Protein Kinase Activity at the Inner Membrane of Mammalian Mitochondria

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This paper reports on the discovery of a protein kinase activity associated with the inner membrane of mammalian mitochondria. The enzyme does not respond to addition of cyclic AMP or cyclic GMP and has a preference for whole histone as phosphate acceptor. Some standard assay systems for the cyclic nucleotide-dependent cytosol protein kinases would be unable to pick up this activity if the orthophosphate concentration is higher than 25 mM and the pH of the assay lower than pH 6.5. The enzyme described here has an apparent pH optimum of 8.5. Activity in liver mitochondria is not evident unless the mitochondria are disrupted by either sonication or freezing and thawing. Distribution of kinase activity in centrifugal fractions of both liver and heart mitochondrial sonicates was parallel to that of the two inner membrane marker enzymes succinic dehydrogenase and cytochrome oxidase and quite different from that of the matrix enzyme malic dehydrogenase. Experiments with preparations enriched in outer or inner membranes confirmed the contention that this enzyme is located on the inner membrane. Since disruption of the inner membrane by a freeze-thaw treatment (after the outer membrane had been disrupted by swelling in phosphate) was necessary for full expression of activity by this enzyme, the tentative conclusion was reached that substrate is accepted only from the matrix side of the inner membrane.

Protein phosphorylation and dephosphorylation is emerging as a possible method of control of the functional characteristics of a variety of proteins. In certain cases where the proteins in question are enzymes, there is ample documentation of the physiological importance of such phosphorylation, activity being determined by existence in either a phosphorylated or a dephosphorylated form. Well known examples are phosphorylase, phosphorylase kinase, and pyruvate dehydrogenase. In the case of enzymes associated with glycogen metabolism there is detailed information concerning the characteristics of phospho-dephospho transformations, brought about by hormonal stimuli through cyclic nucleotide-activated kinases. (For a comprehensive review of the recent literature on the subject of protein phosphorylation, see Ref. 1.)

A variety of biological membranes have been shown to exhibit protein kinase activity and contain endogenous phosphate acceptor protein(s). These include erythrocyte plasma membranes (2, 3), polymorphonuclear leukocyte and platelet membranes (4), brain microsomes (5), synaptosomes (6), cardiac sarcoplasmic reticulum (7, 8), and skeletal muscle microsomal membranes (9). The physiological importance of membrane phosphorylation is not established, although distinct correlations have been found in certain cases, between phosphorylation of specific membrane protein components and specialized membrane function (4, 6, 8-14).

Surprisingly little work has been reported about mitochondrial kinases and phosphorylation of mitochondrial membranes. A single report of a mitochondrial kinase that phosphorylates the acidic phosphoprotein phosvitin (15) was recently followed by a communication describing the phosphorylation of mitochondrial membrane protein that had been partially denatured by heat (16). Zahlten et al. (17) have reported on the in vivo phosphorylation of mitochondrial membrane of rat liver and the stimulating effect of glucagon.

EXPERIMENTAL PROCEDURES

Materials - [γ-32P]ATP was obtained from New England Nuclear. Histone fractions, DPN+, DPNH, ATP, coenzyme A, cytochrome c, phenazine methosulfate, and oligomycin were purchased from Sigma Chemical Co. Cocarboxylase was obtained from Pierce. All other reagents were obtained from regular commercial sources and were of the highest purity.

Preparation of Mitochondria - Mitochondria were prepared from mouse liver and mouse heart using a standard procedure detailed by Chefurka (18) except that final suspensions were 20 to 25 mg of protein/ml and 3 to 4 mg of protein/ml for the liver and heart preparations, respectively. Beef hearts were obtained from a local abattoir and mitochondria were prepared according to Procedure 1 of Smith (19).

Sonication of Mitochondrial Suspensions - Mitochondrial suspensions (1.6 to 2.0 ml) were subjected to sonic vibration at 0° at an MSE ultrasonic power unit tuned to maximum amperage. To avoid localized temperature increases, we gave three treatments of 1-min duration with 30-s cooling periods between them. The sonicated mitochondria were then subjected to simple centrifugal fractionation as described under "Results."

Preparation of Outer and Inner Membrane of Mouse Liver Mitochondria - Mitochondria were prepared and subjected to swelling in phosphate exactly as described by Parsons and Williams (20). Crude outer and inner membrane fractions obtained by the above procedure were dialyzed for 2 h against sucrose to eliminate orthophosphate which inhibited the protein kinase. The fractions were also subjected to freeze-thaw treatment and centrifugal fractionation as detailed under "Results."

Enzyme Assays - Protein kinase assays were performed by measuring incorporation of 32P from [γ-32P]ATP into 10% trichloroacetic acid-precipitable material. Initially, the following two standard incubation mixtures were tried at a temperature of 37° and in a total volume of 75 μl: A. 50 mM potassium phosphate (pH 6.0), 20 mM NaF, 0.2 mM EDTA, 0.3 mM EGTA, 1.0 mM caffeine, 12 μg/ml of histone II and...
of assay system A. At pH 6.0, which is much below the optimal pH for this kinase, 50 mM orthophosphate totally inhibits the enzyme.

Optimal conditions for the assay were next sought using a high speed supernatant solution from sonicated mouse liver mitochondria. The pH optimum was found to be 8.5 (Fig. 1). At this pH rates were constant for 20 min, and were directly proportional to enzyme concentration up to 300 μg of protein (Fig. 2). However, with crude sonicated mitochondrial preparations rates declined after only 10 min of incubation and the response of activity (measured as incorporation in a 15-min period) to enzyme concentration was not linear. We felt that a likely explanation for this behavior would be substrate depletion, since it was likely that ATP would be fairly readily hydrolyzed by the mitochondrial preparations that we were using. It was therefore decided to investigate the effects of NaF (a general inhibitor of phosphatases) and oligomycin, (a specific inhibitor of the F1 ATPase of mitochondria and submitochondrial particles) on the activity of our mitochondrial protein kinase. The sonicated mitochondrial preparation was also subjected to simple centrifugal fractionation and the distribution of protein kinase activity in these fractions was investigated. The first fraction, an 8700 x g for 10 min pellet was intended to contain mitochondria that were either undamaged or incompletely sheared by the treatment. The second fraction, obtained by centrifuging the supernatant of the fraction above at 100,000 x g for 60 min would presumably contain all inside-out inner membrane vesicles produced by the treatment while the third fraction, the final supernatant, would of course contain the matrix. The results of these experiments showed that the addition of either NaF or oligomycin led to higher rates of histone phosphorylation, probably through inhibition of reactions that lead to ATP depletion. For example, the oligomycin effect was more pronounced in the fractions that would be expected to contain the oligomycin-sensitive F1 ATPase. Since with at least some of the fractions tested, the effects of NaF and oligomycin were more or less additive it was decided to incorporate both of these substances into the incubation mixture of the protein kinase assay system C with which all of the results that follow were obtained.

RESULTS

Initial Observations — In this section a series of preliminary experiments are described which demonstrated that a protein kinase occurs in mitochondria and which established suitable experimental conditions for the study of this enzyme. Initially, the two standard assay systems indicated as A and B under "Experimental Procedures" were used with intact or sonicated preparations from mouse liver mitochondria. Enzyme activity was observed with system B, although not with system A. Activity was almost totally dependent on addition of histone as phosphate acceptor. There were two findings that immediately seemed to be of importance. First, most of the activity was latent, with 10- or 15-fold activation in mitochondrial particles. The pH optimum was 8.5 (Fig. 1). At this pH rates were constant for 20 min, and were directly proportional to enzyme concentration up to 300 μg of protein (Fig. 2). However, with crude sonicated mitochondrial preparations rates declined after only 10 min of incubation and the response of activity (measured as incorporation in a 15-min period) to enzyme concentration was not linear. We felt that a likely explanation for this behavior would be substrate depletion, since it was likely that ATP would be fairly readily hydrolyzed by the mitochondrial preparations that we were using. It was therefore decided to investigate the effects of NaF (a general inhibitor of phosphatases) and oligomycin, (a specific inhibitor of the F1 ATPase of mitochondria and submitochondrial particles) on the activity of our mitochondrial protein kinase. The sonicated mitochondrial preparation was also subjected to simple centrifugal fractionation and the distribution of protein kinase activity in these fractions was investigated. The first fraction, an 8700 x g x 10 min pellet was intended to contain mitochondria that were either undamaged or incompletely sheared by the treatment. The second fraction, obtained by centrifuging the supernatant of the fraction above at 100,000 x g for 60 min would presumably contain all inside-out inner membrane vesicles produced by the treatment while the third fraction, the final supernatant, would of course contain the matrix. The results of these experiments showed that the addition of either NaF or oligomycin led to higher rates of histone phosphorylation, probably through inhibition of reactions that lead to ATP depletion. For example, the oligomycin effect was more pronounced in the fractions that would be expected to contain the oligomycin-sensitive F1 ATPase. Since with at least some of the fractions tested, the effects of NaF and oligomycin were more or less additive it was decided to incorporate both of these substances into the incubation mixture of the protein kinase assay system C with which all of the results that follow were obtained.

Nature of Observed Latency of Mitochondrial Protein Kinase — As shown above very little activity was evident with intact mitochondria, as compared to mitochondria disrupted by sonic vibration. The simplest explanation for this finding is that the kinase is separated from the suspending medium by a membrane impermeable to at least one of the two substrates. Since histone is the macromolecular substrate in this reaction it is probably the one that does not penetrate, and the barrier could be the outer or inner membrane, the latter being the most likely candidate. In the assay of cytochrome oxidase in mitochondria the same situation in fact exists, since the inner membrane is impermeable to the substrate ferrocyanochrome c. It was therefore decided to do a series of experiments in order to compare the effects of mitochondrial disruption on protein kinase and cytochrome oxidase activity. Two methods of disrupting were used, sonication or a freeze-thaw treatment. These experiments were extended to include mitochondria from heart muscle. The results of two representative experiments, given in Table I, demonstrate close similarity of the effects of disruption on the two enzyme activities. With mitochondria from liver for example there was, as expected, a manifolds increase in protein kinase activity when mitochondria were disrupted by either sonication or freeze-thawing. This observation also held true for cytochrome oxidase activ-
Mitochondrial Protein Kinase

FIG. 1. Response of protein kinase activity to pH. The enzyme was a 100,000 × g × 60 min supernatant of a sonicated preparation of mitochondria from liver. Assay system B was used except for the buffer which was 10 mM phosphate (○), 10 mM Tris (×), or 10 mM glycine HCl (●). Incubation time was 30 min.

TABLE I
Parallel effects of mitochondrial disruption on protein kinase and cytochrome oxidase activities

Mitochondria were prepared and sonicated as described under "Experimental Procedures." The freeze-thaw treatment consisted of three cycles of freezing in an acetone dry ice bath and thawing at room temperature. System C with 25 μl of enzyme was used for the assay of protein kinase and 5 μl of enzyme from the same preparation was assayed for cytochrome oxidase as described under "Experimental Procedures."

| Tissue | Treatment of mitochondria | Protein kinase activity | Cytochrome oxidase activity |
|--------|---------------------------|-------------------------|-----------------------------|
|        |                           | Experiment I            | Experiment II                |
|        |                           | pmol 32 P incorporated/mg protein/min | μmol ferrocytochrome c oxidized/min/mg protein |
| Mouse  | None                      | 0.06                    | 0.092                       |
| liver  | Sonication                | 2.73                    | 0.269                       |
|        | Freeze-thaw               | 25.33                   | 0.810                       |
| Mouse  | None                      | 39.33                   | 0.990                       |
| heart  | Sonication                | 24.49                   | 0.633                       |
|        | Freeze-thaw               | 56.89                   | 1.136                       |
|        |                           | 76.35                   | 1.80                        |

ity. In the case of heart muscle mitochondria the untreated preparation has much higher levels of both protein kinase and cytochrome oxidase activities, which were approximately doubled by disruption.

Association of Protein Kinase with Inner Membrane—The three centrifugal fractions of sonicated mitochondria described above were assayed for cytochrome oxidase as well as another enzyme known to be associated with the inner membrane, succinic dehydrogenase, and an enzyme present in the matrix, malic dehydrogenase. The observations on the distribution of these enzymes made with liver mitochondria and heart mitochondria are presented in the form of histograms, following De Duve et al. (27), in Figs. 3 and 4. As can be seen the distribution of protein kinase coincides with that of the two inner membrane marker enzyme.

Next we prepared fractions from mitochondria enriched in outer or inner membranes by using the technique of swelling in phosphate followed by centrifugal fractionation (20). Monamine oxidase was used as a marker of outer membrane (24), while cytochrome oxidase activity was taken to indicate the presence of inner membrane. The orthophosphate had to be eliminated from all enzyme fractions since it was found to inhibit the protein kinase. Preliminary experiments had shown that even after swelling, which disrupts the outer membrane, it was necessary to subject the swollen inner membrane matrix particles to freeze-thaw extraction in order to measure maximum protein kinase and cytochrome oxidase activities. For the above reasons the experimental regime adopted in these experiments was as follows: swelling and separation of crude outer membrane and inner membrane-matrix fractions, freeze-thaw extraction of all fractions, pelleting of membrane fractions at 100,000 × g for 60 min, resuspension of pellets to original volume, dialysis against 0.25 M sucrose to eliminate orthophosphate, and assay of all fractions for the three enzyme activities mentioned above. The distribution of activity is shown in Fig. 5. Protein kinase followed rather closely the distribution of cytochrome oxidase and was different from that of monamine oxidase.

A systematic attempt to separate outer from inner membrane-matrix in mouse liver mitochondria using digitonin was
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Table IV. Of interest is the fact that the sum of activities in the
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Greenawalt (28). Whenever significant mobilization of mem-
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tonin/mg of mitochondrial protein in the absence and presence
of albumin, respectively), it was found to be a mixture of outer
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marker enzymes, and was always accompanied by loss of
respiratory control in the treated particles.1

Substrate Specificity and Effects of Cyclic Nucleotides—
Table II shows results obtained from experiments with differ-
ent substrate proteins as acceptors. Only minor differences
were obtained in the relative utilization of these proteins by
the different fractions, with histone II being the best acceptor
with all fractions. The one exception was the very small
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It was essential to confirm the absence of an effect of cyclic
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Mitochondria were prepared and sonicated as described in "Exper-
imental Procedure." The sonicated mitochondrial preparation was
then subjected to centrifugal fractionation as outlined below. Assay
of protein kinase was done in system C except for the type of acceptor
protein used which was as shown. The histone fractions are
Sigma designations. All acceptor proteins were added at a final
concentration of 6.6 mg/ml.

| Substrate | Protein kinase activity |
|-----------|------------------------|
| Histone II | 146.0 | 100.1 | 80.4 |
| Histone II-A | 94.1 | 74.1 | 70.9 |
| Histone III | 40.5 | 47.0 | 35.7 |
| Histone IV | 57.6 | 22.3 | 14.6 |
| Casein | 38.0 | 38.2 | 3.9 |
| Protamine | 12.2 | 19.9 | 5.0 |

The digitonin experiments were done in collaboration with Dr.
W. Chefurka of our Institute.

The digitonin experiments were done at three digi-
tonin concentrations (0.12, 0.15, and 0.20 mg of digitonin/mg of
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Next we investigated the effect of histone addition with each
centrifugal fraction. The results of a typical experiment with
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Table IV. Of interest is the fact that the sum of activities in the
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1 2.0 Succinic dehydrogenase 1.0 Cytochrome oxidase
2.0 Protein kinase 1.0 Malic dehydrogenase

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Nonidentity of Present Protein Kinase and Pyruvate Dehydrogenase Kinase—Although unlikely, the possibility exists that the activity under study could be due to pyruvate dehydrogenase kinase. This regulatory system, originally elucidated by Reed and his associates (25, 29, 30), seems to be insensitive to cyclic AMP (31). Since the pyruvate dehydrogenase complex is located in the matrix, the distribution of pyruvate dehydrogenase (and hence its kinase by virtue of its tight binding to the complex) in the various centrifugal fractions was compared with that of our protein kinase. As pyruvate dehydrogenase has been most thoroughly studied, and its assay is simplest, in heart muscle preparations, our experiments were carried out with this tissue. Unfortunately, pyruvate dehydrogenase activity in intact mitochondria from mouse heart was (for reasons not yet determined) lost upon sonicaton. We therefore prepared mitochondria from bovine heart and compared the distribution of these two enzymes. Starting with the supernatant of a 15,000 × g × 10 min spin, we obtained two pellets and a final supernatant. As can be seen from the results (Table V) most of the pyruvate dehydrogenase activity was recovered in the high speed pellet, in agreement with the known properties of the complex. In contrast, the two pellets contained similar amounts of protein kinase activity, with smaller but significant amounts in the final supernatant.

**DISCUSSION**

The main conclusion reached in this paper is that mammalian mitochondria contain a protein kinase capable of catalyzing the phosphorylation of histone, and possibly of endogenous proteins as well, by ATP. The possibility that the activity is due to contamination of the mitochondrial preparations by endoplasmic reticulum or cytosol enzymes can be reasonably excluded. First, in initial experiments, the postmitochondrial supernatant solution was, as expected, found to contain considerable protein kinase activity. Highest activity in this case was obtained with assay system A (where our kinase was completely inactive) and, in addition, 5- to 6-fold increases in activity were obtained by addition of cyclic AMP or cyclic GMP (in contrast to the results with the mitochondrial protein kinase). The second reason for discounting contamination is the fact that most of the mitochondrial kinase activity is latent (see Tables I, III, and IV). This is particularly evident with preparations of liver mitochondria which show a 5- to 55-fold increase in activity upon disruption by either sonic vibration or freezing and thawing. Lastly, we have positive evidence (see below) that the activity is associated with the inner mitochondria membrane and is therefore unlikely to have originated from either endoplasmic reticulum or cytosol.

The simplest explanation for the latency exhibited by this activity is that the enzyme is located on the other side of a

**Table III**

Protein kinase activity in centrifugal fractions of a freeze-thaw preparation of mouse liver mitochondria and effect of cyclic nucleotides

The mitochondrial preparation was subjected to three cycles of freezing in an acetone-dry ice bath and thawing at room temperature. It was then fractioned with the centrifuge as described below. Pellets were made up to original volume with 0.25 M sucrose before assay. Assay system C was used in the presence or absence of 0.01 mM cyclic AMP or 0.1 mM cyclic GMP as shown.

| Mitochondrial fraction used | Protein kinase activity |  
|----------------------------|-------------------------|
|                            | Control +Cyclic AMP +Cyclic GMP | pmol 32P incorporated/mg protein/min |
| 1. Intact                  | 1.0 2.2 2.8             |
| 2. Freeze-thaw             | 22.7 25.2 22.1          |
| 3. Pellet, 8,700 × g (10 min), from Fraction 2 | 25.0 27.4 27.2 |
| 4. Pellet, 8,700 × g, 100,000 × g (60 min), from Fraction 2 | 128.5 128.6 125.8 |
| 5. Final supernatant       | 32.4 25.1 33.2           |

**Table IV**

Effect of histone addition on protein kinase activity of mouse liver mitochondrial fractions and presence of possible endogenous acceptor

Preparation and freeze-thawing of mitochondria as in Table III. Assay system C was used as described under "Experimental Procedures" except for the presence or absence of histone as shown.

| Fraction used | Protein kinase activity |  
|---------------|-------------------------|
|               | −Histone +Histone       | pmol 32P incorporated/mg protein/min |
| 1. Intact mitochondria | 167 3.1 53 1.0  |
| 2. Freeze-thaw mitochondria | 214 4.1 1336 25.3 |
| 3. Pellet, 8,700 × g (10 min), from Fraction 2 | 157 3.3 1336 28.7 |
| 4. Pellet, 8,700 × g–100,000 × g (60 min), from Fraction 2 | 451 141.0 788 246.4 |
| 5. Final supernatant | 38 4.2 369 40.6 |

**Table V**

Protein kinase and pyruvate dehydrogenase activity in centrifugal fractions of sonicated beef heart mitochondria

Mitochondria were prepared and sonicated as described under "Experimental Procedures." The preparation was then spun at 15,000 × g for 10 min and the supernatant was subjected to further centrifugal fractionation. All pellets were resuspended to original volume before assay. Assay system C was used for protein kinase. Pyruvate dehydrogenase activity was assayed as described under "Experimental Procedures."

| Fraction used | Protein kinase activity | Pyruvate dehydrogenase activity |  
|---------------|-------------------------|
|               | Specific | Total in fraction | % | Specific | Total in fraction | % |
| Pellet, 30,000 × g (30 min) | 34.0 305.5 | 41 | 9.9 55.8 | 14 |
| Pellet, 30,000 × g–100,000 × g (60 min) | 102.6 330.3 | 44 | 158.7 611.1 | 79 |
| Final supernatant | 17.3 111.6 | 15 | 6.9 44.4 | 7 |

* Measured in picomoles of 32P incorporated/mg of protein/min.

* Measured in nanomoles of DPNH produced/mg of protein/min.
membrane impermeable to the substrate histone. Consistent with this explanation is the finding that with heart mitochondria which are more likely to be damaged because of the necessity of a somewhat harsher initial homogenization procedure, approximately half-maximal activity is obtained with "intact" mitochondria before intentional membrane disruption (Table I). The argument is strengthened by observations on the extent of the increase in the oxidation of ferrocyanochrome c brought about by sonication or freeze-thawing of these mitochondrial preparations from mouse liver and heart (Table I.) Due to the anatomical complexity of the mitochondrial particle, with two membranes and two distinct spaces bounded by these membranes, several possibilities existed as to the location of our protein kinase, while either membrane could be the barrier to substrate histone. The data obtained initially did not eliminate any of these possibilities. Later results revealed a characteristic distribution of protein kinase activity that allowed us to place the enzyme on the inner membrane and identify this membrane as the barrier to histone. First, simple centrifugal fractionation of sonicated mitochondrial preparations of liver and heart led us to believe that the kinase is membrane-bound, since the distribution of its activity followed rather closely that of the two inner membrane marker enzymes succinic dehydrogenase and cytochrome oxidase in both liver and heart mitochondria, and was very different to that of the matrix enzyme malic dehydrogenase (Figs. 3 and 4). Some protein kinase activity is detected in the final supernatant fraction of the liver preparation (Fig. 3), in contrast to the case with the heart preparation (Fig. 4), but this is likely to be the result of the sonication treatment; for after freezing and thawing a much smaller proportion of the total activity is recovered in this fraction (See Table IV). The results of the swelling experiments (Fig. 5) confirmed the suspicion that the kinase resides at the inner membrane since it followed the distribution of cytochrome oxidase rather than monoamine oxidase. In addition, the fact that freeze thaw treatment of the inner membrane-matrix fraction is necessary for measuring the full activity of the kinase indicates that the inner membrane is the barrier to substrate histone and that the latter can be accepted by the kinase only from the matrix side of the membrane.

The next group of experiments was designed to investigate some of the general properties of the mitochondrial kinase. In these experiments, performed with mixtures of mitochondrial enzymes we had to recognize the possibilities of: (a) more than one kinase being present and (b) the presence of other enzymes such as phosphoprotein phosphatases or histone-degrading proteinases (39) that could interfere with our assay. For these reasons we examined all three of the centrifugal fractions mentioned above, of mitochondrial preparations disrupted by either freezing and thawing or sonication. The hope was that differential distribution of interfering enzyme activities would reveal properties that may have been missed in the crude preparation. It was found (Table V) that the distribution of pyruvate dehydrogenase complex activity in centrifugal fractions of a sonicated mitochondrial preparation after the freeze-thaw treatment. I feel that the most likely explanation for this finding is that the endogenous acceptor is subject to rapid dephosphorylation by a phosphoprotein phosphatase present in the crude enzyme (Fraction 2) and at least partly absent in the high speed pellet preparation (Fraction 4). This endogenous acceptor activity was also obtained in the same fraction in preparations where the mitochondria were disrupted by sonic vibration rather than freezing and thawing. The isolation of this acceptor protein and a study of its dephosphorylation is being undertaken in our laboratory. Another observation, reported in Table IV, was that the sum of total activity obtained in all centrifugal fractions was significantly higher than the total activity of the crude preparation. This finding is probably due to significant dephosphorylation of phosphohistone in the crude preparation coupled with unequal distribution of the kinase and phosphatase activities in the centrifugal fractions.

One possible endogenous acceptor protein is pyruvate dehydrogenase, which is known to be phosphorylated by a specific kinase (25, 29-31, 33, 34). Indeed, the argument could be proposed that the kinase reported here was pyruvate dehydrogenase kinase. This possibility is not likely for several reasons. The latter kinase does not phosphorylate histone (33) and is not strongly bound to any membrane, but is an integral part of the pyruvate dehydrogenase complex, which is found in the mitochondrial matrix. Heart muscle has a much higher content of pyruvate dehydrogenase complex than liver (34). All of these properties are in complete contrast to what we have found with the kinase described in this paper. Additional evidence for the separate identity of the two enzymes was obtained in the present experiments. This was done with bovine heart muscle for the reasons outlined under "Results." It was found (Table V) that the distribution of pyruvate dehydrogenase complex activity in centrifugal fractions of a sonicated mitochondrial preparation was quite different from the histone kinase activity. Since pyruvate dehydrogenase kinase binds tightly to the pyruvate dehydrogenase complex it can be safely assumed to be distributed with the dehydrogenase and is therefore, not likely to be the enzyme under study.

Any suggestion about the possible physiological function of the mitochondrial protein kinase described in this paper would at this stage be pure speculation. An attractive possibility would be involvement of this kinase with ion and metabolite
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transport phenomena controlled by the inner membrane. It is indeed interesting that in vivo, of the two mitochondrial membranes, the inner membrane is preferentially phosphorylated (17). The apparent preference of this kinase for histone as phosphate acceptor is surprising especially since there seems to have been no reports of mitochondrial histones. In this respect, we find the recent report by Heinrich et al. (32) of a mitochondrial histone-degrading enzyme very intriguing. They find that the only subcellular organelle containing a histone degrading neutral protease activity in liver was the mitochondrion.

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