Determining Fatty Acid Wall Binding

Synopsis

ADIFAB and ADIFAB2 can be used to determine the fraction of fatty acid that binds to cuvette walls by simply transferring a solution of fatty acid and ADIFAB(2) from one cuvette to another and measuring the change in free fatty acid concentration.

Procedure

ADIFAB

For details on measuring the ADIFAB ratio see Determining the ADIFAB Ratio. To determine R_0, add 0.2 μM ADIFAB to a cuvette containing buffer, and measure the fluorescence ratio (505/432 upon excitation at 386 nm). Add a small amount of fatty acid to the cuvette, mix gently, wait 5-10 minutes for equilibrium and then measure R_1. Transfer the contents of this cuvette into a second cuvette, again wait for equilibrium and measure R_2. To determine the approximate fraction of fatty acid bound to the cuvette walls ($\%_{bound}$), substitute R_0, R_1 and R_2 into Eq. (1):

$$\%_{bound} = (R_1 - R_2)/(R_1 - R_0) \quad (1)$$

ADIFAB2

For details on measuring the ADIFAB2 ratio and calculating [FFA] and [$ADIFAB2_{bound}$] see Determining the ADIFAB2 Ratio. Measure R_0, R_1 and R_2 according to the ADIFAB procedure above, except substitute 0.5 μM ADIFAB2 and measure the fluorescence ratio 550/457 upon excitation at 375 nm. Equation (1) approximates $\%_{bound}$ by assuming that the *free* fatty acid concentration in the cuvettes is much larger than the fraction of FA *bound to ADIFAB* ([FFA] >> [$ADIFAB_{bound}$]) and that the value of R when ADIFAB is saturated with FA is much greater than R_1 or R_2 (R_{max} >> R_1 & R_2). These assumptions do not hold true for ADIFAB2 because of its high affinity for fatty acids and small R_{max} values. For ADIFAB2, calculate $\%_{bound}$ with Eq. (2):

$$\%_{bound} = 1 - \left(\frac{[FFA]_2 - [ADIFAB2_{bound}]_2}{[FFA]_1 - [ADIFAB2_{bound}]_1} \right) \quad (2)$$

where $[FFA]_1$ and $[ADIFAB2_{bound}]_1$ are calculated using R_0 and R_1, and $[FFA]_2$ and $[ADIFAB2_{bound}]_2$ are calculated using R_0 and R_2.

Notes

- Eq. (1) is an approximation and only applies to ADIFAB; Eq. (2) is a general equation applicable to both ADIFAB and ADIFAB2.
- $\%_{bound}$ is dependent on fatty acid type, temperature and cuvette material but it is fairly constant over a large fatty acid concentration range (0-4 μM).