Interaction of Myelin Basic P2 Protein and Its Peptide Fragments with Anionic Detergent Micelles

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P2 protein is one of the major proteins found in peripheral nervous system (PNS) myelin. It is localized on the cytoplasmic side of Schwann cells where it behaves as a peripheral membrane protein. The P2 protein (131 residues) is basic with an isoelectric point in the vicinity of pH 10, water-soluble, and exists primarily as a β-structure with some α-helix in aqueous solution as studied by circular dichroism (CD), x-ray crystallography and Fourier transform infra-red spectroscopy.[1]

P2 complexed with myelin lipids forms mainly multilayered structures of lipid bilayers with P2 sandwiched between them.[2] It has been reported that no significant changes in the circular dichroism spectra accompany the protein association with either of the zwitterionic bilayer-forming lipids, phosphatidylethanolamine and phosphatidylcholine.[3] On the other hand, major conformational changes occur when P2 is complexed with anionic lipids, such as phosphatidylserine or gangliosides.[3] Along with the basic character of the protein, the relative abundance of negatively charged lipids in myelin membranes[4] and the asymmetric distribution of myelin, it is speculated that the major interactions between P2 and lipid bilayers most likely occur through the interaction of P2 with anionic lipids.

In order to better understand the interactions between lipids and P2 that maintain and stabilize the myelin sheath, the conformational changes induced in P2 and its peptide fragments by the interaction with anionic detergent micelles have been studied by employing circular dichroism. These investigations would permit the identification of protein segments involved in the interactions with negatively charged detergent micelles.

P2 protein was prepared from bovine intradural spinal roots by the method of James and Moore.[5] Cleavage of P2 at the methionine residues with cyanogen bromide in 70% formic acid yields three peptides, peptide 1-20, peptide 21-113 and peptide 114-131, and the resulting three peptides were separated on G-50 and G-25 Sephadex columns as described by Weise *et al.*[6] CD spectra were recorded with a JASCO model J-500C spectropolarimeter at room temperature. The CD spectrum of detergent alone were negligible over the spectral range recorded. The least-squares curve-fitting method was used to determine the content of the secondary structure as described previously.[1a]

CD spectra of bovine P2 and derived peptides were measured in aqueous solution with increasing concentrations of SDS up to detergent/protein molar ratios of 225/1. Conformational changes occurred in the protein and the peptides interacting with detergent micelles. Figures 1 and 2 show the mole percentage α-helix and β-sheet induced in P2 and peptide 21-113 as function of [SDS]/[protein or peptides]. Major conformational changes occur in the SDS/P2 molar ratios between 75/1 and 175/1, where α-helix gradually increases and β-sheet proportionally decreases (Figure 1). Changes in the CD spectra are complete when the SDS/P2 molar ratio is about 175, indicating that each P2 molecule binds with about 2 micelles. At ionic strength of 0.16

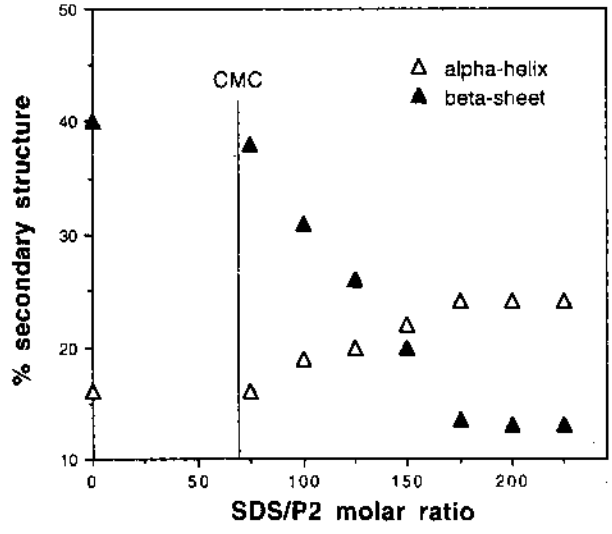

Figure 1. Calculated percent α-helicities and β-sheets of P2 protein as functions of SDS/protein molar ratios at pH 7.0. Protein concentration=0.14 mM. Path length=0.01 cm. Critical micelle concentration (CMC)=8.3 mM.

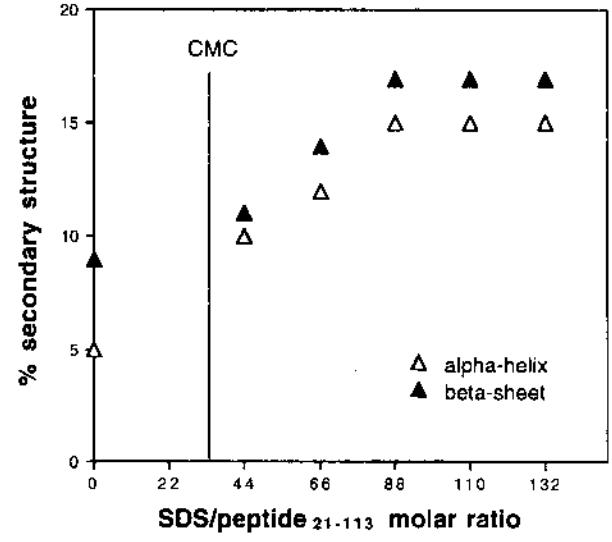

Figure 2. Calculated percent α-helicities and β-sheets of Peptide 21-113 as functions of SDS/peptide molar ratios at pH 7.0. Peptide concentration=0.30 mM. Path length=0.01 cm.

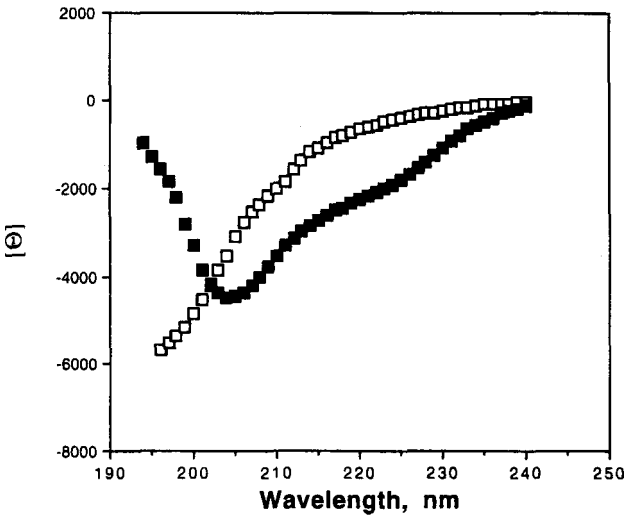

Figure 3. The circular dichroism spectra of Peptide 114-131 in water (open square) and of Peptide 114-131 bound to SDS micelles (closed square). Peptide concentration=0.30 mM. SDS concentration=10 mM. Path length=0.01 cm.

where the spectra were measured, about 90 SDS molecules form 1 micelle.[7] Figure 2 shows the changes in the secondary structures in the peptide 21-113 at increasing molar ratio of SDS/peptide$_{21\text{-}113}$. The α-helix and β-sheet gradually increase until the changes in the CD spectra are complete when the SDS/peptide$_{21\text{-}113}$ molar ratio is about 88/1, indicating that each peptide 21-113 binds with about 88 SDS molecules, *i.e.* 1 micelle. Figure 3 shows the CD spectra of peptide 114-131 in aqueous solution and in the presence of SDS micelles. The α-helix and β-sheet gradually increase until the changes in the CD spectra are complete when the SDS/peptide$_{114\text{-}131}$ molar ratio is about 50/1 (data not shown). Peptide 1-20 is hardly soluble, thus no proper signal was detected in the CD spectra. The solubility did not increase in the presence of SDS micelles.

The calculated percent of α-helix measured for P2 and derived peptides in suspensions of SDS micelles are given in Table 1. In SDS suspensions the total number of residues in helical conformations was 32 for the intact protein and 18 for the sequenctial peptides 21-113 and 114-131. The smaller number of residues in helices in the peptides suggest that the remaining 14 residues of the helix could be formed in segment 1-20.

Detergents have been useful ligands to probe for the presence or absence of amphiphilic binding sites on proteins.[8,9] The most extensively studied detergent has been sodium dodecyl sulfate (SDS), and several generalizations have emerged with respect to the binding of dodecyl sulfate to water-soluble, globular proteins.[9,10] Binding of dodecyl sulfate to these proteins tends to occur in a cooperative fashion, reaching saturation levels of 1.2-1.7 g dodecyl sulfate/g protein, depending on the protein. Water-soluble, globular proteins seem to bind only monomeric dodecyl sulfate, since saturation can be achieved prior to the onset of micelle formation. On the contrary, membrane proteins like cytochrome b5 or myelin basic protein (MBP) can bind dodecyl sulfate micelles. Compared to the globular proteins, dodecyl sulfate binding to P2 protein is unusually large (≥3.5/1) at the ionic strenth 0.16, which is similar to the binding of SDS to myelin basic protein (MBP), being 3.58 and 2.30 for SDS at ionic strengths 0.3 and 0.1, respectively.[11] MBP is found in the central nervous system myelin and is strongly implicated in the formation and maintenance of myelin membranes in the CNS.

Table 1. Mole percentage of α-helix and number of residues in helical conformation in P2 and peptides derived from it in the presence of SDS

Peptides	mol % of α-helix		no. of residues	
	H_2O	SDS	H_2O	SDS
21-113	5	15	5	14
114-131	0	20	0	4
1-131	16	24	21	32

The present finding that P2 can bind two dodecyl sulfate micelles provides strong evidence for two discrete binding sites on the P2 protein and indicates that lipid bilayer cross-linking by this protein may be effected by single molecules. From the results of SDS/peptides binding studies, it seems very likely that one binding site resides in the region defined by residues 21-113 and the other in combined region of residues 1-20 and 114-131.

Besides the role in the maintenance of myelin, P2 protein has been identified as an antigen responsible for induction of experimental autoimmune neuritis (EAN),[12] which is an animal model for a human demyelinating disease, the Guillain-Barre syndrome.[13] The protein, when used alone, exhibits only weak antigenic activity. But when the protein was injected along with lipid components of myelin such as phosphatidylseine[14] or gangliosides,[15] the severity of the disease became much more intense, with a degree comparable to that of whole PNS myelin. These findings have led to the speculation that lipids could alter the conformation of the isolated protein so as to approximate that of P2 when present in myelin. According to the conformation of the antigenic determinant (residues 53-78),[16] three turns exist and it is suggested that these turns form the foci of multiple epitopes. This antigenic region is highly acidic, thus it is not likely to interact with acidic lipids or detergents. Taken together, these results suggest that the antigenic epitopes may be less exposed in the unbound protein, but more exposed by the conformational change of the protein when bound to anionic lipids.

In the future studies, the interactions of P2 protein and derived peptides from it with neutral detergent micelles and with mixed micelles of the neutral/anionic detergents will be investigated. The combined results will provide a detailed picture of the balance between hydrophobic and ionic forces in the interactions of the P2 protein with the detergent micelles.

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Reexamination of *Al*-Isopropoxydiisobutylalane as a Stereoselective Reducing Agent for Reduction of Cyclic Ketones to Thermodynamically More Stable Alcohols

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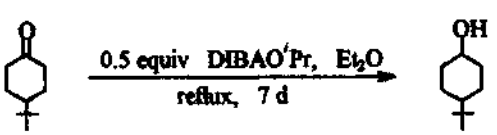
Very recently, we reported that *Al*-isopropoxydiisobutylalane (DIBAO^{i}Pr) reveals an excellent stereoselectivity in the reduction of representative cyclic ketones in ethyl ether at 25° to provide the corresponding thermodynamically more stable alcohols.[1] In this report, we examined only the reaction in which an equimolar mixture of reagent and ketone is involved. However, we soon realized that, like triisobutylaluminum (TIBA),[2] the isobutyl group of DIBAO^{i}Pr is also involved in this reduction. Thus, when 2 equiv of ketone was treated with 1 equiv of DIBAO^{i}Pr in refluxing ethyl ether, the first equiv of ketone was reduced readily and the second one was also reduced in a relatively slow rate. This apparently improves the procedure appeared in the previous report.[1] Herein we describe an improved procedure for reduction of cyclic ketones to thermodynamically more stable alcohols using DIBAO^{i}Pr.

The reactivity of DIBAO^{i}Pr in a half stoichiometric amount toward representative cyclic ketones under reflux in ethyl ether and the isomeric ratio of the product mixture are summarized in Table 1.

When the reduction of excess cyclic ketone with the reagent was carried out at 25°, 1 equiv of ketone was reduced in a relatively fast rate but the reduction of further equiv of ketone was insignificant, indicating the involvement of only the isopropoxy moiety of DIBAO^{i}Pr in this reduction. However, when the reduction was repeated in refluxing ethyl ether, the unreacted ketone was also reduced slowly. Finally, all of the cyclic ketones examined, with the exception of camphor, underwent complete reduction with a half stoichiometric amount of DIBAO^{i}Pr in 10-15 days. Camphor is resistant to reduction under the reaction conditions.

The most interesting feature of the Table is that the stereochemistry of reduction with DIBAO^{i}Pr is apparently dependent on the reaction time, as described in the previous paper.[1] The stereoselectivity increases consistently with increase of reaction time to afford the thermodynamically more stable isomer alcohols exclusively (Eq. 1).



(1)

98 % *trans*
100 % total yield

This seems to be a phenomenon that must rise where the thermodynamically less stable alcohol isomer, one of the two isomers produced by reduction with DIBAO^{i}Pr, is converted to the more stable one by thermodynamically con-