Fatty-acid-binding protein facilitates the diffusion of oleate in a model cytosol system

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The proposed function of fatty-acid-binding proteins in facilitating the diffusion of their ligand has been examined in a cytosolic model using a steady-state diaphragm cell apparatus. The white muscle of ocean pout (*Macrozoarces americanus*) does not have a fatty-acid-binding protein; nor does it exhibit detectable β -oxidative capacity, and as such is a good source of cytosol preparation. After determining the diffusion coefficient of oleate in this medium, fatty-acid-binding protein, isolated from the ventricle of this animal, was added and the apparent diffusion coefficient was again determined. The presence of the fatty-acid-binding protein increased the apparent diffusion coefficient of this long-chain fatty acid about 6-fold, from $0.087 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$ to $0.58 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$. This confirms the facilitated diffusion hypothesis as one role of fatty-acid-binding protein. The diffusion coefficients of oleate in 50 mM-sodium phosphate buffer, pH 7.4, at 10 °C and 25 °C ($0.15 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$ and $0.28 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$ respectively) were also measured.

INTRODUCTION

Diffusion is a limiting factor in the chemistry and architecture of biological systems (e.g. Krogh, 1919; Jacobs, 1935). The rate of diffusion is dependent on the size, shape and physicochemical nature of the molecules, the temperature and the resistance of the medium to movement by the molecules (i.e. viscosity) and the presence of aqueous and non-aqueous compartments. Diffusion coefficients measured in low-viscosity aqueous media provide only a first approximation insight into cellular diffusion *in vivo*. Estimations of diffusion coefficients, assuming values of parameters and various forms of phenomenological equations, have also been used but can yield values that differ from measured values by as much as three orders of magnitude (Yam *et al.*, 1988).

Hazel & Sidell (1987) have developed a steady-state diaphragm cell apparatus to measure the diffusion of low- M_r hydrophilic molecules in small volumes. The reliability of the apparatus for diffusion measurements in a buffered aqueous medium led to the investigation of the diffusion of hydrophilic metabolites in cytosol preparations under different temperature regimes (Sidell & Hazel, 1987). The reported results indicated that fish white skeletal muscle provides a cytosol preparation with properties very similar to the aqueous phase of the cytoplasm and, as such, provides a good model with which to study the intracellular movement of metabolites.

As most intracellular diffusion studies have focused on proteins and hydrophilic molecules, left unaddressed is the movement of poorly soluble hydrophobic metabolites. The question arises: how fast do non-polar compounds, such as fatty acids, move in an aqueous cytosolic environment? The amphiphilic, but predominantly non-polar, nature of long-chain fatty acids results both in poor solubility and in a potential to form detergent micelles in an aqueous environment. The combination of these characteristics results in the critical metabolic problem that insoluble hydrophobic molecules must move in an aqueous environment in quantities sufficient for cellular energy and synthetic demands.

A focus of several investigations of fatty acid metabolism is an abundant intracellular protein (M_r about 14000) that binds long-

chain fatty acids (Ockner *et al.*, 1972; Mishkin *et al.*, 1972). These fatty-acid-binding proteins (FABPs) have been found in mammals (Chan *et al.*, 1985), birds (Katangole & March, 1979) and fish (Stewart & Driedzic, 1988), and their presence is correlated with the capability to oxidize fatty acids. Isolated FABPs represent 3–5% of soluble cytosolic protein in tissues such as the heart and liver (Bass, 1988).

Knowledge of FABP structure, physical properties and binding characteristics has produced suggestions of three broad functions for the protein. One hypothesis implicates FABP as a specialized intracellular 'buffer', attenuating the inhibitory effect of fatty acids on enzymes by sequestering the fatty acids in a protein envelope (Bass, 1988). Protective effects of FABPs on monoacylglycerophosphocholine acyltransferase and the stimulation of glycerophosphate acyltransferase resulting from elevated levels of non-esterified fatty acids during ischaemia and reperfusion lends support to this hypothesis (Samanta *et al.*, 1989). A second hypothesis for FABP function suggests that this protein increases the influx of fatty acids into the cell. Weisiger *et al.* (1989) showed oleate flux through a lipid–water interface to be increased 3-fold by the presence of purified rat liver FABP. Recently, rat liver and heart FABPs have been shown to transfer anthroxyloxy-fatty acids to model membranes (Storch & Bass, 1990). The third hypothesis states that FABP facilitates the diffusion of fatty acids in the aqueous phase of the cytosol. An increase in the rate of diffusion to membrane-bound enzymes or to organelles would maintain the concentration of these low-solubility metabolites and subsequently maintain required metabolic activity. The main experimental support for this putative function is the observation of FABP stimulation of enzymes using fatty acids as substrates (Bass, 1988). However, data supporting the putative FABP function in facilitating fatty acid diffusion to organelles are in need of direct experimental evaluation.

Our objective was to investigate the facilitated diffusion hypothesis of FABP function by determination of diffusion rates of oleate, a representative fatty acid, in a cytosol preparation in the presence and absence of FABP. The white skeletal muscle of a teleost fish, ocean pout (*Macrozoarces americanus*), containing neither an endogenous FABP (Stewart & Driedzic, 1988) nor a

β -oxidative capacity (Driedzic & Stewart, 1982), was used as a cytosol source. The myoglobin-free heart of this animal was used as a source of FABP (Stewart & Driedzic, 1988). We saw in this experimental approach a unique opportunity to examine the ability of FABP to affect the diffusion rate of long-chain fatty acids and thus provide insight into the facilitated diffusion hypothesis, as well as to yield important baseline data on fatty acid diffusion. If FABP facilitates the diffusion of fatty acids, the flux across the diffusion volume should be greater than in the absence of FABP. An increase in the flux should be noted as an increase in the effective diffusion coefficient of the fatty acid. The salient finding of this study was that addition of FABP to a muscle cytosol preparation resulted in a 6-fold increase in the rate at which oleate diffused. This is the first direct evidence that FABP can function to facilitate fatty acid diffusion. Furthermore, the diffusion of oleate is less dependent on temperature than is the diffusion of hydrophilic metabolites in similar experiments.

EXPERIMENTAL

Theory and data treatment

The experimental procedures follow those of Hazel & Sidell (1987) and Sidell & Hazel (1987). Fick's First Law predicts a linear relationship (eqn. 1) between the instantaneous concentration gradient, ΔC_i ($\mu\text{mol} \cdot \text{cm}^{-3}$) and the instantaneous flux, J_i , across a unit area ($\mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$):

$$J_i = \frac{1}{d} \times D \Delta C_i \quad (1)$$

The diffusion distance (cm) is d , and D is the diffusion coefficient ($\text{cm}^2 \cdot \text{s}^{-1}$). Since the effective surface area of the membrane pores through which diffusion occurs is not known, this equation was modified (eqn. 2) to include a cell constant, K , which is the ratio of the effective area of the diffusion surface to the effective diffusion distance ($\text{cm}^2 \cdot \text{cm}^{-1}$):

$$J_i = K D \Delta C_i \quad (2)$$

KD , the slope of the line (J_i versus ΔC_i), was determined by calibration of the diffusion apparatus with a solute of known diffusion coefficient, KCl ($D = 1.83 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$; Harned, 1947). K was determined from the slope value.

The instantaneous concentration gradient was determined directly from the difference in solute concentration between the source and sink chambers. The instantaneous flux was derived from the accumulated flux (J_{acc}), which is the cumulative amount of solute that has entered the sink chamber at the sampling time. The experimental data were fitted to an exponential equation (eqn. 3) relating the accumulated flux and time and allowed the determination of A and B , two empirical values:

$$t = A e^{B J_{\text{acc}}} \quad (3)$$

Since the instantaneous flux is the first derivative of the accumulated flux with respect to time (eqn. 4), J_i can be calculated from the amount of diffusant accumulated in the sink chamber:

$$J_i = dJ_{\text{acc}}/dt = 1/(A B e^{J_{\text{acc}} B}) = K D \Delta C_i \quad (4)$$

The slope (KD) was determined by linear regression and the value of D was calculated from the slope ($D = \text{slope}/K$).

If FABP facilitates the diffusion of oleate in this system, this will be reflected in an increase in the rate at which the solute moves into the sink chamber. This larger flux will also be reflected in a larger value of J_i at a given concentration gradient, a greater slope KD , and thus a larger effective diffusion coefficient.

In short, if FABP facilitates the diffusion of oleate in this system, the observed diffusion coefficient in the presence of FABP will be larger than in its absence.

Diffusion experiments

Apparatus. A detailed description of the three-chambered diffusion chamber is given in Hazel & Sidell (1987). The major structural difference was the decrease of the length of the central chamber to 0.5 cm from 1.0 cm. This central chamber was separated from each terminal chamber by cellulose membranes cut from SpectraPor dialysis tubing (M_r cut-off 6000–8000; Spectrum Medical Industries Inc.) held in place by silicon gaskets. The membranes do not allow the passage of FABP and provide an unstirred volume through which diffusion occurs.

General experimental procedure. The membranes were boiled in a 1 mM-EDTA solution for 2 h to extend pores and remove any heavy metal contaminants, and were then stored in 50 mM-sodium phosphate buffer, pH 7.2, until mounted in the apparatus. Fresh membranes were prepared for each trial. Both terminal chambers received 4.0 ml of the medium under test, ensuring that no air bubbles remained trapped on the membranes. The central chamber was filled to a level equal to those in terminal chambers, and the magnetic stirring system (Diamond Electro-Tech Inc.) was positioned. At time zero, a sample (50 μl) of the diffusion medium was placed in the sink chamber, and a sample (50 μl) of a concentrated solution of the diffusant was placed in the source chamber. After each compartment was thoroughly mixed (< 15 s), a sample representing time zero was removed from each terminal chamber (50 μl). Subsequent 50 μl samples were taken at intervals during the experiment, and sampling time and temperature were recorded.

Apparatus calibration. An initial KCl concentration of 10 mM was used in the source chamber in a diffusion medium of double-distilled water. Three calibration experiments were performed under identical conditions. Samples taken during the time course of the experiments were diluted to 2 ml with distilled water before the potassium concentration was measured with an EEL flame photometer calibrated with known concentrations of KCl (0–0.25 mM). The slope of J_i versus ΔC_i (eqns. 3 and 4) was determined from these data, allowing the calculation of the cell constant upon dividing by the diffusion coefficient of KCl.

Oleate diffusion. Oleate diffusion experiments were performed at 25 °C and 10 °C in 10 mM-sodium phosphate buffer, pH 7.2, at 10 °C in a cytosolic medium and at 10 °C in a cytosol preparation to which FABP had been added. The oleate stock-solutions were made by accurately weighing unlabelled oleate into a 1.00 ml volumetric tube and adding 5 μCi of radioactively labelled oleate. Tris buffer (100 mM, pH 9) was added to a total volume of 1 ml. At a pH of 9, oleate is completely ionized, facilitating its dispersion in the buffer to produce a homogeneous solution. This procedure ensures that the stock solutions, when delivered to the diffusion apparatus, will produce accurately known concentrations of the fatty acid. The initial concentrations in the source chamber were 0.1 mM for diffusion in buffer and 0.23 mM in the cytosol experiments.

The apparatus containing the diffusion medium was equilibrated at the diffusion temperature for 4 h before the addition of stock oleate to the source chamber. The plastic pipette tips used in sampling were rinsed twice in saturated unlabelled oleate solution to decrease subsequent binding of oleate to surfaces during the experiment. Samples (50 μl) were transferred to glass scintillation vials and counted for radioactivity.

Oleate diffusion in a cytosol medium containing FABP was performed using an identical procedure, except for the additional preparation step of adding FABP. The protein was added to a concentration of 1.5 mg/ml of cytosol, with a binding capacity of 6.7 nmol of oleate/ml of cytosol. Thus the concentration of oleate present was always in excess of the total binding capacity of the added FABP, and established conditions that would allow for any facilitated diffusion, i.e. bound and unbound flux of diffusant.

Animal collection

Ocean pout (*Macrozoarces americanus*), ranging in length from 45 to 68 cm, were captured by otter trawl in the Passamaquoddy Bay, off St. Andrews, New Brunswick, between May and August 1989. Some animals were used at the Huntsman Marine Science Center, St. Andrews, and the remaining animals were transported to Mount Allison University and maintained in recirculated sea water at 10 °C.

Preparation of muscle cytosol

The preparation of cytosol was based on the procedure for white perch muscle (Sidell & Hazel, 1987). An ocean pout was killed by a blow to the head and the white skeletal muscle was removed from the lateral fins to the tail fin. The muscle was immediately placed on foil over ice and any parasites were removed. The muscle was minced, weighed and homogenized to a paste with a Waring blender. The resulting paste was centrifuged at 4 °C for 90 min at 20000 g. The supernatant, constituting the crude cytosol, was maintained at this low temperature and passed through syringe filters of pore size 1.2 µm (Millipore). The final cytosol volume was recorded and several parameters were measured. Before diffusion experiments crystalline Na₂S₂O₈ was added to prevent microbial growth (0.2%, w/v) and 65 µg of aprotinin/ml, a proteinase inhibitor, was added to deter protein degradation which, among other things, would alter the viscosity. The viscosity of the cytosol did not change over the course of the experiments.

The protein concentration of cytosol preparations was measured using the Bradford dye-binding technique (Bradford, 1976), with BSA as standard (Sigma, fraction V). The density of cytosol was determined picnometrically at 25 °C and 10 °C in a 1.0 ml volumetric. Cytosol viscosity was determined using Ostwald viscometers (Cannon–Manning; 1 ml and 0.5 ml) and expressed in stokes (1 St = 10⁻⁴ m²·s⁻¹). Ten viscosity measurements of three separate cytosol preparations and of double-distilled water were taken at both 25 °C and 10 °C. The viscosity of water at the measurement temperature was calculated from an empirical equation (CRC Handbook of Chemistry and Physics, 1985) and used to calculate cytosol viscosity. The non-esterified fatty acid concentration of the cytosol preparations was determined by the copper soap method of Duncombe (1963) and compared with palmitic acid standards.

FABP isolation. Ocean pout heart was used as an FABP source as it has been shown to contain an FABP ($M_r = 12800$; Stewart & Driedzic, 1988). In addition, this teleost has a white heart containing no myoglobin, which facilitates the isolation. Hearts were excised and exsanguinated by perfusion with 0.1 M-sodium phosphate buffer, pH 7.2. The bulbous was removed and the remaining ventricle was placed on ice. The combined heart tissue of 17 animals was weighed (8.2 g), minced and suspended (1:1, w/v) in cold 0.1 M-sodium phosphate buffer, pH 7.2, containing 65 µg of aprotinin/ml and 0.1 M-phenylmethanesulphonyl fluoride (both from Boehringer–Mannheim). Tissues were homogenized with a Tissumiser (Teckmar, Cincinnati, OH, U.S.A.) using 3 × 10 s bursts at maximum speed, and centrifuged

at 4 °C for 90 min at 20000 g. After sampling for protein and fatty acid binding assays the supernatant was incubated with 100 µmol of oleate that had been spiked with 1 µCi of [1-¹⁴C]oleate (59 mCi/mmol; NEN Research Products, Dupont) for 15 min at room temperature. The incubated supernatant was applied to a Sephadex G-75 column (2.5 cm × 70 cm), calibrated for M_r , and eluted with the phosphate buffer at a flow rate of 0.3 ml/min. The void volume was determined with Blue Dextran (Sigma). Absorbance at 280 nm was measured continuously (LKB Bromma 21138 UVICORD S). Fractions of 2 ml were collected and a portion (50 µl) of each fraction was counted for radioactivity with an LKB Wallac 1211 Rackbeta liquid scintillation counter in PCS II cocktail (Amersham). The external standard channels ratio method and quenched ¹⁴C standards (Amersham) were used to determine counting efficiencies. Co-elution of a protein fraction and labelled oleate before elution of the unbound ligand indicated the FABP (M_r about 13000). A portion of the pooled FABP fraction was removed for protein and fatty-acid binding assays.

The effectiveness of the isolation was ascertained by monitoring protein concentration and fatty-acid binding capability. The binding capability was assessed by incubating a sample (0.15 ml) in 2 µM-[1-¹⁴C]oleate (final volume 0.45 ml) and using the Lipidex assay (Glatz & Veerkamp, 1983). Before the binding assays the samples were passed through a column (1 cm × 5 cm) of Lipidex 1000 (United Technologies Company) at room temperature to remove any bound lipid material (Dahlberg *et al.*, 1980). There was a high protein concentration (26.16 ± 0.26 mg/ml, total amount 375 mg) in the heart homogenate, but only a small proportion of this bound fatty acids (0.088 ± 0.005 nmol of oleate/mg of protein). Centrifugation resulted in a supernatant with lower protein concentration (7.9 ± 0.4 mg/ml), and a slightly higher fatty-acid binding capacity of 0.11 ± 0.03 nmol of oleate/mg of protein. The fraction (15 ml) containing FABP contained a lower concentration of protein (2.83 ± 0.05 mg/ml), representing about 10% of extractable protein, but a 50-fold increase in the binding capacity to 4.50 ± 0.19 nmol of oleate/mg of protein. FABP fractions were pooled, defatted as above and concentrated prior to diffusion experiments using Centricon ultrafiltration units (Amicon), with an M_r cut-off of 10000. The procedure decreased the protein sample volume from 15 ml to 1.7 ml (25 mg/ml).

RESULTS AND DISCUSSION

Cytosol preparation

The cytosolic preparation represents the fluid through which solutes diffuse. It is devoid of the impediments to diffusion such as organelles, cell membranes and contractile proteins. The physical characteristics of the cytosol preparation are summarized in Table 1. The cytosolic pH for all preparations (6.6 ± 0.2) was consistent with most cellular pH values and did not change during the course of the experiments. The protein concentration was high (80 mg/ml) and varied from animal to animal. At 25 °C the cytosol preparation had a kinematic viscosity of 1.55 × 10⁻² St. In comparison, white perch muscle cytosol (Sidell & Hazel, 1987) had a kinematic viscosity of about 3 × 10⁻² St. The white perch had been acclimated to 25 °C, and the authors suggested that cold acclimation could result in decreased cytosol viscosity by alterations in the state of tissue hydration. Ocean pout skeletal muscle cytosol viscosity increased to 2.7 × 10⁻² St with a temperature decrease to 10 °C. The Q_{10} [(value at T)/(value at $T - 10$)] for kinematic viscosity (1.15) is comparable with that of preparations made from white perch, which had a Q_{10} of 1.35. In cytosol preparations maintained at

Table 1. Physical characteristics of cytosol prepared from ocean pout white skeletal muscle

Values are the means $\pm$ S.E.M. of the numbers of samples given in parentheses.

Parameter	Value
Protein concentration ($n = 3$)	80.0 ± 6.0 mg/ml
pH ($n = 3$)	6.6 ± 0.2
Kinematic viscosity ($n = 10$)	
25 °C	$(1.55 \pm 0.02) \times 10^{-2}$ St
10 °C	$(2.68 \pm 0.18) \times 10^{-2}$ St

25 °C a protein precipitate was formed after 24 h, making measurement of diffusion under these conditions impossible. Cytosol maintained at a temperature of 8–10 °C showed no observable protein precipitation up to 7 days. The non-esterified fatty acid concentration in the cytosol preparations was 26.2 ± 2.3 μ M (mean $\pm$ S.E.M., $n = 6$).

Diffusion experiments

Calibration: KCl diffusion. The high diffusion rate of KCl produced a rapid approach to equilibrium (approx. 1500 min per trial) as compared with the diffusion of oleate, where experiments were conducted over 4–5 days. According to the derivation of Fick's Law used here, the slope of the J_i versus ΔC_i line is the product of the apparatus cell constant and the diffusion coefficient. Using the known diffusion coefficient for KCl (1.83×10^{-5} $\text{cm}^2 \cdot \text{s}^{-1}$; Harned, 1947) the cell constant was 2.20 ± 0.11 cm.

Oleate diffusion in an aqueous buffer. The diffusion of oleate in buffered aqueous medium was used to compare the results of this method with those derived from other experimental techniques. Typical results of J_i versus ΔC_i determined at 10 °C and 25 °C are shown in Fig. 1. The diffusion coefficient for oleate in buffer was 0.28×10^{-5} $\text{cm}^2 \cdot \text{s}^{-1}$ at 25 °C and 0.15×10^{-5} $\text{cm}^2 \cdot \text{s}^{-1}$ at 10 °C (Table 2). The decrease in diffusion rate at 10 °C to approx. one-half of the rate observed at 25 °C was statistically significant ($P = 0.002$; Student's t test). The Q_{10} value for diffusion (1.24) was greater than the Q_{10} for water viscosity (0.98). This suggests that there may be factors in addition to viscosity which affect the diffusion rate as the temperature changes. The observation was also made by Sidell & Hazel (1987), who suggested that the depression of diffusion rates at colder temperatures could result from a reduced kinetic energy of the system and an increase in the strength of non-covalent interactions.

From a series of desorption and time-lag experiments at 25 °C in aqueous solution (Patil *et al.*, 1973), palmitate and oleate were shown to have diffusion coefficients of $(0.37 \pm 0.06) \times 10^{-5}$ $\text{cm}^2 \cdot \text{s}^{-1}$ and $(0.33 \pm 0.02) \times 10^{-5}$ $\text{cm}^2 \cdot \text{s}^{-1}$ respectively. Linoleate has a diffusion coefficient of 0.556×10^{-5} $\text{cm}^2 \cdot \text{s}^{-1}$ under similar experimental conditions (Barclay *et al.*, 1987). This measurement was accomplished using a method (Burkey *et al.*, 1984) which relates increases in peak half-heights of a bolus of diffusant as it moves through small-diameter tubing to the diffusion coefficient. Thus the method used here is adequate for estimating the values of the diffusion coefficients of hydrophobic molecules and is appropriate for the comparative purposes of this work.

Oleate diffusion in a cytosolic medium. The diffusion coefficient of oleate in a cytosolic medium was $(0.087 \pm 0.003) \times 10^{-5}$ $\text{cm}^2 \cdot \text{s}^{-1}$ at 10 °C (Table 2). Typical results of J_i versus ΔC_i are showed in Fig. 2. The 1.7-fold decrease in D ($P = 0.006$) observed in going

Fig. 1. Examples of the diffusion of oleate in aqueous buffer at 10 °C and 25 °C

Slope at 10 °C ($\square$): $y = (-1.71 \times 10^{-6}) + (2.17 \times 10^{-4})x$; $r = 0.99$;
slope at 25 °C ($\blacksquare$): $y = (-1.81 \times 10^{-6}) + (3.27 \times 10^{-4})x$; $r = 0.97$.

Table 2. Oleate diffusion coefficients in each experimental medium

Values are means $\pm$ S.E.M.; n is given in parentheses.

Experimental medium	$10^5 \times D$ ($\text{cm}^2 \cdot \text{s}^{-1}$)	
	25 °C	10 °C
Buffer	0.28 ± 0.02 (4)	0.15 ± 0.01 (3)
Cytosol	—	0.087 ± 0.003 (3)
Cytosol + FABP	—	0.58 ± 0.07 (3)

Fig. 2. Examples of diffusion of oleate at 10 °C in cytosol ($\square$) and in cytosol to which FABP was added ($\blacksquare$)

Slopes: $y = (-5.21 \times 10^{-6}) + 1.23 \times 10^{-4}x$; $r = 0.95$ ($\square$) and
 $y = (-1.03 \times 10^{-4}) + (7.07 \times 10^{-4})x$; $r = 0.93$ ($\blacksquare$).

from buffer to cytosol is comparable with the reported 2-fold decrease in diffusion rate of various hydrophilic pseudometabolites in cytosol when compared to an aqueous buffer (Sidell & Hazel, 1987). The white perch cytosol used in the experiment of Sidell & Hazel (1987) would have had a kinematic viscosity of about 4.6×10^{-2} St at 10 °C (estimated from their value and a Q_{10} of 1.35), which is higher than that of the ocean pout skeletal muscle cytosol. The decreased resistance of a lower viscosity

cytosol would result in a smaller decrease in diffusion rate compared with a buffer environment, as observed in our experiment. In addition, hydrophobic molecules may not respond to a change in viscosity in an identical fashion as hydrophilic molecules. The possibility of differential responses to viscosity as a function of physicochemical characteristics of the diffusant requires further investigations of diffusion in buffer and cytosolic media.

Oleate diffusion in a cytosolic medium containing FABP. Since the validity of this cytosolic model was confirmed, it was possible to test the facilitated diffusion hypothesis of FABP action. J_i versus ΔC_i for a typical diffusion rate measurement in a cytosol preparation containing FABP is illustrated in Fig. 2. The offset in the plot is a result of the presence of both a protein-bound and an unbound fraction of oleate. The effective diffusion coefficient was $0.58 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$ (Table 2), representing a 6-fold increase in the rate of oleate diffusion in cytosol without exogenous FABP. The diffusion rate with FABP was even higher than the rate in a less viscous aqueous buffer environment at 25 °C.

The observed increase in the oleate diffusion rate in the presence of FABP supports the proposed function of FABP in the facilitation of fatty acid diffusion. As with the facilitated diffusion of O_2 by myoglobin, upon initial consideration it seems incredible that FABP with a diffusion coefficient (about $0.13 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$; Tipping & Ketterer, 1981) very similar to that of the ligand is able to increase fatty acid diffusion by over 6-fold. However, facilitated diffusion is attributed to many factors, including the concentrations of binding protein and ligand, the number of ligand-binding sites on the protein and the formation constant of the protein-ligand complex. Wittenburg (1970) brings to the fore the importance of the high concentration of protein relative to the concentration of its ligand, directly compensating for the diffusion coefficient differences. Mathematical models predict diffusion rate increases greater than an order of magnitude under optimal conditions (Tipping & Ketterer, 1978, 1981). Thus the observed 6-fold increase in diffusion rate via FABP facilitated diffusion supports their suggestions. Facilitated fatty acid diffusion could also affect cellular lipid metabolic systems substantially. Since mitochondria of cells in heart and liver tissue use fatty acids as a major source of metabolic fuel, the rate of fatty acid movement from the non-aqueous phase to the organelle may limit the rate of energy production. Further experimentation varying fatty acid and FABP concentrations are necessary to elucidate the precise magnitude of facilitation occurring *in vivo*.

The present observation of FABP functioning in the facilitation of fatty acid transport does not eliminate the possibility of its functioning in other capacities such as protective 'buffering' of fatty acid concentrations in cellular systems or increasing the flux across the membrane barrier. It is probable that a protein present universally in tissues capable of fatty acid oxidation would provide multiple advantages to these cells. It is also likely that

each class of FABP functions in a slightly different manner, tailored to the specific metabolic needs of their respective tissues.

Conclusion

The determination of the diffusion coefficient in buffer medium supports the reliability of this model system in providing data comparable with previous reports. The inclusion of an exogenous FABP in the cytosol preparation caused a 6-fold increase in the apparent diffusion coefficient. In addition, the results suggest the involvement of non-viscosity determinants of diffusion rate at lower temperatures.

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REFERENCES

- Barclay, L. R. C., Baskin, K. A., Locke, S. J. & Schaefer, T. D. (1987) *Can. J. Chem.* **65**, 2529–2540
- Bass, N. M. (1988) *Int. Rev. Cytol.* **111**, 143–184
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Burkey, T. J., Griller, D., Lindsay, D. A. & Scaiano, J. C. (1984) *J. Am. Chem. Soc.* **106**, 1983–1985
- Chan, L., Wei, C., Li, W., Yang, C., Rather, P., Pownall, H., Gotto, A. M. & Smith, L. C. (1985) *J. Biol. Chem.* **260**, 2629–2632
- CRC Handbook of Chemistry and Physics, 66th edn. (1985) CRC Press Inc., Washington
- Dahlberg, E., Snochowski, M. & Gustafsson, J.-A. (1980) *Anal. Biochem.* **106**, 380–388
- Driedzic, W. R. & Stewart, J. M. (1982) *J. Comp. Physiol.* **154**, 593–599
- Duncombe, W. G. (1963) *Biochem. J.* **88**, 7–10
- Glatz, J. F. C. & Veerkamp, J. H. (1983) *Anal. Biochem.* **132**, 89–95
- Harned, H. S. (1947) *Chem. Rev.* **40**, 461–502
- Hazel, J. R. & Sidell, B. D. (1987) *Anal. Biochem.* **166**, 335–341
- Jacobs, M. H. (1935) *Ergebnisse der Biologie*, Julius Springer-Verlag, Berlin
- Katangole, J. B. D. & March, B. E. (1979) *Poult. Sci.* **58**, 272–275
- Krogh, A. (1919) *J. Physiol. (London)* **52**, 391–415
- Mishkin, S., Stein, L., Gatmaitan, Z. & Arias, I. M. (1972) *Biochem. Biophys. Res. Commun.* **47**, 997–1003
- Ockner, R. K., Manning, J. A., Poppenhausen, R. B. & Ho, W. K. L. (1972) *Science* **177**, 56–58
- Patil, G. S., Matthews, R. H. & Cornwell, D. G. (1973) *J. Lipid Res.* **14**, 26–31
- Samanta, A., Prasad, M. R., Engelman, R. M. & Das, D. K. (1989) *J. Lipid Mediators* **1**, 243–255
- Sidell, B. D. & Hazel, J. R. (1987) *J. Exp. Biol.* **129**, 191–203
- Stewart, J. M. & Driedzic, W. R. (1988) *Can. J. Zool.* **66**, 2671–2675
- Storch, J. & Bass, N. M. (1990) *J. Biol. Chem.* **265**, 7827–7831
- Tipping, E. & Ketterer, B. (1978) *Proc. FEBS Symp.* **58**, 369–385
- Tipping, E. & Ketterer, B. (1981) *Biochem. J.* **195**, 442–452
- Weisiger, R. A., Pond, S. M. & Bass, L. (1989) *Am. J. Physiol.* **257**, G904–G916
- Wittenburg, J. B. (1970) *Physiol. Rev.* **50**, 559–636
- Yam, K. L., Anderson, D. K. & Buxbaum, R. E. (1988) *Science* **241**, 330–332

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