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Thermodynamics of Intersubunit Interactions in Cholera Toxin upon Binding to the Oligosaccharide Portion of Its Cell Surface Receptor, Ganglioside G_{M1}[†]

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ABSTRACT: The binding and the energetics of the interaction of cholera toxin with the oligosaccharide portion of ganglioside G_{M1} (oligo-G_{M1}), the toxin cell surface receptor, have been studied by high-sensitivity isothermal titration calorimetry and differential scanning calorimetry. Previously, we have shown that the association of cholera toxin to ganglioside G_{M1} enhances the cooperative interactions between subunits in the B-subunit pentamer [Goins, B., & Freire, E. (1988) *Biochemistry 27*, 2046–2052]. New experiments presented in this paper reveal that the oligosaccharide portion of the receptor is by itself able to enhance the intersubunit cooperative interactions within the B pentamer. This effect is seen in the protein unfolding transition as a shift from independent unfolding of the B promoters toward a cooperative unfolding. To identify the origin of this effect, the binding of cholera toxin to oligo-G_{M1} has been measured calorimetrically under isothermal conditions. The binding curve at 37 °C is sigmoidal, indicating cooperative binding. The binding data can be described in terms of a nearest-neighbor cooperative interaction binding model. In terms of this model, the association of a oligo-G_{M1} molecule to a B protomer affects the association to adjacent B promoters within the pentameric ring. The measured intrinsic binding enthalpy per protomer is −22 kcal/mol and the cooperative interaction enthalpy −11 kcal/mol. The intrinsic binding constant determined calorimetrically is 1.05×10^6 M^{-1} at 37 °C and the cooperative Gibbs free energy equal to −850 cal/mol. These studies provide the first direct thermodynamic description of the cooperative binding of a protein to its cell surface receptor and have allowed us to place energetics constraints on the putative changes in protein conformation triggered by the intrinsic receptor binding process.

Cholera toxin consists of a five-subunit ring, the B-subunit pentamer (M_r = 58 000), which surrounds the dimeric A-subunit (M_r = 27 000) (Gill, 1976). The B-subunit pentamer binds to ganglioside G_{M1} present on the outer surface of the cell membrane, and subsequently the A-dimer penetrates the cell membrane, where it activates adenylate cyclase. Ganglioside G_{M1} plays an active part in the processing of the toxin after binding. Previous studies in this laboratory have shown that the association of cholera toxin to ganglioside G_{M1} enhances the cooperative interactions within the B-subunit pentamer (Goins & Freire, 1988). In the absence of ganglioside G_{M1} the unfolding transition of intact cholera toxin or isolated B pentamer in aqueous solution is a process characterized by little or no intersubunit cooperative interactions. Upon binding to ganglioside G_{M1} micelles or to lipid membranes containing ganglioside G_{M1}, the unfolding process becomes highly cooperative and the pentameric B-subunit ring effectively behaves as a single cooperative unit. The molecular origins of this cooperative enhancement have not been identified and could be triggered either by the attachment of the toxin to the membrane or micellar surface or by intrinsic interactions arising from the binding of the oligosaccharide region of the ganglioside to the toxin molecule. To address this issue, we have isolated the oligosaccharide region of ganglioside G_{M1} and studied its association to cholera toxin as well as the effects of this association on the behavior of the protein. This experimental system allows one to dissect in-

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trinsic oligosaccharide protein interactions insulating them from interactions arising from the protein-membrane interface. A newly developed high-sensitivity isothermal titration calorimeter was used to measure directly the energetics of the association and to construct complete binding isotherms. These experiments were complemented by protein stability analysis using high-sensitivity differential scanning calorimetry and by fluorescence spectroscopy measurements. These studies have permitted us to identify the oligosaccharide–protein interaction as the site of origin of the intersubunit cooperative enhancement in the toxin molecules and to evaluate the magnitude of the forces that characterize the binding and cooperative interactions of the toxin to the oligosaccharide region of its cells surface receptor.

Materials and Methods

Protein. Intact cholera toxin and cholera toxin B-subunit were purchased from Sigma (St. Louis, MO). The lyophilized powder was reconstituted with water to give the desired concentration of protein in 50 mM Tris-HCl, 0.2 M NaCl, 3 mM NaN_3, and 1 mM Na_2–EDTA, pH 7.5. For the experiments carried out by differential scanning calorimetry the protein was made free from EDTA by dialyzing overnight at 4 °C against 4000 volumes of Tris buffer without EDTA. For isothermal titration microcalorimetry and fluorescence measurements the buffer was changed by dialyzing overnight at 4 °C against 4000 volumes of 10 mM phosphate buffer with 3 mM NaN_3, pH 7.5. The protein concentration in all experiments was determined by the method of Lowry (Lowry et al., 1951). Analysis of the binding data revealed that approximately 80–90% of the toxin molecules in each lot were active and able to bind ganglioside G_{M1}.

Preparation and Purification of the Oligosaccharide of Ganglioside G_{M1}. Ganglioside G_{M1} was prepared from the Folch extract of bovine brain (Avanti Biochemicals, Birmingham, AL) as described earlier (Goins & Freire, 1988). The oligosaccharide part (oligo-G_{M1}) was cleaved off from the ceramide by use of endoglycoceramidase (Genzyme, Boston, MA). The ganglioside was dissolved in 0.1 M acetate buffer, pH 6.0, with 1 g/L of taurodeoxycholic acid, and the solution was sonicated for 3 min. The enzyme was added, and the solution was incubated at 37 °C for 24 h (Ito & Yamagata, 1986). After the enzymatic cleavage, the hydrophobic fraction was removed by addition of chloroform to the water mixture. The water-soluble fraction was run through a column packed with Sephadex G-25–150 (Sigma, St. Louis, MO), and the different eluted fractions were checked for purity by TLC. An aliquot from each sample was evaporated and then dissolved in chloroform:methanol (1:1) before spotting it on a Merck silica gel 60 plate (Alltech Associates, Deerfield, IL). The plate was developed in propanol:0.2% $CaCl_2$ (80:20, and detection was made with resorcinol reagent. Pure G_{M1} (Suppelco, Bellefonte, PA) was used as a reference. By use of this solvent the presence of oligosaccharide can easily be distinguished from G_{M1} due to the higher mobility of the more nonpolar ganglioside (Fishman et al., 1978). Pure oligosaccharide containing fractions were pooled together, and the water was evaporated. The oligosaccharide was finally dissolved in the desired buffer. The concentration of the stock solution was determined spectrophotometrically by using resorcinol reagent (Spiro, 1966).

Fluorescence Measurements. Fluorescence measurements were made with a Perkin-Elmer Model LS-5 spectrofluorometer equipped with a thermostated cell holder. The excitation wavelength was 280 nm, and the emission spectrum was recorded between 300 and 400 nm. The protein concentration was around 10^{-7} M in all experiments, and additions of oligosaccharide were made with a 10-μL Hamilton syringe to a thermostated cuvette. All measurements reported here were made at 37 °C.

Isothermal Reaction Calorimetry. For the calorimetric study of the heat associated with the binding of oligo-G_{M1} to the protein, a high-sensitivity twin titration calorimeter developed in this laboratory (Myers et al., 1987) was used. It consists of a microcomputer-controlled dual titration system fitted to the original twin channel of the thermal activity monitor (Thermometric AB, Järfälla, Sweden). Two Hamilton syringes, each equipped with a 330-mm needle fitted to a Teflon stirrer, are rotated simultaneously by a stepping motor and are used to deliver the reactants into the calorimetric cells, already preequilibrated with the other reactants. Usually, 30 μL are injected automatically at predetermined time intervals. In these experiments the two delivering syringes contained the oligosaccharide and identical aliquots were simultaneously delivered to the sample cell (containing cholera toxin or isolated B-subunit pentamer) and the reference cell (containing only buffer). With this configuration the experiments could be considered as being performed in a completely differential mode; i.e., both the heat of dilution and mechanical heat of injection are subtracted automatically during the experiment. With this configuration the base-line noise is less than 10 ncal/s at 25 °C, and heat effects as small as 20 ncal/s could be measured with good accuracy. The temperature stability of the instrument is better than $\pm 10^{-4}$ °C over a period of 8 h. The calorimeter operation and data acquisition are under computer control by means of a Data Translation DT-2801 board. In all experiments the sample cell contained 1.4 mL of the protein at a concentration of 2–5 μM, and the reference cell contained the same volume of buffer. The concentration of oligosaccharide in the syringes was about 0.10 mM. All measurements were made at 37 °C.

High-Sensitivity Differential Scanning Calorimetry. All temperature-scanning experiments were performed with a Microcal MC2 instrument interfaced to an IBM-PC microcomputer system using a Data Translation DT-2801 board for data acquisition and instrument control. For these experiments only the thermal behavior of the B-subunit, and not the intact toxin, was investigated. The sample cell was filled with 1.3 mL of the protein–oligosaccharide solution, and the concentration of protein was around 2 mg/mL in all scans. Each sample was scanned between 20 and 100 °C at a rate of 60 °C/h.

Results

Isothermal Reaction Calorimetry. The results of a typical calorimetric titration of cholera toxin B-subunit pentamer with oligo-G_{M1} at 37 °C are shown in Figure 1. In this figure the differential power output of the calorimeter unit has been plotted as a function of time for 14 consecutive additions of oligo-G_{M1} (~3 nmol/injection) into the calorimeter cell containing ~5 nmol of cholera toxin B-subunit pentamer. After each addition, an exothermic heat effect is observed. The magnitude of this effect is a function of the amount of oligo-G_{M1} that binds to the protein after each injection and becomes zero when the system reaches saturation. The area under each peak is equal to the heat released after each injection. The calorimetric binding isotherm is constructed by plotting the cumulative heat effect as a function of the total concentration of ligand.

The cumulative heat, Q, associated with the binding of oligo-G_{M1} to the cholera toxin B-subunit as a function of the total concentration of oligo-G_{M1} is shown in Figure 2. In this

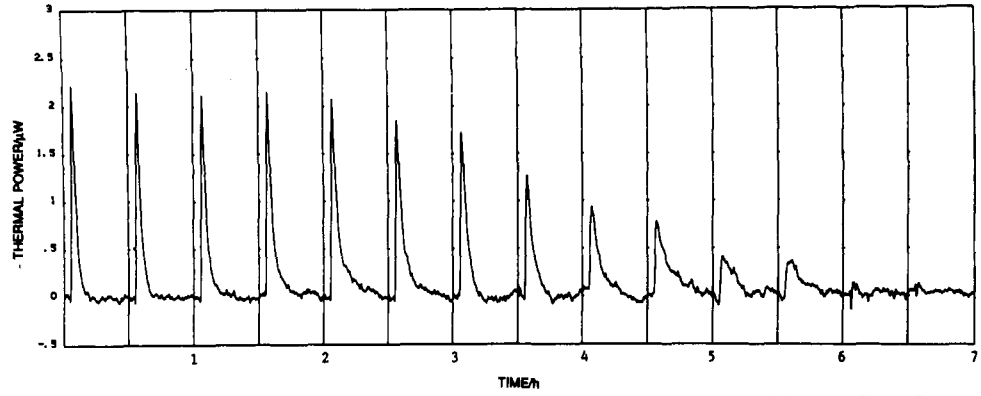

FIGURE 1: High-sensitivity isothermal calorimetric titration of cholera toxin B-subunit pentamer with oligo-G_{M1}. Shown in the figure is the power output in microwatts as a function of time for successive injections of oligo-G_{M1} (3 nmol/injection) at equal intervals of 30 min. The amount of protein in the calorimeter cell is 4.9 nmol in a total volume of 1.4 mL.

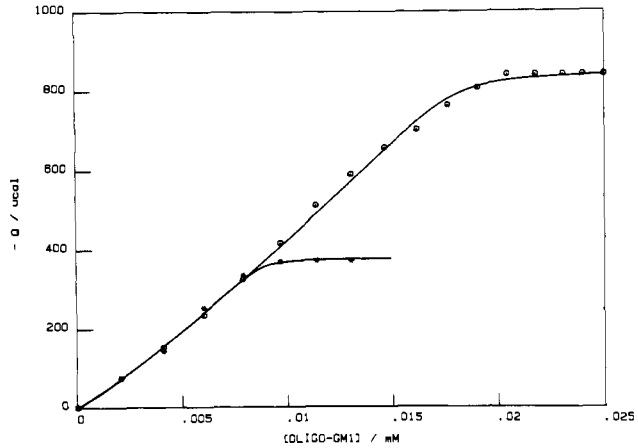

FIGURE 2: Calorimetric binding isotherm of oligo-G_{M1} to cholera toxin B-subunit pentamer. The cumulative heat, Q, has been plotted versus the total concentration of oligo-G_{M1} for two titration experiments, at different protein concentrations. The solid lines are the theoretical curves calculated with the parameters in Table I. (O) [B-subunit] = 3.5 μM; (*) [B-subunit] = 1.7 μM.

figure, the two curves represent experiments performed at two different toxin concentrations. No significant difference was observed when the titration was made with intact toxin. These experiments are consistent with a total binding enthalpy of −163 ± 7 kcal/mol for the binding of the five oligo-G_{M1} to the toxin. The sigmoidal shape of the curve indicates that the five binding sites are not independent and that the total enthalpy cannot be equally partitioned among the five binding sites. This behavior is indicative of positive cooperativity, in agreement with earlier observations (Sattler et al., 1978; Schafer & Thakur, 1982). Since the B-subunit pentamer is made up of five identical subunits, the origin of the cooperative binding behavior can be attributed to a modification of the intersubunit interactions within the B pentamer triggered by the binding of oligo-G_{M1}. The net result of this effect is the enhancement of the binding affinity as the degree of saturation increases. The most conservative model for this cooperative behavior is one in which the binding of one oligo-G_{M1} molecule to a B-subunit modifies the binding affinity of adjacent subunits. This model assumes that the cooperative binding interactions propagate through nearest-neighbor B-subunits. Figure 3 illustrates the eight different binding states of the B-subunit pentamer as well as the Gibbs free energy of each state relative to that of the unligated species. According to this model, the Gibbs free energy of each state can be expressed in terms of an intrinsic binding free energy, ΔG, and a cooperative free energy Δg. For any given state characterized by

STATE	LIGANCY	CONFIGURATION	$\omega_{i,j}$	$\Delta G_{i,j}$	$i\Delta G + j\Delta g$
1	0		1	REF	0
2	1		5	$\Delta G_{1,0}$	ΔG
3	2		5	$\Delta G_{2,0}$	$2\Delta G$
4	2		5	$\Delta G_{2,1}$	$2\Delta G + \Delta g$
5	3		5	$\Delta G_{3,1}$	$3\Delta G + \Delta g$
6	3		5	$\Delta G_{3,2}$	$3\Delta G + 2\Delta g$
7	4		5	$\Delta G_{4,3}$	$4\Delta G + 3\Delta g$
8	5		1	$\Delta G_{5,5}$	$5\Delta G + 5\Delta g$

FIGURE 3: Thermodynamics of ganglioside G_{M1} binding to cholera toxin; the eight different binding configurations of the B-subunit are shown. Each solid circle in the pentamer represents one ligated B-subunit monomer. For each state, the Gibbs free energy and degeneracy are indicated.

i oligo-G_{M1} molecules bound and j adjacent bound subunits, the Gibbs free energy ΔG_{ij} can be written as $i\Delta G + j\Delta g$ as illustrated in the figure. According to this model the average excess enthalpy of binding, $\langle \Delta H \rangle$, can be written

$$\langle \Delta H \rangle = \sum_{ij} F_{ij} \Delta H_{ij} \quad (1)$$

where F_{ij} is the fraction of B-subunit pentamers with i occupied sites and j nearest-neighbor occupied sites. The calorimetrically determined heat of binding, Q, is simply the product of the average excess enthalpy, the macromolecule concentration, and the reaction volume ($Q = V[\mathrm{CT}]\langle \Delta H \rangle$). The enthalpy change, ΔH_{ij}, consists of the intrinsic enthalpy of binding and the enthalpy of interaction between subunits:

$$\Delta H_{ij} = i\Delta H + j\Delta h \quad (2)$$

Table I: Thermodynamic Parameters for the Binding of Oligo-G_{M1} to the B-Subunit Pentamer of Cholera Toxin at 37 °C

ΔG (kcal/mol)	Δg (kcal/mol)	ΔH (kcal/mol)	Δh (kcal/mol)	ΔS (cal/K·mol)	Δs (cal/K·mol)
−8.54 ± 0.1	−0.85 ± 0.07	−22.0 ± 2	−11.4 ± 1	−43.4 ± 6	−34.0 ± 3

F_{ij} can be expressed in terms of the intrinsic and cooperative free energies as

$$F_{ij} = \omega_{ij}[G]^i e^{-\Delta G_{ij}/RT}/Z \quad (3)$$

where the ω_{ij}'s are the degeneracies of each state and are defined in Figure 3. Z is the binding partition function defined as

$$Z = \sum_{ij} \omega_{ij}[G]^i e^{-\Delta G_{ij}/RT} \quad (4)$$

The above equations define the binding process in terms of [G], the free concentration of oligo-G_{M1}. Since the experimental data are obtained in terms of the total concentration of oligo-G_{M1}, the transformation

$$[G]_{total} = [G] + [G]_{bound} = [G] + [CT]\sum iF_{ij} \quad (5)$$

is used, where [CT] is the total protein concentration. Equation 5 can be numerically solved for [G] for known experimental values of $[G]_{total}$ and [CT] and any desired values for ΔG and Δg. The calorimetric data (Q versus $[G]_{total}$) were analyzed within the framework of the above model by using a global nonlinear least-squares procedure directed to determine the set of parameters (ΔG, Δg, ΔH, and Δh) that best fit simultaneously the entire set of data obtained at different protein concentrations (Masserini & Freire, 1987). The results of this analysis are summarized in Table I. According to these parameters, the intrinsic association constant for oligo-G_{M1} at 37 °C is 1.05×10^6 M^{-1} and increases by a factor of 4 if an adjacent site is already occupied. The intrinsic binding enthalpy of −22 kcal/mol is of the same magnitude as that found for the binding of oligosaccharides to other proteins (Dani et al., 1981). The cooperative enthalpy of −11 kcal/mol is suggestive of a relatively small protein conformational change upon binding. It should be noted that the total folding enthalpy for each B-subunit monomer is ~−100 kcal/mol (Goins & Freire, 1988) and that energetically the cooperative enthalpy represents approximately 10% of the total. Additional experiments performed by monitoring the dependence of the intrinsic fluorescence emission spectrum (either intensity or maximum emission wavelength) of the B-subunit pentamer on the concentration of oligo-G_{M1} were also consistent with an association constant of 10^6 M^{-1} at 37 °C as shown in Figure 4. This association constant is also consistent with the cholera toxin toxicity inhibition constant of 1.66×10^6 M^{-1} obtained by Fishman et al. (1978) for oligo-G_{M1} at 37 °C. An important conclusion from the calorimetric experiments is related to the temperature dependence of the association constant. Provided that the binding enthalpy is not strongly temperature dependent, the association constant is expected to increase to about 10^8 M^{-1} at 4 °C. This is important since many cell biological experiments are usually performed at 4 and 37 °C to discriminate between intrinsic binding and metabolic processes. The difference of almost 2 orders of magnitude indicates that care must be observed when these comparisons are performed.

Figure 5 presents a plot of the molecular population of the eight different binding states of the B-subunit pentamer as a function of the degree of saturation. This figure illustrates the effects of the cooperative behavior on the mechanism of binding. From this figure it is clear that the states with

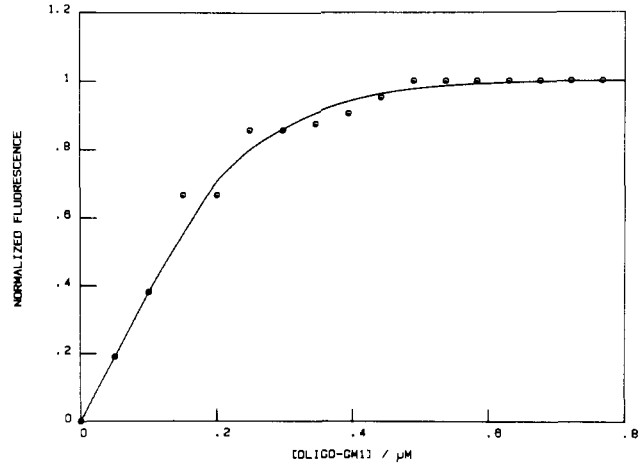

FIGURE 4: Normalized change in fluorescence emission wavelength maximum in cholera toxin B-subunit pentamer at 37 °C. The excitation wavelength was 280 nM and the concentration of cholera toxin 6.92×10^{-8} M. In the absence of oligo-G_{M1} the maximal emission wavelength is 350 nm and shifts to 336 nm under saturation conditions (Fishman et al., 1978; De Wolf et al., 1981).

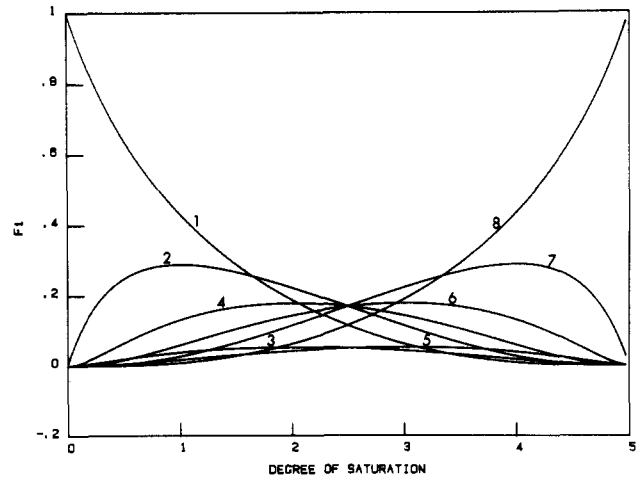

FIGURE 5: Population of the eight different binding states of the cholera toxin B-subunit pentamer as a function of the degree of saturation. Note that states 3 and 5 never become significantly populated.

nonadjacent bound oligo-G_{M1} molecules (3 and 5 in Figure 3) never become significantly populated. In fact, their population is never larger than 2% of the total.

The binding of the toxin molecule to a cell surface containing ganglioside G_{M1} can be thought of as a sequential process in which the protein attaches first to a receptor molecule and subsequently to the remaining ones. This sequential mechanism is supported by the observation that ganglioside G_{M1} diffuses freely within the membrane surface and is not likely to be geometrically distributed in a way that structurally matches the location of the binding sites in the pentameric ring (Goins et al., 1986). Within this context, the cooperative character of the binding will facilitate and optimize the proper attachment to the membrane after the initial contact.

Thermal Stability of the B-Subunit. It has been shown earlier that ganglioside G_{M1} stabilizes and enhances the cooperative melting behavior of the B-subunit pentamer of cholera toxin (Goins & Freire, 1988). Figure 6 shows a series of differential scanning calorimetric scans for the B-subunit

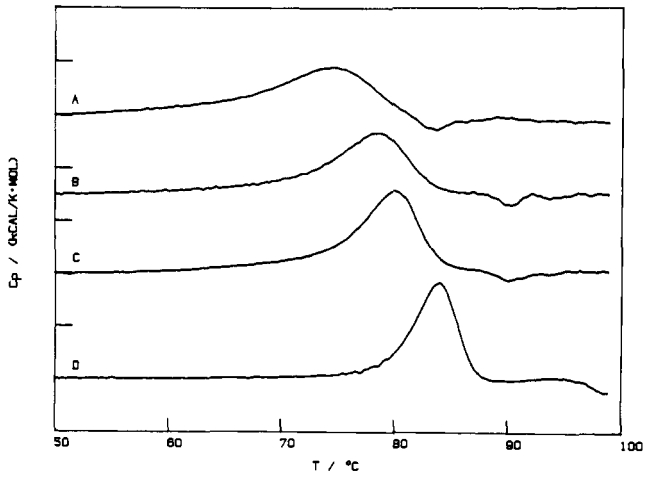

FIGURE 6: Excess heat capacity versus temperature profiles for the cholera toxin B-subunit incubated pentamer with increasing amounts of oligo-G_{M1}. Scans are offset in the y axis for presentation purposes. Each interval on the y axis represents 50 kcal/K·mol. The concentration of protein in these experiments was 2 mg/mL. The molar ratios of oligo-G_{M1} to B-subunit monomer are 0 (A), 3.6 (B), 5.7 (C), and 18.0 (D).

pentamer of cholera toxin at different concentrations of oligo-G_{M1}. In the absence of oligo-G_{M1}, the unfolding transition is centered at 74.6 °C and characterized by an enthalpy change, ΔH, of 90 kcal/mol of B-subunit monomer, in agreement with previous results (Dalziel et al., 1984; Goins & Freire, 1988). As shown in the figure, oligo-G_{M1} by itself is able to stabilize the native conformation of the B-subunit pentamer as evidenced by the increase in transition temperature, T_m, upon oligo-G_{M1} binding. Furthermore, the transition profiles become increasingly sharper, and the ratio of the van't Hoff to calorimetric enthalpies ($\Delta H_{VH}/\Delta H$) increases as the concentration of oligo-G_{M1} increases. These results indicate that the oligosaccharide portion of ganglioside G_{M1} is by itself able to increase the cooperative melting behavior of the B-subunit pentamer. In the absence of oligo-G_{M1} or ganglioside G_{M1}, the unfolding transition is characterized by a $\Delta H_{VH}/\Delta H$ of 0.24, indicating that each subunit within the B pentamer unfolds independently of the others. The association with intact ganglioside G_{M1} increases the $\Delta H_{VH}/\Delta H$ ratio to approximately 1, indicating that under saturating conditions the entire B-subunit pentamer essentially behaves as a single cooperative unit. At equivalent concentrations the effect of oligo-G_{M1} alone is not as pronounced as that of the intact ganglioside G_{M1}. For example, at the concentrations used in scan D of Figure 6, oligo-G_{M1} increases the transition temperature by 10 °C, whereas intact ganglioside G_{M1} increases the transition temperature by 20 °C. Also, under these conditions the $\Delta H_{VH}/\Delta H$ ratio is close to unity for the intact ganglioside but only 0.6 for the isolated oligosaccharide. This difference is probably due to the higher binding affinity of the intact ganglioside molecule (Fishman et al., 1978), since the nature of the effect is qualitatively similar in both cases, or might reflect an additional effect arising from the constraints and interactions imposed by being membrane or micellar bound. This latter possibility is strengthened by the observation that at intermediate degrees of saturation only a single calorimetric peak is observed with oligo-G_{M1}, whereas multiple peaks are observed with membrane-bound (Goins & Freire, 1988) or micellar ganglioside (Dalziel et al., 1984).

Discussion

The interaction of cholera toxin with the cell surface occurs when the B-subunit pentamer recognizes and binds with high affinity and specificity to ganglioside G_{M1}. This association triggers the sequence of events that lead to the release of the A-subunit from the B-subunit pentameric ring, its subsequent membrane insertion, and ultimately the activation of adenylate cyclase by the A-subunit. The molecular events that culminate in the membrane insertion of the A-subunit arise as a result of the interaction of ganglioside G_{M1} and the B-subunit pentamer as demonstrated by experiments performed by using artificial membrane systems (Wisnieski & Bramhall, 1981; Goins & Freire, 1985; Ribi et al., 1988). The exact nature of the interaction and the sequence of events that result in the A-subunit membrane insertion are still largely unknown.

In a previous investigation (Goins & Freire, 1988) it was observed that the association of cholera toxin to its cell surface receptor, ganglioside G_{M1}, resulted in an enhancement of the cooperative interactions between B-subunits. This effect also occurs with isolated B-subunit pentamers, indicating that the cooperative enhancement is not mediated by the A-subunit. The association of cholera toxin or its isolated B-subunit pentamer to micellar or membrane-bound ganglioside involves the formation of a protein–membrane interface in addition to the specific association of the oligosaccharide portion of one ganglioside G_{M1} to each B-subunit monomer within the pentamer. As such, with intact ganglioside G_{M1} it is not possible to unequivocally attribute the origin of the cooperative effect to a specific event. This assignment has been addressed directly in this paper by investigating the interaction of the isolated oligosaccharide portion of ganglioside G_{M1} with the B-subunit pentamer. Since this reaction occurs in solution, it directly probes the interaction at the binding site and the propagation of the cooperative effect through the protein backbone. The experiments presented in this paper demonstrate that the oligosaccharide portion of ganglioside G_{M1} is by itself able to enhance cooperative interactions within the B-subunit pentamer as observed by differential scanning calorimetry.

The calorimetric binding experiments indicate that the binding of oligo-G_{M1} to the B-subunit pentamer displays positive cooperativity. The most conservative model to account for this behavior is one in which the binding to one subunit enhances the affinity of adjacent subunits; i.e., cooperative interactions propagate only to nearest-neighbor B-subunit monomers. Other more complicated models can also be used to fit the data; however, with the information currently available it is not possible to statistically decide on their validity. For example, an allosteric model requires two additional fitting parameters with no statistically significant improvement in the fit. The nearest-neighbor model accounts reasonably well for the entire set of binding data and is consistent with a cooperative free energy of −850 cal/mol. This cooperative free energy is composed of a cooperative enthalpy of −11 kcal/mol and a cooperative entropy of −35.7 cal/K·mol. If these cooperative parameters are primarily associated to a change in conformation, the magnitude and sign of the cooperative enthalpy and entropy would suggest a small structural tightening of the B-subunits upon oligo-G_{M1} binding. This is consistent with the observed changes in the circular dichroism spectra of the B-subunit pentamer upon binding of oligo-G_{M1} (Fishman et al., 1978).

Positive cooperativity facilitates a complete attachment of the toxin to the membrane surface once the initial contact has occurred. In addition, the interaction of the oligosaccharide region of ganglioside G_{M1} with the toxin modifies the interactions within the B-subunit pentamer in a manner that should facilitate the subsequent release of the A-subunit into the

membrane. Presumably, this is accomplished via a local conformational change in the B-subunits. This paper has addressed the changes in the toxin molecule induced by the oligosaccharide portion of ganglioside G_{M1}. The other aspect of this association is the effect on the lipidic portion of the ganglioside, its propagation to the lipid bilayer, as well as the effects resulting from the formation of a protein–membrane interface. These topics are currently under investigation in this laboratory. Preliminary calorimetric experiments performed with intact ganglioside G_{M1} incorporated into phospholipid vesicles are consistent with an overall enthalpy of binding on the order of −85 kcal/mol of toxin (A. Schön, unpublished results). These results suggest that the binding of the B-subunit to the oligosaccharide region of ganglioside G_{M1} affects both the intersubunit protein interactions and the lipid interactions. The additional endothermic effect of ~70 kcal/mol observed with membrane-bound intact ganglioside is consistent with the conclusion of Ribi et al. (1988) of a reduced lipid packing density beneath the B-subunit pentamer. Whether these additional effects are transduced through the lipidic portion of ganglioside G_{M1} or arise as a direct result of the attachment of the protein to the membrane surface is still unknown.

Registry No. Ganglioside G_{M1}, 37758-47-7.

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Carbon-13 NMR of Glycogen: Hydration Response Studied by Using Solids Methods†

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ABSTRACT: The carbon-13 NMR spectra of glycogen are reported by using the methods of magic-angle sample spinning and high-power proton decoupling to provide a dynamic report on the glucose monomer behavior as a function of hydration. Although the glycogen behaves as a typical polymer in the dry state, addition of water makes a significant difference in the spectral appearance. Water addition decreases the carbon spin–lattice relaxation times by 2 orders of magnitude over the range from 7% to 70% water by weight. The proton–carbon dipole–dipole coupling, which broadens the carbon spectrum and permits cross-polarization spectroscopy, is lost with increasing hydration over this range. By 60% water by weight, scalar decoupling methods are sufficient to achieve a reasonably high-resolution spectrum. Further, at this concentration, the carbon spin–lattice relaxation times are near their minimum values at a resonance frequency of 50.3 MHz, making acquisition of carbon spectra relatively insensitive to intensity distortions associated with saturation effects. Though motional averaging places the spectrum in the solution phase limit, the static spectrum shows a residual broader component that would not necessarily be detected readily by using high-resolution liquid-state experiments.

Glycogen is a branched glucose polymer of variable molecular weight that typically ranges from 400 000 to 700 000 in vivo. The glycogen backbone is an α-1,4-glycosidic structure with branch points α-1,6 approximately every 8–12 glucose residues (Metzler, 1977). Since glycogen is a glucose storage molecule, it is important for metabolic studies of carbohydrate metabolism (Cohen et al., 1981; Stevens et al., 1982; Canioni et al., 1983; Sillerud & Shulman, 1983; Neurohr et al., 1984; Reo et al., 1984). As a branched polysaccharide, this molecule represents an important class of molecules that are significantly different from more collapsed structure macromolecules such

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