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Interactions between Bovine Myelin Basic Protein and Zwitterionic Lysophospholipids†

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ABSTRACT: The binding of myelin basic protein to lysolauroylphosphatidylcholine (lysoLPC) and lysolauroylphosphatidylethanolamine was investigated at neutral pH using gel partition chromatography and equilibrium dialysis at 20 and 37 °C. The results show that the protein–lysolipid interactions are highly cooperative and that the free lysolipid concentration at which the binding commences is markedly influenced by both the chemical structure of the lysolipids and the temperature. The binding begins just below the critical micelle concentration for both lysolipids, which suggests that the forces governing micellization and the binding are similar. Circular dichroism (CD) spectroscopy was used to follow changes in the conformation of the protein caused by lysomyristoylphosphatidylcholine and lysoLPC. The CD results indicate that lysolipid association with the protein commences below the critical micelle concentration and continues above this concentration. Mechanisms for the lysolipid–protein interaction, which are consistent with the binding and CD data, are discussed.

Myelin basic protein (MBP)[1] is an extrinsic membrane protein which is believed to play a major role in the compaction and stabilization of central nervous system myelin through protein–protein and protein–lipid interactions (Smith, 1977; Braun, 1977; Readhead et al., 1987). However, as yet, there is no clear evidence of the mechanism by which MBP stabilizes myelin. The complex multilamellar structure of the myelin membrane limits the strategies which can be employed and complicates the interpretation of experiments designed to examine the interaction between MBP and other molecules in its native environment. Thus, investigations of this nature have largely been confined to in vitro studies using purified components.

The binding of zwitterionic lysolipids to MBP has been examined by a number of groups, using techniques such as nuclear magnetic resonance (NMR) (Littlemore & Ledeen, 1979; Smith, 1982a; Hughes et al., 1982; Mendz et al., 1984), circular dichroism spectroscopy (Anthony & Moscarello, 1971; Keniry & Smith, 1981; Mendz et al., 1984), ultracentrifugation (Smith, 1982b; Mendz et al., 1988), and electron microscopy (Mendz et al., 1988). These studies have revealed many aspects of lysolipid–MBP interactions, including regions of the protein which may be involved in the binding and the nature of the induced protein conformational changes.

Isotherms for the binding of lysomyristoylphosphatidylcholine (lysoMPC) to MBP have previously been measured by using a recycling gel partition technique (Gow et al., 1987). In the present study, we have extended our examination of zwitterionic amphiphile–MBP interactions to evaluate the dependence of this binding upon temperature, acyl chain length, and headgroup size of the lysolipid. Circular dichroism (CD) experiments were used to determine whether binding occurred above the critical micelle concentration (cmc) of the lysolipid as has been shown for other MBP–lysolipid interactions (Mendz et al., 1984). These results have enabled us to comment on the mechanism of the association of micelle-

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[1] Abbreviations: CD, circular dichroism; cmc, critical micelle concentration; DLPE, dilauroylphosphatidylethanolamine; DPC, dodecylphosphocholine; lysoLPC, lysolauroylphosphatidylcholine; lysoMPC, lysomyristoylphosphatidylcholine; lysoLPE, lysolauroylphosphatidylethanolamine; MBP, myelin basic protein; NMR, nuclear magnetic resonance; SDS, sodium dodecyl sulfate.

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forming lipids with the protein.

Materials and Methods

Lipids. Dilauroylphosphatidylethanolamine (DLPE), lysolauroylphosphatidylcholine (lysoLPC), and lysoMPC were obtained from Sigma Chemical Co (St. Louis, MO) and used without further purification. All other chemicals used were of analytical grade.

Buffers. Two different buffers containing Tris-HCl have been used in the present study: 5 mM Tris buffer–0.154 M NaCl, $I = 0.16$ (low-salt buffer), and 5 mM Tris buffer–0.48 M NaCl, $I = 0.48$ (high-salt buffer). The temperature at which the buffers were adjusted to pH 7.4 corresponded to that at which they were used.

Phosphorus Analyses. Two phosphorus assays were used to determine the amount of lysolipid in binding experiments. An assay employing malachite green as the chromophore (Van Veldhoven & Mannaerts, 1987) was used over the range of 1–20 nmol of lysolipid per sample, and the method described by Chen et al. (1956) was used to measure lysolipid in the range of 10–70 nmol.

Synthesis of Lysolauroylphosphatidylethanolamine (LysoLPE). LysoLPE was prepared from its parent diacyl compound by a procedure previously described for the synthesis of [1-^{14}C]lysoMPC (Gow et al., 1987). DLPE was deacylated by using porcine phospholipase A_2 (Boehringer-Mannheim, Mannheim, FRG) and the progress of the reaction monitored by using thin-layer chromatography on silica gel 60 F_{254} plates (Merck, Darmstadt, FRG) with chloroform/methanol/28% ammonia (1:5:1 by volume) as the solvent. The constituent lipids of the reaction were visualized by using the Zinzadze stain for phosphorus, ninhydrin stain for free amines, bromo-cresol green stain for organic acids, and charring with 40% (by volume) H_2SO_4 (Kates, 1972). Chromatographically pure lysoLPE was dissolved in a small volume of low-salt buffer for use in binding experiments at 37 °C.

Purification of Bovine MBP. MBP was purified according to the procedures described by Oshiro and Eylar (1970) and Diebler and Martenson (1973). Three major protein fractions were routinely eluted from the ion-exchange column with increasing ionic strength, designated components 4–6 in the order of elution (Chou et al., 1976). According to Chou et al. (1976), these components represent forms of MBP which differ by one net positive charge. The most cationic form of MBP (component 6) and component 5 were used in experiments, separately, after dialysis for 24 h at 4 °C against several changes of the appropriate buffer. Below, unless a distinction needs to be drawn, the two forms of the protein have been referred to collectively as MBP. The concentration of MBP was determined either spectrophotometrically on the basis of an absorption coefficient ($A^{1\%}_{1cm}$) of 5.44 at 280 nm and a monomeric molecular weight of 18 400 (Smith, 1980) or using the method of Lowry et al. (1951) with MBP as the standard.

Micellar Characteristics of LysoLPC and LysoLPE. The cmc for lysoLPC was determined at 20 and 37 °C by monitoring the rate of increase in the lysolipid concentration of buffer dialyzed against a concentrated solution of the lysolipid. The breakpoint in these curves occurs at the cmc (Abu-Hamdiyyah & Mysels, 1967). Values of the cmcs for lysoLPC and lysoLPE were obtained from the breakpoints in the standard curves used for the gel partition binding experiments.

The micellar size of lysoLPC (10 g/L) in high-salt buffer was determined at 20 °C by sedimentation equilibrium at 15 000 and 20 000 rpm in a Beckman Model E ultracentrifuge as described by Gow et al. (1987). The partial specific volume of lysoLPC was calculated to be 0.90 mL/g [from Table 2 of Tanford and Reynolds (1976)].

Binding Studies. Isotherms for MBP binding to lysoLPC and to lysoLPE were determined by using a recycling gel partition technique (Ford & Winzor, 1981; Gow et al., 1987) at 20 and 37 °C, respectively. LysoLPC–MBP binding was also measured by equilibrium dialysis at both temperatures.

For the recycling gel partition studies, high-salt buffer was used in binding experiments at 20 °C, and low-salt buffer was used at 37 °C. Approximately 10 mL of buffer, 2.00 g of Bio-Gel P-2 resin (Bio-Rad, Richmond, CA), and varying amounts of component 6 were added to a glass column with a sintered-disk base. Circulation of the mobile phase was achieved by using a peristaltic pump connected to the base and the top of the column. Small aliquots of a stock lysolipid solution, with volume V_a and concentration $(m_S)_a$, were added and allowed to equilibrate between the mobile and stationary phases of the stirred gel slurry for at least 15 min. Thereafter, 30–300-μL duplicate samples of the mobile phase were removed, to determine the total lysolipid concentration.

After each addition of lysolipid to the system, the amount of lysolipid associated with the stationary phase, $V_g(\bar{m}_s)_g$, was calculated by using the equation:

$$V_g(\bar{m}_S)_g = \sum V_a(m_S)_a - V_m(\bar{m}_S)_m - \sum V_r(\bar{m}_S)_r$$

where V_g and $(\bar{m}_S)_g$ represent the volume and total lysolipid concentration for this phase, respectively. The first term on the right-hand side of the equation represents the cumulative amount of lysolipid added to the system. The second term represents the total amount of lysolipid measured in the mobile phase at equilibrium, derived from the product of the volume, V_m, and the total lysolipid concentration $(\bar{m}_S)_m$. The final term is a correction for the cumulative amount of lysolipid removed for the phosphorus analyses; V_r and $(\bar{m}_S)_r$ respectively represent the volume and total lysolipid concentration of the mobile phase removed at equilibrium. The free lysolipid concentration in the mobile phase, $(m_S)_m$, for each addition of stock lysolipid solution was determined from the amount of lysolipid in the stationary phase, using a standard curve constructed from the results of several partition experiments conducted in the absence of protein.

Before and after each partition experiment, the total MBP concentration, $(\bar{m}_A)_m$, was determined from centrifuged samples of the mobile phase. This enabled calculation of the intermediate concentrations of $(\bar{m}_A)_m$ by taking into account the volume changes in the system. Isotherms were then constructed by graphing the binding function (Klotz, 1946), $r = [(\bar{m}_S)_m - (m_S)_m]/(\bar{m}_A)_m$, against $(m_S)_m$.

Dialysis equilibrium experiments were conducted by using a multichamber dialysis cell (Technilab Instruments Inc., Pequannock, NJ) and a Spectrapor dialysis membrane with a 3500 molecular weight cutoff (Spectrum Medical Industries Inc., Los Angeles, CA). The procedure below describes an experiment for one chamber in the dialysis cell.

Two solutions (each 450 μL), one containing lysoLPC in buffer and the other containing lysoLPC plus MBP in buffer, were prepared in separate test tubes. Sodium azide (2 mM) was added to the solutions to arrest bacterial growth. The lysolipid-to-protein ratio, approximately equal to that expected at equilibrium, was chosen to minimize the time required for dialysis. Samples of these solutions (each 250 μL) were placed in the dialysis cell, on opposite sides of the membrane, and rotated continuously at the desired temperature for at least 24 h.

Attainment of dialysis equilibrium was ensured by utilizing two dialysis chambers in which the protein solutions approached the same lysolipid-to-protein ratio from opposite

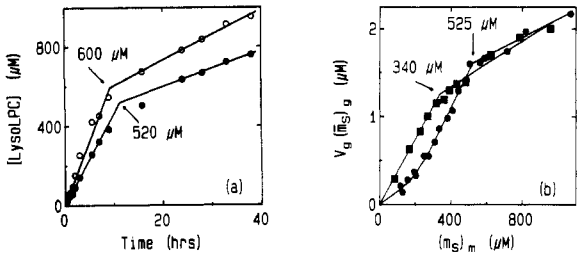

FIGURE 1: Measurement of the cmcs for lysoLPC and lysoLPE. (a) Rate of dialysis of lysoLPC in high-salt buffer at 20 °C (●) and in low-salt buffer at 37 °C (○). (b) Gel partition data in the absence of protein. $V_g(\bar{m}_S)_g$ represents the amount of lysolipid associated with the stationary phase, and $(m_S)_m$ is the free lysolipid concentration in the mobile phase. LysoLPC in high-salt buffer at 20 °C (●) and lysoLPE in low-salt buffer at 37 °C (■).

directions. For some measurements, equilibrium was approached from one direction; this method was used only after establishing that 24 h was sufficient time to reach equilibrium.

After dialysis, the solutions were recovered to determine the concentrations of protein and lysolipid present. The free lysolipid concentration was measured in the protein-free solution, and values for the total lysolipid and protein concentrations were obtained from the solution containing protein. The binding function was then calculated for different free lysolipid concentrations, which were obtained by varying the initial lysolipid-to-protein ratios.

Conservation of lysolipid and protein in the experiments was routinely checked, and the integrity of the protein was also examined before and after dialysis by electrophoresis on sodium dodecyl sulfate (SDS)–polyacrylamide gels (Laemmli, 1970). A small amount of breakdown in some protein samples was evident after dialysis by the presence of a protein band with apparent molecular weight 20000 ± 1000. For the intact protein, M_r = 21000 ± 1000 which is consistent with previously reported values for MBP (Campagnoni & Magno, 1974). This proteolysis had no detectable effect on the lysolipid binding (curve d in Figure 2).

Three separate experiments were carried out using dialysis equilibrium. First, dialysis data were obtained for lysoLPC binding to component 6 in high-salt buffer at 20 °C and compared with the equivalent data from gel partition experiments. Second, the binding of lysoLPC to component 5 was measured in high-salt buffer at 20 °C and compared with the equivalent data for component 6. Finally, dialysis experiments were carried out in low-salt buffer at 37 °C to compare lysoLPC–component 6 binding data with the lysoLPE–component 6 data obtained by using the gel partition technique.

Circular Dichroism. The CD of duplicate samples of MBP, in the absence and presence of lysolipid, was measured at 222 nm in a 1-mm path-length cell at 20 °C using a Jobin Yvon Dichrographe III (Jobin Yvon, Roussel-Jouan, France). The spectrometer was calibrated at 304 nm using an epiandrosterone standard, as recommended by the manufacturers. A 2-nm band-pass was used with a time constant of 20 s. All samples were optically clear; however, they were passed through a 0.22-μm filter prior to measurement of the spectra. The spectra were corrected for the minor contribution arising from the optical activity of the lysolipids.

RESULTS

Micellar Characteristics of the Lysolipids. From the present work, the cmc for lysoLPC was found to be 520 ± 10 μM in both high-salt buffer (Figure 1a) and low-salt buffer (data not shown) at 20 °C. The cmc of lysoMPC was previously found to be 58–65 μM in high-salt buffer at 20 °C (Gow et al., 1987). These values are in accord with previously

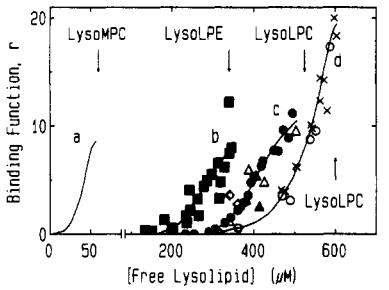

FIGURE 2: Isotherms for MBP binding to lysoMPC, lysoLPC, and lysoLPE at protein concentrations of 0.5–1.3 g/L. Curve a, lysoMPC binding to component 6 in high-salt buffer at 20 °C [from Figure 4a in Gow et al. (1987)]. Curve b, gel partition data for lysoLPE binding to component 6 in low-salt buffer at 37 °C (■). Curve c, lysoLPC binding to components 5 and 6 in high-salt buffer at 20 °C: gel partition data (●) and dialysis equilibrium data approached from both directions (◇), for component 6; dialysis equilibrium approached from one direction (△) and both directions (▲), for component 5. Curve d, dialysis equilibrium data approached from both directions, for lysoLPC binding to component 6 in low-salt buffer at 37 °C: no protein breakdown during the experiment (○) and a small amount of breakdown (×). The arrows indicate the cmcs of the lysolipids.

published data for lysoLPC and lysoMPC of 500 and 63 μM, respectively, in 20 mM Tris-HCl buffer, pH 7.0 at 20 °C (Nakagaki et al., 1986). These data highlight the invariance of the cmc of zwitterionic lysolipids over a wide range of ionic strengths (I = 0.02–0.48). The cmc of lysoLPC was also measured at 37 °C in low-salt buffer using dialysis equilibrium and was found to be 600 ± 15 μM. This value is significantly greater than the cmc measured at 20 °C and is consistent with the effect of temperature on many lysolipids (Bourrel & Schechter, 1988).

LysoLPE was found to have limited solubility in low-salt buffer at room temperature; thus, all experiments with this lysolipid were carried out at 37 °C. The cmc in low-salt buffer was 340 ± 10 μM, from the recycling gel partition standard curve (Figure 1b).

The z-average molecular weight of lysoLPC micelles in high-salt buffer at 20 °C was found to be 27600 ± 1500 from sedimentation equilibrium experiments, which corresponds to a micelle size of 63 ± 3 molecules. Under similar conditions, the average micelle size for lysoMPC was previously found to be 103 ± 3 molecules (Gow et al., 1987). Lipids which form micelles containing greater than approximately 50 molecules have well-defined cmcs (Tanford, 1980).

Binding Studies. The gel partition technique (Ford & Winzor, 1981) was used to measure lysoLPE binding to component 6 and also for the initial lysoLPC–component 6 experiments. However, equilibrium dialysis experiments were found to be more convenient for the latter system and yielded data in accord with the gel partition data.

The isotherms for the binding of lysoLPC and lysoLPE to MBP at protein concentrations of 0.6–1.3 g/L are shown in Figure 2. The curve for lysoMPC–component 6 binding at protein concentrations of 0.5–0.9 g/L [Figure 4a from Gow et al. (1987)] has also been included. Comparison of curves a and c illustrates the marked effect of lysolipid acyl chain length on the protein–lysolipid interaction. The effect of the lysolipid headgroup size can be seen by comparing curve b with curve d while comparison of curves c and d shows the effect of temperature on the binding.

The curves in Figure 2 show that the protein–lysolipid interactions are highly cooperative and that the binding commences just below the cmc for each lysolipid. The maximum value of the binding function, r, at the cmc (r_{cmc}) obtained with lysoLPC at 37 °C varies from those obtained with the other

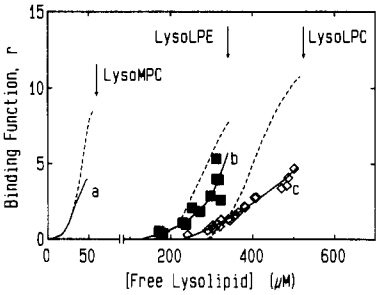

FIGURE 3: Isotherms for component 6 binding to lysoMPC, lysoLPC, and lysoLPE at protein concentrations of 2.3–3.6 g/L. The dashed lines represent the corresponding data for the lysolipids from Figure 2. Curve a, lysoMPC binding in high-salt buffer at 20 °C [from Figure 4a in Gow et al. (1987)]. Curve b, gel partition data for lysoLPE binding in low-salt buffer at 37 °C (■). Curve c, dialysis equilibrium data approached from both directions (◇), for lysoLPC binding in high-salt buffer at 20 °C. The arrows indicate the cmcs of the lysolipids.

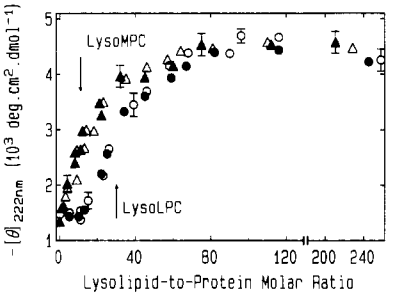

FIGURE 4: Mean residue ellipticities at 222 nm for 0.4–0.48 g/L MBP binding to lysoMPC (△, ▲) and lysoLPC (○, ●) in high-salt buffer at 20 °C. The open symbols represent data for component 5, and the closed symbols are for component 6. Estimates of experimental errors are indicated by the vertical bars. The arrows mark the lysolipid-to-protein molar ratios corresponding to r_{cmc}.

systems. However, the slope of the isotherms near the cmc is high, and the uncertainty in r_{cmc} is correspondingly large.

Isotherms for lysoLPC and lysoLPE binding to component 6 at protein concentrations of 2.3–3.0 g/L at 20 and 37 °C, respectively, are presented in Figure 3, and the curve for lysoMPC–component 6 binding [Figure 4a from Gow et al. (1987)] at a protein concentration of 3.6 g/L has also been included. The dashed lines represent the corresponding binding data for the lysolipids from Figure 2, which illustrate that for each lysolipid, r has decreased with increasing protein concentration.

Circular Dichroism. Measurement of the mean residue ellipticity at 222 nm is a convenient method for following changes in protein secondary structure (Sears and Beychok, 1973). The MBP spectra show an inflection point (i.e., $d[\theta]/d\lambda = 0$) at 222 ± 4 nm over the range of lysolipid concentrations used; for each spectrum, the value of $[\theta]_{222nm}$ was within 3% of $[\theta]$ at the inflection point. The data presented in Figure 4 show the changes to components 5 and 6 in the presence of increasing amounts of lysoMPC and lysoLPC. The equivalence of these data for both forms of the protein, and the similarity of the binding data in curve c of Figure 2, indicates that the posttranslational modifications in component 5 (Chou et al., 1976) have little effect on lysoLPC and lysoMPC binding. Moscarello et al. (1986) and Wood and Moscarello (1989) have previously observed differences in lipid binding between components 5 and 6. However, these studies, which investigated MBP binding to lipid vesicles comprised of mixtures of zwitterionic and acidic diacylphospholipids, are not comparable with the present lysolipid–MBP interactions because of the different properties of the lipids.

The sigmoidality of the MBP–lysoLPC data in Figure 4 is consistent with the binding isotherms shown in Figure 2 (curve c) and Figure 3 (curve c). Furthermore, the lysolipid-to-protein ratio at which $[\theta]_{222nm}$ starts decreasing corresponds to the free lysolipid concentration at which binding commences. The CD data for lysoMPC–MBP may also be sigmoidal; however, the low concentration at which this lysolipid begins binding to MBP makes it difficult to observe any plateau at the beginning of the graph.

The lysolipid-to-protein molar ratios at which the cmc for each lysolipid was reached, calculated from the binding isotherms in Figure 2, have been indicated. These ratios also correspond to the points at which approximately half of the total mean residue ellipticity changes have occurred. As the protein concentration used was below that at which MBP has been shown to self-associate in the presence of lysoMPC (Smith, 1982b), the spectral changes observed are probably attributable to lysolipid binding. The changes in ellipticity at 222 nm are likely to arise from alterations in the secondary structure of the protein, although it has been demonstrated that movement of aromatic side chains into a more hydrophobic environment may also contribute to this region of the spectrum (Cann et al., 1986).

Discussion

The binding of zwitterionic lysolipids to MBP has been examined to assess the effects of temperature, lysolipid acyl chain length, and headgroup size on this protein–lysolipid interaction. The results show that while the highly cooperative nature of the binding is unchanged, the free lysolipid concentration at which binding occurs is markedly influenced by the structure of the lysolipid and the temperature. In each instance, binding commences at lysolipid concentrations just below the cmc. We therefore conclude that the hydrophobic interactions which promote micellization are similar to those which govern the binding of these lysolipids to the protein.

Another feature of the results is that association of the lysolipids with MBP commences below the cmc (Figures 2 and 3) and continues above it (Figure 4); the former feature is consistent with monomer binding to the protein, whereas the latter is suggestive of association with micelles. However, interpretation of these observations relies on an understanding of the behavior of micelle-forming lipids in the region of the cmc. For lipids such as lysoMPC and lysoLPC, which form large micelles in solution, the cmc is defined as a narrow range of concentrations within which lysolipid monomers begin to self-associate. Below this concentration, lysolipid is present almost exclusively as monomer, and above the cmc essentially all added lysolipid is incorporated into micelles with the monomer concentration remaining almost constant (Mukerjee & Mysels, 1971). The results in Figures 2–4 may be explained by the existence of nucleation sites on MBP which act as the focus for micelle formation on the protein, or by the binding of preformed micelles.

The first explanation presumes that MBP binds lysolipid monomers and that this complex behaves as a mixed micelle which competes for lysolipid with protein-free micelles. The view that monomeric, surface-active molecules adsorb to hydrophobic surfaces in preference to micellization is widely accepted (Mittal & Mukerjee, 1977; Hartley, 1977; Tanford, 1980). MBP is known to exist in solution at neutral pH as a highly flexible coil with most of its amino acid residues exposed to the solvent (Liebes et al., 1975; Martenson, 1978). Thus, any of the small hydrophobic segments dispersed throughout the polypeptide chain could act as nucleation sites for micelle formation.

Another consequence of the competition between the protein–lysolipid complex and micellization has been considered by Tanford (1980; Figure 14-2b). If the free energies of transfer for the monomeric lysolipid in solution to the complex and to the micelle are approximately equal, saturation of the binding sites on the protein would not be achieved before the cmc. Above the cmc, the remaining exposed hydrophobic sites on the protein would still compete for lysolipid with the protein-free micelles. The important implication for the binding and CD data is that binding may continue above the cmc of the lysolipid even though the free monomer concentration is virtually constant.

For a system in which MBP interacts with monomeric lysolipid, binding below the cmc may be interpreted on the basis of classical ligand–acceptor binding theory. Such an approach was used by Gow et al. (1987) to determine the minimum number of sites on the protein. The mathematical model used to fit the data has proved useful at free lysoMPC concentrations below the cmc; however, limitations in the experimental methods used to obtain the data preclude measurement of the binding above this concentration and thus provide an incomplete description of the interaction.

An alternative explanation for the binding data is based on MBP binding to the small concentration of micelles which is known to exist at concentrations just below the cmc (Tanford, 1980). Sculley et al. (1981) have shown that exclusive binding of micellar ligand to a single site on an acceptor gives rise to sigmoidal isotherms comparable to those presented in Figures 2 and 3.

The high protein concentration binding data in Figure 3 can also be rationalized in terms of both explanations above. Sedimentation equilibrium analyses (Smith, 1982b) revealed the existence of a critical protein concentration of approximately 1 g/L, above which lysoMPC causes protein self-association. Hydrophobic surfaces involved with this aggregation would not be accessible to the lysolipid, and the value of r_{cmc} would decrease accordingly. In this way, MBP self-association may be viewed as a process in competition with the protein–lysolipid interaction even though the former is enhanced by the lysolipid (Smith, 1982b). Other explanations may also account for the binding at high protein concentration.

It is not possible to distinguish between the two modes of binding discussed above, on the basis of the results in the present study. However, these results do suggest that the binding may arise from nonspecific protein–lysolipid interactions. This is further illustrated by the diverse nature of the hydrophobic molecules reported to interact with the protein. These include azo dyes (Liebes et al., 1976), heme (Vacher et al., 1984), and fatty acids (Anthony & Moscarello, 1971).

Previous attempts at measuring the stoichiometry of lysophosphatidylcholine–MBP binding have been inconclusive. Smith and McDonald (1979), using gel filtration chromatography, found that similarity in the sizes of the micelle and the protein–lysolipid complex precluded accurate determination of the amount of lysolipid bound to the protein.

Another zwitterionic lysolipid for which binding to MBP has been examined in detail is dodecylphosphocholine (DPC) (Mendz et al., 1984, 1988). From ultracentrifugation experiments, MBP was found to bind two micelles per polypeptide. In CD experiments, the protein conformation was affected below the cmc of the lysolipid and continued to change above this concentration, behavior which parallels the behavior of the data in Figure 4. NMR experiments indicated that the protein backbone was largely exposed to the solvent and that several amino acid residues penetrated a small distance into the micelle core. On the basis of their results, Mendz et al. (1988) proposed explanations for the MBP–DPC interactions which are similar to those discussed above.

The interaction between MBP and SDS has also been examined in considerable detail. The binding stoichiometry was found to be approximately two detergent micelles per polypeptide over a wide range of ionic strength and pH, which is an unusually high ratio for a water-soluble protein (Smith & McDonald, 1979; Burns et al., 1981; Burns & Campagnoni, 1983).

In conclusion, the results presented here show that MBP binds lysolipids at concentrations below and above their cmcs. The cmcs of these lysolipids and binding to the protein show a parallel dependence upon the length of the acyl chain, the headgroup size, and the temperature. Therefore, MBP appears to bind these zwitterionic lysolipids nonspecifically, through hydrophobic interactions similar to those which promote micelle formation.

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Registry No. DLPE, 42436-56-6; lysoLPC, 20559-18-6; lysoMPC, 20559-16-4; lysoLPE, 120578-22-5.

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Purification and Characterization of Corticosteroid Side Chain Isomerase†

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ABSTRACT: Corticosteroid side chain isomerase of rat liver catalyzes the interconversion of the ketol (20-oxo-21-ol) and aldol (20-hydroxy-21-al) forms of the corticosteroid side chain. The enzyme has now been purified to apparent homogeneity from rat liver cytosol by sequential chromatography on anionic, hydroxylapatite, and gel filtration columns. Ketol–aldol isomerization is followed by measuring the exchange of tritium from 21-tritiated steroids with water. The native enzyme is a dimer of MW 44000. The isoelectric point is 4.8 ± 0.1 pH units. The purified enzyme is stimulated by Co^{3+} or Ni^{2+}. The enzyme utilizes 11-deoxycorticosterone, corticosterone, and 17-deoxycortisol as substrate but not cortisol, tetrahydrocortisol, and prednisolone. Tritium–water exchange of (21*S*)-[21-^{3}H]DOC is a pseudo-first-order reaction; 21-^{3}H exchange from the 21*R* isomer proceeds with first-order kinetics only after a lag associated with its epimerization to the 21*S* form.

The structures of the neutral urinary metabolites of cortisol in humans are well established. There are additional metabolites, which make up to 20% of the total excretion products in adults, that have been shown by us to be acids. The major acidic metabolites are 20-hydroxy-21-oic acids, to which we have given the collective name cortoic acids (Bradlow et al., 1973). The 21-oic acids are major metabolites of cortisol and corticosterone in other mammals as well (Monder & Bradlow, 1980). Mice convert corticosterone to acid end products that are structurally similar to the cortoic acids in a sequence of steps that parallel those that occur in man (Han et al., 1983). This pathway of corticosterone metabolism is quantitatively important. About half of the corticosterone secreted by the mouse adrenal is converted to epimeric 20-hydroxy-21-oic acids (Marandici & Monder, 1983; Han et al., 1983). We have found that the main metabolic pathway to the hydroxy acids utilizes a 20-hydroxy-21-aldehyde intermediate formed by rearrangement of the ketol side chain (Martin et al., 1977; Monder et al., 1980a). This is a central intermediate since it sits at a metabolic junction where the choice is made between the conversion of corticosteroids to metabolites with acidic (20-hydroxy-21-oic acid) and those with neutral (20,21-diol) side chains (Monder et al., 1980a,1982; Han et al., 1981; Wermuth & Monder, 1983). This relationship is illustrated in Figure 1. The ketol–aldol interconversion that initiates this process is catalyzed by corticosteroid side chain isomerase (Monder et al., 1980b). In this paper we describe the purification and properties of this enzyme.

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