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A COMPARISON OF MICROSOMAL ($Na^+ + K^+$)-ATPase WITH K^+-ACETYLPHOSPHATASE

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SUMMARY

Similar distributions of radioactivity were observed upon paper electrophoresis of pepsin-digested beef brain microsomes previously exposed to either [^{32}P]ATP or acetyl[^{32}P]phosphate in the presence of Na^+ and Mg^{2+}. ATP and acetylphosphate were each competitive inhibitors of the other as substrate for a K^+-requiring acetylphosphatase (acylphosphate phosphohydrolase, EC 3.6.1.7) and for a ($Na^+ + K^+$)-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3).

Despite these similarities, there were marked differences in the inhibitory effect of ouabain, oligomycin, and exposure to *N*-ethylmaleimide on the two enzymes. The data suggest that the K^+-acetyl-phosphatase activity of the beef brain may represent a different entity than the ($Na^+ + K^+$)-ATPase.

INTRODUCTION

Considerable evidence has accumulated that the (Na^+-K^+)-activated ATPase (ATP phosphohydrolase, EC 3.6.1.3) found in microsomal fractions of different tissues plays a part in the active transport of Na^+ and K^+ across the cell membrane. This evidence has recently been reviewed[1]. Microsomal preparations can accept the terminal phosphate from ATP in the presence of Na^+ and Mg^{2+}, but the products are rapidly dephosphorylated if K^+ are introduced into the reaction mixture[2–9]. Studies by HOKIN *et al.*[6] and NAGANO *et al.*[7] suggest that the acid stable phosphorylated intermediate is an acylphosphate group in the microsomes.

BADER AND SEN[8] and YOSHIDA, IZUMI AND NAGAI[9] have observed a K^+-stimulated acetylphosphatase (acylphosphate phosphohydrolase, EC 3.6.1.7) in kidney and brain microsomal fractions that also contained the ($Na^+ + K^+$)-ATPase. The acetylphosphatase was inhibited by ouabain and in the presence of Mg^{2+} was activated by K^+, Rb^+, Cs^+, NH_4^+ or Li^+ but not by Na^+.

On the basis of these and other observations, BADER AND SEN[8] suggested that

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the K^+-acetylphosphatase activity could be the same as the K^+-stimulated part of the ($Na^+ + K^+$)-ATPase that hydrolyzes the phosphorylated intermediate. BOND, BADER AND POST[10] reported that acetylphosphate could reduce the incorporation of ^{32}P from [^{32}P]ATP into guinea pig kidney microsomes and that acetyl[^{32}P]phosphate also formed a labeled phosphorylated intermediate whose formation was activated by $Na^+ + Mg^{2+}$ and reduced by K^+. Although it was not demonstrated whether the labeled compound formed from acetyl[^{32}P]phosphate is the same as that formed from [^{32}P]ATP, it was suggested that acetylphosphate interacts at two sites with the ($Na^+ + K^+$)-ATPase system.

The present communication describes work to determine the nature of the intermediate formed by acetyl[^{32}P]phosphate in the presence of Mg^{2+} and Na^+ in beef brain microsomes and to study whether the K^+-stimulated acetylphosphatase activity is the same as the K^+-stimulated part of the ($Na^+ + K^+$)-ATPase.

METHODS

Enzyme preparation

Beef brain microsomal ATPase was prepared as described by GIBBS, RODDY AND TITUS[4]. In order to reduce the activity of Mg^{2+}-ATPase, the suspension obtained by this procedure was diluted to 12 times its volume with cold 1.0 M NaI in 0.01 M Tris–HCl buffer, pH 7.0, containing 1.0 mM EDTA[11]. This mixture was allowed to stand 15 min at 0°, then centrifuged at 100 000 × *g* for 60 min. After removal of the supernatant, the pellet was washed by resuspension in cold 1.0 mM Tris–HCl buffer, pH 7.0, containing 1.0 mM EDTA and again centrifuged. The pellet was then resuspended in 1.0 mM Tris–HCl buffer, pH 7.0, containing 0.25 M sucrose to a final concentration of about 10 mg of protein per ml and stored at −20°.

Preparation of [^{32}P]ATP and acetyl[^{32}P]phosphate

Terminally labeled [^{32}P]ATP was prepared enzymatically by the method of PFLEIDERER[12] as described in a preceding publication[4].

Acetyl[^{32}P]phosphate was prepared by overnight incubation at room temperature of 0.1 ml of carrier-free [^{32}P]phosphate in 1.0 M HCl (approx. 4 mC), 0.35 ml of 0.1 M Tris–HCl, pH 8.5, 0.10 ml of phosphotransacetylase (Calbiochem; 1500 units/ml), 2.5 mg of Li^+ salt of acetyl-coenzyme A (2.5 μmoles), 0.5 ml of water, and 0.1 ml of 1.0 mM K_2HPO_4. By this method, approximately 15–20% of the ^{32}P was incorporated into acetylphosphate, as determined by thin-layer chromatography in 10% formic acid. Labeled acetylphosphate was not separated from inorganic ^{32}P for these experiments. Preliminary experiments had shown that $^{32}P_i$ does not incorporate into the microsomal protein.

Preparation of Tris–acetylphosphate

0.5 g of dilithium salt of acetylphosphate (Sigma, 75–80% pure, as determined by the acetohydroxamate method)[13] was mixed with 10 ml of water and added to 5 g of Dowex 50-H^+ (Bio-Rad, AG 50W-X8; 200–400 mesh, 2.6 mequiv/g). The mixture was stirred at 0° for 2–3 min, filtered through fritted glass and washed 3 times with 5 ml of cold water. The filtrates were combined and the pH was promptly adjusted to 7.0 with Tris base. The final concentration was determined by the aceto-

hydroxamate method as described by STADTMAN[13]. The yield after resin treatment was 99–100%. At −20° the Tris salt of acetylphosphate was stable for at least 2 months.

ATPase assay

Reaction mixtures containing 2 mM ATP, 5 mM $MgCl_2$ and 0.10 M Tris–HCl, pH 7.4, in a total volume of 1.0 ml were incubated for 4 min at 37°. Included in the 2 μmoles of total ATP in each tube was enough [^{32}P]ATP to provide approximately 100 000 counts/min. ($Na^+ + K^+$)-ATPase was measured by the increase in the rate of P_i liberation when Na^+ and K^+ were present in the reaction mixture. The reaction was started by addition of the enzyme and stopped by addition of 0.5 ml of non-radioactive phosphate reagent[14,15]. The latter was prepared fresh for each experiment by mixing 2 parts of 10% ammonium molybdate, 2 parts of concentrated H_2SO_4 and 1 part of refluxed 0.1 M silicotungstic acid. The solution was shaken vigorously with 3.0 ml of isobutanol for 20 sec and then centrifuged briefly. A 1.0-ml aliquot of the isobutanol was transferred into 5 ml of a phosphor solution. This latter was a mixture of 7 parts of 0.4% bis-(5′-butylbenzoxazolyl (2′))-thiophene solution in toluene, *plus* 3 parts of ethanol. ^{32}P in this solution was determined by counting in a scintillation spectrometer.

Acetylphosphatase

Acetylphosphatase activity was determined by incubating total volume of 1.0 ml containing 2 mM Tris–acetylphosphate; 5 mM $MgCl_2$ and 0.10 M Tris–HCl, pH 7.4, for 10 min at 37°. K^+-Acetylphosphatase was measured by the increase in the rate of hydrolysis when K^+ was added to the reaction mixture. The reaction was started by addition of enzyme and stopped by addition of 0.5 ml of 2 M hydroxylamine, pH 7.0, at 37° (prepared by mixing 1 vol. of 4 M hydroxylamine hydrochloride with 1 vol. of 3.0 M NaOH). By this method acetylphosphate is rapidly converted into the stable acetohydroxamate[13]. More than 90% of the acetylphosphate was converted into acetohydroxamate in 20 sec. After 4 min at 37°, 1.5 ml of acid $FeCl_3$ reagent was added (made by mixing equal volumes of 5% $FeCl_3$ in 0.1 M HCl, 12% trichloroacetic acid and 3 M HCl). Precipitated protein was removed by centrifugation for 5 min at 4000 × *g* and the absorbance of the supernatant was determined at 540 mμ in the Beckman DU spectrophotometer.

Incorporation experiments

Incorporation of ^{32}P from labeled ATP into brain microsomal preparations was determined at 0° as described by GIBBS, RODDY AND TITUS[4]. Oligomycin was added to the reaction tubes as a solution in ethanol, which was evaporated under a stream of N_2 before the addition of the other reagents.

In the experiments in which the electrophoretic patterns of the phosphoprotein formed from [^{32}P]ATP and acetyl[^{32}P]phosphate were studied, 8 mg of enzyme were incubated in 0.5 ml containing 3 mM $MgCl_2$, 60 mM NaCl and $5 \cdot 10^{-5}$ M ATP ($1.8 \cdot 10^7$ counts/min) or $2.0 \cdot 10^{-6}$ M acetylphosphate ($1.2 \cdot 10^8$ counts/min). After 45 sec of incubation, the reaction was stopped with 0.5 ml of 10% trichloroacetic acid and centrifuged at 0–2° for 15 min at 27 000 × *g*. The precipitate was

then washed and centrifuged 3 times with 0.5 ml of a cold solution containing 310 mg Na^+-ATP, 2.5 ml of 0.2 M KH_2PO_4, 1.5 ml of 50% trichloroacetic acid, 2.5 ml of 0.080 M Tris–acetylphosphate, 7.5 ml of water, then washed and centrifuged twice with 0.5 ml of cold 0.01 M HCl. The precipitate was finally resuspended in 0.5 ml of cold 0.01 M HCl and mixed with pepsin (1 mg pepsin/mg microsomal protein). An aliquot was immediately centrifuged (15 min at 27 000 × *g* at 0–2°) and was labeled "zero time". Another aliquot was left at room temperature for 30 min and then centrifuged as above. The supernatant was labeled "pepsin 30 min". Aliquots (50 μl) of supernatant solution corresponding to approximately 0.8 mg of microsomal protein were placed on Whatman No. 1 paper and subjected to high-voltage electrophoresis for 90 min at 4650 V, 150 mA, 8°, in 1% formic acid, pH 2.0. The paper was then dried at room temperature and scanned for radioactivity in a Vanguard Scanner.

RESULTS

Acetylphosphate hydrolysis was Mg^{2+}-dependent. This activity was increased by 6–10 times by addition of K^+ but only by a small fraction by Na^+ (Table I).

TABLE I

EFFECT OF CATIONS AND ATP ON ACETYLPHOSPHATASE ACTIVITY

Acetylphosphatase was measured as described in the text. Concentration of additives were: acetylphosphate, 1.5 mM; $MgCl_2$, 5 mM; KCl, 30 mM; NaCl, 30 mM; ATP, 0.5 mM.

Experiment I	
Acetylphosphate	0.0
Acetylphosphate + K^+	0.0
Acetylphosphate + Na^+	0.0
Acetylphosphate + Mg^{2+}	0.035
Acetylphosphate + Mg^{2+} + K^+	0.240
Experiment II	
Acetylphosphate + Mg^{2+}	0.033
Acetylphosphate + Mg^{2+} + K^+	0.220
Acetylphosphate + Mg^{2+} + Na^+	0.046
Acetylphosphate + Mg^{2+} + K^+ + Na^+	0.171
Acetylphosphate + Mg^{2+} + ATP	0.025
Acetylphosphate + Mg^{2+} + K^+ + ATP	0.094
Acetylphosphate + Mg^{2+} + K^+ + Na^+ + ATP	0.060
Acetylphosphate + Mg^{2+} + Na^+ + ATP	0.029

As can be seen, ATP, in the absence of Na^+, inhibited the K^+-acetylphosphatase. When Na^+ was added to the medium, the inhibitory effect of ATP was only slightly enhanced. Na^+ added in the presence of K^+ also reduced the acetylphosphatase activity. Trinucleotides other than ATP inhibited to lesser extents. The inhibitions in one experiment where 0.5 mM ATP, CTP, UTP and ITP were in turn added to the same preparation were 56, 9, 10 and 38%, respectively. It should be noted that this preparation does not hydrolyze ITP[4].

Comparison of the effects of inhibitors on $(Na^+ + K^+)$-ATPase and K^+-acetylphosphatase

Ouabain inhibits both the K^+-acetylphosphatase and the $(Na^+ + K^+)$-ATPase. The half maximal inhibition concentration of ouabain were determined for both

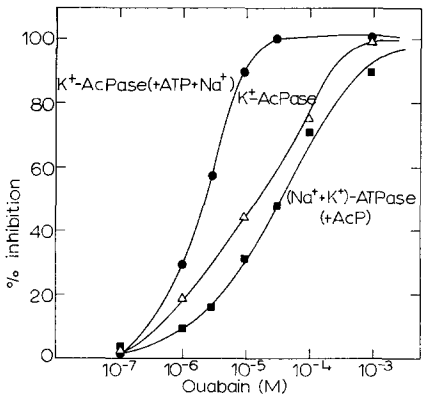

Fig. 1. Effect of ouabain on the $(Na^+ + K^+)$-ATPase and K^+-acetylphosphatase activities (K^+-AcPase). ATP = 0.5 mM, NaCl = 30 mM, KCl = 30 mM, acetylphosphate (AcP) = 2 mM.

enzyme activities in the same tube containing 0.5 mM [^{32}P]ATP, 2 mM acetylphosphate, 5 mM $MgCl_2$, 30 mM KCl and 30 mM NaCl. An aliquot was used to determine residual acetylphosphate and another to determine ^{32}P liberated from [^{32}P]ATP (see METHODS). In Fig. 1 these results are compared with the effect of ouabain on acetylphosphatase when K^+ was the only monovalent cation. $Na^+ + ATP$ decreased the concentration of ouabain needed to produce half maximal inhibition of the acetylphosphatase. When acetylphosphatase and ATPase were measured simultaneously as described above, the K^+ for ouabain was $2.2 \cdot 10^{-6}$ for the K^+-acetylphosphatase and $4.0 \cdot 10^{-5}$ M for the $(Na^+ + K^+)$-ATPase (Fig. 1). This is in disagreement with the findings of BADER AND SEN[8] who found very similar sensitivities

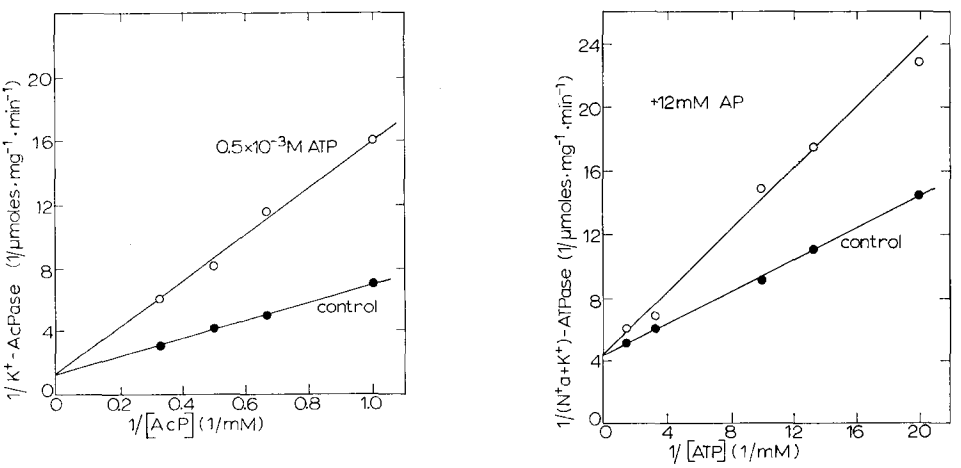

Fig. 2. Effect of substrate concentration on K^+-acetylphosphatase (K^+-AcPase) activity in the presence and absence of $0.5 \cdot 10^{-3}$ M ATP. Data are plotted reciprocally according to the Lineweaver–Burk method. K^+ = 30 mM.

Fig. 3. Effect of substrate concentration on the $(Na^+ + K^+)$-ATPase activity in the presence and absence of acetylphosphate (AcP) Data are plotted as in Fig. 2. Na^+ = 30 mM, K^+ = 30 mM.

to ouabain for both activities on kidney microsomes. This will be discussed later.

The inhibition of K^+-acetylphosphatase by ATP was studied in the absence of Na^+. ATP inhibited competitively, the K_i being 0.31 mM (Fig. 2). When ATP served as substrate in the $(Na^+ + K^+)$-ATPase, the K_m was 0.11 mM (Fig. 3). Acetylphosphate inhibited the $(Na^+ + K^+)$-ATPase competitively with a K_i of 11.4 mM. The K_m for this compound as substrate in the K^+-acetylphosphatase was 5.9 mM (Fig. 2). These crossed competitive inhibitions were also observed by BADER AND SEN[8] in the preparation from kidney.

The effect of oligomycin was also studied on both $(Na^+ + K^+)$-ATPase and K^+-acetylphosphatase (Fig. 4). Concentrations up to 100 μg/ml of oligomycin in the incubation medium did not inhibit the K^+-acetylphosphatase but did inhibit the $(Na^+ + K^+)$-ATPase by more than 50%.

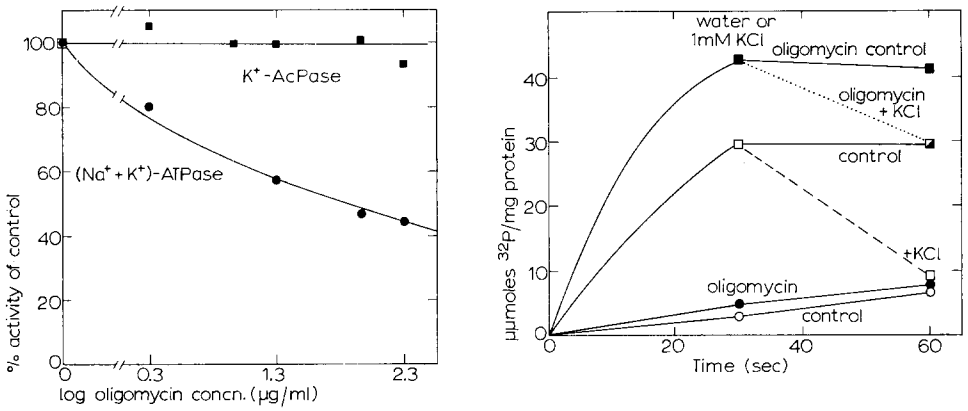

Fig. 4. Effect of oligomycin on $(Na^+ + K^+)$-ATPase and K^+-acetylphosphatase (K^+-AcPase). ATP = 2 mM, acetylphosphate = 2 mM, Na^+ = 30 mM, K^+ = 30 mM.

Fig. 5. Incorporation into microsomal protein of the terminal phosphate of [^{32}P]ATP. Trichloroacetic acid precipitates of microsomes incubated at 0° were prepared for counting as described by GIBBS, RODDY AND TITUS[4]. At 30 sec either KCl or an equal volume of water was added. Oligomycin, when used, was added before incubation at 100 μg/ml. ■, [^{32}P]ATP = 200 mμM ($2 \cdot 10^5$ counts/min per tube), $MgCl_2$ = 50 μM, NaCl = 120 mM; ●, [^{32}P]ATP = 200 mμM, $MgCl_2$ = 50 μM.

The possibility was considered that oligomycin might have inhibited the brain $(Na^+ + K^+)$-ATPase by preventing the formation of the phosphorylated intermediate, rather than by inhibiting the K^+-activated dephosphorylation. Only the latter reaction is inhibited by the compound in ATPase preparations from the electric organ of the eel and from kidney[5,16]. The effect of oligomycin was therefore studied on both the Na^+-dependent phosphorylation and the K^+-dependent dephosphorylation of the labeled trichloroacetic acid-stable intermediate formed from [^{32}P]ATP. As can be seen (Fig. 5), oligomycin did not inhibit the formation of the intermediate but inhibited its dephosphorylation.

The effect of *N*-ethylmaleimide was tested on both $(Na^+ + K^+)$-ATPase and K^+-acetylphosphatase activities. Microsomes were preincubated at 37° at pH 7.4 with Tris–HCl in media containing 10^{-3} M *N*-ethylmaleimide, this compound *plus* 3 mM ATP, or in one without additions. Preincubation was stopped at 5, 13 and

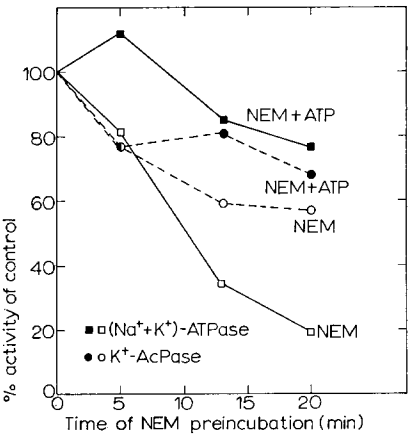

Fig. 6. Effect of preincubation with *N*-ethylmaleimide (NEM) on the activities of $(Na^+ + K^+)$-ATPase and K^+-acetylphosphatase (K^+-AcPase). The enzyme was preincubated at 37° for different times at pH 7.4 adjusted with 50 mM Tris–HCl buffer with either water (control), 1 mM *N*-ethylmaleimide or 1 mM *N*-ethylmaleimide + 3 mM Tris–ATP. Enzyme concentration was approximately 7 mg/ml in the preincubation medium of 3 ml. Preincubation was stopped by removing an aliquot of 0.8 ml and mixing it with 1.2 ml of 20 mM mercaptoethanol ice cold. $(Na^+ + K^+)$-ATPase and K^+-acetylphosphatase were then assayed as described in METHODS. The activity is expressed as percent of the activity of the enzyme preincubated for the same length of time but in the absence of *N*-ethylmaleimide (control).

20 min by mixing an aliquot of 0.8 ml of the microsome mixture with 1.2 ml of ice-cold mercaptoethanol, $2 \cdot 10^{-3}$ M. The resulting enzyme suspension was then assayed for $(Na^+ + K^+)$-ATPase and K^+-acetylphosphatase as described in METHODS. The results in Fig. 6 show some differences between the two activities after the *N*-ethylmaleimide treatment. After 20 min of preincubation with this compound, the K^+-acetylphosphatase activity was 58% of that of the untreated enzyme. Most of the inhibitory effect was already established at 13 min. Under the same conditions the $(Na^+ + K^+)$-ATPase had only 19% of the control activity. ATP, when present in the

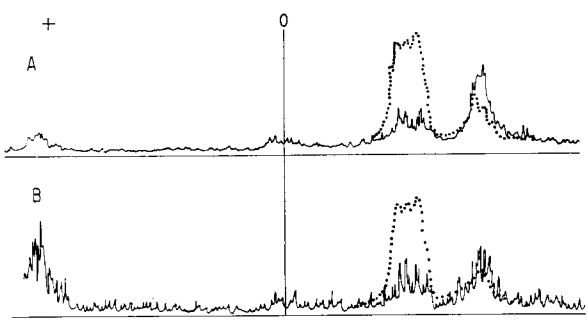

Fig. 7. Paper electrophoresis of radioactivity in soluble fraction obtained by pepsin treatment of microsomes previously incubated with [^{32}P]ATP or acetyl[^{32}P]phosphate. Signs + and − indicate polarity. o indicates point of application of the sample. Solid line indicates radioactivity obtained after treatment of the microsomes with pepsin for 15–20 min at 0° (zero time). The dotted lines represent tracing after treatment of the microsomes with pepsin for 30 min at room temperature (see METHODS). A = [^{32}P]ATP, B = acetyl [^{32}P]phosphate.

preincubation medium together with the *N*-ethylmaleimide had a strong protective effect on the ($Na^+ + K^+$)-ATPase but a much lesser one on the K^+-acetylphosphatase.

Labeling of microsomal protein by acetyl[^{32}P]phosphate

As reported by BOND, BADER AND POST[10] Na^+ in the presence of Mg^{2+} increases the incorporation of ^{31}P from acetyl[^{32}P]phosphate into microsomal protein, the effects being reminiscent of that observed when [^{32}P]ATP is the source of radioactive label. Experiments were performed, therefore, to determine electrophoretically whether intermediate formed from acetylphosphate is the same as the one formed from [^{32}P]ATP.

As can be seen in Fig. 7, two distinct radioactive peaks appear in electrophoretograms of pepsin-treated microsomes previously labeled with either [^{32}P]ATP or acetyl[^{32}P]phosphate. The mobilities were the same, regardless of the substrate used. In the longer incubation with pepsin at room temperature, the faster running peak was reduced and the slow running one increased. This change in proportions again was independent of the substrate used, indicating the similarity between the labeled compound formed from AT^{32}P or acetyl[^{32}P]phosphate.

DISCUSSION

The structural similarity between acetylphosphate and the enzyme-bound acylphosphate postulated to be an intermediate in ($Na^+ + K^+$)-ATPase action would suggest that the two phosphates might compete for hydrolysis at a K^+-activated site on the ATPase. ATP and acetylphosphate each inhibit the hydrolysis of the other. The reduction in acetylphosphatase activity from 0.171 to 0.060 μmole/min per mg in the presence of ATP and Na^+ (Table I) is in agreement with earlier observations in kidney preparations by BADER AND SEN[8]. It is somewhat puzzling, however, that both these authors and ourselves find ATP to inhibit acetylphosphatase in the absence of Na^+, when the phosphorylated protein is not formed from ATP. It is conceivable that adsorption of ATP might stabilize the enzyme in one conformation (presumably the kinase) in which it had no hydrolytic activity and that phosphorylation of the protein or adsorption of acetylphosphate would restore the phosphatase conformation.

Despite the apparent competitive relationship between ATP and acetylphosphate, results presented here incidate that K^+-acetylphosphatase activity and ($Na^+ + K^+$)-ATPase activity are unlikely to be manifestations of the same enzyme. In our experiments ouabain produced half maximal inhibition at $2 \cdot 10^{-6}$ M for the K^+-acetylphosphatase. Under comparable conditions half maximal inhibition occurred at $4 \cdot 10^{-5}$ M for the ($Na^+ + K^+$)-ATPase. BADER AND SEN[8] reported only 20% difference, the acetylphosphatase being less sensitive. However, in their experiments Na^+ was present at 100 mM and K^+ at 25 mM in the ($Na^+ + K^+$)-ATPase assay, whereas in the K^+-acetylphosphatase experiments K^+ was only 10 mM and ATP and Na^+ were absent. Reports by SCHATZMAN[17] have shown that Na^+ increases the sensitivity of the ($Na^+ + K^+$)-ATPase to ouabain. Also, K^+ can overcome part of the ouabain effect[18]. Thus, a strict comparison should be sought in one of two ways. Either K_i for ouabain in the ATPase reaction can be obtained at different concentra-

tions of Na^+ and extrapolated to zero Na^+ or the difficulty can be obviated as it was in the experiments of Fig. 2 by determining the inhibitory effects of ouabain on ATPase and acetylphosphatase in the same medium.

Oligyomycin, as well as ouabain, differentiated between acetylphosphatase and ATPase. The former compound is known to inhibit the dephosphorylation of the intermediate produced from ATP[16]. Its effects were studied in this investigation because of the possibilities shown in the following reaction sequence, namely that acetylphosphate might interact with the ATPase at both of the two steps shown below, not only contributing phosphate to an enzyme-bound intermediate but also serving as a substrate for that part of the enzyme which hydrolyzes the intermediate.

$$\text{Enzyme} + \text{ATP} \xrightarrow[\text{Acetylphosphate}]{Na^+,\ Mg^{2+}} \text{Enzyme} - P + \text{ADP}$$

$$\text{Enzyme} - P \xrightarrow[K^+,\ Mg^{2+}]{\text{Acetylphosphate}} \text{Enzyme} + P_i + \text{acetate}$$

Since Fig. 4 shows that there was no appreciable inhibition of K^+-acetylphosphatase activity by oligomycin, it appears that the enzyme responsible for the second reaction shown above is not that which hydrolyzes acetylphosphate.

Also, different sensitivities to *N*-ethylmaleimide were found. Work by FAHN *et al.*[19] with microsomes prepared from eel electric organ showed that *N*-ethylmaleimide does not inhibit the incorporation of ^{32}P from [^{32}P]ATP in the presence of Mg^{2+} and Na^+. In this respect brain enzyme resembles the eel enzyme and it might be expected that *N*-ethylmaleimide would inhibit both the ATPase and the acetylphosphatase to the same extent. As can be seen in Fig. 6, after 20 min of preincubation with 10^{-3} M inhibitor the microsomes had only 19% of the original ($Na^+ + K^+$)-ATPase activity, while the residual activity of the K^+-acetylphosphatase was 3 times higher (58%).

Perhaps the foregoing observations could be reconciled with the assumption that the hydrolysis of acetylphosphate is carried out by the K^+-activatable entity of ATPase if one postulated a second intermediate of ($Na^+ + K^+$)-ATPase whose formation from the protein-bound acylphosphate was inhibited by oligomycin and *N*-ethylmaleimide. Both this intermediate and acetylphosphate but not enzyme-bound acylphosphate would be hydrolyzed in a K^+-requiring step. Since there is as yet no evidence for such a second intermediate, the more conservative hypothesis is to assume that the K^+-activated acetylphosphatase represents a different entity from the K^+-dependent activity that hydrolyzes the phosphorylated intermediate of the ($Na^+ + K^+$)-ATPase.

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