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PROTEIN KINASES ASSOCIATED WITH PERIPHERAL NERVE MYELIN

1. PHOSPHORYLATION OF ENDOGENOUS MYELIN PROTEINS AND EXOGENOUS SUBSTRATES

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SUMMARY

When highly purified myelin from rat sciatic nerve was incubated with [γ-^{32}P]ATP, protein components of the membrane were phosphorylated indicating the presence of both the substrate (receptor protein) and an endogenous kinase in the membrane. Polyacrylamide gel electrophoresis of the phosphorylated membrane proteins followed by scintillation counting of gel slices and autoradiography showed that the polypeptides of molecular weights 28000, 23000 and 19000 were phosphorylated, and ^{32}P from [γ-^{32}P]ATP having been incorporated into serine residues of the substrate proteins. Phosphorylation of purified myelin was Mg^{2+}-dependent, was optimal at pH 6.5 and was not stimulated by adenosine 3′,5′-monophosphate. We found that proteins other than those in myelin, such as phosvitin, casein, protamine and histones, can also act as a substrate for the membrane associated kinase. Muscle protein kinase inhibitor had no effect on the endogenous phosphorylation of myelin proteins or on the phosphorylation of phosvitin by peripheral nerve myelin protein kinase. However, the phosphorylation of histone by peripheral nerve myelin protein kinase was inhibited by the protein kinase inhibitor. After washing the membrane with 150 mM KCl the protein kinase that utilizes histone as substrate was found in the supernatant. In contrast, the endogenous phosphorylation of membrane proteins or the phosphorylation of phosvitin by the membrane associated kinase was not affected by washing.

From these findings we conclude that at least two protein kinase systems exist in purified peripheral nerve myelin. One system is not inhibited by muscle kinase inhibitor, is tightly bound to the membrane and utilizes as its receptor proteins either exogenous phosvitin or endogenous membrane proteins. The second system is inhibited by muscle kinase inhibitor, is removable from the membrane and utilizes histones as its receptor proteins.

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*′-tetraacetic acid.

INTRODUCTION

Membrane associated protein kinase systems have been described in several tissues (refs. 1–11; for a review see ref. 12). In this report we should like to describe the protein kinase systems present in myelin obtained from peripheral nerve of the rat. We found, as described for other membranes, that when a highly purified preparation of myelin is incubated with [γ-^{32}P]ATP, certain of the membrane proteins are phosphorylated, indicating the presence in the membrane of both the kinase and the substrate proteins. We also found that exogenous proteins could be phosphorylated when incubated with myelin and [γ-^{32}P]ATP, indicating that they can also act as a substrate for myelin associated kinase. On the basis of the specific effect of protein kinase inhibitor [13–15] and the ability to selectively wash one of the kinases from the membrane, at least two protein kinase systems have been shown to be present in myelin, neither of which can be stimulated by adenosine 3′,5′-monophosphate (cyclic AMP).

It is of particular interest that we find important differences between the protein kinase system which has been recently described for the central nervous system myelin [7, 10] and the peripheral nervous system myelin which is described in this communication. The implications of the differences as well as the function of the enzymatic systems awaits further definition.

MATERIALS AND METHODS

Materials. Male albino rats were used throughout and were obtained from Zartman Farms, Douglassville, Pa. [γ-^{32}P]ATP was obtained from New England Nuclear Corp. Histone (Type 11A), casein, protamine, albumin and phosvitin, beef heart protein kinase and cyclic AMP were obtained from Sigma Chemical Co. Reagents for gel electrophoresis were obtained from Eastman Organic Chemicals. All other chemicals used were of reagent grade. Rabbit skeletal muscle protein kinase inhibitor was a gift from Dr. J. D. Corbin of Vanderbilt University, Nashville, Tenn., and was also isolated in this laboratory from rabbit skeletal muscle [16], and was purified up to trichloroacetic acid precipitation step.

Preparation of myelin. Myelin was isolated by a modification of the method of Autilio et al. [17] which we used previously for the isolation of myelin from mouse brain [18] and human, rat and rabbit peripheral nerve [19–21], using a Beckman L2-65B preparative ultracentrifuge. The methods for the isolation of myelin and the criteria for its purity have been discussed in our recent publications [19–21]. The method is modified only in that in the final purification step we used, instead of cesium chloride, a continuous gradient of sucrose, starting from 0.3 M and increasing up to 1.3 M. Briefly, the method includes a series of flotations in a discontinuous gradient, "osmotic shock" for the separation of myelin from other neuronal material, as well as the final isolation of myelin by flotation in a continuous sucrose gradient. The myelin was then washed with water to remove sucrose and suspended in water, and was used immediately for the determination of protein kinase activity.

Enzyme assays. Phosphorylation of the myelin proteins was assayed in a total volume of 0.2 ml at 37 °C for 5 min. The standard assay, unless otherwise indicated, contained 70–125 μg of membrane protein, 50 mM sodium phosphate

buffer (pH 6.5), 12.5 mM magnesium acetate, 0.3 mM ethyleneglycol-bis-(β-amino-ethylether)-*N,N'*-tetraacetic acid (EGTA), and 5 μM [γ-^{32}P]ATP, (1.5–2.5 · 10^6 cpm). The reaction was started by the addition of [γ-^{32}P]ATP and similar results were obtained when the reaction was started by the addition of enzyme. The reaction was stopped by the addition of 1.5 ml of ice cold 10 % trichloroacetic acid; and 0.2 ml of albumin (1 mg/ml) was added as carrier protein. After standing at 0 °C for 10 min the precipitate was recovered by centrifugation at 2500 × *g* for 10 min and the supernatant was decanted. The precipitate was suspended in 0.2 ml of ice cold 1.0 M NaOH and reprecipitated by the addition of 1.5 ml of ice cold 10 % tricholoroacetic acid. The procedure of centrifugation, dissolving the precipitate in NaOH and reprecipitation with trichloroacetic acid was repeated once. The final precipitate was dissolved in 0.2 ml of 1.0 M NaOH and 10 ml of scintillation counting solution containing 0.4 % (w/v), 2,5-diphenyloxazole (PPO), 0.01 % (w/v) of 1,4-bis-[2-(4-methyl-5-phenyl-1,3-oxazolyl)]benzene (dimethyl POPOP), 9.5 % (v/v) of BIO-SOLV BBS-3 (Beckman instruments) in toluene. All assays were done at least in triplicate. The blank value obtained in the absence of Mg^{2+} was subtracted from the value obtained in the presence of magnesium acetate. Similar blank values were obtained if boiled enzyme was used or if ATP was added after the addition of trichloroacetic acid. All results are expressed as pmol ^{32}P incorporated/mg of membrane protein per 5 min. The reaction was linear with an increasing amount of membrane protein in both the endogenous phosphorylation of myelin proteins or when phosvitin was used as an exogenous substrate. Initial rate of incorporation was also linear with time for 3 min and remained at a maximal level in incubations up to 10 min, at which time a decrease was noted possibly indicating the presence of a phosphatase.

The specific activity of heart muscle protein kinase under the assay condition described above for myelin was 13.4 nmol of phosphate transferred/5 min per 1.0 μg of protein kinase using 100 μg of histone as substrate. This activity was stimulated more than 3-fold in the presence of 1 μM cyclic AMP.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In experiments in which proteins were analyzed by polyacrylamide gel electrophoresis, the assay described above was scaled up 5–10 times.The reaction was stopped by the addition of trichloroacetic acid and the addition of albumin as carrier protein was omitted. The procedure for delipidation of myelin, gel electrophoresis in sodium dodecyl sulfate, staining with Coomassie blue, destaining, scanning of gels and the determination of radioactivity in gel slices, have been described previously [20, 21].

Autoradiography of gel slices. Gels after staining and destaining were sliced longitudinally with a device using three thin wires essentially as described by Fairbanks, et al. [22]. One of the central 1.5 mm thick slices was dried on a filter paper and was covered with saran wrap paper. Autoradiography using an RP/SS-14 medical X-ray film was performed for 1–4 days and the film was then developed using Kodak X-omat automatic developer.

Acid hydrolysis of [γ-^{32}*P*]*ATP-labeled peripheral nerve myelin proteins.* After the labeling of the myelin with ^{32}P the proteins were isolated as described [20]. The final protein pellet was suspended in 0.3–0.5 ml of 6 M HCl and hydrolyzed for 4 h at 110 °C in ampoules sealed under nitrogen. After drying in vacuo, the samples were dissolved in water and applied to 3 MM paper and the components were separated by high voltage electrophoresis in 2.5 % formic acid/7.8 % acetic acid

(pH 2.1) at 3.0 kV for 1.5 h [23]. Standard phosphoserine, phosphothreonine and phosvitin were treated with 6 M HCl as described for the myelin sample and were simultaneously run as markers. The amino acids were visualized by staining with cadmium-ninhydrin spray. The radioactivity in amino acids was determined by two methods. In one method the paper was cut into 1-cm strips following electrophoresis and radioactivity of each strip was counted by scintillation spectrometry. In the other method the radioactivity was located by autoradiography.

RESULTS

In vitro phosphorylation of peripheral nerve myelin

Fig. 1 shows the labeling pattern obtained after peripheral nerve myelin was incubated with [γ-^{32}P]ATP. Polypeptides of molecular weight 28000, 23000 and 19000 were all labeled under the experimental conditions used (see Materials and Methods). The phosphorylation of endogenous proteins was not simply proportional to the amount of each protein present. The polypeptide of molecular weight 19000 present in relatively small amounts was the most extensively phosphorylated. Autoradiography of the gels confirmed that these were the only protein components

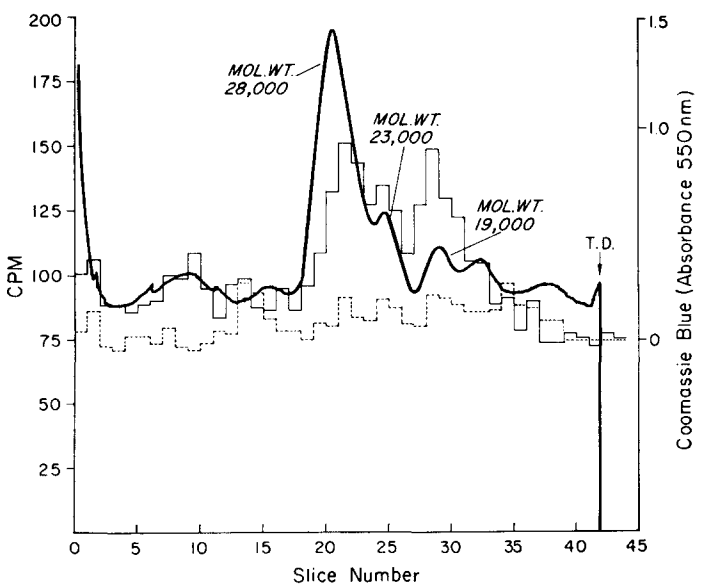

Fig. 1. Distribution of radioactivity and relative intensity of Coomassie blue stain after incubation of rat sciatic nerve myelin with [γ-^{32}P]ATP for 5 min at 37 °C. The assay system (2.0 ml) contained 50 mM phosphate buffer (pH 6.5), 12.5 mM magnesium acetate, 0.3 mM EGTA and 5 μM [γ-^{32}P]-ATP, (15–25 · 10^6 cpm). After incubation the proteins were precipitated with 10 % trichloroacetic acid, delipidated and separated on 15 % polyacrylamide gels containing 0.1 % sodium dodecyl sulfate. The gel was stained with Coomassie blue and the intensity of stained bands recorded on Gilson spectrophotometer with a scanning attachment. The gels were then sliced into 2 mm thick sections, dissolved in hydrogen peroxide and counted. Blocked solid trace, ^{32}P counts; blocked broken trace, ^{32}P counts in incubation done in the absence of Mg^{2+}; curved trace, absorbance at 550 nm. The molecular weights of various peptides on the curve are also shown [20].

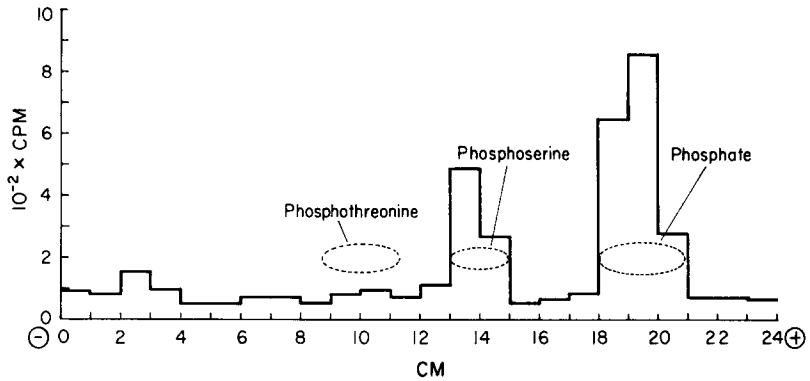

Fig. 2. High voltage electrophoresis following limited hydrolysis of phosphorylated sciatic nerve myelin proteins after incubation with [γ-^{32}P]ATP. The phosphorylated myelin was hydrolysed in 0.5 ml of 6 M HCl for 4 h at 110 °C in ampoules sealed under nitrogen. The hydrolysate was subjected to electrophoresis on Whatman No. 3MM paper for 1.5 h at 3.0 kV at pH 2.1 (2.5 % formic acid/ 7.8 % acetic acid). Phosphothreonine and phosphoserine hydrolysed under the same conditions were used as standards. After electrophoresis the radioactive components were detected by autoradiography or the paper was cut into 1-cm strips and radioactivity was determined by scintillation counting. Phosphoserine and phosphothreonine used as standards were visualized by staining with cadmium ninhydrin spray.

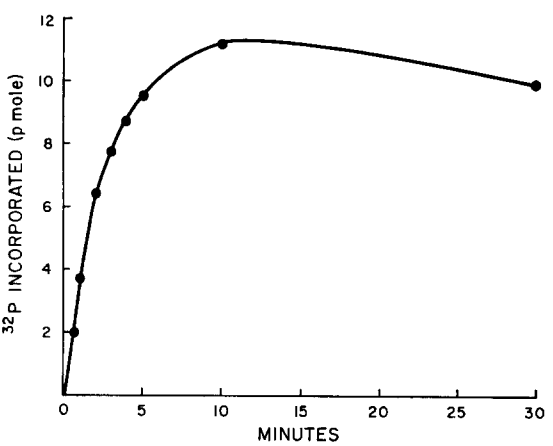

Fig. 3. Time course of incorporation of phosphate from [γ^{32}P]ATP into endogenous proteins by rat sciatic nerve myelin associated protein kinase. The complete assay system (0.2 ml) contained 50 mM sodium phosphate buffer (pH 6.5), 12.5 mM magnesium acetate, 0.3 mM EGTA, 5 μM [γ^{32}P]ATP (1.5–2.5 · 10^6 cpm) and 70–125 μg of membrane protein. Incubations were carried out at 37 °C for the time period indicated. Reaction was stopped by the addition of 1.5 ml of ice cold 10 % trichloroacetic acid and 0.2 ml of albumin (1 mg/ml) was added as carrier protein. Values are corrected for the blank values obtained in the absence of magnesium acetate.

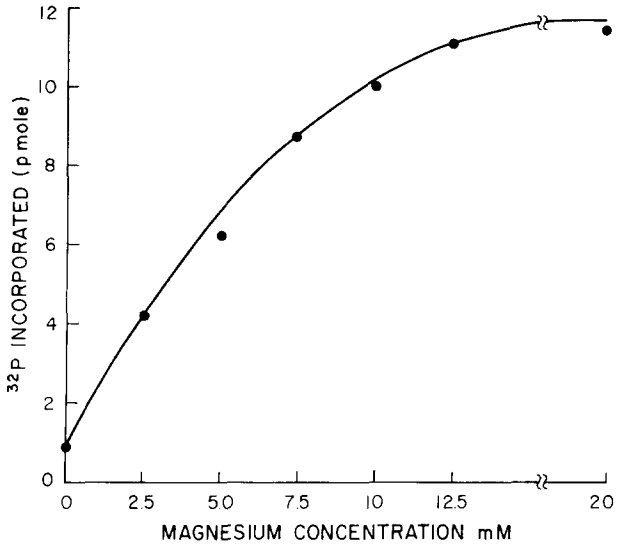

Fig. 4. Effect of Mg^{2+} concentration on the phosphorylation of endogenous proteins by rat sciatic nerve myelin associated protein kinase. The assay system was the same as described in the legend to Fig. 3 except that the concentration of Mg^{2+} was varied as indicated. Incubations were carried out at 37 °C for 5 min.

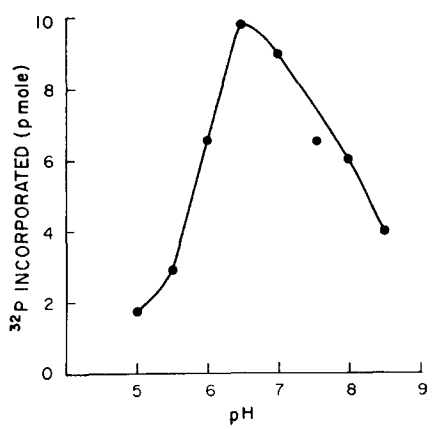

Fig. 5. Effect of pH on phosphorylation of peripheral nerve myelin proteins. Assay conditions are described in the legend to Fig. 3 except that pH of the system was varied as indicated and reaction was carried out at 37 °C for 5 min.

TABLE I

PHOSPHORYLATION OF VARIOUS SUBSTRATES BY RAT PERIPHERAL NERVE MYELIN ASSOCIATED PROTEIN KINASE

Experiments were performed as stated in Fig. 3 except that when exogenous substrate was used each tube contained 100 μg of protein as indicated. The reaction was terminated by the addition of 10% trichloroacetic acid. Activity is expressed as pmol of ^{32}P incorporated per mg of membrane protein per 5 min incubation and represents the acid precipitable protein. All values represent average of triplicate determinations on each of 5 different preparations of myelin.

Substrate	Activity (pmol/mg per 5 min)
Myelin	8.5
Myelin+cyclic AMP (1 μM)	7.7
Myelin+cyclic AMP (50 μM)	7.7
Myelin+cyclic AMP (1 μM)+theophylline (2 mM)*	7.7
Myelin+NaF (10 mM)	8.5
Myelin+cyclic GMP (1 μM)*	8.7
Myelin+cyclic GMP (100 μM)*	8.9
Myelin+histone Type IIA	26.2
Myelin+histone Type IIA+cyclic AMP (1 μM)	23.4
Myelin+albumin	9.7
Myelin+casein	14.0
Myelin+phosvitin	19.6
Myelin+protamine	18.4

* Determinations on one preparation of myelin.

TABLE II

EFFECT OF RABBIT MUSCLE PROTEIN KINASE INHIBITOR ON PROTEIN KINASE OF BEEF HEART AND ON PROTEIN KINASE OF RAT SCIATIC NERVE MYELIN

The activity of beef heart protein kinase was determined by using 100 μg of histone (Type IIA) as substrate and the experiments are performed as described in Table I.

Reaction mixture	Relative specific activity
Beef heart protein kinase	
Protein kinase	100
Protein kinase+cyclic AMP, (1 μM)	326
Protein kinase+cyclic AMP, (1 μM)+inhibitor (10 μl)	37
Protein kinase+cyclic AMP, (1 μM)+inhibitor (20 μl)	15
Protein kinase+inhibitor (10 μl)	12
Protein kinase+inhibitor (20 μl)	5
Myelin protein kinase	
Myelin	100
Myelin+cyclic AMP, (1 μM)	91
Myelin+inhibitor (10 μl)	92
Myelin+inhibitor (20 μl)	90
Myelin+cyclic AMP (1 μM)+inhibitor (20 μl)	113
Myelin+histone (100 μg)	308
Myelin+histone (100 μg)+inhibitor (10 μl)	93
Myelin+phosvitin (100 μg)	230
Myelin+phosvitin (100 μg)+inhibitor (10 μl)	207

phosphorylated. In the absence of Mg^{2+}, radioactivity throughout the gel did not differ significantly from the blank values and is shown in Fig. 1. In order to get further evidence that the phosphate was incorporated into proteins, and to identify the amino acids being phosphorylated, the membrane obtained after incubation with [γ-^{32}P]ATP was delipidated and hydrolyzed. The hydrolysate was then analyzed by high voltage paper electrophoresis [23]. Phosphoserine, phosphothreonine and phosvitin hydrolyzed under identical conditions were used as standards. As shown in Fig. 2, limited hydrolysis of the ^{32}P-labeled membrane protein gives products which move electrophoretically with phosphoserine. The position of the radioactive compounds was also confirmed by autoradiography.

Characteristics of the myelin protein kinase

Time course. The initial rate of incorporation of ^{32}P into endogenous substrate was linear with time for 3 min and is shown in Fig. 3. It remained at maximal level in incubation up to 10 min, after which a decrease in label occurred. Addition of NaF (10 mM) had no effect on the kinetics of the reaction (not shown).

pH and Mg^{2+} optimum. The phosphorylation reaction had requirements similar to those described for other protein kinases and there was an absolute requirement for magnesium [2, 24–26]. The variation of enzyme activity with Mg^{2+} concentration is shown in Fig. 4, and the maximal activity was observed at a concentration of 12.5 mM. The effect of pH on the phosphate incorporation into endogenous substrate is shown in Fig. 5. Maximal activity was observed at pH 6.5.

Effect of cyclic AMP and phosphorylation of exogenous substrates

No significant stimulation of phosphorylation occurred in the presence of cyclic AMP* (1 μM, and 50 μM) or cyclic AMP plus theophylline or guanosine 3′,5′-monophosphate (cyclic GMP) (see Table I). As shown in Table II, utilizing the same assay conditions, we were able to demonstrate a more than 3-fold stimulation of heart muscle protein kinase activity by 1 μM cyclic AMP. The ability of peripheral nerve myelin associated protein kinase to phosphorylate different exogenous protein substrates with ATP as the phosphate donor was tested (Table I). It is interesting to note that both acidic proteins, such as phosvitin, and basic proteins, such as histone, were phosphorylated. Phosphorylation of exogenous substrate was not stimulated by cyclic AMP. [γ-^{32}P]GTP did not serve as a phosphate donor for the kinase associated with peripheral nerve myelin. When myelin was incubated with protein kinase from beef heart, no enhancement of phosphorylation occurred and only the endogenous phosphorylation was observed.

Effect of protein kinase inhibitor from rabbit skeletal muscle on peripheral nerve myelin protein kinase

This protein kinase inhibitor is very effective when used with the cyclic AMP dependent protein kinase of heart muscle (Table II), using histone as the substrate [13–15]. The amount of inhibitor which decreased the heart muscle kinase activity to 10 % of that in the presence of cyclic AMP had no effect on the endogenous phosphorylation of myelin (Table II). However, the phosphorylation of the histone by

* Our preliminary experiments on cyclic AMP-binding by peripheral nerve myelin show that this activity is absent in this membrane (unpublished results).

peripheral nerve myelin kinase was inhibited. It is interesting to note that phosphorylation of histone by myelin protein kinase was completely inhibited by inhibitor and the value obtained in the presence of inhibitor was due to the endogenous phosphorylation of myelin. In contrast, the inhibitor had no effect on the phosphorylation of phosvitin by peripheral nerve myelin protein kinase.

Effect of washing peripheral nerve myelin with 150 mM KCl

The effect of washing peripheral nerve myelin with 150 mM KCl on the protein kinase activity associated with KCl supernatant and particulate fraction are shown in Table III. Endogenous phosphorylation of myelin proteins or phosphorylation of phosvitin by KCl washed myelin was similar to that of water washed myelin and protein kinase inhibitor had no effect on these activities. The phosphorylation of histones by myelin after KCl washing was considerably reduced and this activity was recovered in the KCl supernatant. KCl supernatant did not phosphorylate phosvitin to any significant extent. Cyclic AMP had no effect on phosphorylation of histone by KCl soluble enzyme and the protein kinase inhibitor inhibited this reaction.

Since the protein kinase activity of myelin towards histone was readily removed with KCl, experiments were designed to examine whether this activity is a component of myelin or was present in the peripheral nerve homogenate and had adsorbed to it during the isolation procedure. Myelin was isolated from rat sciatic nerves and divided into two fractions. One fraction of myelin was washed with water and the other with 150 mM KCl. The washed myelins were then added to fresh nerve homogenates and the myelin was reisolated and the protein kinase activity was deter-

TABLE III

SOLUBILITY CHARACTERISTICS OF PROTEIN KINASE ASSOCIATED WITH PERIPHERAL NERVE MYELIN

Peripheral nerve myelin (4.0 mg protein) was extracted with ice-cold 150 mM KCl (2.0 ml) for 10 min. The particulate (membrane) and soluble fractions were then separated by centrifugation for 20 min at 80 800 × *g*. The membrane fraction was suspended in water and the protein kinase activity in the particulate and supernatant was determined. The activity in the KCl supernatant was related back to the amount of mg of myelin protein that was used for its extraction. The recovery of protein kinase activity towards histone in KCl-washed myelin and KCl supernatant was 85–95 % of that of water washed myelin. The data is the average of 3 determinations on 4 different preparations of myelin. Protein kinase assay was performed as described in the legend to Fig. 3.

Additions	Water-washed myelin (pmol/mg per 5 min)		KCl-washed myelin (pmol/mg per 5 min)	
	Particulate fraction	Supernatant	Particulate fraction	Supernatant
None	8.5	(−)	7.9	1.8
Inhibitor (10 μl)	9.8	(−)	7.2	0.2
Histone (100 μg)	26.2	(−)	10.2	15.9
Histone (100 μg)+inhibitor (10 μl)	7.9	(−)	7.1	0.3
Histone (100 μg)+cyclic AMP (1 μM)	(−)	(−)	(−)	16.1
Phosvitin (100 μg)	19.6	(−)	19.6	1.9
Phosvitin (100 μg)+inhibitor (10 μl)	17.6	(−)	17.6	1.8

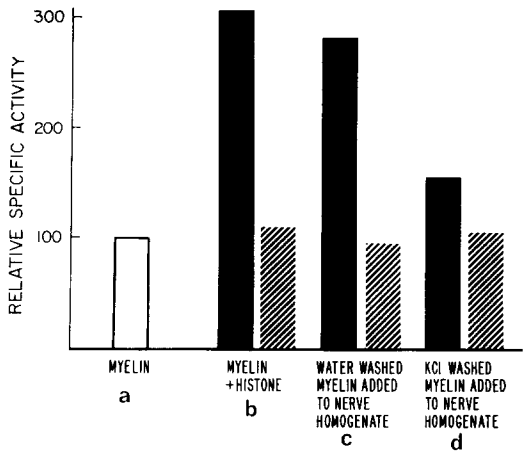

Fig. 6. Effect of adding water-washed and KCl-washed myelin to nerve homogenate on the protein kinase activity. Water-washed and KCl-washed myelins were added to fresh nerve homogenates. Myelin was reisolated and protein kinase specific activity using histone as substrate in the absence of inhibitor of muscle protein kinase (solid bars) and in its presence (hatched bars) was determined as described in the legend to Fig. 3. (a) Endogenous phosphorylation of myelin (open bar) in the absence of histone. (b) Water-washed myelin. (c) Myelin reisolated after adding water-washed myelin to the nerve homogenate. (d) Myelin reisolated after adding KCl-washed myelin to the nerve homogenate.

mined. As shown in Fig. 6, the protein kinase specific activity towards histone in the material to which KCl washed myelin was added was half that of the group to which water washed myelin was added. These specific activity values indicate that KCl washed myelin does not bind any protein kinase activity when homogenized with rat sciatic nerve. Protein kinase activity, in the absence of cyclic AMP and using histone as substrate, was also determined in total nerve homogenate and it was 4 times the total activity of that present in isolated myelin. This suggests that sufficient protein kinase activity was present in total nerve homogenate to have bound to KCl-washed myelin, had the removable activity represented the material nonspecifically adsorbed to myelin.

DISCUSSION

Protein kinase systems have been classified on the basis of their ability to be stimulated by cyclic AMP and by the effect of the inhibitor of muscle protein kinase [14, 15]. According to this proposal, the cyclic AMP dependent protein kinase system (Type I) consists of a regulatory and a catalytic subunit which is activated by cyclic AMP due to a complex formation between the regulatory subunit and cyclic AMP with the release of active catalytic subunit (Type II protein kinase), which no longer requires cyclic AMP for activation but which is inhibited by the inhibitor of muscle protein kinase. Protein kinases that are apparently distinct from this system, that are neither activated by cyclic AMP nor inhibited by muscle protein kinase inhibitor, have been designated as Type III. We find that at least two protein kinase systems are present in myelin isolated from rat peripheral nerve, one categorized as Type II and the other as Type III. No protein kinase activity that can be stimulated

by cyclic AMP was found in myelin, indicating the absence in myelin of Type I protein kinase.

The Type II protein kinase system present in myelin utilized histone as substrate. It could be removed from the membrane by washing it with 150 mM KCl and by definition was inhibited by rabbit muscle protein kinase inhibitor. It does not phosphorylate endogenous myelin proteins. These findings would suggest that a catalytic subunit without its regulatory subunit is present in purified peripheral nerve myelin. This finding is subject to several interpretations. It is possible that this enzyme is not a constituent of myelin but is non-specifically adsorbed to it during isolation of myelin from the nerve homogenate. We provide evidence from recombination experiments that from whole nerve homogenates, protein kinases do not adsorb to purified myelin under the conditions used for the isolation of the membrane, making this an unlikely possibility. It is also possible that while the catalytic subunit is a bonafide constituent of peripheral nerve myelin, it was, prior to membrane isolation, associated with its regulatory subunit. This explanation is unlikely since dissociation of this complex in other systems has not occurred under the conditions utilized in our studies for the isolation of membrane [11]. The possibility exists, however, that a regulatory subunit is present in peripheral nerve which under different circumstances is complexed to or is free of the myelin catalytic subunit.

The Type III protein kinase activity is apparently tightly bound to the membrane. It phosphorylates both endogenous membrane proteins as well as phosvitin and this reaction was neither stimulated by cyclic AMP nor inhibited by the inhibitor of muscle protein kinase. Washing the membrane with 150 mM KCl had no effect on these activities. The phosphorylation of phosvitin, an acidic protein, has been shown to be associated with Type III kinase in other systems [14] and its substrate specificities are different from those observed for Type II kinase.

The properties of the protein kinases associated with peripheral nerve myelin are different from those of the central nervous system myelin [7–10] in at least two respects: (a) protein kinases associated with the central nervous system myelin phosphorylates only endogenous basic proteins of mol. wt. 19000 and 16000, while major proteolipid proteins or any other protein(s) were not phosphorylated. In contrast, the peripheral nerve myelin associated protein kinase phosphorylated a basic protein (mol. wt. 19000) and two other major polypeptides of this membrane (mol. wt. 28000 and 23000). (b) Histone was phosphorylated by brain myelin protein kinase and this reaction was slightly stimulated by cyclic AMP [9]. In the case of sciatic nerve myelin, no stimulation of histone phosphorylation occurred when cyclic AMP was added to the reaction mixture. Further, the myelin from the central nervous system could be phosphorylated by an exogenous soluble protein kinase [7–10]. In the present study no enhancement of phosphorylation of intact peripheral nerve myelin protein was observed using a protein kinase from bovine heart*. This, however, could be due to differences in the structure of these two membranes or to the source of the enzyme used.

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* Although the intact rat sciatic nerve myelin is not phosphorylated by heart muscle protein kinase, we have found that (i) boiled myelin (both rat and rabbit), (ii) native rabbit myelin (iii) both rat and rabbit myelin treated with phospholipase A or C are excellent substrates for this enzyme (manuscript in preparation).

The biological role of these kinase systems in vivo is unknown*. In other tissues the cyclic AMP dependent protein kinase systems have been shown to be a part of the mechanisms by which hormones exert their effects on cells [27]. We have shown that insulin and other hormones can affect certain metabolic aspects of myelin [21] and the possibility exists that the kinase system we have found in myelin, particularly the Type II system, may play a role in the effects of hormones on myelin. While the role of the Type II kinase is unknown, that of the Type III system is even more uncertain. The presence of the tightly membrane-associated protein kinase suggests that the degree of phosphorylation of myelin proteins is variable and is under the control of the type III kinase system. This variation in the degree to which membrane proteins are phosphorylated could be an important determinant of the spatial relationships between these protein molecules and the lipids of this membrane.

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* In some experiments, intact sciatic nerve was incubated with [γ-^{32}P]ATP and myelin proteins were analyzed by polyacrylamide gel electrophoresis. In these experiments, the labeling of proteins was qualitatively similar to that observed when purified myelin was incubated with [γ-^{32}P]ATP indicating that this reaction can occur in intact cells. We have also shown that myelin proteins are phosphorylated when intact sciatic nerves are incubated with $Na_2H^{32}PO_4$ (unpublished results).

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