Does fatty acid-binding protein facilitate the diffusion of oleic acid?

Recently an interesting paper by Stewart and coworkers was published in this journal (Stewart *et al.*, 1991), showing that heart fatty acid-binding protein (FABP) from ocean pout (*Macrozoarces americanus*) enhances the diffusion coefficient of oleate in a cytosolic muscle preparation by a factor of 6. Although the notion that FABP presumably has a function in the intracellular trafficking of fatty acids is generally accepted, it was felt that the experiments described by Stewart *et al.* do not allow unequivocal conclusions about this putative transport function of FABP. Our main concern is that appropriate control experiments are lacking.

The investigators have measured the diffusional flux of oleate using an apparatus made out of plexiglass originally described by Hazel & Sidell (1987). This device consists of three compartments which are separated by cellulose membranes through which oleate can diffuse but FABP cannot. In an aqueous solution, devoid of fatty acid binding factors, they found that the diffusion coefficient of oleate was of the order of $0.28 \times 10^{-5} \text{ cm}^2/\text{s}$ at 25 °C and $0.15 \times 10^{-5} \text{ cm}^2/\text{s}$ at 10 °C. In our opinion these values are far too low considering the molecular mass of oleate (285 Da), which is a major determinant of the diffusion coefficient (Jacobs, 1967). For sucrose, a substance with a comparable molecular mass (342 Da), a diffusion coefficient of $0.46 \times 10^{-5} \text{ cm}^2/\text{s}$ at 20 °C has been reported (Van Holde, 1971). The latter value is in the normal range for substances with molecular masses of 200–1000 Da (Setlow & Pollard, 1962). In the presence of FABP, Stewart and colleagues measured a substantial increase in the diffusion coefficient of oleate. However, when oleate is complexed to a macromolecular substance such as FABP, theoretically its apparent diffusion coefficient can only decrease, as already mentioned by Tipping & Ketterer (1981). The diffusion coefficient should not be confused with the diffusional flux of oleic acid which, in the presence of FABP, may largely increase, due to an increased total solubility of the fatty acid in the aqueous compartment. In fact, an increase in apparent diffusion coefficient, as measured by Stewart *et al.* (1991), would imply that FABP is capable of catapulting oleate molecules through the solution. This mechanism seems extremely unlikely to us.

Recently it has been described by our group (Vork *et al.*, 1990) and by others (Mailman & Rose, 1991) that oleic acid in an aqueous environment shows a high affinity for binding to surfaces. This affinity is probably due to the amphiphilic nature of long chain fatty acids. We showed that for oleic acid solutions ranging from 1 to 10 μM the concentration decrease amounted to 75 %, 35 % and 58 % for glass, polystyrene and polypropylene, respectively. Mailman & Rose (1991) showed that a substantial concentration decrease also occurred in the presence of a plexiglass surface. Unfortunately, Stewart *et al.* (1991) did not measure the loss of oleate in the compartment in which oleate diffusion actually took place. Hence, it cannot be excluded that, in their experiments conducted in the absence of FABP, a significant portion of the oleate had bound to the membranes and the walls of the diffusion cell. This phenomenon may explain the measured relatively low diffusion coefficient of oleate in an aqueous buffer. Patil *et al.* (1973), who earlier measured a diffusion coefficient for oleate of similar magnitude, had also not taken into account the aspecific binding of oleate to surfaces.

At the pH at which the measurements were performed (pH 7.2), oleic acid exists in two phases, i.e. an aqueous phase and a lamellar phase. Moreover, the lamellar phase may be present as crystals at temperatures below the melting temperature of the hydrocarbon chains (Cistola *et al.*, 1988). The formation of

ordered structures may have caused an additional effect on the measured value of the diffusion coefficient by Stewart *et al.* It may also explain the differences in diffusion coefficient measured at 25 °C and at 10 °C.

Similarly, the 6-fold increase in the apparent diffusion coefficient of oleate observed after the addition of FABP to the muscle cytosol preparation may have been caused by the fact that an appreciable part of the oleate is then bound to FABP, thus resulting in a lower percentage of aspecific binding to the cell surfaces. This would subsequently lead to an increase in diffusional flux but not to an increase of the diffusion coefficient.

In conclusion, experimental support for the putative FABP function in facilitating intracellular fatty acid diffusion is intriguing to pursue. However, in our opinion, evidence for this hypothesis does not unequivocally appear from the data presented by Stewart *et al.* (1991). The suggestion is made that the extent of aspecific binding of oleate to the wall of the diffusion chamber should be monitored in order to calculate the values of diffusion in the absence and presence of FABP in a more appropriate way. These data will enable us to judge the true physiological value of these observations.

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Received 19 June 1991

Fatty acid-binding protein and facilitated diffusion of fatty acids

In the preceding letter, Vork *et al.* (1991) point out, correctly, that the binding of a ligand to a macromolecular carrier reduces the apparent diffusion coefficient of the ligand, but only if all the ligand is bound. By introducing a carrier protein to a system, the diffusion coefficient of the free ligand is unchanged. In fact, with a given set of physical parameters (concentration of diffusant, temperature, viscosity etc.) a diffusion coefficient is constant. What is augmented in the presence of a carrier is the diffusional flux because of the combinatorial nature of the separate diffusional fluxes of free ligand and carrier-bound ligand. This augmentation can be observed only under conditions in which the saturation of the carrier leaves unbound ligand. This is the situation that pertains to and is expressly stated in our paper: the ligand concentration is far in excess of the binding capacity of the carrier and two diffusional fluxes are eventually established. The