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Analysis of claims of enhanced enzyme catalysis by inorganic colloidosomes

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ABSTRACT

Ester hydrolysis reactions in the presence of enzymes have been reported to occur within inorganic colloidsomes that contain water in organic solvents. Analysis of reported data plots that were used to obtain values for K_m and V_{max} that led to those conclusions show that there is a linear dependence of the initial rates on the concentration of substrate, with no evidence of saturation that is necessary to establish binding of reactants and enzymes. The results do establish that there is no Michaelis–Menten complex formed between the substrates and enzymes. Therefore, any calculations of enzyme kinetic parameters from these data and the resulting conclusions should be reconsidered.

1. Introduction

In a recent paper, Li et al. reported the formation of silicon nanoparticles that produce colloidosomes with inorganic membranes [1]. These are reported to be cell-like structures that contain localized aqueous media within an organic solvent that are remarkable for their ability to promote activity that can mimic biological processes. The systems also present the possibility of producing inorganic materials for novel biomimetic catalysis in a solvent environment. In particular, that report includes descriptions of the particles and their effectiveness as sites for catalytic involvement of localized enzymes within them. The activities of three enzymes (lipoprotein lipase, chymotrypsin, and alkaline phosphatase) are assessed with a labile chromophoric ester so that the localized products can be detected accurately. The data are presented as Lineweaver–Burk plots [2,3] ($1/v$ vs. $1/S$) from which enzyme kinetic parameters are derived [2]. However, it is of some concern that the results as presented in those plots present no evidence for even partial saturation, a minimum condition that would indicate binding to an enzyme. Without evidence of binding, the reported parameters that depend on its kinetic consequences binding are clearly not valid.

2. Results and discussion

If a reaction is subject to saturation of a catalyst, in the most general formulation the results will be the observation of Michaelis–Menten kinetics [2,4], leading to a nonlinear plot of initial rate (v) as a function of substrate concentration (S) at a constant enzyme concentration: $v = V_{max}S/(K_m + S)$. In order to obtain values for V_{max} and K_m, which generally characterize the enzyme-substrate interaction and turnover, it is common to do a nonlinear least squares fit of the data to the equation [2]. However, earlier graphical methods employ the inverse plot of the equation so that the slope and intercept can be used to obtain V_{max} and K_m[2].

$$l/v = K_m/V_{max}(S) + l/V_{max}$$

In order to obtain meaningful values for V_{max}, the inverse plot must intersect the $1/v$ access far enough from $1/v = 0$ so that the inverse is finite. Similarly, the intercept of the S axis must also be far enough from 0 as that intercept is equal to $-1/K_m$. Clearly, where the intercept is at or near the origin (0, 0), no saturation is observed and neither parameter can be obtained. Reactions without saturation, with normal first order behavior, give the rate law $v = k[S]$ and the inverse plot $1/v = 1/(k[S])$, is also linear but with the intercept necessarily at (0, 0).

The data for enzyme catalysis by lipoprotein lipase are presented Fig. 1. Direct plot and trend-line fit of data derived from Li et al. [1] for catalysis by lipoprotein lipase in colloidsomes by Li et al. [1] as a plot of $1/v$ vs. $1/S$ with an extension of the best fit of the data as shown extending through (0, 0). Thus, there is no evidence from the plots that can be used for kinetic analysis

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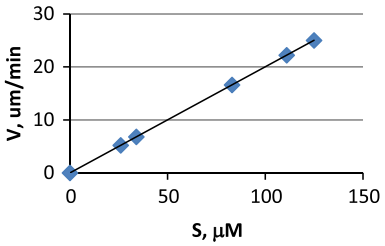

Fig. 1. Direct plot of data for hydrolysis in presence of lipoprotein lipase. There is no deviation from linearity that would be necessary in order to obtain valid parameters for assessing enzymic activity.

to obtain K_m and V_{max}. A conventional plot of v vs. [S] (measured from the reported plots) gives an excellent fit to the linear equation of $v = 3.3 \times 10^{-3}\ s^{-1}$ [S] (Fig. 1). Furthermore, since there is no saturation, finite values for V_{max} or K_m are not accessible. Other plots of the data that are presented are shown in the Supplemental Information to the present paper and show a similar lack of saturation kinetics, which would be necessary to obtain meaningful values of K_m or V_{max} for an enzymic reaction. On the other hand, an ordinary hydrolytic process should depend on only on the concentration of reactant in a first order manner.

Li et al. report values for V_{max} and K_m but it is not apparent how these could be obtained: "The large increase in V_{max} indicated a much greater turnover number (k_{cat}) under conditions of substrate saturation for the colloidosome system ($302\ s^{-1}$) compared with bulk aqueous solution ($0.35\ s^{-1}$)." It is also reported that the specific activity of the enzyme is higher in the colloidosome than in bulk solution along with an analysis of the effect of particle size on rate. While important conclusions may eventually be inferred, it is difficult to understand the role of the enzyme in the process as presented.

Li et al. also present inverse plots (their Fig. S4) for reactions with chymotrypsin and alkaline phosphatase in dodecane as well as representative plots of time courses of the plotted reactions of chymotrypsin (their Fig. S3). The plots in Fig. S3 show some irregularities that make the results difficult to assess. For the inverse plots in Fig. S4, extending each of the lines that are presented as a fit to the data as $1/v$ vs. $1/[S]$ gives lines that pass through (0, 0). This gives a clear indication that neither K_m nor V_{max} could be derived from the plotted data. Reports of kinetic parameters of chymotrypsin in water:ethanol mixtures indicate that there is a large increase in K_M, consistent with structural changes of the enzyme that are reflected in CD spectra [5]. The media of the colloidosomes would have an even greater effect and could make kinetic parameters difficult to obtain.

3. Methods and calculations

Data for plots of rates versus concentrations were obtained by measurements of the reported rates as presented in plots of the inverse values. The values of the data points were inverted and plotted using Microsoft Excel with a point added for (0, 0), which is logically required (there can be no reaction in the absence of reactant). The points were fit by a linear least squares fit using the trendline function.

4. Conclusion

While the production and utilization of colloidosomes with inorganic membranes presents an opportunity for producing novel biomimetic materials, conclusions based on the kinetic parameters of reactions in the presence of enzymes within the colloidosomes suggest that binding and catalysis by the enzymes are not consistent with the available evidence.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2013.06.004.

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