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On choosing a detergent for solution NMR studies of membrane proteins

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Abstract

Translational diffusion coefficients and catalytic activities were measured for the integral membrane protein diacylglycerol kinase (DAGK) in a variety of types of detergent micelles. Despite the structural diversity of the detergents examined, the translational diffusion coefficients observed for DAGK spanned a fairly limited range of values: 2.7 to 4.7 ($\times 10^{-7}$cm^2/s). No general correlation was observed between the diffusion coefficients for the detergent-DAGK aggregates and the sizes of the corresponding protein-free micelles. These results indicate that the effective molecular weights of the DAGK-detergent aggregates were determined more by the structural properties of the protein than by the properties of the detergents. The catalytic activity of DAGK in detergents having medium-length alkyl chains such as dodecylphosphocholine or decylmaltoside was usually observed to be substantially higher than in short-chain detergents such as octylphosphocholine or octylglucoside. Taken together, the diffusion and activity results indicate that medium-chain detergents are generally preferred for use in NMR studies of complex membrane proteins because they are no worse than short-chained detergents in terms of increasing the effective molecular weight of the protein of interest while they are considerably better at maintaining native-like protein conformation. Among the 10 detergents examined, only sodium dodecylsulfate was observed to be unable to support DAGK activity under any conditions examined, suggesting that this well-known protein denaturant should be used with care in studies of complex membrane proteins.

Introduction

Detergent micelles are widely used as lipid bilayer-mimetic media in NMR studies of both peripheral and integral membrane proteins and polypeptides (see reviews by Henry and Sykes, 1994 and Opella et al., 1994). The ideal micelle type for such studies would satisfy two critical criteria. First, it should be a good mimic of an actual phospholipid bilayer in the sense that the structure adopted by the protein of interest should be the same in the micellar system as it is when associated with bilayers. Secondly, the tumbling of the micelle-protein aggregate in solution should be rapid enough to yield spectra of sufficiently high resolution to permit spectral assignments to be made and structurally interpretable data to be extracted.

In this contribution, we examine a cross-section of detergents in order to assess which classes of micelles best satisfy the criteria outlined above. Gradient-based NMR diffusion measurements are reported which shed light upon which classes of micelles tumble most rapidly in the presence of added membrane protein. Because for ideal spheres translational diffusion coefficients scale in proportional to (aggregate molecular weight)$^{-1/3}$, these measurements provide a rough gauge of detergent-protein aggregate size (see Kallick et al., 1995). As a model membrane protein for use in these studies, we have chosen to focus upon *E. coli* diacylglycerol kinase (DAGK). DAGK is a 13 kDa protein which forms 39 kDa homotrimers in which each subunit contains three transmembrane α-helices (Sanders et al., 1996; Vinogradova and Sanders, 1997). DAGK is ideal for this study because it has an assayable catalytic function which allows the suitability of micelle types as model membranes to be assessed based on observed enzyme activity in any given system.

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Materials and Methods

The detergents and lipids used in this study were purchased from Anatrace (Maumee, OH), Avanti Polar Lipids (Alabaster, AL), or Calbiochem (San Diego, CA).

Polyhistidine-tagged *E. coli* diacylglycerol kinase was purified to homogeneity in a D_2O solution of the detergent of interest under acidic conditions as described elsewhere (Vinogradova et al., 1997).

DAGK stability assays were carried out by diluting aliquots of the NMR samples used in the diffusion studies into the standard UV-detected coupled assay system. As described elsewhere (Badola and Sanders, 1997), this assay provides a convenient measurement of DAGK's conversion of MgATP and diacylglycerol to MgADP and phosphatidic acid. Direct assays of DAGK's activity in different detergent micelles were also carried out using either a thin layer chromatography/fluorescence-based assay (Sanders et al., 1996) or the standard UV-detected assay system, in either case substituting the detergent of choice (see Table 1) for the octylglucoside/dimyristoylphosphatidylcholine mixed micelles normally employed.

DAGK's translational diffusion coefficient was measured in a number of micellar systems in acidic D_2O (see Table 1) by a PFG (pulse field gradient) water-suppressed LED (longitudinal eddy-current delay) experiment (Altieri et al., 1995). The intensity of the NMR signals in this experiment have the following dependency upon the field gradient and molecular diffusion coefficient:

$$I = I_0 \exp[-(\gamma\delta g)^2 * (\Delta - \frac{\delta}{3}) * D_t] \qquad (1)$$

where γ is the gyromagnetic ratio of the nucleus, δ and g are the gradient duration (i.e., 8.5 ms) and strength, respectively, Δ is the duration between the gradient pulses (the diffusion time: 0.1 s), D_t is the diffusion coefficient and I_0 is the intensity of the signal when no gradient is applied. The gradient strength in these experiments was stepped as an array of 20 points, ranging from 0 to ca. 30 Gauss/cm, with 32 scans being taken per point and allowing a 5 s delay between scans. Spectra were processed using a shifted sine-bell square apodization function.

Results and Discussion

Determination of diffusion coefficients for DAGK in detergent micelles

A cross-section of biological detergents was chosen for evaluation (Table 1), including non-ionic detergents (the alkyl glycosides) zwitterionic detergents (the phosphocholines and CHAPSO), an anionic detergent (sodium dodecyl sulfate), a long-chain detergent (lyso-myristoylphosphatidylcholine), medium-chain detergents (dodecylphosphocholine, dodecylsulfate, decylmaltoside), short-chain detergents (octylphosphocholine, octylglucoside), a short-chained lipid (dihexanoylphosphatidylcholine), and a chain-less bile salt class detergent (CHAPSO).

Measurements were made at high detergent concentrations and low DAGK concentrations in order to avoid problems with protein aggregation at low micelle to protein ratios (see McDonnell and Opella, 1993). Solutions containing 8% detergent were used, corresponding to molar concentrations of 0.13–0.27. This ensures that the actual concentrations of micelles in these samples ((total detergent conc.–CmC)/aggregation number) is well in excess of the DAGK concentration used (0.1 mM).

Proton NMR spectra of micellar DAGK are invariably very broad, except for the resonances from the N-terminal polyhistidine purification tag (Vinogradova et al., 1997). We therefore integrated the two aromatic spectral regions of DAGK which were dominated by resonances from the histidine peaks (Figure 1A) in a series of gradient strength variation spectra (Figure 1B) in order to acquire data which could be fit to Equation 1 to calculate the translational diffusion coefficients for DAGK in micelles (Figure 2). The results for various micelle types are summarized in Table 1. In all cases except for lyso-myristoylphosphatidylcholine, the data were very satisfactorily fit by a single exponential curve. In the case of lyso-phosphatidylcholine, the integral vs. (gradient strength)^2 data was clearly non-exponential (data not shown), most likely due to the presence of two or more non-rapidly exchanging aggregate populations having different translational diffusion coefficients. Measurements were also made for protein-free micelles, as summarized in Table 1.

As can be seen in Table 1, the range of translational diffusion coefficients measured for DAGK in various micellar mixtures is somewhat limited, spanning from 2.7 to 4.7×10^{-7}cm^2/s. Furthermore, there is no general correlation between the observed transla-

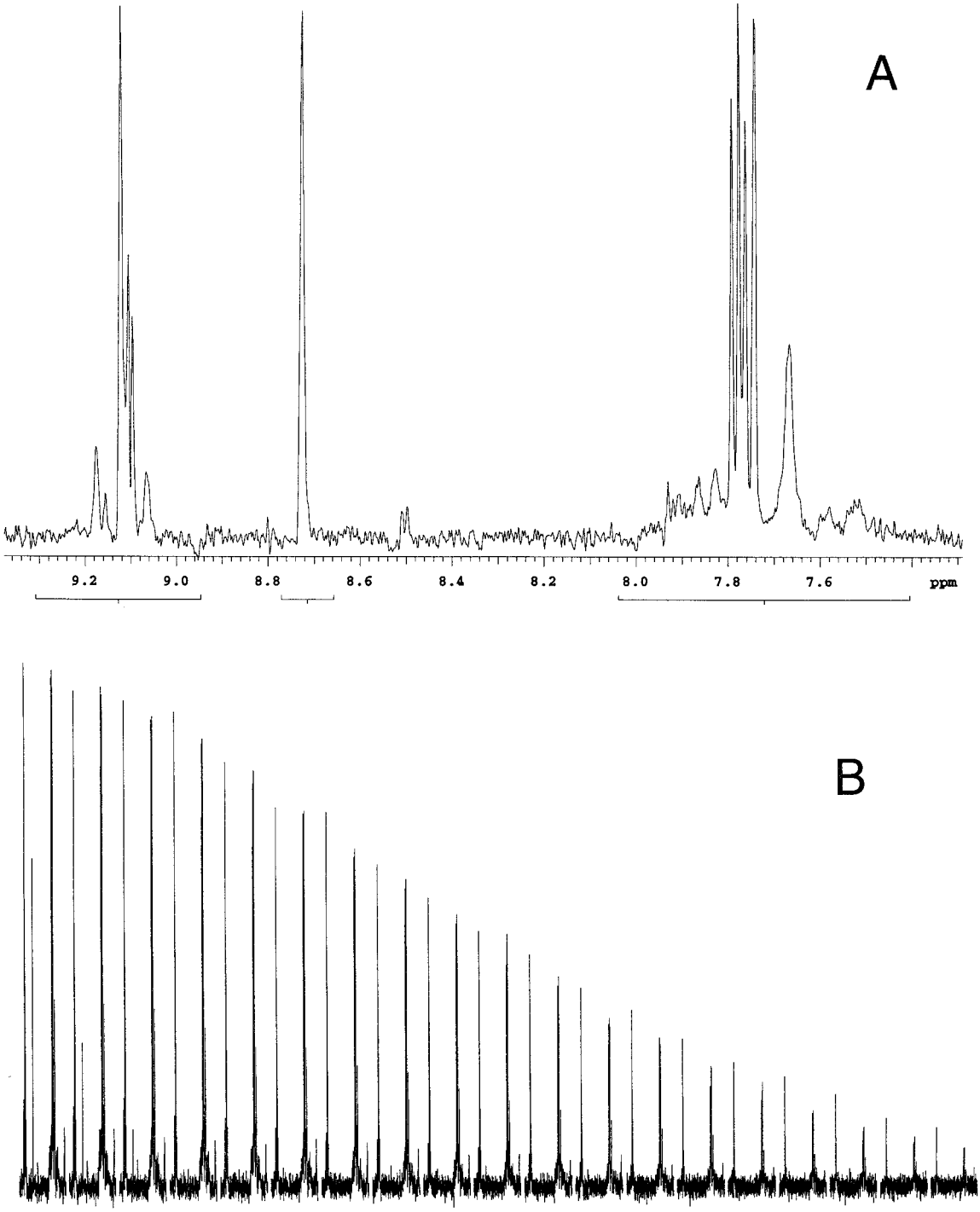

Figure 1. Example of data used for determination of DAGK's diffusion coefficient in a micellar system. (A) Aromatic region of 8% dodecylphosphocholine-DAGK (0.1 mM DAGK, 1% d-formic acid, 100 mM NaCl in D_2O) spectrum at 30 °C. Integrals over the regions ~ 9.3−8.95 ppm and 8.0 − −7.4 ppm, dominated by resonances from the N-terminal poly-His tail were used for calculations. The large peak at 8.72 ppm is from residual protonated formic acid and decays at a much faster rate than do the protein peaks, owing to the acid's rapid diffusion rate. (B) Dependence of the spectral region shown in (A) upon increasing gradient field strength (see Materials and Methods).

Table 1. Translational diffusion coefficients and activity measurements for DAGK in various micelle types

Detergent	MW	CMC (mM)[a]	Protein-free micelle MW[b]	D_t, DAGK-free micelles[c] $\times 10^{-7}$cm^2/s	D_t, DAGK[d] $\times 10^{-7}$cm^2/s	DAGK stability[e]	DAGK activity, no lipid[f]	DAGK activity with lipid[f]
β-decylmaltoside	483	2	50000	6.3 ± 1.0	4.1 ± 1.0	Moderate	Moderate	High
β-(4-Cyclohexyl-butyl)-maltoside	467	7	21000	7.2 ± 0.6	3.9 ± 0.7	Moderate	ND	ND
β-Octylglucoside	292	25	23000	3.7 ± 0.5[g]	2.7 ± 0.5	Low	Low	High
n-Octylphosphocholine	295	100	very low	14 ± 3	4.4 ± 0.7	High	Low	High
Dodecylphosphocholine	316	1	19000	7.7 ± 0.3	4.7 ± 0.9	Moderate	Moderate	High
1,2-Dihexanoyl-phosphatidylcholine	454	14	9000	8.6 ± 0.5	3.1 ± 0.7	Moderate	Low	High
Lyso-1-myristoyl-phosphatidylcholine	468	0.07	90000	5.9 ± 0.3	[h]	Moderate	High	High
Sodium dodecylsulfate	288	10	30000	6.7 ± 0.5	3.2 ± 0.9	ND	Low[i]	Low[i]
Triton X-100	647	0.2	90000	6.8 ± 0.9	ND	ND	ND	High
CHAPSO	631	8	6000	9.0 ± 0.5	4.1 ± 0.9	ND	Low	High

[a] Critical micelle constant (CMC) values were obtained from the literature. References are as follows: octylglucoside (Chattopadhyaya and London, 1984), decylmaltoside (Anatrace, 1997), cyclohexylbutylmaltoside (Anatrace, 1997), octylphosphocholine (Anatrace, 1997), dodecylphosphocholine (Lauterwein et al., 1979), dihexanoylphosphatidylcholine (Burns et al., 1982), lyso-phosphatidylcholine (Stafford et al., 1989), sodium dodecylsulfate (Chattopadhyaya and London, 1984), Triton X-100 (Lichtenberg et al., 1983), and CHAPSO (Hjelmeland et al., 1983).

[b] Micelle molecular weights were calculated based on multiplying the aggregation number by the detergent monomer molecular weight. Aggregation numbers were obtained from the literature with references as follows: octylglucoside (Chattopadhyaya and London, 1984), decylmaltoside (Jhun et al., 1991), cyclohexylbutylmaltoside (Anatrace, 1997), dodecylphosphocholine (Lauterwein et al., 1979), dihexanoylphosphatidylcholine (Lin et al., 1986), sodium dodecylsulfate (Lichtenberg et al., 1983), Triton X-100 (Lichtenberg et al., 1983), and CHAPSO (Hjelmeland et al., 1983).

[c] Translational diffusion coefficients were measured for detergent micelles at a total detergent concentration of 8% at 30 °C, 100 mM NaCl and 25 mM phosphate, pD = 7.0. The reported values have been corrected to remove the contribution to the observed diffusion coefficient (D_{obs}) from the free (non-micellar) detergent population using the equation $D_t = (D_{obs}$ - [critical micelle concentration]/[total detergent concentration] $\times$ $D_{free})$/(1 - [critical micelle concentration]/[total detergent concentration]), where D_{free} is the diffusion coefficient of the free detergent (measured at sub-micellar total detergent concentrations, data not shown). Values for the critical micelle concentrations were taken from the literature (see footnote a). In the case of octylglucoside, we initially suspected that the critical micelle concentration did not provide a good estimate of the free detergent concentration at 8% octylglucoside. Therefore, the micellar diffusion coefficient was confirmed by doping the micelles with 5 mM dilauroylphosphatidylcholine (whose free concentration can be assumed to be effectively zero), measuring the observed diffusion coefficient for this lipid additive, and comparing the diffusion coefficient of the lipid to that determined for octylglucoside micelles by the standard method (see above).

[d] Translational diffusion coefficients were measured for DAGK at 30 °C, 0.1 mM DAGK trimer concentration, 8% (w/v) detergent concentration, and in the presence of 1% formic acid and 100 mM NaCl.

[e] DAGK stability was determined by taking the samples used for the diffusion measurements (ca. 2 h at 30 °C), incubating them at 50 °C for ca. 30 min and then diluting a small aliquot from each sample into the mixed micellar assay system where DAGK activity was measured. 'High' means that 50–100% of the specific activity of fully active DAGK was observed at this point. 'Moderate' means 10–50% activity. 'Low' means <10% activity.

[f] DAGK activity was determined by diluting stock solutions of the enzyme into the DAGK assay mixture in which the usual octylglucoside/dimyristoylphosphatidylcholine (DMPC) mixed micelles were replaced with either the detergent of interest (with no added lipid) or with the detergent of interest plus added DMPC (with lipid). 'High' activity means that the activity is >50% of the activity in the normal assay system. 'Moderate' means 5–50% activity. 'Low' means < 5%.

[g] It is interesting to note that the diffusion coefficient for octylglucoside is much smaller than expected based on the literature value for the micellar molecular weight. The most likely explanation for this discrepancy is that at the very high (8%) concentration at which the diffusion coefficients reported in this paper were measured, the aggregation number for octylglucoside micelles is much higher than it is under the conditions where the literature value was measured.

[h] Data could not be fit to a single diffusion coefficient (see text).

[i] Activity could not be detected within a detection threshold of 0.7% of the specific activity of DAGK in the normal assay mixture.

tional diffusion coefficients for the solubilized protein and the estimated size of the corresponding micelles under protein-free conditions based either upon literature values or upon the measured diffusion coefficient. For example, the diffusion coefficient observed for DAGK in dihexanoylphosphatidylcholine was among the smallest, indicative of a high effective molecular weight, despite the fact that this detergent forms relatively small micelles under protein-free conditions.

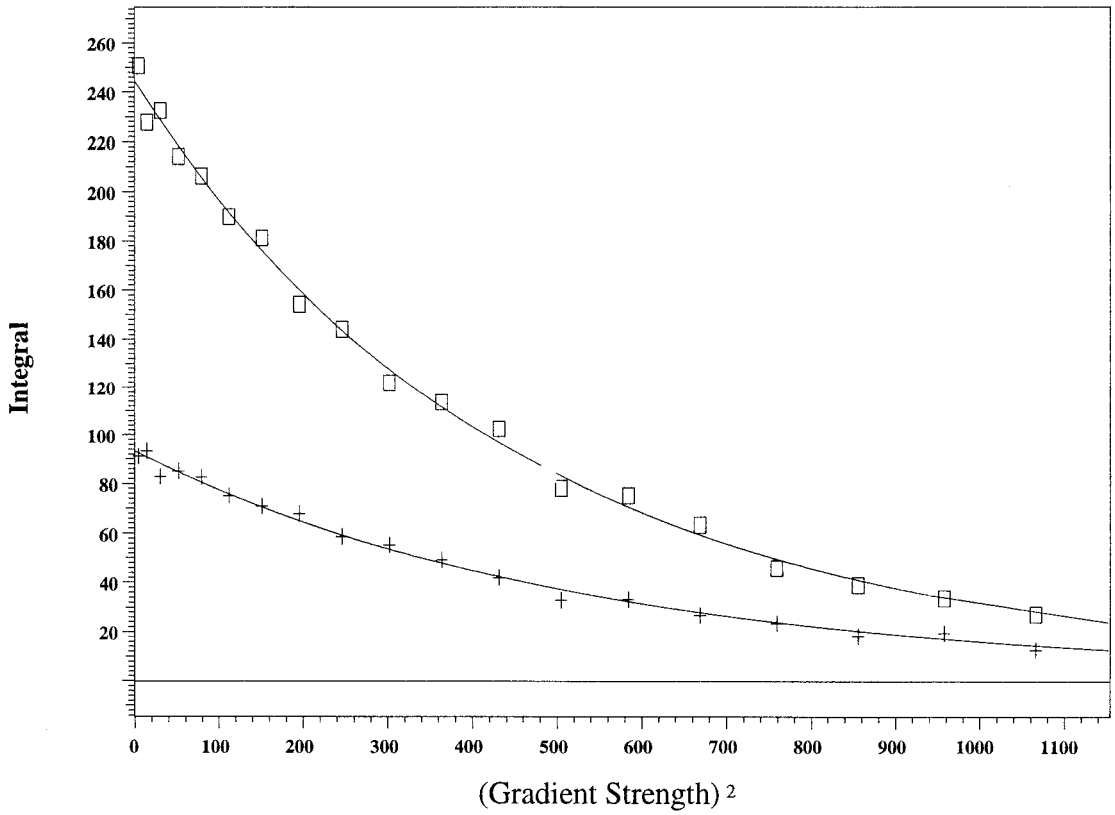

Figure 2. Plots and fits of the data from Figure 1 used to determine the diffusion coefficient. Open squares correspond to the 9.3–8.95 ppm region, while crosses correspond to the 8.0–7.4 ppm region. Diffusion coefficients (D_t) were calculated according to Equation 1 using non-linear regression (VNMR program, Varian, Palo Alto, CA). In this case, the value of D_t reported in Table 1, 4.7 ± 0.4 ($\times 10^{-7}$ cm^2/s), represents the average of the values determined from each curve (4.3 and 5.1).

Stability and activity of DAGK in micelles

DAGK's stability in a given micellar NMR sample was tested by dilution of small aliquots of the DAGK solutions back into a decyl maltoside/dimyristoylphosphatidylcholine mixed micellar assay solution in which DAGK is normally active and stable (see Materials and Methods). The results are summarized in Table 1 and clearly reflect a variety of detergent-specific behavior. Low stability can be taken as evidence that DAGK was irreversibly unfolded in the micellar NMR sample. High stability cannot necessarily be taken as evidence for proper folding in the original NMR sample, but does indicate that if DAGK was unfolded, it was *reversibly* so. A better judge of the state of folding in the micellar samples is DAGK's activity measured directly within a given micelle type.

DAGK was directly assayed in the various micelle types listed in Table 1. As can been seen, DAGK is completely active in most micelle types provided that phospholipid is also present. However, in the absence of added lipid the degree of DAGK activity varied a great deal from detergent to detergent, with the long/medium-chain detergents being generally better than short-chain detergents at maintaining DAGK's active folded conformation. Sodium dodecyl sulfate represents the one clear exception to this latter observation: DAGK is not active in this anionic detergent under any conditions.

Implications for choosing an optimal detergent for NMR of membrane proteins

Based on Table 1, several important observations and conclusions can be made.

(1) *For DAGK and most detergents, the size of the protein-micelle aggregate appears to be determined more by the properties of the membrane protein, than by the detergent type.* This observation explains the general lack of a correlation between the sizes of the protein-free and DAGK-containing micelles. A particularly convincing demonstration of this generalizaton

is provided by comparing results for octylphosphocholine and dodecylphosphocholine: despite the fact that octylphosphocholine has four less carbons in its apolar tail, DAGK exhibits virtually the same diffusion coefficient in both systems. The basis for this observation appears to be that the size of the protein-detergent aggregate is governed primarily by the hydrophobic area of the transmembrane domain of the protein which must be detergent-stabilized (Moller and le Maire, 1993; Vinogradova et al., 1997): if a shorter chained detergent is used then more must bind to cover up the same area which is more easily covered up by detergents having longer hydrocarbon chains. Because DAGK can be regarded as a model integral membrane protein which spans the membrane bilayer multiple times, this observation can likely be extrapolated to other membrane proteins. The single exception to this observation found in Table 1 is lysophosphatidylcholine: a molecule which appears to be too lipid-like to be employed in experiments requiring the monodispersity of classical micellar systems.

(2) *The functional conformational state of DAGK is quite sensitive to detergent type, with longer chained detergents generally being more supportive of the active conformation.* In the absence of added lipid, DAGK activity was low in all of the short-chained detergents examined, but was substantial in all medium-chained detergents examined except for sodium dodecylsulfate. Since there does not appear to be a motional basis for preferring a short-chained detergent for NMR studies and since long-chained detergents appear to be too lipid-like, this suggests that medium-chained detergents are preferred for NMR studies.

(3) *Sodium dodecylsulfate (SDS) was the only detergent which failed to support DAGK activity, even in the presence of added lipid.* This observation reflects the fact that, as for most water-soluble proteins, sodium dodecylsulfate denatures DAGK (Lau and Bowie, 1997). This observation suggests that while sodium dodecylsulfate has been widely used in NMR studies of membranous polypeptides, great care should be taken to verify native-like structure if SDS is to be employed in NMR studies of complex membrane proteins such as DAGK.

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