Biochem. J. (1983) **209**, 789–795
Printed in Great Britain

Partial characterization of the phosphotransferase system of human central-nervous-system myelin

Niou-Ching WU, Jay E. YOURIST,* William D. RECTOR and Fazal AHMAD
Papanicolaou Cancer Research Institute, 1155 N.W. 14th Street, P.O. Box 016188, Miami, FL 33101, U.S.A.

(*Received 5 October 1982/Accepted 22 October 1982*)

The phosphotransferase system of human central-nervous-system myelin was investigated. Evidence obtained indicated the presence of at least two different phosphotransferase systems (cyclic nucleotide-dependent and -independent) in myelin, which were found to be firmly associated with the membrane. The cyclic AMP-dependent kinase of myelin and white-matter cytosol preferentially phosphorylated certain histone fractions and displayed only modest activity with basic protein as substrate. On the other hand, the cyclic nucleotide-independent system showed specificity toward basic protein. Its activity was not only dependent on Mg^{2+} but it was greatly enhanced by this bivalent cation. Whereas the cyclic nucleotide-dependent kinase could be extracted with buffers containing Triton X-100, the bivalent cation-regulated kinase resisted solubilization from myelin under these conditions.

The presence of cyclic AMP-stimulated protein kinase (EC 2.7.1.37) in brain was first described by Miyamoto *et al.* (1969). Weller & Rodnight (1970) and Maeno *et al.* (1971) then found substantial levels of the enzyme in membrane fractions prepared from ox brain and rat cerebrum. Since then a number of investigators have shown that myelin basic protein is phosphorylated under conditions *in vitro* by cyclic AMP-dependent protein kinases purified from different sources (Carnegie *et al.*, 1973; Miyamoto & Kakiuchi, 1974; Steck & Appel, 1974; Yourist *et al.*, 1975). The phosphorylation of basic protein has also been found to occur under conditions *in vivo* and the data indicate that the turnover rate of the protein-bound phosphoryl group is fairly rapid (Carnegie *et al.*, 1974; Miyamoto & Kakiuchi, 1974; Steck & Appel, 1974; Agrawal *et al.*, 1982).

The mode of regulation of myelin-associated kinase(s) has been of interest for some time, but the precise mechanism by which myelin kinases are regulated is not yet firmly established. An attempt was made to compare the properties of the enzyme solubilized from rat myelin with the kinase while it was still associated with the membrane. Whereas phosphorylation of the endogenous basic protein catalysed by the membrane-associated kinase was not affected by cyclic AMP, phosphorylation of histones catalysed by the enzyme extracted from rat myelin with Triton X-100 was activated 6–8-fold by the cyclic nucleotide (Miyamoto & Kakiuchi, 1974; Steck & Appel, 1974). However, addition of cyclic AMP to the soluble enzyme preparations engendered only a modest (20–30%) increase in the rate of phosphorylation of basic protein (Miyamoto, 1975). Subsequently Miyamoto *et al.* (1978) detected multiple forms of protein kinase when the detergent-solubilized extract of bovine brain myelin was subjected to ion-exchange chromatography. The significance of these studies was not clear since proteins (histones, protamine, phosvitin or casein) other than myelin basic protein were employed as the substrate. Besides cyclic AMP-dependent kinase and the kinase whose mode of regulation is not completely understood, Petrali *et al.* (1980*a*,*b*) and Sulakhe *et al.* (1980) have found yet another kinase in rat central- and peripheral-nervous-system myelin preparations. This enzyme requires Mg^{2+} and its activity is greatly enhanced by Ca^{2+} and calmodulin.

We have previously described the presence and purification of a basic protein-specific phosphoprotein phosphatase from human central-nervous-system myelin (Yourist *et al.*, 1978; Wu *et al.*, 1980). The present paper describes some properties of the phosphotransferase system of human central-nervous-system myelin preparations. A preliminary report of these results has been published (Ahmad *et al.*, 1981).

* Present address: Department of Medicine, University of Miami School of Medicine, Miami, FL, U.S.A.

0306-3275/83/030789-07$2.00

Materials and methods

Materials

Benzamidine, different histones, phosphatidic acid, phosphatidylinositol, phosphatidylcholine, leupeptin, antipain, pepstatin A, 1-chloro-4-phenyl-3-L-toluene-*p*-sulphonamidobutan-2-one, 7-amino-1-chloro-3-L-tosylamidoheptan-2-one and protein kinase inhibitor were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Phosphatidylserine and phosphatidylethanolamine were obtained from P.-L. Biochemicals (Milwaukee, WI, U.S.A.). Triton X-100 was from Polysciences (Warrington, PA, U.S.A.) and [γ-^{32}P]ATP (sp. radioactivity 1–3 Ci/mmol) was obtained from New England Nuclear Corp. (Boston, MA, U.S.A.). All other chemicals were of the highest purity commercially available.

Isolation and purification of myelin basic protein

Myelin basic protein was prepared from human brain by the method of Deibler *et al.* (1972). The final step of the purification procedure involves chromatography on a Sephadex G-100 column. Each fraction containing basic protein was subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and only those fractions showing a single protein band were pooled, concentrated and then used in the experiments described below.

Isolation of myelin

We isolated myelin essentially by the method of Uyemura *et al.* (1972) with some modifications. White matter was separated from grey matter by careful scraping of human brain (autopsy material 12–24 h after death) with a razor blade at 4°C. After homogenization in 0.32 M-sucrose (15 strokes in a Teflon/glass homogenizer) the white matter was processed immediately for myelin isolation or it was stored at −70°C for future use. In a typical preparation 5 g of white matter was suspended in 100 ml of 0.32 M-sucrose and then homogenized. The homogenate (12 ml) was layered over 15 ml of 0.85 M-sucrose and the tubes were centrifuged at 50000 ***g*** for 45 min. Myelin was collected from the 0.32 M/0.85 M interface. The contents from eight tubes were pooled, centrifuged and the pellet was suspended in about 200 ml of cold water. After homogenization in a Potter–Elvehjem homogenizer (five strokes) the contents were allowed to stand for 15 min and then centrifuged at 20000 ***g*** for 30 min. The cloudy supernatant solution was discarded. The pellet was rehomogenized in cold water and recentrifuged at 20000 ***g***. The pellet constituted one gradient myelin.

To achieve further purification, myelin is carried through the above steps (discontinuous gradient centrifugation and osmotic shocks) two to four more times. Myelin purified through three successive gradients is usually mostly white in appearance. A number of preparations have been examined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Besides some minor protein bands, the most prominent components were basic protein, proteolipid and the Wolfgram protein. Presence of degraded product(s) of basic protein was not evident in these myelin preparations. The sodium dodecyl sulphate/polyacrylamide-gel electrophoretic profiles observed in our laboratory resemble those reported for human myelin by others (Everly *et al.*, 1977; Zetusky *et al.*, 1979). The specific activity of the cyclic nucleotide phosphodiesterase of three gradient myelin preparations was enriched about 6-fold over white-matter homogenate. Further purification did not lead to additional enrichment of cyclic nucleotide phosphodiesterase.

Protein kinase assay

Unless otherwise indicated, the protein kinase activity was measured in a total volume of 100 μl containing 50 mM-Tris/HCl buffer, pH 8.0, 10 mM-$MgCl_2$, 10 mM-dithiothreitol, 100 μg of basic protein or other substrate proteins, 110 μM-[γ-^{32}P]ATP (100 c.p.m./pmol) and an appropriate amount of protein kinase. The mixture was incubated at 37°C for 5 min and the reaction was terminated by the addition of 20 μl of acetic acid. The incorporation of phosphate into the acceptor protein was measured by the method of Huang & Robinson (1976), which involves separation of the reaction product from unchanged ATP. Radioactivity was determined in a liquid-scintillation spectrometer with the use of the scintillation cocktail described by Fricke (1975). One unit of enzyme activity represents the transfer of 1 nmol of P_i from [γ-^{32}P]ATP to the acceptor protein per min at 37°C.

Preparation of phospholipid vesicles

Individual phospholipids (1 mg/ml) were suspended in 20 mM-Tris/HCl buffer, pH 8.0, and dispersal was achieved by sonication with a W-375 sonicator (Heat System, Plainview, NY, U.S.A.), equipped with a sonic cup. Sonication was performed intermittently at 0°C for 2 min followed by a 30 s cooling period for a total time of 10–20 min in a test tube under an N_2 blanket. The dispersed phospholipids were kept on ice and portions of the individual phospholipids were added to the assay mixture before initiating the phosphorylation reaction.

Other methods

Protein was measured by the method of Bradford (1976). Protein concentration of myelin preparations was determined by the method of Lowry *et al.* (1951) after digestion of the membrane in 0.5%

sodium deoxycholate/0.5 M-NaOH (Petrali *et al.*, 1980*a*). Bovine serum albumin was used as a standard. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was performed by the method of Weber & Osborn (1969).

Results

Properties of myelin-assoiated kinases

Substrate specificity and cyclic nucleotide effects. Despite numerous reports demonstrating the presence of protein kinase activities in myelin preparations, some doubts are still expressed whether these enzymes are indeed intrinsic to myelin membrane. Therefore, in the present study myelin purified through one, three and five successive sucrose-density-gradient centrifugations has been investigated. The distribution of protein kinase activity of different myelin preparations measured with histone V and basic protein as substrate is presented in Table 1.

Table 1. *Protein kinase activity of various myelin preparations in the presence and absence of cyclic AMP*

Myelin purified through one, three and five successive sucrose-density-gradient centrifugations was suspended in buffer A [20mM-Tris/HCl, pH 7.5, containing 20mM-benzamidine, 2mM-dithiothreitol and 0.15% (v/v) Triton X-100] and then assayed for protein kinase activity as described in the Materials and methods section. White matter was homogenized in 10vol. of buffer A without Triton X-100 in a Waring blender for 30s at 4°C and the homogenate was centrifuged at 100000*g* for 1h. Supernatant solution was assayed for protein kinase activity. Cyclic AMP was present at a final concentration of 10μM where indicated. Protein kinase activities are expressed as means ± S.D. Myelin preparations derived from three different brains were examined. Death-to-autopsy time interval varied between 12 and 24h. Storage of myelin at −70°C for 24h results in about 50% loss of the basic protein phosphotransferase activity. The reasons for this loss are not clear. However, myelin isolated from white matter previously homogenized in 0.32M-sucrose and stored at −70°C for up to 2 months contains basic protein phosphotransferase levels comparable with those found in myelin isolated from freshly dissected tissue.

Enzyme source	Cyclic AMP	Kinase activity (units/mg) Histone V	Basic protein
One-gradient myelin	−	2.0 ± 0.2	2.5 ± 0.2
	+	4.2 ± 1.3	2.8 ± 0.3
Three-gradient myelin	−	1.7 ± 0.1	3.3 ± 0.5
	+	3.0 ± 0.6	3.4 ± 0.4
Five-gradient myelin	−	1.5 ± 0.1	3.5 ± 0.4
	+	2.6 ± 0.6	3.6 ± 0.4
White matter cytosol	−	0.8 ± 0.4	0.3 ± 0.1
	+	2.3 ± 0.3	0.5 ± 0.2

Myelin purified through one gradient centrifugation had the highest kinase activity. The activity determined with histone V decreased somewhat in three gradient myelin preparations, whereas the phosphotransferase activity assayable with basic protein as substrate showed some enrichment over the levels found in less pure membrane preparations. Additional purification (up to five gradients) neither enriched nor did it bring about a significant change in the ratios of these activities over the values found in three gradient myelin preparations. Since repeated density-gradient centrifugations and osmotic shocks failed to release the basal and the cyclic AMP-stimulated kinase activities we conclude that these enzymes constitute a tightly associated phosphotransferase system of human central-nervous-system myelin.

Stimulation by cyclic AMP was observed only when the reactions were performed with histone (especially histone V) as substrate. A heat-stable protein has been used as a tool to distinguish cyclic AMP-dependent protein kinases from other protein kinases (Traugh *et al.*, 1974). This inhibitor effectively blocked the stimulatory effect of cyclic AMP during histone V phosphorylation but it had no effect on the rate of phosphorylation of basic protein (Table 2), indicating that perhaps phosphorylation of basic protein was being catalysed by the basal or the cyclic AMP-independent kinase. These and the results presented below support the suggestion that both the cyclic AMP-dependent (histone kinase) and -independent phosphotransferase activities (basic protein kinase) are present in human myelin. Results of Table 1 also show that, had the assays been

Table 2. *Effect of cyclic AMP and protein kinase inhibitor on the phosphorylation of histones and basic protein by whole myelin preparations*

Myelin (1g), purified through three gradients, was suspended in 20ml of buffer A and then gently stirred over a magnetic stirrer for 1min. Portions (10μl) of this suspension were assayed for protein kinase activity with basic protein and histone V as substrate. When added, the concentration of cyclic AMP was 10μM, whereas the inhibitor concentration was 120μg/assay. Protein kinase activities are expressed as means ± S.D. Myelin from three different brains was examined.

Additions	Substrate	Relative activity (%)
None	Histone V	100 ± 7
Cyclic AMP	Histone V	184 ± 25
Cyclic AMP + inhibitor	Histone V	106 ± 10
None	Basic protein	100 ± 28
Cyclic AMP	Basic protein	103 ± 24
Cyclic AMP + inhibitor	Basic protein	103 ± 25

performed with basic protein only, the presence of the cyclic AMP-dependent kinase in myelin preparations would have escaped detection.

Table 3 compares the substrate specificities of the kinase present in whole myelin and in high-speed supernatant solutions derived from myelin extract and white-matter homogenate. Both basic protein and histone V were phosphorylated at nearly equal rates by the phosphotransferases of whole myelin. On the other hand, histone V was phosphorylated at a much higher rate by the enzymes present in myelin extract and white matter cytosol than most other histone fractions tested, including basic protein. As observed with the phosphotransferase system of whole myelin, stimulation by cyclic AMP of the soluble enzymes was noticed only when histones were used as the substrate (histone V > histone III > histone VII etc.).

White-matter cytosol contained high levels of cyclic AMP-dependent kinase (Table 3). However, phosphorylation of the basic protein was catalysed at modest rates by this phosphotransferase system. Thus, in relation to histone fractions V, III and VII, basic protein appears to be a poor substrate for the cyclic AMP-dependent kinase.

Under the standard assay conditions, cyclic GMP did not influence the rate of phosphorylation of basic protein or of histone V catalysed by the myelin enzymes. However, activation of the cyclic GMP-dependent protein kinase by cyclic GMP requires an unusually high concentration of Mg^{2+} (Nishiyama *et al.*, 1975). When phosphorylation of both the histone V and basic protein was measured at 50–100 mM-Mg^{2+} in the presence of 5–10 μM-cyclic GMP no enhancement in the catalytic rate was observed. Thus, according to the procedures used, presence of the cyclic GMP-dependent kinase could not be detected in human myelin preparations.

Effect of time of incubation, enzyme protein concentration and pH on rate of catalysis. The rate of phosphorylation of basic protein was linear for incubation periods extending to 15 min. Similar results were obtained with histones when the assays were performed in the presence or absence of cyclic AMP. Linearity in the rate of phosphorylation was also observed when the enzymic protein concentration was maintained at or below 0.5 mg/ml during assays. By using buffers of overlapping pH values, different buffers (Tris/HCl, 4-morpholineethanesulphonic acid and glycylglycine) of similar pH value gave almost comparable results. Optimal pH range was found between pH 7.5 and pH 8.5.

Effect of bivalent cations and increasing ionic strength on myelin kinase activity. The effects of various concentrations of Mg^{2+} on the phosphorylation of basic protein is shown in Fig. 1. Increasing concentrations activated the kinase and at optimal

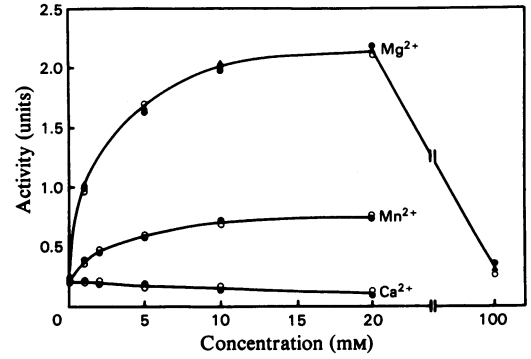

Fig. 1. *Effect of Ca^{2+}, Mn^{2+} and Mg^{2+} on the phosphotransferase reaction*

Protein kinase activity was measured at various concentrations of the cations shown with basic protein as substrate. Assays were performed in the absence of cyclic nucleotides (○), and in the presence of 10 μM-cyclic AMP (●) or 10 μM-cyclic GMP (▲). Three-gradient myelin suspended in buffer A was the enzyme source.

Table 3. *Substrate specificity of kinases present in myelin and white-matter cytosol*

Three-gradient myelin was suspended in buffer A and then assayed for kinase activity. Extraction and preparation of high-speed supernatants (from myelin and white-matter cytosol) is described in the legends to Tables 1 and 4. Assays were performed in the absence or presence of cyclic AMP (10 μM) as described in the Materials and methods section.

	Activity (units/mg)													
Substrate ...	Basic protein		Histone II		Histone III		Histone V		Histone VI		Histone VII		Histone VIII	
Enzyme source / Cyclic AMP ...	−	+	−	+	−	+	−	+	−	+	−	+	−	+
Whole myelin	0.6	0.6	0.1	0.1	0.4	0.5	0.5	0.6	0.1	0.1	0.2	0.2	0	0.1
Myelin extract (100000 *g* supernatant)	0.3	0.3	0.3	0.5	1.0	1.3	2.0	3.0	0.8	1.0	1.0	1.3	0.3	0.6
Cytosol (white matter)	0.5	0.7	0.4	0.7	0.8	1.4	1.6	4.7	0.4	0.7	0.7	1.3	0.3	0.4

concentration of Mg^{2+} (10mM), the rate of reaction was enhanced about 9–10-fold.

Though $MgATP^{2-}$ is the most likely substrate for the kinase, nearly a 100-fold excess of Mg^{2+} over ATP (which was 110μM) is needed to achieve maximum rate of catalysis. This suggests a role for free Mg^{2+} presumably via interaction with a metal-binding site on the enzyme, substrate or both. The phosphorylation reaction was also supported by Mn^{2+}, but this bivalent cation was considerably less potent. Ca^{2+}, on the other hand, could not replace Mg^{2+}, and when included in the reaction mixture at a concentration of 1mM, it was inhibitory.

The rate of phosphorylation of histones catalysed by the cyclic AMP-dependent protein kinases of cytosolic and membranous origin responds differently to changes in the ionic strength (Uno *et al.*, 1977). Similarly, the activation normally observed with cyclic AMP is almost completely blocked when the kinase of membranous origin is assayed in the presence of 0.1M-NaCl.

At different fixed concentrations of Mg^{2+} (0.2, 1 and 10mM), the effect of ionic strength on the phosphotransferase system of myelin has been investigated (Fig. 2). With increasing ionic strength the rate of phosphorylation of basic protein increased only when 10mM-Mg^{2+} was present (results of the experiments conducted at 0.2 and 1mM-Mg^{2+} are not shown in this Figure). This concentration of Mg^{2+} elicits maximum rate enhancement. However, as the concentration of NaCl exceeded 0.15M, phosphorylation of basic protein was inhibited and the rate in the presence of 0.25M-NaCl declined to the rate obtained in the absence of added NaCl. The rate of phosphorylation of basic protein was not affected by the presence of cyclic AMP over the entire range of ionic strength employed. In contrast, increase in ionic strength inhibited the phosphorylation of histone V and a partial masking of the cyclic AMP effect was also observed. For example, in the absence of NaCl, phosphorylation of histones was increased by about 100% by cyclic AMP, whereas in the presence of 150mM-NaCl this stimulation declined to about 50%. These properties of the myelin enzyme (with histone V as substrate) are similar to those described by Uno *et al.* (1977) for a neural membrane (cerebral cortex)-associated cyclic AMP-dependent (type II) protein kinase.

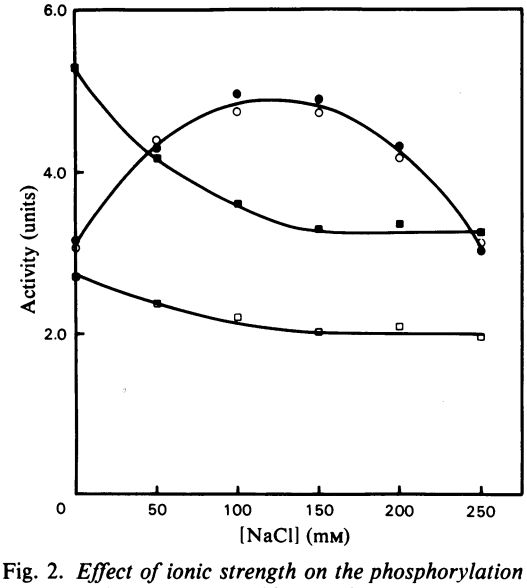

Fig. 2. *Effect of ionic strength on the phosphorylation of basic protein and histone fraction V*

Ionic strength of the medium was varied by adding different concentrations of NaCl. Assays were performed in the absence (○ and □), or presence of 10μM-cyclic AMP (● and ■) with basic protein (○ and ●) or histone fraction V (□ and ■) as substrate. Three-gradient myelin suspended in buffer A was the enzyme source.

Effect of certain lipids on the rate of phosphorylation of basic protein and estimation of K_m values. The effect of a number of lipids (pure and in the form of different brain extracts) on the phosphorylation of basic protein was also studied. Most of the lipids (bovine brain extract fractions V and VII, phosphatidylinositol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, sphingomyelin, lysophosphatidylcholine and lysophosphatidylethanolamine) when included during assays at 100μg/ml had no measurable effect on activity. Modest stimulation (15–30%) was observed in the presence of phosphatidylserine, bovine brain extract fractions III and VI and phosphatidic acid.

From the double-reciprocal plots of initial velocity versus substrate concentration, the apparent K_m values for ATP and basic protein were estimated at 12μM and 40μM respectively. The apparent kinetic constants (K_m and $V_{max.}$) remained essentially the same when the experiments were performed in the presence of phosphatidylserine, phosphatidylinositol or phosphatidylethanolamine.

When the phosphotransferase reactions were conducted with assay mixtures containing [γ-^{32}P]-ATP to which had also been added unlabelled GTP, UTP or CTP (each of the unlabelled nucleotides was tested at a final concentration of 0.1, 0.2 and 0.3mM), the amount of radioactivity transferred to the basic protein remained constant. These findings suggested that ATP could not be replaced by GTP, UTP or CTP during phosphorylation of basic protein catalysed by the myelin kinase.

Solubilization of myelin kinases

Myelin purified through three to five sucrose-density-gradient centrifugations contains almost

comparable levels of histone and basic protein kinase activities (Table 1). Conditions were sought (different concentrations of Triton X-100, duration of extraction etc.) to achieve solubilization of these enzymes from myelin. Maximum histone kinase activity was found in the 100000 *g* supernatants derived from myelin suspension that had been stirred in buffer A for 16h. However, this supernatant solution contained a relatively small amount (15–20%) of the basic protein phosphotransferase activity (Table 4). [Two well resolved peaks of protein kinase activity are obtained when the freshly prepared supernatant is chromatographed over a QAE-Sephadex column. The first peak of activity shows a high degree of specificity for basic protein and it is not activated by cyclic AMP. The second (major) peak of activity is activated by cyclic AMP but basic protein is a poor substrate for this enzyme. Owing to the labile nature of basic protein phosphotransferase it has not been possible to purify it further.] Most of the basic protein phosphotransferase activity remained in the 100000 *g* pellet. Once the basic protein kinase had been separated from myelin and/or its fragments by high-speed centrifugation, its activity declined rapidly on storage at 4°C. Under identical conditions the histone kinase remained stable.

The loss in the basic protein phosphotransferase activity could be due to proteolysis, since myelin is known to contain proteinases (Benjamins & Smith, 1977). Therefore, the effects of a number of proteinase inhibitors (leupeptin, antipain, pepstatin A, 1-chloro-4-phenyl-3-L-toluene-*p*-sulphonamidobutan-2-one and 7-amino-1-chloro-3-L-tosylamidoheptan-2-one), at a final concentration of 0.1mM, either singly or in combination, were examined on the solubilized kinases. Unfortunately, no protective effect on the basic protein phosphotransferase activity was observed. In spite of these observations all the buffers employed contained 20mM-benzamidine to prevent suspected proteolysis.

Table 4. *Solubilization of kinases present in myelin*
Myelin purified through three sucrose density gradients was suspended in buffer A and assayed immediately or it was extracted with buffer A for 16h and then centrifuged at 100000 *g* for 1h. The pellet was suspended in buffer A. Assays were performed in the absence or presence of cyclic AMP (10 μM) as described in the Materials and methods section.

	Activity (units/mg)			
	Basic protein		Histone V	
Cyclic AMP ...	−	+	−	+
Whole myelin	0.7	0.7	0.4	0.8
Myelin extract (100000 *g* supernatant)	0.1	0.1	0.7	1.1
Pellet	0.8	0.9	0.7	0.6

Discussion

The phosphotransferase system of human central-nervous-system myelin was investigated with respect to substrate specificity, differential stability, activation by cyclic AMP and the effects of ionic strength on enzyme activity. The results presented suggest that human myelin contains at least two different kinases. It was shown for the first time that one of the human myelin-associated kinases preferentially phosphorylated basic protein. The activity of the basic protein phosphotransferase was not only dependent on bivalent cations (Mg^{2+}) but it was greatly stimulated by them.

Human myelin has been shown previously to contain a basic protein-specific phosphoprotein phosphatase whose activity was markedly enhanced by bivalent cations (Yourist *et al.*, 1978; Wu *et al.*, 1980). Whereas 10mM-Mg^{2+} was required for optimal phosphorylation, the maximum rate of dephosphorylation of basic protein by myelin phosphatase was achieved at much lower concentrations of Mn^{2+} (0.125mM). Thus there exists the possibility that the relative concentrations of bivalent cations may control the degree of phosphorylation of basic protein in myelin membrane.

Basic protein is known to form stable complexes with acidic phospholipids such as phosphatidylserine and phosphatidylinositol (Palmer & Dawson, 1969). Yet in the presence of these compounds phosphorylation of basic protein was not altered. The lack of stimulatory effect during basic protein phosphorylation might indicate that the lipids tested thus far do not possess the proper structural features (chain length and degree of unsaturation of fatty acyl moieties etc.) that the basic protein and/or the enzyme require for the activation process.

Besides the kinase that preferentially phosphorylated basic protein, human myelin also contained at least an additional kinase. Basic protein was a poor substrate for the latter enzyme. Although its activity was activated by cyclic AMP during histone V phosphorylation, such stimulation was not observed with basic protein as substrate. The biological significance and the relationship of this enzyme to the basic protein phosphotransferase activity of myelin is not yet clear. This kinase could be involved in the phosphorylation of another myelin protein. Additional work is required to substantiate this suggestion.

This work was supported by National Institutes of Health U.S. Public Health Service Grants NS-13646,

NS-14947 and RR-05690. We thank Miss Melissa Harrell for typing the manuscript.

References

Agrawal, H. C., O'Connell, K., Randle, C. L. & Agrawal, D. (1982) *Biochem. J.* **201**, 39–47

Ahmad, F., Wu, N. C. & Yourist, J. E. (1981) *Trans. Am. Soc. Neurochem.* **12**, abstr. 322

Benjamins, J. A. & Smith, M. E. (1977) in *Myelin* (Morell, P., ed.), pp. 233–270, Plenum Press, New York

Bradford, M. W. (1976) *Anal. Biochem.* **72**, 248–254

Carnegie, P. R., Kemp, B. E., Dunkley, P. R. & Murray, A. W. (1973) *Biochem. J.* **135**, 569–572

Carnegie, P. R., Dunkley, P. R., Kemp, B. E. & Murray, A. W. (1974) *Nature (London)* **249**, 147–150

Deibler, G. E., Martenson, R. E. & Kies, M. W. (1972) *Prep. Biochem.* **2**, 139–165

Everly, J., Quarles, R. H. & Brady, R. O. (1977) *J. Neurochem.* **28**, 95–101

Fricke, U. (1975) *Anal. Biochem.* **63**, 555–558

Huang, K.-P. & Robinson, J. C. (1976) *Anal. Biochem.* **72**, 593–599

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Maeno, H., Johnson, E. M. & Greengard, P. (1971) *J. Biol. Chem.* **246**, 134–142

Miyamoto, E. (1975) *J. Neurochem.* **24**, 503–512

Miyamoto, E. & Kakiuchi, S. (1974) *J. Biol. Chem.* **249**, 2769–2777

Miyamoto, E., Kuo, J. F. & Greengard, P. (1969) *Science* **165**, 63–65

Miyamoto, E., Miyazaki, K., Hirose, R. & Kashiba, A. (1978) *J. Neurochem.* **31**, 269–275

Nishiyama, K., Katakami, H., Yamamura, H., Takai, Y., Shimomura, R. & Nishizuka, Y. (1975) *J. Biol. Chem.* **250**, 1297–1300

Palmer, F. B. & Dawson, R. M. C. (1969) *Biochem. J.* **111**, 637–646

Petrali, E. H., Thiessen, B. J. & Sulakhe, P. V. (1980*a*) *Int. J. Biochem.* **11**, 21–36

Petrali, E. H., Thiessen, B. J. & Sulakhe, P. V. (1980*b*) *Arch. Biochem. Biophys.* **205**, 520–535

Steck, A. J. & Appel, S. H. (1974) *J. Biol. Chem.* **249**, 5416–5420

Sulakhe, P. V., Petrali, E. H., Davis, E. R. & Thiessen, B. J. (1980) *Biochemistry* **19**, 5363–5371

Traugh, J. A., Ashby, C. D. & Walsh, D. A. (1974) *Methods Enzymol.* **38**, 290–299

Uno, I., Ueda, T. & Greengard, P. (1977) *J. Biol. Chem.* **252**, 5164–5174

Uyemura, K., Tobari, C., Hirano, S. & Tsukada, Y. (1972) *J. Neurochem.* **19**, 2607–2614

Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412

Weller, M. & Rodnight, R. (1970) *Nature (London)* **225**, 187–188

Wu, N. C., Martinez, J. J. & Ahmad, F. (1980) *FEBS Lett.* **116**, 157–160

Yourist, J. E., Chaviano, A., Ahmad, F., Block, R. & Brady, A. H. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, abstr. 502

Yourist, J. E., Ahmad, F. & Brady, A. H. (1978) *Biochim. Biophys. Acta* **522**, 452–464

Zetusky, W. J., Calabrese, V. P., Zetusky, A. L., Anderson, M. E., Cullen, M. & DeVries, G. H. (1979) *J. Neurochem.* **32**, 1103–1109