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Reverse micelles, a system for antibody-catalysed reactions

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Abstract

This work presents some aspects of the application of catalytic antibodies in water-in-oil microemulsions (reverse micelles) based on sodium bis-2-(ethylhexyl)sulfosuccinate (AOT) in isooctane. The monoclonal antibody (mAb) 9A8 used in this study is a fully characterised acetylcholinesterase-like antibody produced by the anti-idiotypic approach. The effect of various parameters, such as, the size and the concentration of reverse micelles, as well as the concentration and the nature of substrates on abzyme catalytic activity were investigated.

1. Introduction

Catalytic antibodies (or abzymes) were first identified in 1986 as a new class of man-designed biocatalysts with very promising applications. Since then, abzymes have been generated that can catalyse chemical reactions corresponding to all of the major enzyme classes (acyl-transfer, pericyclic, photochemical and redox reactions, etc. [1]).

Abzymes like enzymes are conventionally applied to aqueous reaction systems. However, their versatility would be expanded if reactions could also be performed in nearly anhydrous organic media, aqueous–organic biphasic media or water-in-oil microemulsions [2] (Fig. 1).

Reverse micelles are thermodynamically stable, water droplets dispersed in an organic phase by means of a surfactant. One of the most important properties of reverse micelles is their ability to entrap enzymes and other biomolecules into their water pools [3,4]. To date there are no extensive studies on catalytic antibody function in microemulsions.

In the present work, we studied the catalytic activity of the acetylcholinesterase-like abzyme 9A8 [5] in a reverse micellar reaction system. The effect of various parameters of the system on the abzyme hydrolytic activity (Scheme 1) was investigated.

2. Material and methods

2.1. Preparation of the 9A8 abzyme

MAb 9A8 was produced from ascitic fluid by fractionation on a Sephacryl S-200 2.6 cm × 70 cm column (Pharmacia), equilibrated with 0.1 M phosphate buffer, pH 7.4. Protein concentration was measured by the Bradford method. Purity was assessed by SDS-PAGE.

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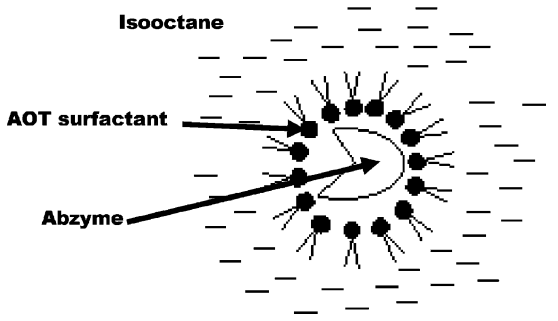


Fig. 1. Schematic presentation of the abzyme entrapped in a w/o micelle.

2.2. Preparation of microemulsions and activities measurements

Microemulsions were typically prepared by the addition of known volumes of catalytic antibody in phosphate buffer 0.1 M, pH 7.4 into an isooctane solution containing 100 mM AOT at 30 °C. The mixture was briefly shaken until an optically clear single-phase solution was formed. Assays designed to measure hydrolytic activity employed microemulsions in which the substrate *ortho*- or *para*-nitrophenylacetate (*o*- or *p*-NPA) was introduced as a stock solution in isooctane. Release of the product was typically measured by mixing in a spectrophotometer cell 0.1 ml of the reaction mixture with 0.9 ml of 167 mM AOT in isooctane solution containing 0.05 M Tris–HCl, pH 8.4 in a molar ratio $w_0 = 56$.

In aqueous media reactions, the abzyme was added in a spectrophotometer cell containing the substrate in 0.1 M phosphate buffer pH 7.4 and the hydrolytic activity was measured at 30 °C.

In both systems the product release was measured at 400 nm for *p*-NP and 412 nm for *o*-NP. The catalytic constants (V_{max} and K_m) of the reactions were determined from background corrected data fit to the Michaelis–Menten equation using non-weighted non-linear least-squares regression analysis. K_{cat} values were calculated by assuming that the molecular weight of IgM is 900 kDa and that all the antigen binding sites per molecule are active.

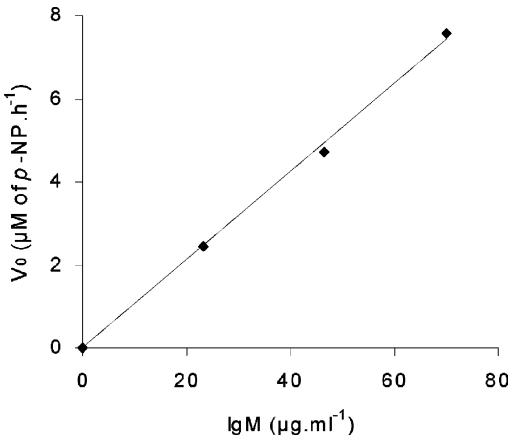


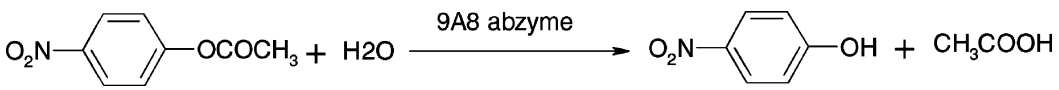
Fig. 2. Effect of abzyme-concentration on the hydrolytic activity in AOT/isooctane reverse micelles (*p*-NPA 0.56 mM); $w_0 = 16.7$; AOT 100 mM).

3. Results

In the microemulsion system, an increase of the 9A8 concentration with an identical water and AOT content shows a linear increase of activity revealing a kinetically controlled reaction (Fig. 2).

We have investigated the effect of the water content of the microemulsions, expressed in terms of the molar ratio $w_0 = [H_2O]/[AOT]$ for the hydrolysis of *p*-NPA. As it can be seen on Fig. 3, the mAb 9A8 hydrolytic activity follow a rather bell-shaped profile, presenting a maximum at $w_0 = 11.1$. Above this value, a constant slight decrease of the enzyme activity was observed.

The effect of the AOT concentration, at a constant w_0 value ($w_0 = 11.1$), on the antibody catalytic behaviour was investigated. It must be noted, that in this case, the increase of the AOT concentration causes an increase also in the number of the reverse micelles in the system. As shown in Fig. 4, the catalytic activity



Scheme 1. Hydrolysis of the *p*-nitrophenyl acetate (*p*-NPA) substrate

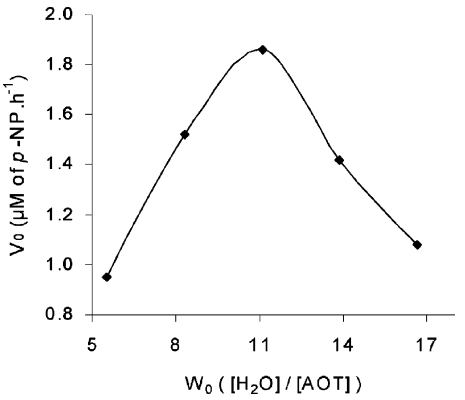


Fig. 3. Effect of the w_0 ratio ([H_2O]/[AOT]) on the 9A8 abzyme hydrolytic activity in reverse micelles (abzyme 10.20 μg ml^{-1}; *p*-NPA 0.56 mM; AOT 100 mM).

of mAb 9A8 was inhibited by the increased number of the reverse micelles.

The effects of substrate (*p*-NPA and *o*-NPA) concentration on the initial rate of hydrolysis were determined in microemulsion system and in aqueous (buffer) solution. It was found that hydrolysis reactions are following Michaelis–Menten kinetics in both systems. For each substrate (Table 1), K_{cat} values in w/o microemulsion and in aqueous media are found to be similar. But catalytic efficiencies (K_{cat}/K_m) in w/o microemulsion are greatly affected because of the increased K_m value. This inhibitory effect could be explained by diffusionnal constrains of the substrates and/or products through the surfactant layer as well as by non-competitive inhibition by the surfactant AOT.

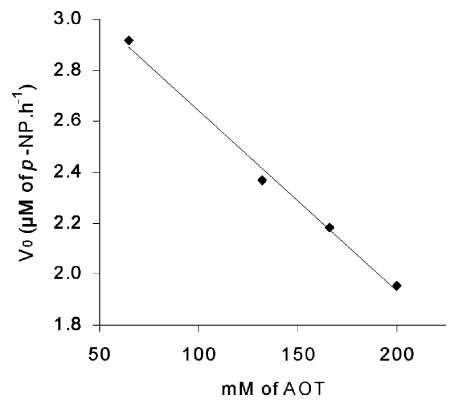


Fig. 4. Effect of AOT concentration on the hydrolytic activity of the 9A8 abzyme in reverse micelles (abzyme 10.20 μg ml^{-1}; *p*-NPA 0.70 mM; $w_0 = 11.1$).

Table 1
Comparison of kinetic results for *p*-NPA and *o*-NPA substrates in aqueous and reverse-micelles systems

	K_{cat} (min^{-1})	K_m (mM)	K_{cat}/K_m (mM^{-1} min^{-1})
p-NPA in aqueous buffer	3.30	0.20	16.50
p-NPA in reverse micelles	3.37	5.88	0.57
o-NPA in aqueous buffer	9.76	0.66	14.79
o-NPA in reverse micelles	7.44	11.82	0.63

4. Conclusion

It has been established that a catalytic monoclonal antibody (9A8) retains its catalytic function after its entrapment in an AOT/isooctane reverse micellar system, catalyzing the hydrolysis of *p*- and *o*-nitrophenyl acetates. Studies are in progress in our lab in order to investigate the ability of catalytic antibodies to be used as catalysts in synthetic reactions in reverse micellar systems. The ability of antibodies to preserve their catalytic activity in low-water organic media is expected to expand their versatility as catalysts in various biotechnological processes.

Acknowledgements

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