 mM Na^+) we observe at pH 6.0 the enhancement of activity in the presence of either Mg^{2+} or Ca^{2+}, where the Mg^{2+} enhancement is

* For studies of salt dependency, the soluble fraction (S100/30) was dialysed for 3 h at 4° against distilled water, 1 : 600.

TABLE I

ATPASE SPECIFIC ACTIVITY IN THE INTACT MEMBRANE AND SOLUBLE FRACTION

All values are the mean of at least three observations. Numbers in parentheses indicate percentage change from zero reference.

Fraction	*Reaction pH*	*ATPase specific activity (μmoles P_i released per h per mg protein)*						
		I = 0.20–0.24			*I = 0.354–0.387*			
		10 mM Na^+	*10 mM Na^+, 7.35 mM Ca^{2+}*	*10 mM Na^+, 7.35 mM Mg^{2+}*	*10 mM Na^+, 7.35 mM Mg^{2+}, 7.35 mM Ca^{2+}*	*100 mM Na^+, 7.35 mM Mg^{2+}*	*100 mM Na^+, 7.35 mM Mg^{2+}, 20 mM K^+*	*10 mM Na^+, 177 mM choline*
Intact membrane						(0)	(22)	
	6	4.0	14.3 (−27)	19.7 (0)	20 (2)	18.1 (−8)	22.0 (12)	7.0
						(0)	(−20)	
	9	2.6	23.0 (83)	12.6 (0)	15 (19)	38.6 (206)	31.0 (146)	8.9
Soluble fraction S100/30	6	644	704 (−4)	730 (0)	652 (−11)	733 (0)	541 (−26)	586
	9	176	403 (34)	300 (0)	271 (−10)	359 (20)	539 (80)	481

TABLE II

ATPASE SPECIFIC ACTIVITY OF SOLUBLE FRACTION AS FUNCTION OF IONIC STRENGTH AND pH

All values are the mean of at least three observations. I(v) = ionic strength of reaction mixture, varied; I(c) = ionic strength of reaction mixture, controlled.

Reaction pH	*ATPase specific activity (μmoles P_i released per h per mg protein)*											
	10 mM Na^+		*10 mM Na^+, 7.35 mM Ca^{2+}*		*10 mM Na^+, 7.35 mM Mg^{2+}*		*10 mM Na^+, 7.35 mM Mg^{2+}, 7.35 mM Ca^{2+}*		*100 mM Na^+, 7.35 mM Mg^{2+}*		*100 mM Na^+, 7.35 mM Mg^{2+}, 20 mM K^+*	
	I(v) 0.20	*I(c)* 0.387	*I(v)* 0.22	*I(c)* 0.387	*I(v)* 0.22	*I(c)* 0.387	*I(v)* 0.24	*I(c)* 0.387	*I(v)* 0.354	*I(c)* 0.387	*I(v)* 0.387	*I(c)* 0.387
6	644	586	704	326	730	537	652	426	733	537	541	541
9	176	481	403	504	300	553	271	454	359	553	539	539

greater. Compared to Mg^{2+}-ATPase, equimolar substitution with Ca^{2+} is suppressive. Increase in the Na^+ concentration (to a high ionic strength) does not enhance activity, but the presence of Na^+ and K^+ (at high ionic strength) does enhance activity further.

At pH 9.0 contrary results obtain. At low ionic strength there is marked enhancement of activity in the presence of either Mg^{2+} or Ca^{2+}, but the Ca^{2+} enhancement is now greater, that is, compared to Mg^{2+} ATPase equimolar substitution with Ca^{2+} increases activity. Increase in the Na^+ concentration (to a high ionic strength) greatly enhances activity while the presence of Na^+ and K^+ (at high ionic strength) suppresses this enhancement.

(*2*) *"Soluble" fraction ATPase.* Again referring to Table I for a summary of specific activity of the "soluble" ATPase, we observe at pH 6.0 an enhancement of the ATPase specific activity in the presence of either Mg^{2+} or Ca^{2+}, where the Mg^{2+} enhancement is greater. At this ionic strength and pH and for the divalent cations, the "soluble" fraction and membrane-bound ATPase respond in similar patterns. Increase in Na^+ concentration (high ionic strength) does not enhance activity further and unlike the response of the membrane-bound ATPase, the presence of both Na^+ and K^+ results in a marked suppression of activity. At this ionic strength and pH and in the presence of monovalent cations the "soluble" fraction and membrane bound ATPase respond in opposite patterns.

At pH 9.0 and low ionic strength and in the presence of divalent cations the soluble ATPase again responds in a pattern similar to the membrane-bound ATPase. There is enhancement of activity in the presence of either Mg^{2+} or Ca^{2+}, and the Ca^{2+} enhancement is greater. Increase in the sodium concentration (high ionic strength) has little effect while the presence of Na^+ and K^+ (high ionic strength) enhances the activity. At this ionic strength and pH and in the presence of monovalent cations the "soluble" fraction ATPase responds in a pattern opposite to that of the membrane-bound ATPase.

In summary, a comparison of the membrane-bound fraction with the soluble fraction (Table I) with $I = 0.20$–0.24 at both pH 6 and pH 9 shows a similarity of response to added divalent cations (At both pH 6 and pH 9, the soluble fraction respond somewhat differently to the simultaneous presence of both Ca^{2+} and Mg^{2+} as does the membrane.) A comparison of the membrane-bound fraction with the "soluble" fraction (Table I) with $I = 0.354$–0.387 at both pH 6 and pH 9 shows an opposite pattern of response to monovalent cations.

Additional and more detailed studies of the affect of constant ionic strength in the presence of various cations at pH 6.0 and pH 9.0 were carried on the "soluble" fraction. These data are presented in Table II. Compare the data along the rows. At pH 6.0, whether at low ($I = 0.20$–0.24) or high ($I = 0.387$) ionic strength, equimolar substitution of Mg^{2+} by Ca^{2+} leads to a reduction in activity. The reduction is far more marked at the higher ionic strength. At the higher ionic strength ($I = 0.387$) there is not the marked suppression of (Na^+-Mg^{2+})-ATPase when K^+ is added but no significant enhancement is observed. On the other hand, at pH 9.0, equimolar substitution of Mg^{2+} by Ca^{2+} leads to an increase in activity at low ionic strength and a slight decrease in activity at high ionic strength. And in similar fashion, at pH 9.0 the enhancement in (Na^+-Mg^{2+})-ATPase observed when K^+ was added for $I = 0.354$ to 0.387, is not observed for $I = 0.387$.

(b) Influence of cations at varying ionic strength

Table I also illustrates the effect of varying ionic strength on the activities of both membrane-bound and "soluble" fraction ATPase. The first and the seventh column compare activities in the presence of 10 mM Na^+ where the ionic strength has been increased by means of choline. The third and fifth columns allow for a comparison (Na^+-Mg^{2+})-dependent activities where the ionic strength has been increased by a ten-fold increase in sodium concentration.

(1) Membrane-bound ATPase activity. At pH 6.0 and pH 9.0 the activity in the presence of 10 mM Na^+ is markedly increased as the ionic strength is increased from $I = 0.20$ to 0.387 by the addition of choline. (The absolute activity is low.) A similar relatively marked increase is noted at pH 9.0 for the (Na^+-Mg^{2+})-ATPase when the ionic strength is increased from $I = 0.22$ to 0.387 by a 10-fold increase in Na^+ concentration. On the other hand there is essentially no change in activity when the same change in ionic strength is carried at in a reaction mixture at pH 6.0

(2) Soluble fraction ATPase. The soluble fraction ATPase exhibits only a limited similarity to the membrane-bound ATPase under similar conditions of increased ionic strength. As shown in columns one and seven of Table I, at pH 6.0 there is a slight decrease in activity in the presence of 10 mM Na^+ when the ionic strength is increased from $I = 0.20$ to 0.387 by the addition of choline. At pH 9.0, however, there is a very marked increase, quite similar to the response of the membrane bound ATPase. As shown in columns 3 and 5, there is little difference at pH 6.0 between (Na^+-Mg^{2+})-ATPase activity when the ionic strength is increased, a pattern similar to that observed for the membrane-bound enzyme. At pH 9.0 there is a slight increase in activity, but in no way as great as the relative increase observed for the membrane-found ATPase.

In summary, at pH 9.0, the membrane-bound and "soluble" fraction ATPases exhibit similar responses in the presence of 10 mM Na^+ as the ionic strength is increased almost 2-fold by the addition of choline chloride. They exhibit opposite responses at pH 6.0. On the other hand, at pH 6.0 they exhibit similar responses in the presence of Na^+-Mg^{2+} as the ionic strength is increased almost 2-fold by an increase in Na^+ concentration. They exhibit responses similar in direction but different in relative magnitude at pH 9.0.

Additional and more detailed studies of the effect of varying ionic strength in the presence of various cations at pH 6.0 and pH 9.0 were carried out on the "soluble" fraction alone. These data are presented in Table II. The ionic strength was adjusted to the high value of 0.387 by the addition of choline chloride when required. What is most striking is the reversal of effects as a function of pH. At pH 6.0, regardless of the ionic species present in the reaction mixture, an increase in ionic strength resulted in a decrease in specific activity. At pH 9.0, regardless of the ionic species present in the reaction mixture, an increase in ionic strength resulted in an increase in specific activity.

Determination of correlation coefficients showed no statistically significant correlation between activity and the concentration of choline chloride used to adjust the ionic strength of the reaction mixtures to a constant value at either pH 6 or pH 9. The correlation between ionic strength and specific activity is significant at the 5 % level for the 'soluble' fraction. Overall rank correlation does not, however, signify that differences between each salt treatment are solely due to the ionic strength differences,

but may also be due to changes in monovalent and divalent cations species, concentrations and concentration ratios of species.

(c) Effect of buffer species variation

Absolute magnitudes of specific activity of both the membrane pieces and soluble fraction varied, at a given pH, according to the buffer used in the reaction mixture. In particular, imidazole buffer enhances activity over Tris–histidine and sodium acetate–acetic acid buffers. This phenomenon is due to a specific ion effect of the buffer species at pH 9 since we demonstrated that the buffer species employed in our assays do not affect the color development reaction in the absence of the enzyme. However, at pH 6, the colorimetric assay of inorganic phosphorus is affected by buffer species, thus confounding any specific ion effect.

(d) Effect of reaction mixture temperature

The influence of the incubation temperature of the reaction mixture on the activity of the membrane pieces and the soluble fraction is presented in Fig. 1. The samples were removed from their 4° storage temperature and added directly to reaction mixtures of the appropriate condition, and incubated at the desired temperature for 30 min. There is a parallel temperature dependency demonstrated by the membrane pieces and the soluble fraction.

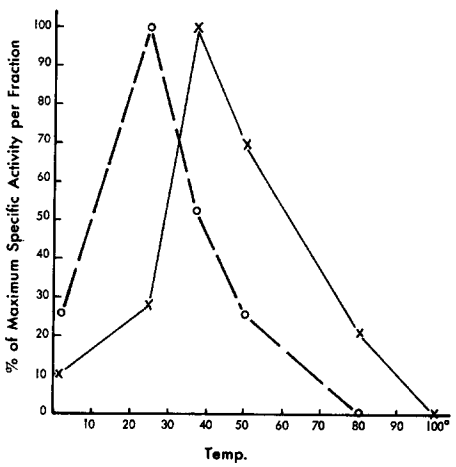

Fig. 1. Variation in (Mg^{2+}-Na^{+}-K^{+})-ATPase relative specific activity with temperature. Maximum specific activity is taken as 100%. ×—×, soluble fraction (S100/30) 0.2 M Tris–histidine buffer (pH 9); ○---○, membrane species, 0.2 M imidazole–HCl buffer (pH 7).

(e) Effects of the reaction pH to specific activity

A typical relationship between the pH of the reaction mixture at constant ionic strength ($I = 0.387$) and the specific activity both of the soluble fraction (S100/30) and of membrane pieces is presented in Fig. 2. Activity maxima coincided at pH 8.5 for the soluble fraction and membrane pieces. The soluble fraction has a second maximum in vicinity of pH 5.0 whereas the membrane-bound enzyme has a second peak in the vicinity of pH 6.0 For the soluble fraction, the observations at pH 5.90 and pH 6.88 are significantly different ($t = 2.37$ *to*.975 (16) = 2.120) and similarly, at pH 7.95 and pH 8.45 ($t = 2.78$ *to*.99 (10) = 2.764). The activity at pH 7.95 does not differ signifi-

cantly from that at pH 6.88, primarily due to the large standard deviation at the pH 7.95 point (approx. 40 % of the mean). However, this large standard deviation at pH 7.95 can be attributed to the extreme sensitivity to pH at that region. The mean probable percentage error for all other determinations was 14 %. A similar distribution of specific activity exists for membrane pieces and the differences between the peaks and the saddle region are significantly different at a 5 % level of confidence,

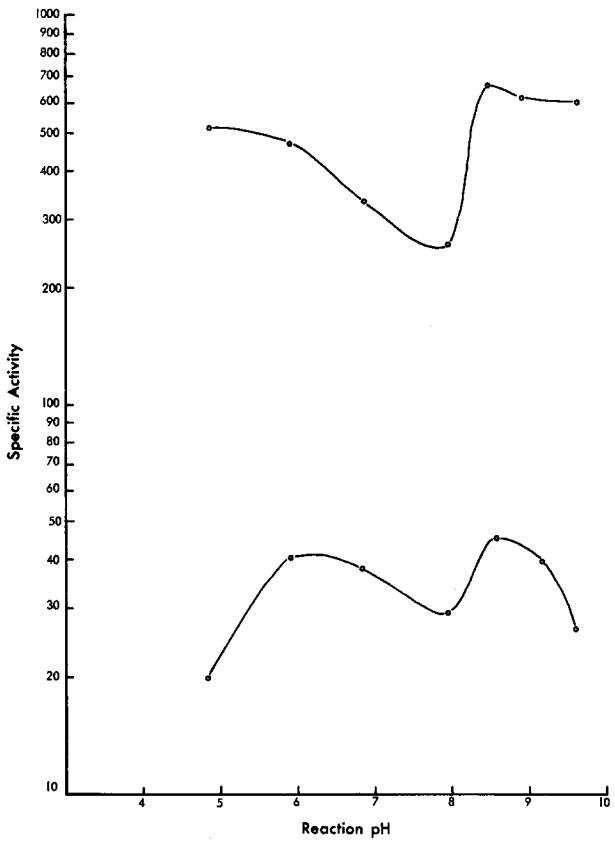

Fig. 2. Variation in (Mg^{2+}-Na^{+}-K^{+})-ATPase specific activity (μmoles P_i per h per mg protein) with reaction pH. Upper curve, soluble fraction (S100/30); lower curve, membrane species.

with a minimum of 5 replications per point. A similar biphasic distribution of maxima around a saddle point has been observed using 0.1 M Tris–acetate and 0.1 M glycine–NaOH buffers.

DISCUSSION

Characterization of an enzyme in its membrane-bound and soluble forms provides unique opportunities for comparison, the goal being the definition of the functional nature of the enzyme–membrane complex. Results of a partial characterization and comparison are reported for an ATPase in the membrane-bound and soluble form.

Effects of salts

The diversity of functions assigned to membrane-bound ATPase suggests a family of ATP phosphohydrolases and this is corroborated by the ATPases "solubilized" to date[1–5,7–16]. The ATPase solubilized from *Streptococcus faecalis* ghosts was Mg^{2+}-dependent, as was the activity of the membrane-bound enzyme; Ca^{2+} was completely ineffective as an activator. There was a non-specific enhancement of activity by large concentrations of Na^+ and/or K^+ (ref. 7). Three ATPases were solubilized from guinea pig brain microsomes but salt characterizations were not reported[8]. A (Mg^{2+}-Na^+-K^+)-ATPase that was inhibited in the presence of Ca^{2+} was solubilized from calf kidney[9]. A Mg^{2+}-dependent ATPase prepared from rat brain demonstrated marked enhancement by Na^+ (ref. 10). An actomyosin-like protein with ATPase activity isolated from whole brains showed Mg^{2+}-dependent activity with Ca^{2+} inhibition, except in the presence of a high KCl concentration where there was a reversal to Ca^{2+} dependency, with suppression when Mg^{2+} was added with Ca^{2+} (see ref. 11).

The divalent cation activation or dependency, and the monovalent cation enhancement of ATPase activity have been widely employed as means of distinguishing among ATPases, and as circumstantial evidence in some cases, for their biological role. Cation effects on ATPase activity are not simply ion-specific, but also reflect absolute concentrations, cation ratios, and the pH of the reaction mixture of the assay. Many of these effects are demonstrated in the classic paper by SKOU[1]. The influence of ionic strength of the reaction mixture on the cation dependency and enhancement of an ATPase are amply demonstrated in our study. The presence, in varying ionic strength conditions, of the classic "transport ATPase" response to Mg^{2+}, Na^+, and K^+ and the loss of this pattern under conditions of constant ionic strength cast some doubt on the validity of salt dependency as an identifying marker. Sodium enhancement of the Mg^{2+}-dependent ATPase activity is considered more specific for the "transport ATPase" since the K^+ increment is not reliable; inhibition by Ca^{2+} is another marker[3]. In our study, Na^+ enhancement of Mg^{2+}-ATPase activity is demonstrated at pH 9 for the membrane pieces and in the varying ionic strength assay of the soluble fraction. Likewise, there is Ca^{2+} enhancement rather than inhibition. With constant ionic strength, these effects are not apparent. There is no evidence for enhancement of Mg^{2+}-ATPase activity by Na^+ in any fraction, assayed at pH 6. However, the Mg^{2+}-activated, Ca^{2+}-suppressed ATPase activity pattern is demonstrated in the membrane pieces and the soluble fraction ($I = 0.387$) at pH 6. As noted above, the reverse pattern holds for the membrane pieces and the soluble fraction at low ionic strength, at pH 9. Thus, at pH 9.0 we observe one marker (Na^+ enhancement) for the presence of the "transport ATPase" and one marker (Ca^{2+} enhancement) for the presence of a "contractile ATPase". At pH 6.0 the situation is reversed. The results are quite similar for both the membrane-bound and "soluble" ATPase fractions. Whether the consistent enhancement and suppression of activity by increasing ionic strength at pH 9.0 and pH 6.0, respectively, are related to these responses remains to be determined.

Effects of temperature

Cold lability has been reported for several enzymes[12–14], among which mitochondrial ATPase is prominent. For mitochondrial ATPase the cold-induced loss of enzymatic activity is attributed to a reversible, partial dissociation of the enzyme into subunits. Dilution to low protein concentration and addition of certain salts accelerate

the dissociation, and pH is critical, with maximum stability at pH 7.5[14]. Our soluble fraction demonstrated marked reduction of catalytic activity at low temperatures, although some activity was retained. When stored in 0.6 M KCl at −28.9° for 12 h, our crude fraction (S27) lost all activity; however, when stored in 0.6 M KCl with 8 mM Na_2ATP and 8 mM $MgCl_2$ the preparation retained its activity. Soluble fractions and membrane pieces were stable for several months when stored at 4° in 0.5 M KCl–0.125 M $NaHCO_3$. Storage of soluble fractions in less than 0.2 M KCl resulted in a marked, loss of activity. This reduction in activity seems attributable to either the decrease of ionic strength of the solution or to the reduction of specific cation concentration, since this effect was demonstrable regardless of the protein content of the sample[6]. On the other hand, the membrane-bound ATPase is strikingly stable. The membrane pieces can be stored at 4° in isotonic buffer or KCl with little loss in activity.

Effects of pH

A striking similarity of Na^+-K^+-enhanced ATPase activity in response to pH changes exists for the intact membrane and the soluble fraction; and, in fact, this biphasic distribution of activity maxima separated by an activity trough was demonstrable in all fractions studied, and in a wide variety of buffers. For all isolated fractions, we observed a similar appearance and separation of activity maxima, consisting of a broad peak in the vicinity of pH 5 to 6 and a sharp peak at pH 8.4 to 8.8. Specific ion effects were obvious in pH–ATPase activity studies performed in different buffers, making comparison of absolute magnitudes of specific activity difficult across differing buffers. While the magnitudes of specific activity varied significantly between the bound and soluble enzyme, the parallel changes of specific activity in responses to pH variation suggests that the bound state is quantitatively, not qualitatively, different than the soluble state. A similar distribution of activity in response to pH variation has been demonstrated for myosin ATPase[15]. A Mg^{2+}-dependent ATPase solubilized from *S. faecalis* ghosts has a single pH optimum of about pH 8.[7] It has been demonstrated that for the ATPase of mitochondrial membrane ghosts the cation condition-of the assay influenced the pH point at which maximum activity occurred[16]. Actomyosin-like proteins isolated from rat and cat brain had single pH optima at 6.8 and 7.6, respectively[11]. A Mg^{2+}-activated ATPase complex from fowl leukemia virus showed two pH optima at pH 7.0 and 8.5[19]. Since a pH range of 7.4 to 7.5 is frequently chosen for the pH conditions of ATPase assay, attention should be given to the possibility that this pH may be a point of minimal activity, a pertinent fact for systems with a low ATPase activity or content. Previous work on the onset of Na^+-K^+-enhanced ATPase activity, assayed at pH 7.5, of the hen's oocyte following ovulation[17], may require re-evaluation in view of the pH dependency reported here.

Functional correlations

We have demonstrated[17] that Na^+-K^+-enhanced ATPase activity increases markedly in the oocyte membrane within 15 min following ovulation. During the first 15 min following ovulation the oocyte traverses the first 15 cm of oviduct, where the outer vitelline membrane and albumen are formed. This movement through the oviduct is accompanied by a drastic ionic environment change, since the pH of the forming albumen is about 9, compared to that of the yolk at pH 6[18]. This difference in pH between the inside and outside environment of the membrane *in vivo* suggests a functional correlation with pH optima demonstrated for the ATPase of the membrane and

the soluble fraction. This possible relationship suggested further that activation of the membrane ATPase might be mediated by the change in ionic environment. In order to test this hypothesis, ovarian oocytes about to ovulate were stripped of their follicle membranes and placed in the albumen of eggs taken from the upper region of the magnum of the oviduct. Parallel experiments using Hanks basal salt solution (pH 7.4, $I = 0.15$) and distilled water were performed. After incubation at 37° for 1 h, the membranes were isolated and assayed for Na^+-K^+-enhanced ATPase activity in the standard fashion. Results of these preliminary experiments indicate that the ovarian membrane Na^+-K^+-enhanced ATPase can be activated by either the albumen or Hanks basal salt solution.

The system reported in this and the preceding paper[6] offers an excellent opportunity to study an ATPase in its membrane-bound and soluble forms, referred to as allotopy. Such comparative studies should help define the nature of enzyme–membrane interaction. In our system we have found similar response patterns to pH, temperature. Response to cation and ionic strength enhancement or suppression has been similar under proper selection of pH and ionic strength. Under other carefully selected environmental conditions, allotopy could be demonstrated. In addition, both the membrane-bound and "soluble" ATPase appear to represent a family of enzymes that have the marker characteristics of "transport ATPase" and "contractile ATPase". The results of our initial comparative studies of this membrane system emphasize the necessity for meticulous definition and control of the assay conditions, since variations in the species, absolute concentrations and ratios of ions, as well as variations in pH and ionic strength of the reaction, can markedly effect the data and their subsequent interpretation.

ACKNOWLEDGMENTS

We thank Mrs. Mary Jeffreys and Mrs. Judith Beckman for technical assistance. This work was supported in part by U.S. National Institutes of Health Grant No. GM 13547.

REFERENCES

1 J. C. Skou, in J. A. V. Butler and H. E. Huxley, *Progress in Biophysics and Molecular Biology*, Vol. 14, Macmillan, New York, 1964, p. 134.
2 M. E. Pullman, H. S. Penefsky, A. Datta and E. Racker, *J. Biol. Chem.*, 235 (1960) 3322.
3 R. W. Albers, *Ann. Rev. Biochem.*, 36 (1967) 727.
4 E. Racker, *Federation Proc.*, 26 (1967) 1335.
5 E. Munoz, M. R. J. Salton, M. H. Ng and M. T. Schor, *European J. Biochem.*, 7 (1969) 490.
6 J. E. Haaland, E. Etheredge and M. D. Rosenberg, *Biochim. Biophys. Acta*, 233 (1971) 137.
7 A. J. Abrams, *Biol. Chem.*, 240 (1965) 3675.
8 F. Medzihradsky, M. H. Kline and L. E. Hokin, *Arch. Biochem. Biophys.*, 121 (1967) 311.
9 R. Rendi and M. L. Uhr, *Biochim. Biophys. Acta*, 89 (1964) 520.
10 K. Ahmed and J. D. Judah, *Biochim. Biophys. Acta*, 104 (1965) 112.
11 S. Puszkin, S. Berl, E. Puszkin and D. D. Clarke, *Science*, 161 (1968) 170.
12 D. J. Graves, R. W. Sealock and J. H. Wang, *Biochemistry*, 4 (1965) 290.
13 E. A. Havir, H. Tamir, S. Ratner and R. C. Warner, *J. Biol. Chem.*, 240 (1965) 3079.
14 H. S. Penefsky and R. C. Warner, *J. Biol. Chem.*, 240 (1965) 4694.
15 W. F. H. M. Mommaerts and I. Green, *J. Biol. Chem.*, 208 (1954) 833.
16 W. Drabikowski and U. Rafalowska, *Acta Biochim. Polonica*, 15 (1968) 45.
17 J. E. Haaland and M. D. Rosenberg, *Nature*, 223 (1969) 1275.
18 J. Needham, *J. Expt. Biol.*, 8 (1931) 330.
19 J. Riman and B. Thorell, *Biochim. Biophys. Acta*, 40 (1960) 565.