Specific Inhibition of Glucokinase by Long Chain Acyl Coenzymes A below the Critical Micelle Concentration*

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Rat liver glucokinase (EC 2.7.1.2) is inhibited specifically by the free form of palmitoyl-CoA (Kᵢ, apparent of approximately 1.8 μM) and other related long chain fatty acyl-CoAs under conditions where the micellar form of these compounds does not exist. This inhibition is instantaneous, and is immediately reversible by dialution, bovine serum albumin, and α- or β-cyclodextrins. Inhibition is specific for long chain fatty acyl-CoAs. Related compounds also form micelles, palmitate and myristate, as well as the structurally related compounds, acetyl-CoA and CoASH, are not inhibitory. The extent of inhibition is independent of the molar ratio of glucokinase to acyl-CoA. The inhibition is competitive with both ATP and glucose and does not affect the positive cooperativity which glucokinase normally displays with glucose (Hill coefficient = 1.5 under all inhibited conditions).

The critical micelle concentrations (cmc) of oleoyl-CoA and the saturated long chain acyl-CoAs (C-12 to C-20) were determined under actual assay conditions by a pinacyanol chloride dye binding method. The cmc for palmitoyl-CoA under several conditions was also estimated by ultracentrifugation and ultramicroscopy. Significant inhibition by these compounds was found to occur well below their cmc values in all cases. The apparent Kᵢ for palmitoyl-CoA, for example, was between 2.5- and 14-fold below its cmc in assay mixtures lacking glycerol and between 10- and 52-fold below its cmc in mixtures containing 30% glycerol. The reversibility, instantaneous, specific, and sensitive nature of this inhibition, coupled with the lack of micelle formation at inhibitory concentrations of these long chain acyl-CoAs, suggest that inhibition of glucokinase is not related to detergent effects but rather represents the binding of monomeric acyl-CoA to the enzyme.

Many enzymes (1-4) including glucokinase (5) have been reported to be inhibited by palmitoyl-CoA and related compounds. Much controversy has surrounded this literature, primarily because a distinction has been difficult to make between a specific, relevant interaction of a long chain acyl-CoA with an enzyme and nonspecific lipid-protein, detergent, or micelle interactions of the amphiphile with an enzyme. Indeed, the reported inhibition in many cases is probably nonspecific. The primary criticisms (6-10) have been that long chain acyl-CoAs have been shown to inhibit numerous unrelated enzymes in diverse metabolic pathways without any possible physiologic explanation and that these powerful detergents often irreversibly inactivate the enzymes or inhibit them in a manner which is not characteristic of classical site-specific inhibition. Other workers (1-4, 11-13) suggest that long chain acyl-CoAs (especially palmitoyl- and oleoyl-CoAs) occupy an ideal position as appropriate effectors of several metabolic pathways and that there are several systems in which their interaction with an enzyme is very likely to be both specific and metabolically appropriate.

In view of this controversy, the present paper presents a thorough investigation of the inhibition of rat liver glucokinase by palmitoyl-CoA and related compounds. The cmc for five long chain acyl-CoAs have been studied under several conditions by different techniques. The onset, reversibility, kinetics, and relationship of the cmc and micelles to the inhibition of glucokinase have been studied. The results indicate a specific and significant interaction between glucokinase and the free, nonmicellar form of long chain acyl-CoA.

MATERIALS AND METHODS*

RESULTS

The Effect of Palmitoyl-CoA on Glucokinase Activity in Assays at Low Magnesium Concentrations—In an assay mixture containing 5 mM MgCl₂ and 5 mM ATP, palmitoyl-CoA inhibited glucokinase (Ref. 5, Fig. 1). The onset of this inhibition was instantaneous and its extent was the same whether palmitoyl-CoA was added before or during the course of the assay. The upward curvature of the double reciprocal plots in Fig. 1 shows that the normal cooperativity of glucokinase with glucose was retained at all levels of inhibition. The extent of inhibition was characteristically less than 1.5 under all inhibited conditions.)

The abbreviations used are: cmc, critical micelle concentration; DTT, dithiothreitol; nₚ, Hill coefficient; MMP, 3-O-methylmannoselcontaining poly saccharide; MGLP, lipopolysaccharide containing 6-O-methylglucose and glucose; PEP, phosphoenolpyruvate; PK/LDH, pyruvate kinase-lactate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; M6SO, dimethyl sulfoxide; Sₐ, substrate concentration for half-maximal activity.

* Portions of this paper (including "Materials and Methods," part of "Results" and a discussion of micelle formation, Table I, and Figs. 1-6) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9560 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-0923, cite authors, and include a check or money order for $5.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Inhibition of Glucokinase by Long Chain Acyl-CoAs

The inhibition by palmitoyl-CoA displayed a competitive kinetic pattern with glucose; the V_max was constant at all inhibitor concentrations and only the glucose concentration for half-maximal velocity (S_0.5) changed with added palmitoyl-CoA. A study of palmitoyl-CoA inhibition versus ATP concentrations showed that palmitoyl-CoA was also competitive with ATP^1 (19) and that the linear Michaelis-Menten kinetics of glucokinase with ATP were retained at all levels of inhibition.

Other long chain acyl-CoAs inhibited glucokinase in a similar way. Fig. 2 shows inhibition curves for lauroyl-, myristoyl-, palmitoyl-, and stearoyl-CoAs in assays with 2 mM glucose, 5 mM ATP, 5 mM MgCl_2. An apparent K_i for each of these acyl-CoAs was calculated from the changes in the S_0.5 (pure competitive inhibition) taken from Hill plots (data not shown) of a more extended form of the data shown in Fig. 2. These apparent inhibition constants are shown in Table I. Generally, the longer the hydrocarbon chain, the better the apparent K_i of the inhibitor for this series.

The cme of Palmitoyl-CoA—An extensive investigation of the critical micelle concentration of palmitoyl-CoA and related acyl-CoA compounds is detailed in the Miniprint. These results indicated that, although the formation of micelles of palmitoyl-CoA is a complex phenomenon, the cme was consistently higher than the K_i for glucokinase inhibition (2.5- to 14-fold higher without glycerol or 10- to 52-fold higher in the presence of 30% glycerol) and that manipulating the cme in the enzyme assays did not affect the properties of the inhibition. Furthermore, manipulating the cme directly in the enzyme assay did not affect the properties of the inhibition. Thus, the inhibition is not due to detergent action of acyl-CoA micelles.

Inhibition in High Concentrations of Free Magnesium—The inhibition of glucokinase by palmitoyl-CoA was investigated in a high magnesium (10 mM MgCl_2, 5 mM ATP) assay mixture. Although the glucose cooperativity and the competitive nature of the inhibition were retained (Fig. 3), the inhibition was unusual in one respect compared with classical competitive inhibition. Under low free magnesium conditions (Fig. 1), successive increases in inhibitor concentration caused successively decreased activity until at very high concentrations of inhibitor the enzyme showed no activity at all. Clearly, this was not the case for glucokinase inhibited by palmitoyl-CoA under high magnesium conditions (Fig. 3); continuing increases in the palmitoyl-CoA concentration above approximately 5 pM failed to produce continued decreases in glucokinase activity at any glucose concentration. Typically, the extent of maximum inhibition in this system was limited to about 50% and, invariably, the reaction rates at palmitoyl-CoA concentrations of 5 pM and above were indistinguishable from each other. In the high magnesium system, inhibition was limited for the other long chain acyl-CoAs (Fig. 4) just as it was for palmitoyl-CoA. In every case, the inhibition up to the limit appeared competitive with glucose and gave double reciprocal plots similar to those in Fig. 3.

There are two reasonable explanations for this behavior. First, at least under high magnesium conditions, the effector could be working through an allosteric site of glucokinase rather than at the active site per se (19). The maximal effect of binding at the allosteric site therefore could be merely to reduce the apparent affinity of glucokinase for glucose and ATP severalfold without actually competing for the same site as the substrate (partial competitive inhibition). Second, the free concentration of the effector, palmitoyl-CoA, may cease to increase above a limit imposed by its critical micelle concentration and micelles which form at concentrations above the cme might neither inhibit nor activate glucokinase. The distinction between these two possibilities is discussed in the following paper (19).

The possibility was investigated that, at relatively high concentrations of acyl-CoA, the addition of magnesium to a low magnesium mixture might actually activate glucokinase by affecting the limiting inhibition. The addition of 5 mM MgCl_2 to an assay inhibited by 10 pM palmitoyl-CoA in a 2 mM glucose, 5 mM MgCl_2, 5 mM ATP mix did partially reverse the inhibition (data not shown). The reversal was instantaneous and gave the same final rate as a parallel assay started in 10 mM MgCl_2. Calcium chloride mimicked MgCl_2 in this regard, although concentrations of 20 mM CaCl_2 were required for maximal reactivation. The range over which free magnesium could modify the cme for palmitoyl-CoA and protect against palmitoyl-CoA inhibition of glucokinase was investigated (Fig. 5). The activation of glucokinase occurred at about the same Mg^{2+} concentration as the binding of Mg^{2+} to palmitoyl-CoA measured by the resultant decrease in the measured cme. This correlation, however, does not prove the mechanism by which Mg^{2+} acts.

Enzyme Concentration Effects—The effect of the ratio of inhibitor to enzyme concentrations was investigated in the low magnesium system by assays in the presence and absence of 5 pM palmitoyl-CoA, over a 20-fold range of glucokinase concentrations (from 0.8 milliunit/ml (5 ng/ml) to 16 milliunits/ml (100 ng/ml)). The inhibition (in 2 mM glucose, 5 mM MgCl_2, 5 mM ATP) was constant over this range with inhibited rates equal to 20.4 ± 2.8% of controls.

Reversibility—In both the high and the low magnesium systems, the inhibition by palmitoyl-CoA could be quantitatively reversed at any time during the assay by dilution, by the addition of bovine serum albumin (1 mg/ml), or by the addition of 1 mM α- or β-cyclodextrins. Control proteins transferrin and lysozyme (at 1 mg/ml) reversed the inhibition by an average of only 3.5%. As mentioned above, the addition of either 5 mM MgCl_2 or 20 mM CaCl_2 could partially and instantly reverse the palmitoyl-CoA inhibition in low magnesium systems.

Specificity—The specificity of glucokinase for inhibitors was generally limited to long chain fatty acyl-CoAs (Table I). Concentrations of acetyl-CoA to 10 mM or free CoASH to 1 mM were not at all inhibitory, nor were free palmitate or myristate up to 1 mM. Palmitoylcarnitine did inhibit glucokinase, but only above its cmc (15 μM, Ref. 26; or 19 μM, Table I). This inactivation was characteristic of nonspecific detergent action; it was maximal (50% inhibition) at 100 μM palmitoylcarnitine, time-dependent and irreversible, and unaffected by the free magnesium concentration.

Yeast hexokinase (P II), which catalyzes the same reaction as glucokinase and is similar in molecular weight and amino acid composition (21), was not affected by palmitoyl-CoA at concentrations through 15 μM when assayed in a 50 μM glucose, 5 mM ATP, 5 mM MgCl_2 mix, pH 7.5.
Inhibition of Glucokinase by Long Chain Acyl-CoAs

All of the data presented here for the palmitoyl-CoA (and the other long chain acyl-CoAs) inhibition of rat liver glucokinase are consistent with classical, site-specific binding. In comparison with the properties of some enzymes reported to be inhibited by palmitoyl-CoA (and related compounds), several characteristics of the inhibition of glucokinase stand out.

(a) The inhibition of glucokinase is instantaneous, with no time dependence whatsoever. Preincubation of glucokinase with palmitoyl-CoA gives the same level of inhibition as adding glucokinase to an assay mix containing the inhibitor or as adding the inhibitor during the course of an uninhibited assay.

(b) The inhibition is instantly reversible either by simple dilution or by the addition of albumin or cyclodextrins.

(c) No evidence exists for any major structural change in glucokinase which might cause inactivation. The V_max and the Hill coefficient for cooperativity with glucose (19), as well as a sulfhydryl-related decay in activity (3) which glucokinase undergoes under specific conditions, are all unaffected by the presence of palmitoyl-CoA. Only the S<sub>v</sub> values for glucose and ATP (19) are affected.

(d) No change occurred in the extent of inhibition by palmitoyl-CoA over a 20-fold range of glucokinase concentrations. A mole ratio-type of inhibition (independent of actual inhibitor concentrations but requiring a specific fold excess of inhibitor) seen with some enzymes (9, 17) is ruled out.

(e) The apparent K<sub>i</sub> for palmitoyl-CoA inhibition is quite low, about 1.8 µM with one substrate saturating. The true K<sub>i</sub>, extrapolated to zero concentrations of both substrates, is even lower. In any case, the dissociation constant for palmitoyl-CoA from glucokinase is significantly below the critical micelle concentration of palmitoyl-CoA measured here by dye incorporation experiments (which give the lowest values of the methods used) at 4.5 µM under the same conditions in which the K<sub>i</sub> was determined without glycerol or at 18.6 µM in the presence of 30% glycerol (see Miniprint). All methods employed to determine the cmc showed at least a 4-fold increase in the cmc for palmitoyl-CoA in 30% glycerol, yet the inhibition of glucokinase under these conditions was essentially unaltered. The proposed mechanisms of nonspecific inhibition may involve either the necessary formation of micelles before a detergent-like inhibition of the enzyme activity of nonspecific attraction between the hydrophobic tail of the amphiphile and relatively hydrophobic regions of the enzyme resulting in nucleation of multimeric amphiphile on the surface of the enzyme and inhibition below the cmc (26). In either case, manipulation of the solvent polarity (e.g., glycerol) should predictably alter this nonspecific inhibition.

(f) The specificity of the inhibition is further demonstrated in the limited group of compounds which inhibit glucokinase (Table I). Neither the CoA moiety as acetyl-CoA or CoASH nor the long fatty acid component as myristic or palmitic acid is at all inhibitory through concentrations of at least 1 mM. The only inhibitory compound tested which is not a long chain fatty acyl-CoA was palmitoylcarnitine, which was inhibitory only above 20 µM. Since the cmc of palmitoylcarnitine in either the high or low Mg<sup>2+</sup> assay mixes is 19 µM and the inhibition is both time-dependent and irreversible, it is probably due to nonspecific detergent action of palmitoylcarnitine on glucokinase. These results differ from those of Dawson and Hales (5), who reported no inhibition of crude preparations of glucokinase at concentrations of palmitoylcarnitine up to 100 µM. Perhaps the much more purified enzyme used in these studies was more sensitive to detergent action; the contaminating proteins in their work may have adsorbed some of the palmitoylcarnitine, thus effectively lowering its free concentration.

(g) The kinetics of the inhibition indicates that palmitoyl-CoA is competitive with respect to both glucose (Fig. 1) and ATP (19). This finding was unusual, but repeated experiments gave competitive patterns even at several concentrations of the fixed substrate. Inhibition competitive with a substrate is good evidence that palmitoyl-CoA is a site-specific effector, as opposed to a generalized, nonspecific inhibitor, especially since neither substrate has any effect on the cmc of palmitoyl-CoA.

Taken together, then, these data on the specificity, kinetics, and character of the inhibition of glucokinase by the long chain acyl-CoAs, which are unaffected by perturbation of micelle formation and the partition of amphiphiles, suggest that the inhibition is site-specific and unrelated either to the formation of detergent micelles or to nonspecific hydrophobic binding of amphiphile to glucokinase. Assessment of any physiological role of palmitoyl-CoA inhibition of liver glucokinase would be premature at this stage. However, the idea of some control over the uptake and storage of blood glucose by the major product of fat metabolism through the glucose-glucose 6-phosphate substrate cycle is very appealing. We might speculate, therefore, that physiological concentrations of palmitoyl-CoA (see Ref. 3 for discussion) perform a regulatory role by shifting this cycle toward release of glucose during starvation or diabetes when lipolysis and lipid metabolism are increasing. Clearly, more information is needed with regard to intracellular free acyl-CoA concentrations and the susceptibility to altering flux through the glucokinase reaction by these metabolites.

REFERENCES

Inhibition of Glucokinase by Long Chain Acyl-CoAs

27. Deleted in proof
Inhibition of Glucokinase by Long Chain Acyl-CoAs

Below the Critical Micelle Concentration

P. L. Tippett and C. W. North

MATERIALS AND METHODS

Glucokinase was prepared by modification (17) of the method of Molineux et al. (18). Like the work of Slover and Grossman (19), most of this work was done on enzyme purified through the affinity column stage having a specific activity of 30 units per milligram. Kinetic comparisons between enzymes from this stage and commercial enzyme (11) (sterile) have always given identical results in this work as well as in other laboratories (5).

Glucokinase was used to prepare a solution of glucokinase dephosphorylase (12843).

The standard assay mixture was in ml: 200 mM triethanolamine pH 7.5, 1 mM MgCl2, 5 mM ATP, 0.2 units of glucokinase, and either 5 or 10 mM MgC2 as indicated. Glucose and other additions were added as described in the figure legends. In some experiments the triethanolamine concentration was 50 or 30 mM, although this did not affect the results. Complete assay procedures were equilibrated to 25°C for 10 minutes; the reaction was started by the addition of 5 microliters of 5 x 10-4 M glucokinase, and the absorption at 400 nm was followed over time on a tension recorder (12). The reaction was stopped by the addition of 0.5 ml of 10% trichloroacetic acid. The formation of free inorganic phosphate was measured.

Glucokinase was inhibited by long chain acyl-CoAs, the coupling enzyme, has been reported to be inhibited by palmitoyl CoA (17). Although the enzyme isolated from liver microsomal membranes used here has been shown (17) to be inhibited by palmitoyl CoA at high concentrations less than 0.05 M of palmitoyl CoA, our results suggest that the enzyme in our system is inhibited by palmitoyl CoA at lower concentrations below the critical micelle concentration.

The concentrations of all the CoA esters were determined in their absence at 297 nm with an extinction coefficient of 13.5 m41 cm-1. Sodium palmitate and sodium stearate were added to 140 mM solution. The weight itself had no effect on the assay.

Measurement of the Critical Micelle Concentration: The absorption spectrum of the long chain acyl-CoAs is similar to the hexadecyltrimethylammonium bromide as a function of its concentration relative to water. In aqueous solutions above the critical micelle concentration, the absorption at 500 nm increases dramatically as the micelle concentration increases and the hypochromicity of the micellar environment. A measurement of the CMC can be obtained from the point of break in the plot of absorbance versus micelle concentration.

METHODS

RESULTS AND DISCUSSION

[GLUCOSE] 1 mM

Many measurements made by several other laboratories suggested that palmitoyl CoA inhibited glucokinase activity (17). However, there was considerable controversy as to the mechanism of inhibition. Only recently has it been shown that palmitoyl CoA inhibits glucokinase activity (17).

The concentration of palmitoyl CoA was also determined by ultracentrifugation of various concentrations of palmitoyl CoA in the sample mixture containing ATP and NADH with additions as indicated. A Beckman model 8 analytical ultracentrifuge equipped with an ultraviolet scanner was set between 230 and 295 nm to minimize contributions from the sample at the wavelengths of the scanner. The ultracentrifuge was used at 50,000 rpm for sedimentation studies. Under these conditions either of the CoA esters was kept to sedimentation at 25°C for 20 hours. The sample from the center portion was taken to be palmitoyl CoA or stearoyl CoA in the sample. The CMC was estimated by interpolation of the sedimentation curves. In the absence of a sedimenting material the CMC was seen to be 0.13 M with a value of 0.15 M, as indicated.

An experimental method was used to determine the effect of palmitoyl CoA on the enzyme activity. The assay system was a 0.1 M solution containing 5 mM MgCl2, 100 nM [1-14C] palmitoyl CoA, and 0.8 mM NADH and palmitoyl CoA was determined.

The results obtained for the inhibition of glucokinase activity at the critical micelle concentration are shown in Figure 1. Inhibition of glucokinase activity was obtained over a range of 0.6 to 1.0 micromolar palmitoyl CoA. The results for palmitoyl CoA were similar at various concentrations of glucokinase. The results obtained for the inhibition of glucokinase activity at the critical micelle concentration are shown in Figure 2. Inhibition of glucokinase activity was obtained over a range of 0.6 to 1.0 micromolar palmitoyl CoA. The results for palmitoyl CoA were similar at various concentrations of glucokinase. The results obtained for the inhibition of glucokinase activity at the critical micelle concentration are shown in Figure 3. Inhibition of glucokinase activity was obtained over a range of 0.6 to 1.0 micromolar palmitoyl CoA. The results for palmitoyl CoA were similar at various concentrations of glucokinase.
Inhibition of Glucokinase by Long Chain Acyl-CoAs

Figure 1—The effect of various fatty acyl CoA compounds on glucokinase activity in the high magnesium (5 mM ATP, 10 mM MgCl₂) system. Other conditions were as follows: 1 mM glucose, 29.8 μM enzyme, and all other conditions were as indicated in Figure 1.

<table>
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<tr>
<th>Acid</th>
<th>Palmitoyl CoA</th>
<th>Stearoyl CoA</th>
<th>Palmitoyl CoA</th>
<th>Palmitoyl CoA</th>
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<td>40 μM</td>
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<td>Stearoyl-CoA</td>
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<td>Palmitoyl-CoA</td>
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<tr>
<td>Palmitoyl-CoA</td>
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<td>60 μM</td>
<td>90 μM</td>
<td>120 μM</td>
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</table>

Table 1—Apparent inhibition constants and critical micelle concentrations for compounds tested as effects of palmitoyl CoA. All measured values are within ±10% except where indicated by ± which are within ±100%.

*The apparent Kᵢ values are the effective concentrations required to double the activity of glucokinase compared to the control at saturating concentrations of ATP, since the acyl-CoAs are competitive inhibitors of both substrates. The experiments were performed using a 30 mM magnesium chloride concentration in the low P₃₀₀₀₀ mixture. The value shown is extrapolated from a log-log versus molar concentration of fatty acyl-CoA used here. The apparent Kᵢ values were calculated using the Hill equation.

Other conditions are as indicated in methods.
Inhibition of Glucokinase by Long Chain Acyl-CoAs

In order to verify the lack of involvement of palmitoyl CoA micelles in the inhibition independent of the uncertainty in the cmc measurements, glucokinase activity was determined by a glucokinase assay in which the assay buffer contained 10% glucose and 10% DMSO. The inhibition of glucokinase by palmitoyl CoA was determined by the increase in the 

cmc determination. The experiment was carried out by incubating the sample with the enzyme for 1 hour at 37°C. The enzyme was then 'corrected' for the event monitored by the dye-related methods and perhaps by the other methods. The addition of high (3-5 µM) or low (25-60 µM) values of palmitoyl CoA has only a slight effect (less than 2 µM) on the cmc measured for palmitoyl CoA under high solubility conditions (20 µM, 2.5 mM). A plot of cmc versus (cmc)2 (not shown) was linear and when extrapolated to infinite cmc concentrations, showed a limiting curve of solute which is equal to 0.4 µM. It is apparent that MPMG, not only lowers the cmc for the long chain acyl CoA but, since information is not available on how to deal with this problem, there is a relatively specific interaction with the micelle.

The micelle concentration in either the low or high cmc system was not affected by the monovalent electrolyte concentration. The micelle concentration was determined from the change in the absorbance of the micelle at 1 hour at 37°C. The cmc values determined in the presence of both (low and high concentration of electrolyte) were found to be constant. The results of this work are shown in Table 3. The cmc values of palmitoyl CoA in both the high and low (2.5 mM, 3.5 µM) free monovalent electrolyte assay were shown in Table 2.

Although this method was shown (25) to give an accurate measure of the cmc for palmitoyl CoA, the original work of (25) indicated a cmc of 2.5 µM using light scattering and polarized light measuring of the micelle formation. The procedure used in this work was shown to be superior.

The question arises as to the actual value of the cmc for palmitoyl CoA. The cmc value of palmitoyl CoA at 37°C was determined by a micelle formation assay using a micelle formation assay. The cmc values determined in the presence of both (low and high concentration of electrolyte) were found to be constant. The results of this work are shown in Table 3. The cmc values of palmitoyl CoA in both the high and low (2.5 mM, 3.5 µM) free monovalent electrolyte assay were shown in Table 2.

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All of the methods used thus far, except light scattering, have the potential to perturb the system enough to alter the cmc. The introduction of dye or spin labels is invasive and may alter the system. In this study, it has been suggested that these probes used for determining the cmc may be in fact perturbing the formation of micelles and giving an artificially low value for the cmc. A cmc determination on the micelle formation at low concentrations was carried out using a polarized light scattering assay. The cmc values determined in the presence of both (low and high concentration of electrolyte) were found to be constant. The results of this work are shown in Table 3. The cmc values of palmitoyl CoA in both the high and low (2.5 mM, 3.5 µM) free monovalent electrolyte assay were shown in Table 2.