Thermodynamic Parameters and Shape of the Mycobacterial Polymethylpolysaccharide–Fatty Acid Complex†

Tadashi Kiho‡ and Clinton E. Ballou*

Department of Biochemistry, University of California, Berkeley, California 94720

Received February 10, 1988; Revised Manuscript Received April 6, 1988

ABSTRACT: Properties of the mycobacterial polymethylpolysaccharide–lipid complex have been investigated by fluorometric techniques. From the dissociation constant for the *O*-methyglucose polysaccharide–parinaric acid complex at 293 K, a Gibbs free energy ($\Delta G°$) of −33.65 kJ/mol was obtained. The K_d decreased with increasing temperature, giving an enthalpy ($\Delta H°$) of 15.4 kJ/mol. From these data, a molar entropy ($\Delta S°$) of 167.4 J K^{-1} was obtained. Thus, the reaction is slightly endothermic, but the large positive entropy change leads to an overall negative free energy favoring complex formation. From fluorescence depolarization measurements, the methylglucose polysaccharide–parinaric acid complex appears to display isotropic rotation with a correlation time of 2.55 ns at 23 °C. This may be compared to a rotational correlation time of 6.17 ps for free parinaric acid in water at 23 °C calculated from the value determined in cyclohexanol at the same temperature, which demonstrates that the mobility of the fatty acid in the complex is restricted. Assuming the complex is spherical, it was calculated to have a diameter of 23–26 Å, whereas a helical methyglucose polysaccharide molecule assembled from space-filling models has the dimensions of a cylinder of 18 × 24 Å. The polysaccharide and fatty acid chain-length dependence of the interaction shows a discontinuity for helical polysaccharide segments shorter than 12 sugars and for fatty acids shorter than palmitate.

The polymethylpolysaccharides (PMPS),[1] which occur in the cytoplasm of all mycobacteria, have lipophilic properties that allow them to form stable complexes with long-chain fatty acid derivatives (Bloch, 1977; Ballou, 1981). Because the polysaccharides alter the activity of the isolated fatty acid synthase (Ilton et al., 1971) and the product chain length distribution (Flick & Bloch, 1974) and because they protect acyl coenzyme A derivatives from the action of a mycobacterial thioesterase (Yabusaki & Ballou, 1979), it is possible that the polysaccharides function both as regulators of fatty acid synthesis and as general lipid carriers in the cell. Moreover, the fact that two kinds of polysaccharide, a methylmannose polysaccharide (MMP) and a methylglucose lipopolysaccharide (MGLP), coexist in the cell suggests a specialization of function between the two.

The dissociation constant for the PMPS–lipid complex is dependent on both the polysaccharide and lipid chain length, but for the *O*-methylglucose polysaccharide (MGP) and β-parinaric acid (*trans*-9,11,13,15-octadecatetraenoic acid) a K_d of 0.4 μM has been determined by fluorometric titration (Yabusaki & Ballou, 1978). From the known affinity of amylose for fatty acids (Mikus et al., 1946) and from analogy with the formation of inclusion complexes between the cyclodextrins and nonpolar substances (Saenger, 1980), it was reasonable to propose that the predominantly α1→4-linked PMPS can assume a helical conformation and include the bound lipid in the resulting nonpolar cavity (Bloch, 1977). This model has been supported by physical measurements that reveal changes in optical rotation (Yabusaki & Ballou, 1979; Bergeron et al., 1975), in fluorescence (Yabusaki & Ballou, 1978), and in ^1H (Yabusaki et al., 1979) and ^{13}C (Maggio, 1980) NMR chemical shifts when a PMPS is titrated with fatty acid.

In the present study, we have evaluated the thermodynamic parameters of the MGP–PA complex by fluorometric titration, and from fluorescence depolarization studies we have determined a rotational correlation time for the complex from which an apparent shape and volume have been derived. The results indicate that complex formation is driven by a large positive entropy change, that the rotational correlation time of parinaric acid is changed from picoseconds when free to nanoseconds in the complex, and that the complex has the apparent dimensions of a sphere 23–26 Å in diameter. The results provide convincing documentation for the helical polysaccharide–lipid inclusion complex suggested by earlier studies.

Experimental Procedures

Materials. Polymethylpolysaccharides MGP, MGLP, and MMP were from a previous study (Hindsgaul & Ballou, 1984), and the various homologues were purified according to Keller and Ballou (1968) and Maitra and Ballou (1977). β-Parinaric acid was from Molecular Probes, whereas glycogen (G-8751, type II), free fatty acid, and their coenzyme A derivatives were from Sigma. Argon gas was from Matheson, and all other reagents were of the highest purity available commercially.

Methods. Fluorescence measurements were made on a SLM Model 4800S subnanosecond spectrofluorometer in 10

† Supported by U.S. Public Health Service Grant AI-12522 and National Science Foundation Grant PCM84-00251.

‡ Present address: Department of Chemical Hygiene, Gifu Pharmaceutical University, Gifu, Japan.

[1] Abbreviations: PMPS, polymethylpolysaccharide; MMP, *O*-methylmannose polysaccharide; MGLP, *O*-methylglucose lipopolysaccharide; MGP, *O*-methylglucose polysaccharide; AGMGP, amylase-digested MGP; PA, β-parinaric acid; FAB, fast atom bombardment; F/F_s, relative fluorescence intensity; τ, fluorescence lifetime; τ_p, lifetime determined by phase lag; τ_m, lifetime determined by relative modulation; τ_c, rotational correlation time; r_0, limiting fluorescence anisotropy; P_0, limiting fluorescence polarization; tan Δ_{max}, maximal differential tangent.

0006-2960/88/0427-5824$01.50/0 © 1988 American Chemical Society

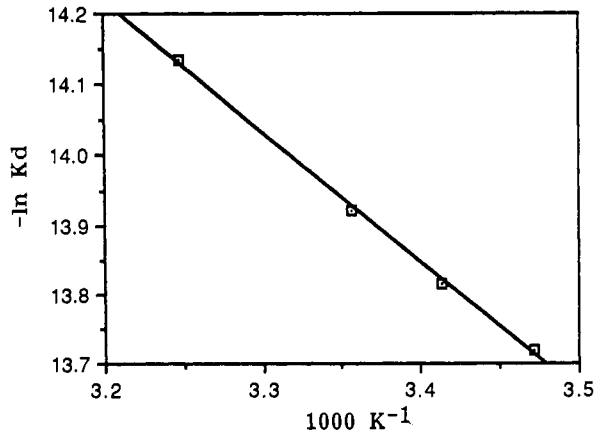

FIGURE 1: Temperature dependence of the dissociation constant (K_d) of the MGP–parinaric acid complex. K_d values were obtained by titration of 0.5 μM MGP with parinaric acid in 10 mM phosphate buffer, pH 7.0. Excitation was at 328 nm (4-nm slit), and emission was at 410 nm (16-nm slit). A molar enthalpy, $\Delta H°$, of 15.4 kJ was obtained.

mM potassium phosphate buffer of desired pH. Fixed concentrations of polysaccharide, determined by the phenol–sulfuric acid procedure (Dubois et al., 1956), were titrated in buffer by adding small portions of β-parinaric acid in methanol from a 1-μL syringe. The stock solution of parinaric acid in methanol was prepared fresh and kept under argon gas to minimize oxidation, and the ultraviolet spectrum (Sklar et al., 1977) was checked routinely to assure that decomposition had not occurred. Polysaccharide solution was added to a 3-mL stoppered cuvet, which was flushed with argon gas before the parinaric acid in methanol was added, after which the cuvet was tightly stoppered to exclude air and the content was mixed by inversion. Temperature was maintained by a Lauda RM6 bath that circulated water around the cell compartment, and the temperature was monitored with a YSI Model 42SC Tele-thermometer immersed in the reference cell. Fluorescence emission was measured at 410 nm after excitation at 328 nm (Yabusaki & Ballou, 1978).

Fluorescence depolarization data were analyzed and interpreted according to Mantulin and Weber (1977), Lakowicz et al. (1979), and the Model 4800S operator's manual (SLM Instruments, Urbana, IL). Measurements of steady-state anisotropy (r) and differential tangent (tan Δ) were performed with Glan-Thompson calcite prism polarizers. Excitation was at 321 nm (4-nm slit) for the steady-state anisotropy and at 321 nm (0.5-nm slit) for the differential tangent, whereas emission was observed at 410 nm (16-nm slit) with the 370-nm cutoff filter. The differential tangent was observed at a modulation frequency of 30 MHz and referenced to a glycogen-scattering solution. For measurements of phase (τ_p) and modulation (τ_m) lifetimes, the polarizers were removed, the sample was excited at 310 nm (0.5-nm slit), and emission was observed at 410 nm (16-nm slit) at frequencies of 18 and 30 MHz. Data were collected and analyzed by computer. Concentrations of parinaric acid and MGP were 1 and 2 μM for the steady-state anisotropy, 12.5 and 25 μM for the differential tangent, and 10 and 50 μM for the lifetimes, respectively, all in 10 mM phosphate buffer, pH 7.0.

Results and Discussion

Thermodynamic Parameters of Methylglucose Polysaccharide–Parinaric Acid Interaction. Previous studies of the methylglucose polysaccharide–parinaric acid interaction by fluorometry (Yabusaki & Ballou, 1978), ^{1}H NMR (Yabusaki & Ballou, 1979), and FAB mass spectrometry (Ballou & Dell, 1985) have demonstrated that a 1:1 molar complex is formed. With β-parinaric acid, an excellent fluorescent analogue of stearic acid (Sklar et al., 1975), a K_d of 0.4 μM was obtained previously by fluorometric titration for the polysaccharide–lipid interaction (Yabusaki & Ballou, 1978). Because the fluorescence of free parinaric acid in water is significant relative to that of the complex, however, correction for the fluorescence of the uncomplexed probe must be made with care. This we have done in the present study, which may account for the slightly higher K_d value for the MGP–PA complex we have found (1.0 μM) than was reported by Yabusaki and Ballou (1978). The K_d decreases with increasing temperature (Figure 1), from which we have calculated a small positive enthalpy for the reaction ($\Delta H°_{293}$ = 15.4 kJ/mol), similar to that observed for the interaction of α-cyclodextrin with p-nitrophenolate (Cramer et al., 1967) and attributed to changes in hydrogen bonding to solvent. From the K_d at 293 K, a Gibb's free energy ($\Delta G°$ = −33.65 kJ/mol) was obtained that yields a molar entropy of 167.4 J K^{-1}, which demonstrates that the negative free energy of complex formation is derived from this term.

That entropy should be the driving force for complex formation is expected if it is assumed that both the free fatty acid and the methyl groups on the uncomplexed polysaccharide would induce order in the water structure surrounding them. In the complex, both the fatty acyl methylene groups and the polysaccharide methyl groups are removed from the solvent (Yabusaki & Ballous, 1979), so that the disorder of the system would increase. From the estimate (Jencks, 1969) that transfer of a methylene group from water to an inert solvent is associated with a change in entropy of 5.86 J K^{-1}, we calculate that the result of complexation between a C_{18} fatty acid (17 methylene groups) and the MGP molecule (10 methyl groups) could be a total increase in entropy of 158 J K^{-1}, close to the observed value. That the methyl groups on the polysaccharide must contribute significantly to the interaction with lipid is supported by the observation that a maltooligosaccharide of the same degree of polymerization does not yield a detectable complex at micromolar concentrations (Yabusaki & Ballou, 1978).

Analysis of the MGP–Parinaric Acid Interaction by Fluorescence Depolarization. Fluorescence lifetimes for the MGP–parinaric acid complex determined by the phase shift and demodulation methods at both 18 and 30 MHz were in good agreement (Table I), and the relative values of τ_p agree

Table I: Phase and Modulation Lifetimes of the MGP–Parinaric Acid Complex

	18 MHz		30 MHz	
temp (°C)	τ_p (ns)	τ_m (ns)	τ_p (ns)	τ_m (ns)
4	4.79	4.78	4.63	4.49
10	3.63	3.96	3.83	3.85
20	2.80	3.25	2.69	2.42
30	2.54	3.19	2.39	2.23

Table II: Relative Values of Lifetimes and Fluorescence Intensities for the MGP–Parinaric Acid Complex

	temp (°C)				
	4	10	15	20	23
fluorescence intensities (F/F_s)[a]	1.19	1.00	0.90	0.81	0.71
τ_p at 18 MHz	1.31	1.00	0.87	0.77	0.70
τ_p at 30 MHz	1.21	1.00	0.87	0.70	0.62

[a] MGP (5 μM) and β-parinaric acid (1 μM) in 10 mM potassium phosphate, pH 7.0, were excited at 321 nm (4-nm slits), and emission was recorded at 410 nm (16-nm slits). For MGLP-I, the corresponding values of F/F_s were 1.14, 1.00, 0.86, 0.76, and 0.71.

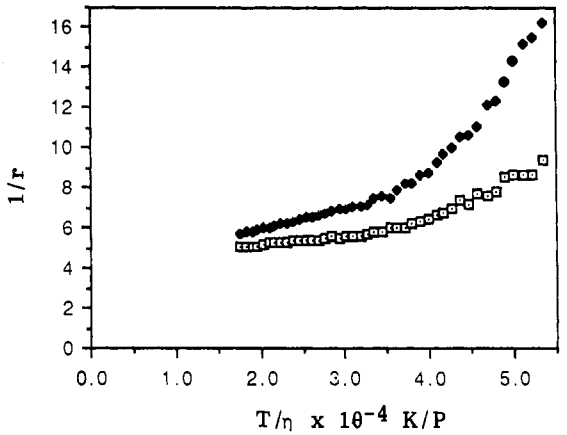

FIGURE 2: Estimation of limiting anisotropy. The steady-state anisotropy of the parinaric acid complex with MGP (open symbols) and MGLP-I (closed symbols) was measured with 1 μM parinaric acid and 2 μM polysaccharide from 5 to 45 °C under conditions given in Figure 1. The limiting anisotropy (r_0) was determined by extrapolation of $1/r$ against temperature/viscosity (T/η) of water, from which P_0 was calculated [$r = 2P/(3 - P)$].

Table III: Maximum Differential Tangent and Rotational Correlation Time of the MGP–Parinaric Acid Complex at 30 MHz[a]

temp (°C)	r_0	P_0	τ_p (ns)	tan Δ_{max} obsd	tan Δ_{max} calcd	τ_c (ns)
4	0.241	0.323	4.63	0.170	0.125	3.29
23			2.39			2.55
4	0.272	0.360	4.63		0.170	2.66
23			2.39			2.06

[a] Values of r_0, P_0, and τ_c calculated from the lifetimes (τ_p) and observed tan Δ_{max}, values which suggest that the parinaric acid has some freedom to rotate in the complex.

with the relative fluorescence intensities (F/F_s), all normalized to the values observed at 10 °C (Table II). Both results suggest that the decays are represented by a single exponential (Lakowicz et al., 1979). From steady-state depolarization measurements, the limiting value $r_0 = 0.241$ was obtained (Figure 2), which yielded a limiting polarization $P_0 = 0.323$ (Table III). From this value and the lifetime at 4 °C, a value for tan $\Delta_{max} = 0.125$ was calculated (Table III) (Mantulin & Weber, 1977). Dynamic depolarization measurements as a function of temperature yielded a maximum value at 4 °C of 0.170 (Figure 3).

A rigidly fixed isotropic rotator is expected to yield calculated and observed values for tan Δ_{max} that are in close agreement, whereas anisotropic rotation gives a calculated value larger than the observed one (Mantulin & Weber, 1977). That we find a calculated value that is *smaller* than the observed one indicates that the parinaric acid, which is not covalently linked to the polysaccharide, has some freedom of rotation within the complex (Cantor & Schimmel, 1980). To obtain a calculated tan Δ_{max} in agreement with the observed one, a value of $r_0 = 0.272$ would be required (Table III).

From the lifetimes and limiting polarization, we have also calculated a rotational correlation time (τ_c) of 3.29 ns at 4 °C and 2.55 ns at 23 °C for the MGP–PA complex (Mantulin & Weber, 1977). These are comparable to a value of 1.2 ns at 37 °C for the MMP–lipid complex determined by NMR (Maggio, 1980). The published rotational correlation time for β-parinaric acid in cyclohexanol at 23 °C, after correction for the difference in viscosity between cyclohexanol and water, gives a value of 6.17 ps in water (Wolber & Hudson, 1981). Thus, the rotational freedom of the complexed parinaric acid is significantly decreased, even though it appears to be rotating

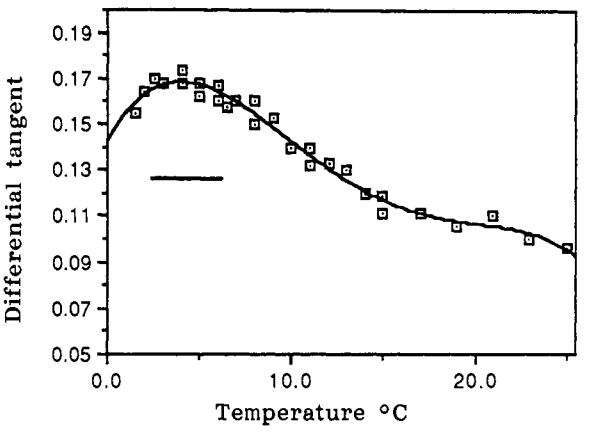

FIGURE 3: Differential tangents for the MGP–parinaric acid complex. Measurements were done at 30 MHz with 12.5 μM parinaric acid and 25 μM MGP under the conditions in Figure 1. The horizontal bar indicates the tan Δ_{max} at 4 °C calculated for an isotropic rotator with $P_0 = 0.323$ and $\tau = 4.63$ ns (Table III) (Mantulin & Weber, 1977).

within the complex (Table III). Assuming that the complex is isotropic, the hydrated molecular volume, V_h, of a spherical molecule can be calculated from τ_c ($V_h = kT\,\tau_c/\eta$) (Cantor & Schimmel, 1980), and from the volume we obtain a diameter of 23–26 Å for the complex.

By X-ray crystallography, Goldsmith et al. (1982) determined the conformation of maltoheptaose in association with the active site of glycogen phosphorylase and found it to assume a left-handed helix with 6.5 glucose units per turn and a rise of 2.4 Å per glucose. Such a molecule possesses a cavity with a diameter of 5 Å, adequate to accept an aliphatic fatty acid chain. If the α1→4-linked section of the MGP molecule, which contains 14 hexose units, were similarly coiled, it would be somewhat more than 33 Å in the long dimension and would have a relatively open conformation. Our results suggest that the MGP–PA complex is more tightly coiled so that the α1→4-linked section has a length closer to that of the included C_{18} fatty acid chain, about 23 Å. The dimensions of a tightly coiled helix of MGP assembled from space-filling models are that of a cylinder of about 18 × 24 Å.

Methylglucose Lipopolysaccharide–Parinaric Acid Interaction. MGP in the mycobacterial cell occurs in acylated forms (Keller & Ballou, 1968), and anomeric proton NMR measurements reveal that the acylation stabilizes a helical conformation even in absence of added fatty acid (Hindsgaul & Ballou, 1984). This suggests that the polysaccharide exists as an intramolecular inclusion complex with the attached octanoyl group that is esterified to position 3 of the glyceric acid of all MGLP isomers, which differ only in the amount of esterified succinic acid (Keller & Ballou, 1968). If this is so, then it is probable that parinaric acid would tend to displace the octanoyl group when forming a complex with MGLP, which could modulate the interaction. The measured K_d at 20 °C for MGLP-I (1.5 μM) and MGLP-IV (1.4 μM) with parinaric acid are consistent with this idea (Table IV), although the differences in K_d may not be significant. On the other hand, the MGLP isomers give lower K_d values with palmitoyl-CoA than does MGP, a confirmation that the coenzyme A moiety is also importantly involved in the interaction (Bergeron et al., 1975).

In steady-state anisotropy measurements, MGP gives a nearly linear curve of increasing values of r from 45 to 3 °C (Figure 2). In contrast, MGLP-I gives a biphasic curve with an apparent break at about 27 °C that suggests a phase transition occurs at that temperature, which could reflect a

Table IV: Polysaccharide–Lipid Interaction

polysaccharide	lipid	K_d (μM) (20 °C)[a]
MGP	parinarate	1.0
	palmitate	0.96
	myristate	2.9
	palmityl-CoA	0.60
AGMGP	parinarate	6.0
MGLP-I[b]	parinarate	1.5
	palmityl-CoA	0.40
MGLP-IV[b]	parinarate	1.4
	palmityl-CoA	0.40
MMP-II[c]	parinarate	1.1
MMP-III[c]	parinarate	0.9
MMP-IV[c]	parinarate	5.0

[a] Complex formation with parinaric acid was determined by direct titration and for the other lipids by displacement of parinaric acid from the complex. [b] MGLP isomers I–IV contain respectively 0–3 molecules of esterified succinate in addition to 3 mol of acetate and 1 mol each of propionate, isobutyrate, and octanoate (Keller & Ballou, 1968). [c] MMP homologues I–IV contain respectively 14–11 hexose units.

change in the fluorescence lifetime or in the apparent volume of the complex. Since the lifetime is proportional to fluorescence intensity, which shows a linear temperature dependence for both MGP and MGLP-I (Table II), the effect may be due to a change in volume. Such a change could result from a transition in which the octanoyl group attached to MGLP changes its position, perhaps by moving into or out of the polysaccharide cavity of the MGLP–parinaric acid complex.

Dependence of Complex Formation of Polysaccharide and Lipid Chain Length. Qualitative evidence was presented earlier that the polysaccharide chain length was important for complexation of long-chain fatty acids by PMPS (Yabusaki & Ballou, 1978). Here we show that the K_d for complex formation between the methylmannose polysaccharide homologues and parinaric acid increases 5-fold when the polysaccharide chain length decreases from 12 to 11 hexose units, while the homologues with 12 and 13 hexoses give similar K_d values (Table IV). A comparable difference is observed between the complexing abilities of MGP and AGMGP, in which instance the unbranched chain of α1→4-linked hexose units is decreased from 14 in MGP to 10 in AGMGP (Forsberg et al., 1982). Thus, it appears that 12 α1→4-linked hexoses give optimum interaction, which is the number required for two complete turns of the amylose-like helix and twice the number found in the smallest naturally occurring cyclodextrin (French, 1957). It is apparent from inspection of space-filling models that two turns of the polysaccharide chain would just encompass a lipid chain the size of palmitic or stearic acid. Interestingly, MMP is found in the mycobacterial cell as a mixture of homologues in which those with 12 and 13 hexoses predominate (Maitra & Ballou, 1977), and it has been suggested that this is a consequence of a chain termination mechanism that is regulated by the enhanced fatty acid complexing ability of MMP homologues as they reach this size (Weisman & Ballou, 1984).

The fatty acid chain length is also known to affect complex stability (Yabusaki & Ballou, 1978), and here we show that the K_d is increased 3-fold when myristic acid (C_{12}) replaces palmitic acid (C_{16}) in the complex. Palmityl coenzyme A also binds to MGP better than free palmitic acid does (Yabusaki & Ballou, 1978; Bergeron et al., 1975), which implies that the coenzyme A part must enhance the interaction, possibly owing to the hydrophilic outer surface of the coiled polysaccharide. This postulated interaction might also be facilitated by the hairpin conformation of free palmityl coenzyme A (Wilson et al., 1975).

Conclusions

At least five independent types of physical measurement give results that are consistent with a model for polymethylpolysaccharide–fatty acid interaction in which the polysaccharide assumes a helical conformation as the lipid becomes included within the nonpolar interior of the coiled polysaccharide to give a 1:1 molar complex. The temperature dependence of this interaction shows that it is entropy driven, as is also observed for the inclusion complexes of the cyclodextrins, but the K_d for the PMPS–lipid complexes is 3–4 orders of magnitude smaller than those found for the cyclodextrins. This tighter binding is probably a function of the greater flexibility of the linear polysaccharides that allows them to adjust their shape to that of the lipid chain, as well as to the methyl ether groups that increase the polysaccharide hydrophobicity. Because the methyl ether groups in MMP and MGLP occupy different positions and because synthetic 6-deoxymaltooligosaccharides have similar properties (Lu et al., 1987), there is considerable latitude in the structural requirements for the lipid-binding property. Why mycobacteria should synthesize two different polysaccharides with this property is unknown.

Fluorescence depolarization studies indicate that the MGP–PA complex is essentially spherical and has the dimensions expected for an α1→4-glucan coiled more tightly than maltoheptaose when in association with glycogen phosphorylase (Goldsmith et al., 1982). The parinaric acid in the complex retains some rotational freedom, although its movement is restricted relative to that of the free acid in solution. It is expected that the lipid chain in a similar complex involving palmityl-CoA would be even more restricted in its mobility owing to the interaction of the coenzyme A part with the outer surface of the coiled polysaccharide.

The complexing ability of the α1→4-linked *O*-methylhexopyranose segment with parinaric acid appears to optimize at the dodecamer for MMP, since the homologue with one more hexose gives a similar K_d (1 μM) whereas the homologue with one less hexose shows a 5-fold weaker interaction (5 μM). Such a polysaccharide chain length is required for two complete turns of a tightly coiled helix, and the length of such a helix is about that of the extended palmitic acid or stearic acid chain. Thus, it is reasonable that these fatty acids give optimum binding whereas myristic acid shows a 3-fold higher K_d. The coenzyme A derivatives of fatty acids bind better than the free lipids, although we find a difference of only 2–3-fold rather than the 14-fold difference reported by Bergeron et al. (1975). This discrepancy could arise from the fact that the latter estimate was derived from a gel filtration experiment at high lipid concentrations so that the equilibria could have been influenced by micelle formation. The enhanced binding of the coenzyme A derivatives is attributable to a hydrophilic interaction on the outer surface of the coiled polysaccharide (Bloch, 1977).

MGP binds parinaric acid slightly better than MGLP-I does, even though the latter has several short-chain fatty acyl groups esterified at the nonreducing end that should increase the overall hydrophobicity of the polysaccharide. Because MGLP-I probably exists in a helical conformation as an intramolecular complex with its attached octanoyl group (Hindsgaul & Ballou, 1984), however, the parinaric acid may have to displace the included octanoate at least partially before it can form the complex. Thus, the enhanced binding of parinaric acid one might expect from the increased hydrophobicity of MGLP-I may be outweighed by the energy required to displace the included octanoyl group.

ACKNOWLEDGMENTS

We thank Dr. Joseph Falke, of this Department, for helpful comments and criticism.

REFERENCES

Ballou, C. E. (1981) *Pure Appl. Chem. 53*, 107–112.
Ballou, C. E., & Dell, A. (1985) *Carbohydr. Res. 140*, 139–143.
Bergeron, R., Machida, Y., & Bloch, K. (1975) *J. Biol. Chem. 250*, 1223–1230.
Bloch, K. (1977) *Adv. Enzymol. Relat. Areas. Mol. Biol. 45*, 1–84.
Cantor, C. R., & Schimmel, P. R. (1980) in *Biophysical Chemistry*, pp 454–465, Freeman, San Francisco, CA.
Cramer, F., Saenger, W., & Spatz, H.-Ch. (1967) *J. Am. Chem. Soc. 89*, 14–20.
Dubois, M., Giles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956) *Anal. Chem. 28*, 350–356.
Flick, P., & Bloch, K. (1974) *J. Biol. Chem. 249*, 1031–1036.
Forsberg, L. S., Dell, A., Walton, D. J., & Ballou, C. E. (1982) *J. Biol. Chem. 257*, 3555–3563.
French, D. (1957) *Adv. Carbohydr. Chem. 12*, 189–260.
Goldsmith, E., Sprang, S., & Fletterick, R. (1982) *J. Mol. Biol. 156*, 411–427.
Hindsgaul, O., & Ballou, C. E. (1984) *Biochemistry 23*, 577–584.
Ilton, M., Jevans, A. W., McCarthy, E. D., Vance, D., White, H. B., III, & Bloch, K. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 87–91.
Jencks, W. P. (1969) in *Catalysis in Chemistry and Enzymology*, pp 393–463, McGraw-Hill, New York.
Keller, J. M., & Ballou, C. E. (1968) *J. Biol. Chem. 243*, 2905–2910.
Lakowicz, J. R., Prendergast, F. G., & Hogen, D. (1979) *Biochemistry 18*, 508–519.
Lu, D.-p., Ballou, C. E., Defaye, J., & Dell, A. (1987) *Carbohydr. Res. 160*, 171–184.
Maggio, J. E. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 2582–2586.
Maitra, S. K., & Ballou, C. E. (1977) *J. Biol. Chem. 252*, 2459–2469.
Mantulin, W. W., & Weber, G. (1977) *J. Chem. Phys. 66*, 4092–4099.
Mikus, F. F., Hixon, R. M., & Rundle, R. E. (1946) *J. Am. Chem. Soc. 68*, 1115–1123.
Saenger, W. (1980) *Angew. Chem., Int. Ed. Engl. 19*, 344–362.
Sklar, L. A., Hudson, B. S., Peterson, M., & Diamond, J. (1977) *Biochemistry 16*, 813–819.
Sklar, L. A., Hudson, B. S., & Simoni, R. D. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1649–1653.
Weisman, L. S., & Ballou, C. E. (1984) *J. Biol. Chem. 259*, 3464–3469.
Wilson, G. E., Bazzone, T. J., Kuo, C. H., & Rinaldi, P. L. (1975) *J. Am. Chem. Soc. 97*, 2907–2908.
Wolber, P. K., & Hudson, B. S. (1981) *Biochemistry 20*, 2800–2810.
Yabusaki, K. K., & Ballou, C. E. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 691–695.
Yabusaki, K. K., & Ballou, C. E. (1979) *J. Biol. Chem. 254*, 12314–12317.
Yabusaki, K. K., Cohen, R. E., & Ballou, C. E. (1979) *J. Biol. Chem. 254*, 7282–7286.