DISCONTINUITIES IN ARRHENIUS PLOTS DUE TO FORMATION OF MIXED MICELLES AND CHANGE IN ENZYME SUBSTRATE AVAILABILITY

Rolf Kristian BERGE, Erik SLINDE* and Mikael FARSTAD

Laboratory of Clinical Biochemistry, University of Bergen, N-5016 Haukeland Sykehus and **Norwegian Food Research Institute, PO Box 50, N-1432 Aas-NLH, Norway*

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1. Introduction

Discontinuities in Arrhenius plots due to the effects of various lipid species on enzymes (viscotropic regulation), have been used to obtain general correlation between lipid motional parameters and the functional properties of reconstituted enzymes (reviewed [1]). According to [1], the molecular mechanisms underlying bi- or triphasic Arrhenius plots have not been elucidated, and generally the effects of temperature on the rate of enzyme reactions are complex [2]. This study shows that the availability of substrate gives discontinuities in the Arrhenius plots at concentrations equal to the critical micelle concentration (cmc) of mixed micelles between substrate and detergent.

Long-chain acyl-CoA hydrolase (trivial name, palmitoyl-CoA hydrolase, EC 3.1.2.2) activities are found in a number of tissues [3–6]. However, the physiological role of the enzyme is unclear at present, but it has been shown that the enzyme activity is elevated in brown adipose tissue upon cold adaptation [7]. The substrate, palmitoyl-CoA, of the enzyme is hydrophobic and detergent-like, and forms micelles in solution, and mixed micelles in the presence of a detergent above cmc. The formation of micelles is temperature dependent; thus, it is possible to have a solution containing only monomers at one temperature and monomers and micelles at another temperature. This study shows that the enzyme activity is affected by the availability of substrate; that is, the enzyme shows different reaction behaviour towards the substrate in monomeric and micelle form, and discontinuities are observed in Arrhenius plots.

2. Materials and methods

Microsomal palmitoyl-CoA hydrolase was purified and the activity was measured radiochemically as in [8]. The cmc values were determined in a Shimadzu spectrophotometer MPS 5000. In solutions with Triton X-100 the cmc values were determined by measuring the ΔA_{286} [9]. The cmc of palmitoyl-CoA was determined by the pinacyanol chloride dye absorption method [10]. The cmc values were determined in a solution of 10 mM Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid), 50 mM KCl (pH 7.4). Protein was determined by the Lowry method [11].

3. Results and discussion

The cmc of Triton X-100 (a non-ionic detergent) given in table 1, shows that the cmc value of the detergent decreases with increasing temperature, while that of palmitoyl-CoA (an ionic detergent) increases with increasing temperature. The cmc values of solutions of Triton X-100 and palmitoyl-CoA are consistent with the theory of binary surfactant systems [12], which predicts that the cmc of a mixture will fall between the cmc of the two components. The temperature, which affects the cmc of both palmitoyl-CoA and Triton X-100 (table 1) will also affect the concentration of free molecules in equilibrium with the micelles. If both palmitoyl-CoA and Triton X-100 are present in the reaction mixture, and mixed micelles are formed, the monomer concentrations of

Table 1
Critical micelle concentrations of palmitoyl-CoA and Triton X-100

Component added	Critical micellar concentration	
	25°C	37°C
Palmitoyl-CoA	3.4 ± 0.4 μM	4.9 ± 0.3 μM
Triton X-100	(15.7 ± 0.6) × 10^{-3}% (v/v)	(8.4 ± 0.6) × 10^{-3}% (v/v)
Triton X-100 + 2.6 μM palmitoyl-CoA	(8.6 ± 0.4) × 10^{-3}% (v/v)	(4.8 ± 0.4) × 10^{-3}% (v/v)
Triton X-100 + 3.6 μg enzyme	(13.5 ± 0.5) × 10^{-3}% (v/v)	(7.3 ± 0.3) × 10^{-3}% (v/v)

the individual species are determined by the equilibrium constant of the mixed micelle.

Palmitoyl-CoA hydrolase isolated from rat liver microsomes acts on the monomeric species of palmitoyl-CoA, and is stimulated by the monomeric concentration of Triton X-100 [8]. Figure 1 shows the effect of temperature on the activity of palmitoyl-CoA hydrolase. In the presence of 2.6 μM palmitoyl-CoA, only one transition is observed at 38°C. This is due to the effect of temperature on the velocity of the enzyme reaction, and may be a result of several different causes [2].

When 0.005% (v/v) Triton X-100 is added, both

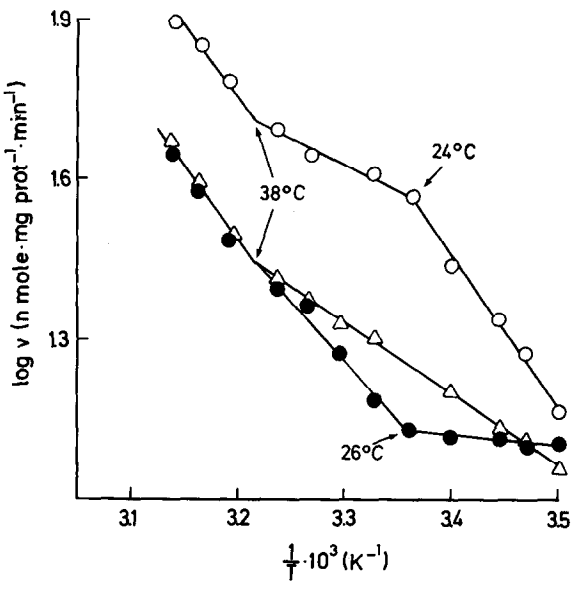

Fig.1. The effect of temperature on the palmitoyl-CoA hydrolase activity. (Δ) Arrhenius plots of 3.6 μg purified microsomal palmitoyl-CoA hydrolase and 2.6 μM palmitoyl CoA. (○) Addition of 0.005% (v/v) of the detergent Triton X-100 or (●) addition of 0.015% (v/v) Triton X-100.

Triton X-100 and the 2.6 μM palmitoyl-CoA are present as monomeric species at low temperatures; however, at higher temperatures micelles are formed (table 1). When the temperature increases the palmitoyl-CoA hydrolase activity shows a transition point at 24°C, where mixed micelles are formed, and the amount of monomeric substrate available becomes limited. This temperature is somewhat lower than expected if only Triton X-100 and palmitoyl-CoA were present (table 1), but it is due to the presence of enzyme which lowers the cmc for Triton X-100 (table 1) and probably also the cmc value of palmitoyl-CoA. This is, however, impossible to measure due to the hydrolase activity of the enzyme.

When 0.015% (v/v) Triton X-100 is added to 2.6 μM palmitoyl-CoA, mixed micelles are formed (table 1). When the temperature increases a transition point is observed at 26°C. From table 1 it is seen that as the temperature increases an increase in the cmc value of palmitoyl-CoA is observed. At <26°C the amount of Triton X-100 is in excess and the micelle size and concentration behaviour are determined mainly by the Triton X-100 molecules. At 26°C the equilibrium constant of the mixed micelles of Triton X-100 and palmitoyl-CoA are changed and a higher amount of monomeric palmitoyl-CoA becomes available and a higher rate is observed.

In both cases where Triton X-100 is added the transition at 38°C is also observed.

Another explanation of the transition points in the presence of Triton X-100 might be that the micelles are inhibitory to the enzyme activity. However, addition of increasing amounts of Triton X-100 and palmitoyl-CoA give the same activity as long as the ratio between the two components is constant (unpublished results).

In general, changes in activation energy are interpreted as changes in membrane lipid fluidity [1,13].

However, this work shows that such changes can be explained as changes in the availability of the substrate of the reaction. This might be an important regulating system in the cell, and in cases where changes in activation energies have been observed for membrane enzymes or enzymes that act on hydrophobic substrates the change may be due to changes in the availability of the substrate.

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References

[1] Sandermann, J. jr (1978) Biochim. Biophys. Acta 515, 209–237.
[2] Dixon, M. and Webb, E. C. (1964) Enzymes, 2nd edn, pp. 145–166, Longmans Green, London.
[3] Kurooka, S., Hosoki, K. and Yoshimura, Y. (1972) J. Biochem. 71, 625–634.
[4] Hashimoto, S. and Dayton, S. (1975) Artery 1, 138–149.
[5] Kako, K. J. and Patterson, S. D. (1975) Biochem. J. 152, 313–323.
[6] Brophy, P. J. and Vance, D. E. (1976) Biochem. J. 160, 247–251.
[7] Berge, R. K., Slinde, E. and Farstad, M. (1979) Biochem. J. 182, 347–351.
[8] Berge, R. K. (1979) Biochim. Biophys. Acta 574, 321–333.
[9] Gratzer, W. B. and Beaven, G. H. (1969) J. Phys. Chem. 73, 2270–2273.
[10] Zahler, W. E., Barden, R. E. and Cleland, W. W. Biochim. Biophys. Acta 164, 1–11.
[11] Lowry, O. H., Rosebrough, H. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[12] Shinoda, K. (1954) J. Phys. Chem. 58, 541–544; 1136–1141.
[13] McMurchie, E. J. and Raison, J. K. (1979) Biochim. Biophys. Acta 554, 264–374.