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Adhesion of acidic lipid vesicles by 21.5 kDa (recombinant) and 18.5 kDa isoforms of myelin basic protein

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Abstract

Myelin basic protein (MBP) is thought to be responsible for adhesion of the intracellular surfaces of compact myelin to give the major dense line. The 17 and 21.5 kDa isoforms containing exon II have been reported by others to localize to the cytoplasm and nucleus of murine oligodendrocytes and HeLa cells while the 14 and 18.5 kDa isoforms lacking exon II are confined to the plasma membrane. However, we show that the exon II$^-$ 18.5 kDa form and a recombinant exon II$^+$ 21.5 kDa isoform both caused similar aggregation of acidic lipid vesicles, indicating that they should have similar abilities to bind to the intracellular lipid surface of the plasma membrane and to cause adhesion of those surfaces to each other. The circular dichroism spectra of the two isoforms indicated that both had a similar secondary structure. Thus, both isoforms should be able to bind to and cause adhesion of the cytosolic surfaces of compact myelin. The fact that they do not could be due to differences in post-translational modification in vivo, trafficking through the cell and/or subcellular location of synthesis, but it is not due to differences in their lipid binding.

Keywords: Myelin basic protein; Aggregation of lipid vesicle; Size isoform; Myelin; Membrane; Liposome

1. Introduction

Myelin basic protein (MBP) is thought to be responsible for adhesion of the intracellular surfaces of the compact multilayered myelin sheath. It is located at the major dense line of myelin [1,2] and it causes aggregation of lipid vesicles containing negatively charged lipids [3–9]. In mice and rats, MBP occurs as four major size isoforms of 21.5, 18.5, 17 and 14 kDa due to alternative splicing of a single primary transcript of the MBP gene which contains seven exons. All isoforms contain exons I, III, IV, V and VII while only the 21.5 and 17 kDa forms contain exon II. The 21.5 and 18.5 kDa forms also contain exon VI. The exon II$^+$ 21.5 and 17 kDa forms are expressed at high levels early in myelination and in immature oligodendrocytes in culture [10], while the exon II$^-$ 14 and 18.5 kDa forms are the primary forms expressed later in myelination, in mature oligodendrocytes and in adult myelin. In human, the

Abbreviations: MBP, myelin basic protein; PS, phosphatidylserine; PC, phosphatidylcholine; LUVs, large unilamellar vesicles; CD, circular dichroism spectroscopy

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18.5 kDa form is the primary form in adult myelin but exon II$^+$ forms are expressed during fetal development and during remyelination [11].

Interestingly, these different isoforms localize to different regions of the cell when expressed individually in transfected cells. Both in Shiverer mouse oligodendrocytes lacking endogenous MBP [12] or in HeLa cells [13], the exon II$^+$ forms were found distributed in the cytoplasm and in the nucleus but not at the plasma membrane, while the exon II$^-$ forms were confined to the plasma membrane. The exon II$^+$ forms were transported into the nucleus by active transport [14]. Entry was directly related to the growth rate of the cells, as found for bona fide nuclear proteins, and was suppressed by cell-cell contact [15]. In wild-type mouse oligodendrocytes containing endogenous MBP, a transfected tagged 14 kDa form was found in the cytoplasm and nucleus in addition to being bound to the plasma membrane, leading to the suggestion that the exon II$^+$ forms might help to transport exon II$^-$ forms into the nucleus [16].

The absence of the exon II$^+$ forms at the plasma membrane of cultured OLs and non-glial cells has led to the suggestion that the presence of the exon II domain suppresses membrane binding and that the exon II$^+$ forms do not participate in compaction of myelin [15,17]. However, exon II$^+$ forms are found equally in compact myelin and in the radial component (a junctional specialization within internodal myelin), although the exon II$^-$ forms are found more preferentially in compact myelin compared to the radial component [18]. Furthermore, like the rest of the MBP sequence, the exon II domain is also highly basic and would contribute an additional net positive charge of four to the net positive charge of 20 for the 18.5 kDa form, giving a total net positive charge of 24 for the 21.5 kDa form at neutral pH. Thus, unless the presence of exon II causes a significant change in the conformation, degree of oligomerization or degree of post-translational modifications of MBP in vivo, it is expected to have little effect on electrostatic interactions of MBP with negatively charged lipids at the cytosolic surface of the myelin or oligodendrocyte membrane. The single Cys^{81} of exon II has been found to cause some dimerization of a recombinant 21.5 kDa form on gels but only of a fraction of the protein [19].

MBP probably causes adhesion of the cytosolic surfaces of myelin by binding to acidic lipids. The only other membrane protein present in sufficient quantity to bind a significant amount of MBP is the transmembrane proteolipid protein (PLP). PLP is also positively charged at the cytosolic surface [20] and has been shown to bind only to the least positively charged isomer of MBP in which six Arg residues have been deiminated to citrulline [21]. Aggregation of lipid vesicles containing negatively charged lipids by MBP is a useful model system to study factors which might affect its ability to cause adhesion of the intracellular surfaces of myelin. This approach has been used to study the effect of post-translational charge modifications to the 18.5 kDa form on its ability to cause adhesion of lipid vesicles [6,21,22]. It is not known whether MBP causes this adhesion as a monomer or as a dimer but it can do so quite effectively in the absence of any other proteins. In the present study, we compare the ability of the recombinant 21.5 kDa form of human MBP [19] and the 18.5 kDa form of bovine MBP, using the least modified, most positively charged form of the latter, C1, to aggregate lipid vesicles.

2. Materials and methods

2.1. Lipids and proteins

Bovine MBP was isolated from bovine brain white matter according to the method of Cheifetz and Moscarello [6]. It was separated into its charge isomers C1–C5 by ion exchange chromatography at alkaline pH on CM52 as described [23,24]. C1, the least modified component, was used. The recombinant 21.5 kDa isoform of human MBP bearing a C-terminal histidine tag was expressed in *Escherichia coli* and purified as described [19]. Egg L-α-phosphatidylcholine (PC) was purchased from Sigma (St. Louis, MO, USA) and bovine brain L-α-phosphatidylserine (PS) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Lipids were chromatographically pure and were used as supplied. [^{14}C]dipalmitoylphosphatidylcholine ([^{14}C]DPPC), specific activity 110 mCi/mmol, was from Dupont NEN (Boston, MA, USA).

2.2. Preparation of large unilamellar vesicles (LUVs)

Chloroform solutions of the lipids were combined in a PC/PS 4:1 mol ratio and the solvent evaporated under a stream of nitrogen. The lipid was redissolved in 1 ml benzene, frozen and lyophilized overnight. The dry lipid film (42 μmol) was hydrated in 1.5 ml of 10 mM HEPES buffer containing 0.1 mM EDTA at pH 7.4 and dispersed by vortex mixing. LUVs were prepared by extrusion of the lipid suspension through a 100 nm pore filter using an Avestin LiposoFast extruder (Ottawa, Canada) as described earlier [22,25].

2.3. Aggregation assay

Solutions of 21.5 and 18.5 kDa MBP were made in distilled water at concentrations of 1.5, 0.75 and 0.5 mg/ml. The LUVs were diluted to a final concentration of 0.6 μmol lipid/ml in 10 mM HEPES buffer, pH 7.4, containing 0.1 mM EDTA and varying NaCl concentrations from 10 to 210 mM as described earlier [9,22]. After addition of NaCl, the LUVs were sonicated briefly in a bath sonicator and left overnight at 4°C to allow for equilibration of the salt concentration inside and outside the LUVs. Although the cytosol of oligodendrocytes contains more K^+ than Na^+, in a previous study, we found that both cations had similar effects on MBP-induced aggregation [9]. An aliquot (10 μl) of the protein solutions was added to 1 ml of diluted LUVs, giving MBP to lipid weight ratios of 1:90, 1:60 and 1:30 in Sarstedt acrylic cuvettes (Newton, NC, USA) as described earlier [9]. The cuvette was gently inverted three times to mix, left at room temperature for 10 min and aggregation was measured from the absorbance at 450 nm with a Hitachi U-2000 spectrophotometer at room temperature. Absorbance of protein-free LUVs at different Na^+ concentrations was also measured and subtracted from that of protein-containing LUVs to give ΔAbs. The absorbance of protein-free LUVs was low (0.05) and addition of 210 mM NaCl increased it to only 0.07.

2.4. Binding assay

PC/PS 4:1 LUVs containing [^{14}C]DPPC (7500 cpm/μmol lipid) were prepared as described above in 80 and 210 mM NaCl at a lipid concentration of 0.6 μmol/ml. A small aliquot of protein solution containing 75 μg protein was added (initial weight% protein of 3.4) and the lipid-protein vesicles were sedimented by centrifugation at $95\,000 \times g$ in a Beckman Optima L-90K ultracentrifuge at 4°C for 2 h. The supernatant was removed and aliquots were taken for counting [^{14}C]DPPC and for protein analysis by the Peterson assay [26]. Virtually all (99%) of the lipid at 80 mM NaCl sedimented under these conditions. The pellet was resuspended in 450 μl buffer, aliquots were taken for counting and protein assay and the weight% protein bound to the lipid pellet was determined as the ratio of protein weight to the total weight of lipid plus protein multiplied by 100.

2.5. Circular dichroism (CD) spectroscopy

CD spectra of the two isoforms were measured at 190–250 nm on a Jasco J720A spectropolarimeter using a 0.01 cm pathlength cell at ambient temperature. Appropriate dilutions of solutions of the two isoforms in deionized water were made with 10 mM phosphate buffer, pH 7.4, with or without 2 mg/ml sodium dodecyl sulfate (SDS). Protein concentrations were in the range of 1 mg/ml and were quantitated by amino acid analysis or the Peterson assay [26]. CD spectra of the buffers alone were subtracted from the protein spectra. The CD spectra are expressed as the mean residue ellipticity, Θ (degree $\times$ cm^2/dmol).

3. Results and discussion

The lipid composition of the cytosolic surface of myelin is thought to contain about 15 mol% negatively charged lipid, mostly PS with a small amount of phosphatidylinositol [27]. The remainder consists mainly of the electrostatically neutral zwitterionic lipids PC, phosphatidylethanolamine and sphingomyelin. In order to resemble the lipid content of the cytosolic surface of myelin, LUVs of PC containing 20 mol% PS were used for this study. The ability of the two size isoforms of MBP to cause aggregation of PC/PS 4:1 (m/m) LUVs at different Na^+ concentrations was compared at three lipid to protein

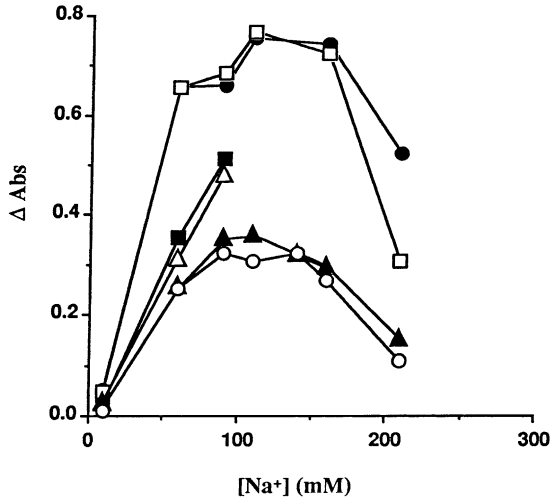

Fig. 1. Dependence on the NaCl concentration of absorbance of PC/PS 4:1 LUVs in the presence of 21.5 kDa isoform (●) and 18.5 kDa isoform (□) at a lipid to protein weight ratio of 30:1; 21.5 kDa isoform (■) and 18.5 kDa isoform (△) at a lipid to protein weight ratio of 60:1; 21.5 kDa isoform (▲) and 18.5 kDa isoform (○) at a lipid to protein weight ratio of 90:1. An increase in absorbance is due to aggregation of the LUVs by the protein. The decrease in absorbance at high salt concentrations is due to dissociation of the protein from the LUVs, resulting in decreased aggregation. ΔAbs is the difference in absorbance at 450 nm of protein-bound LUVs from protein-free LUVs. The latter ranged from 0.05 to 0.07.

weight ratios, 30:1, 60:1 and 90:1, higher than the ratio in central nervous system compact myelin, about 14:1 [28]. At these higher lipid to protein ratios, the negatively charged lipid is in excess relative to the number of positively charged residues of the protein (21, 14 and 7 PS/net positive charge). Thus, the protein cannot neutralize all of the charged lipid and the vesicles have a net negative charge. At these lipid to protein ratios, the vesicles repel each other and the protein cannot aggregate them unless the lipid charge is shielded by salt. The ability of the protein to aggregate the vesicles is more dependent on modifications to the protein under these conditions than at the low lipid to MBP ratio in compact myelin, as shown previously for charge isoforms of MBP [22].

Fig. 1 shows that the absorbance of the lipid-protein vesicles increases with an increasing Na^+ concentration up to about 160 mM Na^+ for all three lipid to protein ratios, indicating that aggregation increases with an increasing salt concentration. Absorbance decreases at 210 mM Na^+. This has been shown to be due to dissociation of some of the protein at higher salt concentrations, thus resulting in a decreased aggregation [9]. Both the 21.5 and 18.5 kDa forms of MBP show a similar dependence on the Na^+ concentration up to 160 mM Na^+. At 210 mM

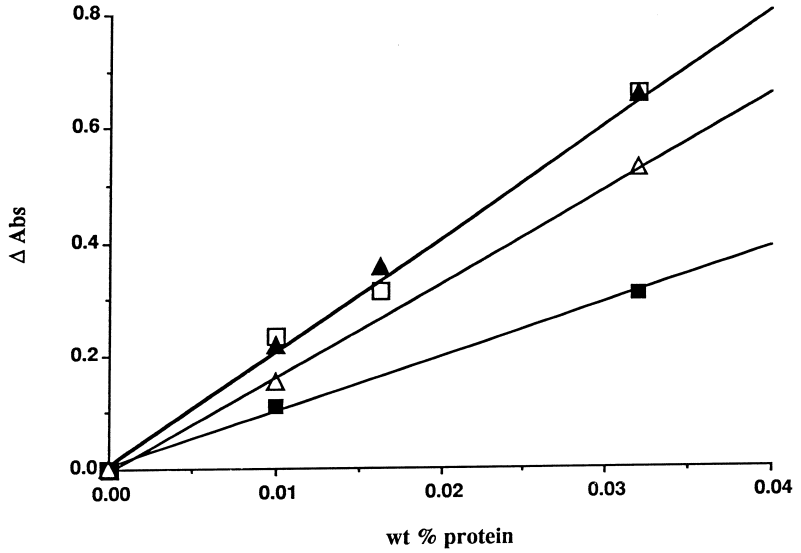

Fig. 2. Dependence of absorbance on the protein concentration expressed as weight% of the total amount of lipid and protein in the vesicles, for the 21.5 kDa isoform (▲) and 18.5 kDa isoform (□) at 60 mM NaCl and for the 21.5 kDa isoform (△) and 18.5 kDa isoform (■) at 210 mM NaCl.

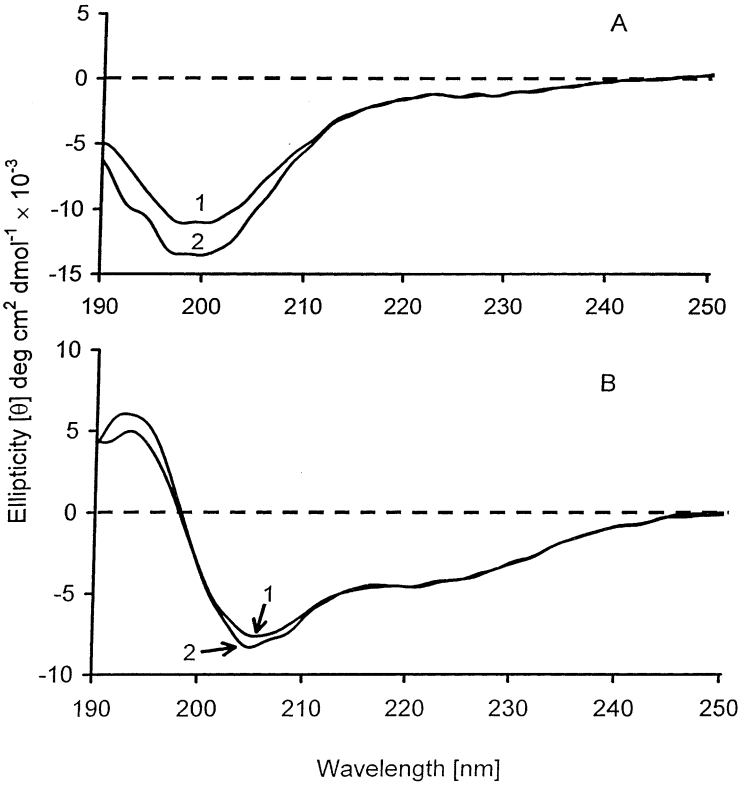

Fig. 3. Representative CD spectra of 21.5 kDa (curve 1) and 18.5 kDa (curve 2) isoforms in (A) 10 mM phosphate buffer, pH 7.4, and (B) in the presence of 2 mg/ml SDS in the same buffer. The CD data are expressed as the mean residue ellipticity, Θ (degree × cm^2/dmol). Relative differences and similarities of the spectra of the two isoforms were reproducible.

Na^+, however, absorbance is less for the 18.5 kDa form than the 21.5 kDa form, particularly at the 30:1 weight ratio, suggesting that the 18.5 kDa form is dissociated more readily by salt than the 21.5 kDa form. This was confirmed by a binding assay at 80 and 210 mM NaCl as shown in Table 1. The total intracellular monovalent cation concentration of oligodendrocytes is 75–90 mM [9]. All of the protein added to the lipid vesicles is bound at the physiological intracellular salt concentration of 80 mM NaCl for both isoforms. However, at 210 mM NaCl, some of the protein dissociates due to shielding of electrostatic interactions with the lipid. More of the 21.5 kDa form remains bound than the 18.5 kDa form, indicating a tighter association of the 21.5 kDa form due to its greater net positive charge.

The dependence of aggregation on the protein concentration is shown in Fig. 2 at two different salt concentrations. At 60 mM Na^+ where aggregation is not yet maximal, there is no difference in the ability of the two size isoforms to cause aggregation of the vesicles. At 210 mM Na^+, where protein dissociation has started, the 21.5 kDa form causes greater aggregation than the 18.5 kDa form. Thus, the 21.5 kDa form of MBP binds to negatively charged lipid bilayers as well as or better than the 18.5 kDa form and causes similar aggregation of vesicles. The high lipid to MBP ratio used for these studies should magnify any differences in their ability to bind to and

Table 1
Effect of the salt concentration on the amount of 18.5 and 21.5 kDa forms of MBP bound to lipid[a]

Size isomer	NaCl (mM)	MBP bound (weight%)
18.5 kDa	80	3.4
21.5 kDa	80	3.4
18.5 kDa	210	1.7
21.5 kDa	210	2.5

[a]75 μg protein was added to 2.15 mg lipid, PC/PS 4:1 LUVs (3.4 weight% protein), and the weight% MBP bound to the lipid was determined as described in Section 2.

aggregate lipid vesicles. Thus, at the low lipid to MBP ratio in compact myelin and at physiological monovalent salt concentrations, no difference in the ability of the two size isoforms to bind to and adhere acidic lipid surfaces would be expected.

Although we compared bovine brain 18.5 kDa MBP with recombinant human 21.5 kDa MBP, we have never observed any difference in the ability of human and bovine MBP to aggregate vesicles (Boggs, unpublished). There are only 16 residues which differ in the sequences of human and bovine 18.5 kDa MBP and no difference in net positive charge [28]. However, the recombinant 21.5 kDa form also has six His residues at the C-terminus. These are expected to be unprotonated at pH 7.4. If protonated, they may contribute to the ability of the 21.5 kDa isoform to bind and aggregate vesicles. If all the His residues in the proteins are protonated, then, the 21.5 kDa His-tagged isoform would have a net positive charge of 41 compared to 35 for the native 21.5 kDa isoform and 31 for the 18.5 kDa isoform. It is very unlikely that the extra six His residues of the His-tagged protein would convert it from a non-membrane-binding protein into a binding one, particularly since the C-terminal end of the native form of both charge isomers is already positively charged with two Arg residues at the C-terminus.

CD spectra of the two isoforms show that in aqueous solution, they both have a mostly random structure. The mean residue elipticity of the 21.5 kDa form is reproducibly less negative (15% ± 3) at 198–200 nm but similar at 215–240 nm to that of the 18.5 kDa form, indicating that the 21.5 kDa form has a little more secondary structure in solution than the smaller form (Fig. 3). The small difference in elipticity observed is unlikely to be due to the difference in purification procedures of the recombinant and native forms since two quite different purification procedures were found to have no effect on the CD spectrum of MBP [29]. It is more likely due to the His tag at the C-terminal than due to the presence of exon II, since exon II of human MBP contains five prolines in a segment of 26 amino acids, which is not expected to result in an increased secondary structure. In the presence of the anionic detergent SDS, the CD spectra are more similar for the two isoforms. The elipticity greatly increases at wavelengths below 200 nm and decreases at wavelengths above 205 nm, indicating that SDS induces a significant amount of secondary structure for both isoforms as reported previously for the 18.5 kDa form [29–31]. The % difference in elipticity for the two isoforms in SDS is 10 ± 2. Thus, in the presence of lipid, the two isoforms probably have a relatively similar secondary structure.

These results indicate that the greater nuclear targeting of the 21.5 kDa form of MBP and the greater plasma membrane binding of the 18.5 kDa form in mouse oligodendrocytes and in HeLa cells must be due to some reason other than a difference in the ability of the unmodified forms of these size isoforms to bind to and cause adhesion of membranes. A difference in secondary structure also is not likely to account for these results. The studies of cellular localization of the 21.5 kDa isoform of MBP were carried out using mouse MBP while our aggregation studies utilized the 21.5 kDa form of human MBP. The sequence of exon II in mouse MBP differs from human at four out of 26 residues, with Pro-67 → Glu, Gly-7 → Ser, Gln-18 → Arg and Asn-23 → His substitutions occurring in mouse MBP [11]. These would not affect the net charge of the 21.5 kDa form at neutral pH and thus would be unlikely to affect the ability of the mouse 21.5 kDa isoform to bind to and aggregate acidic lipid vesicles.

Possibly some post-translational modifications of the exon II^+ forms cause their targeting to the nucleus, although phorbol ester inhibited it, suggesting that phosphorylation of MBP may inhibit nuclear targeting [14]. The extent of post-translational modifications of the exon II^+ forms of MBP in oligodendrocytes or in non-glial cells where they have been expressed is not known. Post-translational modifications which decrease the net positive charge of MBP decrease its ability to cause aggregation of acidic lipid vesicles [6,21] and increase its susceptibility to dissociation from the lipid at high salt concentrations [22]. They may also affect the secondary structure. Phosphorylation increases the secondary structure of the protein in solution [32], although the effect on the lipid-bound protein has not been determined. Deamidation, however, has no effect on the secondary structure [33]. Another possible cause of different localization of the exon II^+ isoforms is the presence of a nuclear targeting sequence in exon II or unmasking of a nuclear targeting sequence identified

in exon I by the presence of exon II, as suggested by Staugaitis et al. [17]. Alternatively, targeting may be due to the localization of MBP synthesis. The mRNAs for exon II$^-$ forms of MBP are transported far into oligodendrocyte processes while exon II$^+$ mRNAs have been detected only in the cell body [34]. Thus, it may be the mRNA exon II sequence which regulates sorting and trafficking of MBP rather than the MBP protein sequence.

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References

[1] F.X. Omlin, H.F. Webster, C.G. Palkovits, R. Cohen, J. Cell Biol. 95 (1982) 242–248.
[2] D.A. Kirschner and A.E. Blaurock, in: R.E. Martenson (Ed.), Myelin: Biology and Chemistry. CRC Press, Boca Raton, FL, 1992, pp. 1–70.
[3] R. Smith, Biochim. Biophys. Acta 470 (1977) 170–184.
[4] P.D. Lampe, G.J. Wei, G.L. Nelsestuen, Biochemistry 22 (1983) 1594–1599.
[5] A.G. Walker, M.G. Rumsby, Neurochem. Int. 7 (1985) 441–447.
[6] S. Cheifetz, M.A. Moscarello, Biochemistry 24 (1985) 1909–1914.
[7] W.K. Surewicz, R.M. Epand, R.F. Epand, F.H. Hallet, M.A. Moscarello, Biochim. Biophys. Acta 863 (1986) 45–52.
[8] M.B.A. terBeest, D. Hoekstra, Eur. J. Biochem. 211 (1993) 689–696.
[9] E. Jo, J.M. Boggs, Biochemistry 34 (1995) 13705–13716.
[10] E. Barbarese, J.H. Carson, P.E. Braun, J. Neurochem. 31 (1978) 779–782.
[11] J. Kamholz, J. Toffenetti, R. Lazzarini, J. Neurosci. Res. 21 (1988) 62–70.
[12] B. Allinquant, S.M. Staugaitis, D. D'Urso, D.R. Colman, J. Cell Biol. 113 (1991) 393–403.
[13] S.M. Staugaitis, P.R. Smith, D.R. Colman, J. Cell Biol. 110 (1990) 1719–1727.
[14] L. Pedraza, L. Fidler, S.M. Staugaitis, D.R. Colman, Neuron 18 (1997) 579–589.
[15] L. Pedraza, J. Neurosci. Res. 50 (1997) 258–264.
[16] R.J. Hardy, R.A. Lazzarini, D.R. Colman, V.L. Friedrich Jr., J. Neurosci. Res. 46 (1996) 246–257.
[17] S.M. Staugaitis, D.R. Colman, L. Pedraza, BioEssays 18 (1996) 13–18.
[18] J. Karthigasan, J.S. Garvey, C.V. Ramamurthy, D.A. Kirschner, J. Neurocytol. 25 (1996) 1–7.
[19] S.H. Nye, C.M. Pelfrey, J.J. Burkwit, R.R. Voskuhl, M.J. Lenardo, J.P. Mueller, Mol. Immunol. 14/15 (1995) 1131–1141.
[20] T. Weimbs, W. Stoffel, Biochemistry 31 (1992) 12289–12296.
[21] D.D. Wood, M.A. Moscarello, J. Biol. Chem. 264 (1989) 5121–5127.
[22] J.M. Boggs, P.M. Yip, G. Rangaraj, E. Jo, Biochemistry 36 (1997) 5065–5071.
[23] F.C.-H. Chou, C.-H.J. Chou, R. Shapira, R.F. Kibler, J. Biol. Chem. 251 (1976) 2671–2679.
[24] S. Cheifetz, M.A. Moscarello, C.M. Deber, Arch. Biochem. Biophys. 233 (1984) 151–160.
[25] R.C. MacDonald, R.I. MacDonald, B.P.M. Menco, K. Takeshita, N.K. Subbarao, L. Hu, Biochim. Biophys. Acta 1061 (1991) 297–303.
[26] G.L. Peterson, Anal. Biochem. 83 (1977) 346–356.
[27] H. Inouye, D.A. Kirschner, Biophys. J. 53 (1988) 247–260.
[28] J.M. Boggs, M.A., Moscarello and D. Papahadjopoulos, in: P. Jost and O.H. Griffith (Eds.), Lipid-Protein Interactions, Vol. 2, John Wiley and Sons, New York, 1982, pp. 1–51.
[29] A. Gow, R. Smith, Biochem. J. 257 (1989) 535–540.
[30] J.S. Anthony, A. Moscarello, Biochim. Biophys. Acta 243 (1971) 429–433.
[31] L.F. Liebes, R. Zand, W.D. Phillips, Biochim. Biophys. Acta 427 (1973) 392–409.
[32] J.J. Ramwani, R.M. Epand, M.A. Moscarello, Biochemistry 28 (1989) 6538–6543.
[33] G.E. Deibler, A.L. Stone, M.W. Kies, Proteins Struct. Funct. Genet. 7 (1990) 32–40.
[34] H. deVries, J.C. deJonge, C. Schrage, M.E. vanderHaar, D. Hoekstra, J. Neurosci. Res. 47 (1997) 479–488.