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Equilibrium Binding of Myristoyllysophosphatidylcholine to Bovine Myelin Basic Protein: An Example of Ligand-Mediated Acceptor Association†

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ABSTRACT: The interaction of myristoyllysophosphatidylcholine with bovine myelin basic protein at pH 7.4 and 4.5, I = 0.48, has been investigated by a recycling partition equilibrium technique with Bio-Gel P-2 as the gel phase. Important points to emerge from this direct binding study are (i) that it is a monomeric (not micellar) amphiphile that binds to myelin basic protein, (ii) that the amphiphile binds preferentially to the monomeric form of myelin basic protein, (iii) that this binding to monomer is highly cooperative, (iv) that the similarity of binding behavior in the two environments tested is consistent with the dominance of a hydrophobic contribution to the protein–amphiphile interaction, and (v) that the self-association of myelin basic protein in the presence of phospholipid [Smith, R. (1982) *Biochemistry 21*, 2697–2701] must reflect the aggregation of a protein–amphiphile complex(es) coupled with concomitant release of some lipid. These findings are then related to earlier nuclear magnetic resonance and circular dichroism studies in which the results were interpreted on the basis that myelin basic protein bound preferentially to micellar phospholipid.

Central nervous system myelin contains two predominant proteins in association with an unusually high lipid content. One of these proteins, the proteolipid protein, displays many of the properties considered characteristic of an intrinsic membrane protein that is retained within the lipid bilayer by hydrophobic interactions. The other major protein, myelin basic protein (MBP),[1] is less easily classified. Evidence for its classification as an extrinsic membrane protein associated

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[1] Abbreviations: cmc, critical micelle concentration; DMPC, dimyristoylphosphatidylcholine; lysoMPC, myristoyllysophosphatidylcholine; MBP, myelin basic protein; NMR, nuclear magnetic resonance.

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with the polar head groups of the lipid has emerged from X-ray diffraction studies, which have been interpreted as showing little protein penetration beyond the head groups in complexes with acidic lipids (Sedzik et al., 1984; MacNaughtan et al., 1985). On the other hand, NMR studies have provided substantial evidence for the interaction of MBP with the lipid acyl chains rather than the head group. Thus, the ^1H NMR peak of the methylene chains in myristoyllysophosphatidylcholine (lysoMPC) is shifted upfield by the binding of this amphiphile to MBP, whereas resonances from the lipid head group are unaffected (Smith, 1982a); the ^{31}P NMR spectrum of the lipid is also narrowed (Littlemore & Ledeen, 1979). In a similar vein, NMR spectroscopy has provided evidence for a hydrophobic interaction of MBP with ganglioside G_{M1} (Ong & Yu, 1984) and with sodium dodecyl sulfate (Liebes et al., 1976). The possibility therefore arises that, even though MBP may be bound at the bilayer surface, the protein may also interact with the hydrophobic segments of lipids either within or external to the lipid bilayer.

Interaction with simple amphiphiles (Anthony & Moscarello, 1971; Liebes et al., 1976; Keniry & Smith, 1981; Mendz & Moore, 1983) and with vesicles of acidic lipids (Keniry & Smith, 1979, 1981) causes alterations in the secondary structure of MBP, whose state of association is also enhanced by lysoMPC (Smith, 1982b). From the viewpoint of biological relevance, lipid vesicles provide the better model of a membrane environment, but the presence of large lipid aggregates severely restricts the range of experimental methods by which such systems may be examined. The use of single-chain amphiphiles thus offers distinct advantages for probing quantitative aspects of the lipid–protein interaction.

In this investigation a recycling gel partition technique for the study of ligand binding (Ford & Winzor, 1981) is used to study the interaction of lysoMPC with MBP at pH 7.4 and 4.5. These direct binding studies indicate preferential interaction of lysoMPC with monomeric MBP, the enhanced association of which in the presence of amphiphile (Smith, 1982b) is therefore considered to reflect lipid-mediated association, i.e., the aggregation of MBP–lysoMPC complex(es).

Materials and Methods

Lipids. Dimyristoylphosphatidylcholine (DMPC) and lysoMPC were obtained from Sigma Chemical Co., St. Louis, MO, and a radioactive analogue of the former, 1,2-di[1-^{14}C]myristoyl-*sn*-glycero-3-phosphocholine with a specific activity of 120 mCi/mmol, was supplied by the Radiochemical Center, Amersham.

Purification of MBP. After isolation of MBP from bovine brain white matter by the method of Oshiro and Eylar (1970), the covalently modified forms of the protein were removed by ion-exchange chromatography on (carboxymethyl)cellulose (CM-32, Whatman, Maidstone, Kent) in accordance with the procedure described by Diebler and Martenson (1973). Prior to use, this purified, unmodified MBP was dialyzed for 24 h at 4 °C against several changes of either phosphate–chloride buffer (0.1 M Na_2HPO_4–0.02 M HCl–0.2 M NaCl–0.002 M NaN_3), pH 7.4, I = 0.48, or acetate–phosphate–chloride buffer (0.1 M CH_3COONa–0.13 M CH_3COOH–0.1 M NaH_2PO_4–0.28 M NaCl–0.002 M NaN_3), pH 4.5, I = 0.48. The integrity of the MBP was confirmed before and after the binding experiments by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Laemmli, 1970). Concentrations of the dialyzed MBP solutions were determined spectrophotometrically on the bases of an absorption coefficient ($A^{1\%}_{1cm}$) of 5.44 at 280 nm and a monomeric molecular weight of 18 400 (Smith, 1980).

Synthesis of [1-^{14}C]LysoMPC. Radioactive lysoMPC was prepared from the parent diacyl lipid by an adaptation of the standard procedure for such deacylation (Kates, 1972). To a methanolic solution (0.4 mL) containing [1-^{14}C]DMPC (10 μCi) and unlabeled DMPC (40 mg) were added peroxide-free diethyl ether (19.6 mL) and 0.1 M tetraborate buffer (pH 7.4) containing 16 mM $CaCl_2$ (2 mL). A small volume (30 μL) of porcine phospholipase A_2 (Boehringer-Mannheim GmbH, West Germany) was quickly added to the vigorously shaken mixture, which was then stirred continuously for 70 h at room temperature. The progress of deacylation was monitored by thin-layer chromatography (silica gel 60 F_{254} plates; Merck, Darmstadt, West Germany) with chloroform/methanol/ammonia (65:45:10, v/v) as solvent, authentic samples of DMPC, lysoMPC, and myristic acid being chromatographed concurrently for identification purposes. The aqueous phase (which contained no detectable [1-^{14}C]DMPC) was freeze-dried and the residue resuspended in chloroform/methanol (2 mL; 19:1 v/v) prior to removal of salts by chromatography on a column (2 × 20 cm) of Sephadex G-25 (Pharmacia, Uppsala, Sweden) equilibrated with the chloroform/methanol solvent. Desalted reaction products (2 mL) were then applied to a column (1 × 15 cm) of (diethylaminoethyl)cellulose (Whatman DE-52) equilibrated with the same solvent (Rouser et al., 1961) and eluted with 7:1 (v/v) chloroform/methanol: thin-layer chromatography was used to monitor the column eluate. Those fractions (5 mL) containing solely [1-^{14}C]lysoMPC were pooled, dried by rotary evaporation, and dissolved in the appropriate buffer (pH 7.4 or 4.5). This solution was then combined with a solution of unlabeled lysoMPC in the same buffer to yield lipid with a specific activity of 50–100 pCi/mmol.

Micellar Characteristics of LysoMPC. The critical micelle concentration (cmc) of lysoMPC was determined by measuring the surface tension acting on a mica plate (2 × 3 cm) suspended vertically from a transducer and dipping 10 mm into 20 mL of phosphate–chloride buffer (pH 7.4, I = 0.48) thermostatically maintained at 20 °C and supplemented with lysoMPC (0–200 mg/L). The maximal transducer response observed during removal of the plate from the liquid was correlated with surface tension by use of the corresponding response with water for calibration purposes.

The micellar sizes of lysoMPC in the phosphate–chloride buffer (pH 7.4, I = 0.48) and acetate–phosphate–chloride buffer (pH 4.5, I = 0.48) were determined by sedimentation equilibrium in a Beckman model E ultracentrifuge operated at 20 °C and a rotor speed of either 15 000 or 20 000 rpm. Equilibrium distributions from these experiments with an amphiphile concentration of 10 g/L were recorded by the schlieren optical system and analyzed by the Lamm (1929) procedure, the partial specific volume of lysoMPC being taken as 0.92 mL/g [Table II of Tanford and Reynolds (1976)].

Binding Studies. A recycling gel partition technique (Ford & Winzor, 1981) was used to study the binding, at room temperature (21 ± 1 °C), of lysoMPC to MBP in both the phosphate–chloride buffer (pH 7.4) and the acetate–phosphate–chloride buffer (pH 4.5). Bio-Gel P-2 (2 g; Bio-Rad, Richmond, CA.) and buffer (10 mL) were placed in a glass column with sintered-disc base, which was then connected to a recycling system to ensure adequate mixing of the gel slurry. An aliquot (0.1 mL) of radiolabeled stock lysoMPC solution (1.0–4.5 g/L) was then added to the slurry and ample time (10 min) allowed for attainment of partition equilibrium. After removal of a sample (0.1 mL) of mobile (liquid) phase for assessment by scintillation counting, further aliquots of

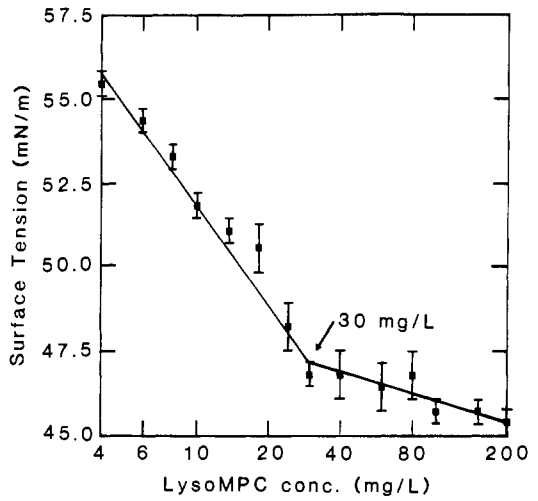

FIGURE 1: Evaluation of the cmc for lysoMPC from surface tension measurements on the amphiphile in phosphate–chloride buffer, pH 7.4, $I = 0.48$.

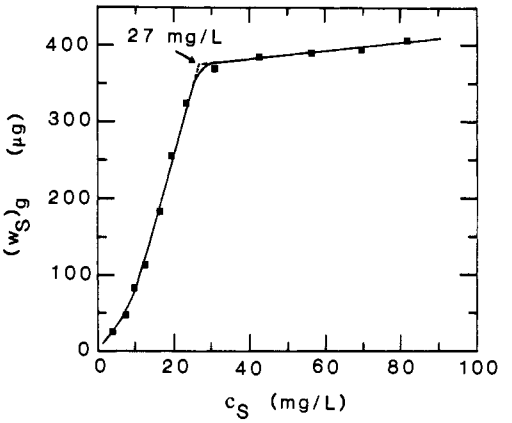

FIGURE 2: Effect of lysoMPC concentration on the distribution of the amphiphile between aqueous and gel phases in partition experiments with Bio-Gel P-2 (2 g) equilibrated with phosphate–chloride buffer (10 mL), pH 7.4, $I = 0.48$. The discontinuity in this calibration plot for MBP–amphiphile binding studies is taken as a second estimate of the cmc for lysoMPC.

[1-^{14}C]lysoMPC stock solution were added, and the process was repeated after each addition. By this means, a calibration curve was constructed to relate the amount $(w_S)_g$ of lysoMPC in the gel phase to its weight concentration c_S in mobile phase with volume V_m. Specifically, $(w_S)_g$ is given by

$$(w_S)_g = V_a(c_S)_a - V_m c_S - (w_S)_r \quad (1)$$

where V_a and $(c_S)_a$ are the cumulative volume and concentration, respectively, of stock solution added and the final term, $(w_S)_r$, takes into account the cumulative amount of lipid removed in the process of determining preceding values of c_S.

For experiments with MBP (0.9–5.8 g/L) included in the initial gel slurry, the assay of mobile phase yielded the total concentration of lysoMPC in that phase, $\bar{c}_S$. Calculation of $(w_S)_g$ from eq 1 then allowed the determination (via the relevant calibration plot) of the corresponding free lipid concentration, c_S. The binding function r, in mol/mol (Klotz, 1946), could thus be determined as $r = (\bar{c}_S - c_S)/M_S\bar{m}_A$, where M_S denotes the molecular weight of the ligand: $\bar{m}_A$, the total base molar concentration of acceptor (MBP) pertaining to the particular $(\bar{c}_S, c_S)$ combination, was calculated on the basis of the initial amount present and the cumulative amount removed in assays of lysoMPC concentrations.

Scintillation Counting. Samples taken from the mobile phase of the binding experiments were mixed with 15 mL of Triton X-100 scintillant (Turner, 1968) and, after storage in the dark for 6–12 h to eliminate chemiluminescence, were counted in a Beckman LS 5800 liquid scintillation spectrometer. To avoid possible errors due to inadequate allowance for quenching, spectral analyses were performed to check that the measured counts reflected the entire, unimodal, energy spectrum associated with ^{14}C. The uncertainty in c_S values arising from counting errors was estimated to be 2–5%.

RESULTS

Micellar Characteristics of LysoMPC. LysoMPC is a typical single-chain amphiphile in that it forms micelles in aqueous solution. Since knowledge of the state of the lipid is essential for quantitative interpretation of binding studies, the micellar characteristics of lysoMPC in the particular environments of interest were determined.

Results of surface tension measurements on solutions of lysoMPC in phosphate–chloride buffer (pH 7.4, $I = 0.48$) are summarized in Figure 1, which signifies a lipid concentration in the vicinity of 30 mg/L (65 μM) for the onset of a significant extent of micellization. A similar value (27 mg/L or 58 μM) is obtained from the calibration curve used for the gel partition studies of lipid binding (Figure 2), a plot which indicates the amount of lysoMPC in the gel phase as a function of amphiphile concentration in the mobile (liquid) phase surrounding the Bio-Gel P-2 beads. Increases of the latter concentration beyond 30 mg/L clearly have less effect on the amount of lysoMPC in the gel phase. Both monomeric and micellar lysoMPC apparently bind to the polyacrylamide matrix, but monomeric amphiphile is the only species to gel partition. Consequently, the abrupt change in the concentration dependence of $(w_S)_g$ is taken to signify the onset of micellization and essential constancy of monomer concentration thereafter. A cmc of 20 mg/L was inferred from the calibration plot obtained at the more acidic pH. For binding studies the important point to emerge from Figures 1 and 2 is the need for caution in interpreting results from experiments with free lipid concentrations greater than the cmc, because of the existence of ever-increasing proportions of the amphiphile in the micellar state.

To comment on the likely size of micellar lysoMPC under the present conditions, a relatively high concentration of amphiphile (10 g/L) in phosphate–chloride buffer (pH 7.4, $I = 0.48$) or acetate–phosphate–chloride buffer (pH 4.5, $I = 0.48$) was subjected to sedimentation equilibrium. Plots of ln $[(1/r)(dn/dr)]$ vs. r^2 were linear, giving z-average M_r's of 49 600 ± 1700 and 48 200 ± 1400 from experiments conducted in the neutral buffer at 15 000 and 20 000 rpm, respectively, and corresponding values of M_r 53 900 ± 1400 and 56 500 ± 1100 in the more acidic environment. Because of the deliberate analyses in terms of the z-average to weight the contribution of micellar species, a value of 100–105 may reasonably be inferred for the aggregation number in phosphate–chloride buffer (pH 7.4), the corresponding parameter being 115–120 in the more acidic buffer system.

Binding Studies. Equilibrium dialysis and ultrafiltration were the initial methods selected for studying the binding of lysoMPC to MBP. However, the former method proved too time consuming, several days of dialysis being required for attainment of transmembrane equilibrium: results obtained by ultrafiltration were inconsistent. In contrast, results obtained by the recycling gel partition technique (Ford & Winzor, 1981) were very reproducible, and equilibrium (both chemical and partition) was reached within minutes, irrespective of the direction from which it was approached.

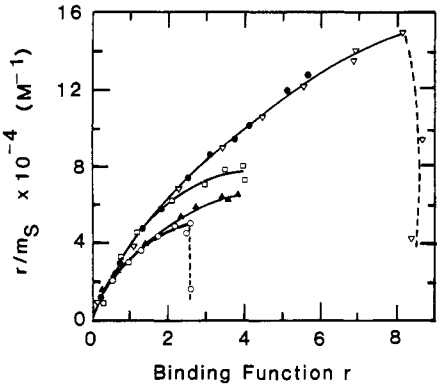

FIGURE 3: Scatchard plots of binding data for the interaction of lysoMPC with MBP in phosphate–chloride buffer, pH 7.4, $I = 0.48$, the initial concentrations of MBP being as follows: (▽) 0.9, (●) 1.6, (□) 3.6, (▲) 4.4, and (○) 5.8 g/L.

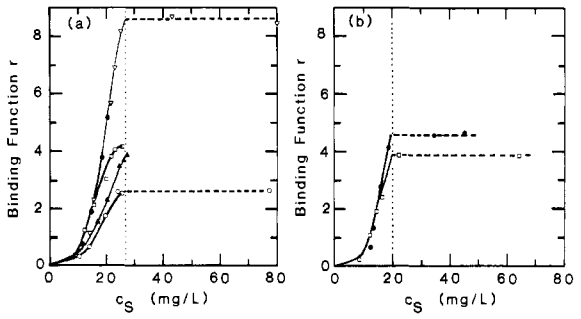

FIGURE 4: Classical binding curves for the interaction of lysoMPC with MBP: (a) replot of the results from Figure 3; (b) corresponding results obtained in acetate–phosphate–chloride buffer (pH 4.5, $I = 0.48$), together with the binding curves (—) from (a).

The interaction of lysoMPC with MBP was first investigated at pH 7.4 with a series of protein concentrations (0.9–5.8 g/L), at the highest of which some 14% of the MBP would have been polymeric in the absence of lipid (Smith, 1980). Scatchard plots of the results exhibited pronounced cooperativity and also a dependence on the MBP concentration used for their determination (Figure 3). Since the extent of amphiphile binding decreases progressively with increasing protein concentration, these results signify the preferential interaction of lysoMPC with monomeric MBP. Furthermore, although there is correspondence between the existence of critical points in Scatchard plots and the sigmoidal nature of the associated binding curves (Baghurst et al., 1978), the maxima observed in Figure 3 do not reflect that correspondence but rather the onset of micellization. This effect is more clearly evident from Figure 4a, which presents the results in direct binding format (r vs. c_S), together with the cmc (vertical dotted line) deduced from the calibration plot (Figure 2). The fact that the binding function increases monotonically with lysoMPC concentration up to the cmc but remains constant thereafter is considered to indicate that MBP exhibits little if any affinity for micellar amphiphile. Thus, the extent of binding remains constant because of the relatively constant concentration of monomeric amphiphile despite increases in the concentration of free lipid. Consequently, the down-turn in the Scatchard plots (Figure 3) is an artifact reflecting use of the latter concentration instead of its monomeric counterpart in evaluating the ordinate parameter, r/m_S.

Essentially identical results were obtained below the cmc for the binding of lysoMPC to MBP at pH 4.5, a factor evident from Figure 4b, which compares the experimental results with the corresponding binding curve taken from Figure 4a: the lower limiting value of r reflects the lower cmc of lysoMPC

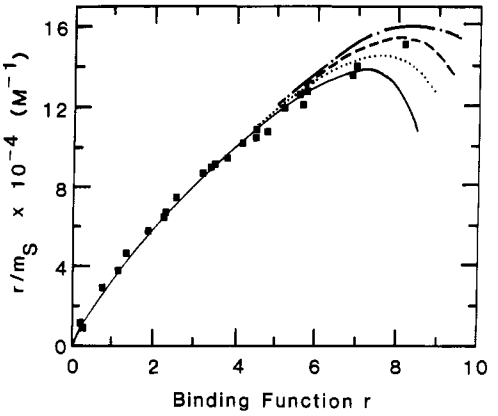

FIGURE 5: Curve fitting of binding data for the MBP–lysoMPC system (0.9 g/L) in phosphate–chloride buffer (pH 7.4, $I = 0.48$) to the allosteric model of Monod et al. (1965). Theoretical plots were calculated from eq 2 with the following magnitudes of parameters: (—) $p = 10$, $Y = 7.35 \times 10^5$, $K_A = 7.35 \times 10^4$ M^{-1}; (···) $p = 11$, $Y = 6.71 \times 10^5$, $K_A = 6.10 \times 10^4$ M^{-1}; (– –) $p = 12$, $Y = 6.24 \times 10^5$, $K_A = 5.20 \times 10^4$ M^{-1}; (–·–) $p = 13$, $Y = 5.88 \times 10^5$, $K_A = 4.52 \times 10^4$ M^{-1}.

in the more acidic environment. Ligand binding is thus not sensitive to the net charge on the protein, which changes substantially over this pH range.

The form of the binding curves has been explained satisfactorily, but there still remains a problem with the extent to which the binding curves exhibit dependence on MBP concentration. Although this concentration dependence is qualitatively consistent with preferential ligand binding to the monomeric form of a self-associating acceptor (Nichol et al., 1967), its magnitude is far too great for interpretation of the results solely in such terms. Furthermore, the preferential binding of amphiphile to monomeric protein must be reconciled with the observation that the association of MBP at pH 7.4 is enhanced in the presence of lysoMPC (Smith, 1982b). A similar situation pertains at pH 4.5, where inclusion of a 10-fold molar excess of lysoMPC has been found to cause the $s_{20,w}$ of a 4.6 g/L solution of MBP to increase from the essentially monomeric value (Smith, 1980) of 1.5 S to 2.6 S. These findings make it necessary to postulate the occurrence of ligand-mediated association of monomer–ligand complex(es), AS_i. In this regard the simplest such model, involving self-association of AS_i, is also precluded since it predicts positive dependence of r upon MBP concentration (the opposite of that observed in Figures 3 and 4). We therefore conclude that the binding of lysoMPC to MBP reflects the interaction of monomeric amphiphile with monomeric protein to form complex AS_i that reversibly associates but with the release of some of the amphiphile [$nAS_i \rightleftarrows CS_q + (ni - q)S$].

In the absence of any definitive means of determining the number of lipid-binding sites (p) on monomeric MBP, an attempt has been made to curve fit the binding data obtained at the lowest acceptor concentration (0.9 g/L), where the protein is essentially monomeric not only in the absence of amphiphile [Figure 6 of Smith (1980)] but also in the presence of lysoMPC concentrations exceeding the cmc [Figure 4 of Smith (1982b)]. For this purpose, the results, replotted in Figure 5, have been considered in terms of the expression (Monod et al., 1965; Nichol et al., 1967]

$$r/m_S = pK_A(1 + K_A m_S)^{p-1}/[(1 + K_A m_S)^p + Y] \quad (2)$$

which attributes the sigmoidal behavior to exclusive binding by one form, A, of acceptor undergoing reversible isomerization ($A \rightleftarrows C$) governed by equilibrium constant Y; K_A is the intrinsic association constant (Klotz, 1946) for the interaction

of ligand with p equivalent and independent sites on A. From the right-hand side of eq 2, the limiting value of r/m_S as $m_S \rightarrow 0$ is $pK_A/(1 + Y)$, which, from any estimate of the ordinate intercept of Figure 3, is a very small number: the additional assumption has been made that this ordinate intercept is unity (for purposes of illustration), whereupon $pK_A = 1 + Y \simeq Y$. With that restriction the substitution of a set of (r, m_S) values into eq 2 then allows calculation of an apparent K_A for any designated value of p, the point ($r = 3.5$, $m_S = 38$ μM) being used for this purpose.

Figure 5 presents the results together with theoretical plots for systems with 10 (—), 11 (···), 12 (– –), and 13 (–·–) amphiphile-binding sites on the monomer, a range of p considerably larger than the value of 4 inferred previously (Smith, 1982b) for the stoichiometry of the MBP–lysoMPC interaction. That estimate was based on the lipid/protein ratio at which the sedimentation coefficient of a 3 g/L MBP solution increased from that of 1.5 S for monomer to a limiting value of 4 S. In light of Figure 4, the constancy of $s_{20,w}$ at high lipid/protein ratios could rationally be explained as signifying that the cmc had been exceeded, and indeed, we note that for a 3 g/L solution of MBP the value of r is in the vicinity of 4 (Figure 4a). Irrespective of the correctness or otherwise of these illustrative descriptions of the binding results, the important points to emerge from this direct binding study are (i) that it is monomeric rather than micellar lysoMPC that binds to MBP, (ii) that the amphiphile binds preferentially to the monomeric form of MBP, (iii) that this binding to monomer is highly cooperative, (iv) that the similarity between binding data obtained at pH 7.4 and 4.5 is consistent with the interaction between MBP and amphiphile being largely hydrophobic, and (v) that the increased association of MBP in the presence of lysoMPC (Smith, 1982b) must reflect aggregation of MBP–amphiphile complex(es).

Discussion

The binding of lysoMPC to MBP is undoubtedly one of the most unusual positively cooperative systems studied to date inasmuch as preferential binding of amphiphile to monomeric protein had to be reconciled with enhanced association of MBP in the presence of lipid (Smith, 1982b, 1985). Since the direct evidence for monomeric MBP being the preferential lipid-binding form is unequivocal, the enhanced protein association in the presence of lipid has been attributed to aggregation of MBP–amphiphile complex(es) coupled with concomitant release of some lysoMPC. However, the sedimentation equilibrium results (Smith, 1982b, 1985) still require rationalization in terms of the formation of polymeric MBP–amphiphile complexes with lower lipid content, a concept postulated to accommodate the extent of the negative dependence of binding curves upon MBP concentration (Figures 3 and 4). In that regard, the major obstacle to surmount is the fact that $\Omega(r)$ analyses (Milthorpe et al., 1975) of sedimentation equilibrium distributions for mixtures with 6:1 and 10:1 (but not 3:1) initial lipid/protein molar ratios yielded indistinguishable dependences of monomeric protein concentration upon total MBP concentration (Smith, 1982b). These results were later shown to be consistent with a two-state (nA $\rightleftarrows$ C) monomer–hexamer equilibrium (Smith, 1985). Irrespective of the mechanism responsible for the ligand-dependent association, such description in terms of a constituent association constant X such that

$$X = c_{CS_q}/[c_A + \sum_{j=1}^{j=p} c_{AS_j}]^6 \quad (3)$$

implies that the concentration of free ligand throughout the equilibrium distributions is either constant or in excess of that required to saturate all acceptor sites. Of these two alternatives, the latter is precluded by the demonstration (Figure 3) that monomeric lysoMPC is the binding form of the amphiphile and that saturation of MBP sites is not achieved before the onset of micellization (Figure 4). On the other hand, the former requirement would be met if the concentration of free lysoMPC were to exceed the cmc, thereby rendering the concentration of monomeric amphiphile effectively constant and identical throughout both sedimentation equilibrium distributions, despite the different lipid/protein mixing ratios: the smaller value of X in the sedimentation equilibrium experiment with 3:1 mixing ratio would then signify that the free concentration of lysoMPC was below the cmc in this instance. On the basis that the reported equilibrium constant for hexamer formation at the higher lipid/MBP ratios does refer to the system with free monomeric amphiphile equal to the cmc, the maximum values of r obtained in the binding experiments at pH 7.4 signify that less than one lipid molecule per polypeptide is retained by hexameric MBP.

This direct binding study has indicated the existence of 10–12 amphiphile-binding sites on monomeric MBP (Figure 5), a number some 2–3-fold higher than earlier indirect estimates of lipid-binding stoichiometry. Thus, Ong and Yu (1984) have interpreted NMR data in terms of four saturable sites for monoganglioside G_{M1}, whereas Bach and Sela (1985) favor the existence of six such sites from the molar ratios of G_{M1}/MBP producing maximal changes in tryptophan fluorescence and differential scanning calorimetry. Since the gangliosides differ from lysoMPC in that they contain two acyl chains, a 2-fold difference in stoichiometry may well correlate with the viewpoint (Tanford, 1980) that the lipid-binding site on some proteins is designed to accommodate a fixed volume of hydrocarbon chain. A second feature to emerge from Figure 5 is the extremely large value of the preexisting isomerization constant Y (Monod et al., 1965) required to account for the cooperativity of lysoMPC binding. Because of the thermodynamic equivalence of the two common models of allostery (Nichol & Winzor, 1981), this finding may alternatively signify a correspondingly large value for K_t, the isomerization constant describing ligand-induced transformation of acceptor to the form with greater affinity (Kosland et al., 1966). Indeed, the latter concept may be considered to be a more plausible explanation inasmuch as the difficulty encountered in the initial binding of amphiphile would be consistent with evidence for the interactions between lysoMPC and MBP being largely hydrophobic. Such evidence includes the effects of MBP on the acyl chain proton NMR of lysoMPC (Smith, 1982a) and the effects of lysoMPC on hydrophobic residues of MBP (Littlemore & Ledeen, 1979; Smith, 1982a).

Preferential interaction of the micellar forms of neutral amphiphiles has often been assumed [e.g., Littlemore and Ledeen (1979), Smith and McDonald (1979), Hughes et al. (1982), and Mendz et al. (1984)]. In this regard, the calibration plot for the present binding study (Figure 2) has a finite slope above the cmc, and therefore, the partition method should still provide information about binding of lysoMPC in this concentration region. The observed constancy of r (Figure 4) from measurements in this region signifies either the absence of additional amphiphile binding or the identical behavior of micellar lysoMPC and micelle–MBP complexes in their interactions with the Bio-Gel beads. Since such identity of interactions is considered extremely unlikely for a system in which the micelle and protein are of comparable size (Stokes radii of 2.6 and 3.0 nm, respectively), we have interpreted the

constancy of r as signifying that micellar lysoMPC exhibits little affinity for MBP. This finding is of particular relevance to the above-mentioned NMR studies, which have entailed the assumption that the binding of MBP to micellar lipid should give rise to a larger rotational correlation time. However, in view of the fact that MBP binds to monomeric lipid, the overall rotational rate of the protein may be little affected, whereas the diffusion rate of the lipid may increase and thus be manifested as a decrease in NMR line width. Such an explanation possibly accounts for the reduction in ^{31}P NMR line width observed (Littlemore & Ledeen, 1979) for lysoMPC in the presence of MBP: if the phosphate does not bind directly to the protein, the P nucleus may well have a more rapid reorientation rate in MBP–amphiphile complexes than in the micelle.

Although there may be little or no interaction between MBP and zwitterionic or nonionic micelles, the protein does bind to micelles of negatively charged amphiphiles [e.g., Smith and McDonald (1979) and Burns and Campagnoni (1983)] and to vesicles formed from acidic lipids [e.g., Keniry and Smith (1979, 1981) and Boggs et al. (1982)].

In conclusion, the results reported here reinforce the view that MBP possesses an extensive surface that is capable of binding hydrophobic molecules. Since MBP apparently does not penetrate deeply into the hydrocarbon region of lipid bilayers (Sedzik et al., 1984; MacNaughtan et al., 1985), it may, in vivo, interact with other apolar molecules external to the membrane bilayer. Alternatively, the hydrophobic site on the protein may form the contact region between associating MBP molecules, a possibility favored by the present inference that partial release of lysoMPC accompanies the amphiphile-mediated association of MBP.

Registry No. LysoMPC, 20559-16-4; [1-^{14}C]lysoMPC, 106252-26-0.

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