side arm and nitrogen on the upper. If the frit caked with product and filtration slowed, momentary reversing of the flow of nitrogen lifted the cake away from the frit. The precipitate was washed with deoxygenated toluene (17 mL), and the flask containing the filtrate was exchanged for an empty one. (^{31}P NMR showed that the filtrate contained none of the desired product.) The entire apparatus was put under vacuum, and the product was dried overnight to give 470 mg (75%) of a dark red solid which appeared orange when pulverized: ^{1}H NMR (CD_2Cl_2, 101 MHz) δ 8.07 (t, J = 8.8 Hz, 4 H), 7.82 (t, J = 8.3 Hz, 2 H), 7.65 (t, J = 8.3 Hz, 6 H), 7.55 (t, J = 8.8 Hz, 4 H), 7.47 (dd, J = 11.2, 8.8 Hz, 4 H), 7.4–7.1 (m, 20 H), 6.95 (t, J = 7.5 Hz, 2 H), 6.84 (t, J = 7.4 Hz, 2 H), 6.8–6.7 (m, 4 H), 6.7–6.6 (m, 4 H), 6.6–6.5 (m, 12 H), 3.24 (m, 6 H), 1.45 (t, J = 7.3 Hz, 9 H); ^{31}P NMR (CD_2Cl_2, 101 MHz) δ 56.5 (d, J = 38 Hz), 52.3 (d, J = 38 Hz).

Asymmetric Reductions. *tert*-Butyl acetoacetate (14.5 g, 90 mmol) and methanol (30 mL) were mixed and deoxygenated with flowing nitrogen for 5 min in a septum-covered Parr shaker bottle. The catalyst prepared above (36 mg, 0.041 mmol) was added along with 2 N HCl (0.041 mL, 0.082 mmol). The mixture was transferred to a standard Parr shaker apparatus and flushed by evacuating and refilling with nitrogen and then hydrogen several times. The apparatus was heated at 40 °C with shaking under 50 psi of hydrogen. After 20 min the reaction became a homogeneous clear yellow solution which took up hydrogen for approximately 8 h. At this time the reaction was complete and the mixture was cooled and diluted with hexane (30 mL) to precipitate the catalyst, which was filtered away. The filtrate was concentrated to give *tert*-butyl 3(*R*)-hydroxybutyrate (14.5 g, 97%).

Acknowledgment. We wish to acknowledge the very helpful assistance of Mr. William Benning of the Merck high-pressure laboratory. We thank Professor Taber for providing a preprint of his paper.

Supplementary Material Available: Additional procedures, reduction rates, and NMR spectra (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Antibody Catalysis in Low Water Content Media

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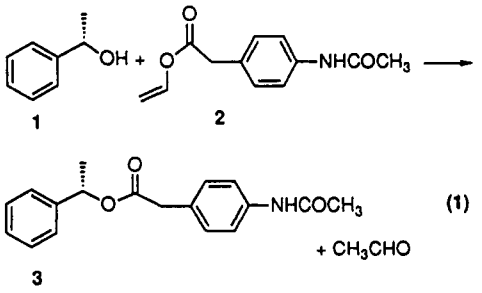
Received September 2, 1992

Summary: An investigation has been performed wherein an immunoglobulin's catalytic function in aqueous–organic biphasic solvent systems was elucidated, and the water requirements necessary to preserve its catalytic competency in an apolar solvent system were determined.

Enzymes can act as catalysts in organic solvents.[1] On the other hand, antibodies have only recently been investigated for their ability to function in organic media.[2] Lately we have focused our efforts on trying to observe antibody catalysis in organic solvents[3] in an effort to make catalytic antibodies more attractive catalysts from a synthetic standpoint. To take full advantage of the possible opportunities afforded by catalytic antibodies[4] in organic solvents it is critical to understand their "abzymology" when they are subjected to these nonnatural environments. Herein, we report on an immunoglobulin's catalytic activity in a variety of apolar solvent systems and elucidate its water requirements in such media.

It has been shown that an antibody can catalyze a hydrolysis reaction in a reverse micelle.[5] However, there have been no reports of abzymes functioning at an aqueous-organic interface or in low water containing media. Previously, we studied a set of antibodies which stereoselectively hydrolyzed either the (*R*) or (*S*) enantiomer of an alkyl ester to its acid and α-methyl benzyl alcohol components.[6] One of these abzymes (PCP 21H3) could catalyze an enantioselective acyl transfer reaction (eq 1)



(1)

via a ping-pong mechanism.[7] However, our attempts to catalyze this transesterification reaction in polar organic solvents proved unsuccessful.[3]

It was quickly determined that octane (log P = 4.5)[8] was an excellent solvent system for the abzyme's catalysis. A

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typical assay system consisted of a lyophilized sample of 0.6 mg of 21H3, which was suspended in octane containing a percentage of water. The reaction was initiated by the addition of (*S*)-alcohol 1 and vinyl ester 2.[9] Steady-state kinetics were observed when a 4% (v/v) water addition was made ($K_{m(alcohol)}$ = 2.3 mM, $K_{m(ester)}$ = 106 μM, k_{cat} = 3.0 min^{-1}), and the data obtained were again consistent with a ping–pong bi–bi kinetic pattern.[12] Our previous findings, conducted under typical "aqueous" assay conditions [90%, ATE (0.1 M aces [2-[(carbamoylmethyl)-amino]ethanesulfonic], 0.052 M tris, and 0.052 M ethanolamine), pH 9.0/10% DMSO], yielded values of $K_{m(alcohol)}$, $K_{m(ester)}$, and k_{cat} to be 7.3 mM, 3.0 mM, 21 min^{-1}, respectively.[7] The aqueous–organic biphasic assay conditions had no effect on the abzymes mechanism of action or stereoselectivity, however a sizable decrease in the Michaelis constant for the vinyl ester was observed. Such differences may in part be explained by the partitioning of the vinyl ester 2, between the bulk solvent phase and the biocatalyst phase.[13] A comparison of the apparent second-order rate contant (k_{cat}/K_m), the so called specificity term,[14] between these two assay systems shows that there were no deleterious effects. What was most intriguing was that the (k_{cat}/K_m) of the vinyl ester was improved by almost an order of magnitude in going from the aqueous to the biphasic solvent system.

To get an idea of the potential utility of this biphasic process we performed the following experiment. Octane was added to a lyophilized sample of 21H3 (2×10^{-9} mol). To this suspension was added 5% (v/v) water, 2% (v/v) chloroform, 1 (1 mM), and 2 (1 mM). The reaction was monitored over time. After 6 h the reaction had slowed considerably; however, over 500×10^{-9} mol of 3 had formed corresponding to a 50% yield and over 250 turnovers. The shortcoming lies in the fact that 3 is rather hydrophilic and tends to partition into the aqueous layer causing product inhibition. It is anticipated that reactions with less hydrophilic products will provide more favorable yields.

It has been shown previously that enzyme specificity and reactivity are related to the hydrophobicity of the organic solvent used as the reaction medium.[1,15] We determined

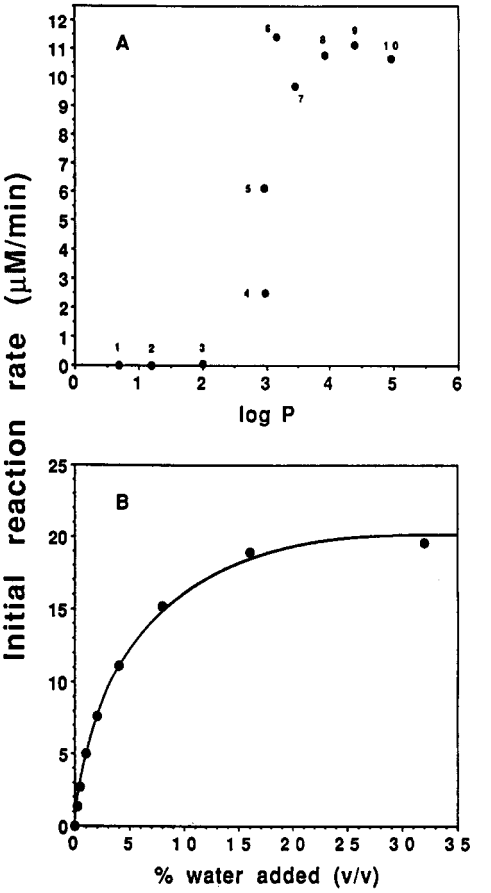

Figure 1. Initial reaction rates of 21H3 in transesterification reaction (eq 1) as a function of log *P* (A) and percent water (buffer) (B). Conditions: in graph A initial rates were all measured using 8 mM 1 and 1 mM 2 with 4% (v/v) water[16] by the assay procedure described in ref 9. The solvents used were as follows: 1, ethyl acetate; 2, propyl acetate; 3, chloroform; 4, carbon tetrachloride; 5, pentane; 6, cyclohexane; 7, hexane; 8, heptane; 9, octane; and 10, nonane. log *P* values are reported and described in ref 8. These values were corrected for the added solvent effects of chloroform. In graph B the bulk solvent used was octane. Initial rates were measured using 8 mM 1 and 1 mM 2 while varying the percentage of water present[16] (assay procedure described in ref 9). The calculated line for graph B was obtained by using $Y = A*X/B + X$.

21H3's initial reaction velocities in a number of organic solvents (Figure 1A). An apparent, sigmoidal-shaped correlation exits between 21H3's transesterification activity and log *P*. Activities are virtually nonexistent in hydrophilic solvents having a log $P < 2.0$, are variable in solvents having a log *P* between 2.0 and 3.5, and are high in hydrophobic solvents having a log $P > 4.0$. Similar trends have been seen in various enzymatic systems.[17]

Enzyme-catalyzed esterification and transesterification reactions may be carried out in either monophasic, nearly anhydrous organic media[1] or, as we have described, an aqueous–organic biphasic system. Although published experimental procedures give no clear indication as to the optimal amount of exogenous water to be added to the

(9) The antibody was stored in, and lyophilized from, 10 mM Bicine buffer, pH 8.5. Apparently it is critical that the antibody be lyophilized from an aqueous solution at the pH optimal for catalytic activity. In our previous report[3] antibody 2H6 was lyophilized from a solution of triethylammonium bicarbonate, pH 7.0. Under such conditions this antibody displayed poor catalytic activity.[10] All reactions for this study (total volume, 1 mL) contained 0.6 mg of lyophilized antibody 21H3. Typically, 90.9 μL of a 6.6-mg antibody/mL buffer solution was lyophilized directly into the eppendorf tube to be used for the transesterification reaction. Lyophilized samples contained less than 1% (v/v) water.[11] The dried antibody was first suspended in organic solvent (e.g., octane). Then, a percentage of 100 mM Bicine buffer, pH 8.5, was added and the suspension allowed to equilibrate 5 min at 23 °C on an IKA-VIBRAX-VXR vortexer shaking at 1800 rpm. For our steady-state kinetics assay we chose to use 4% (v/v) buffer. Reactions were initiated by the addition of varying amounts of substrates 1 and 2 yielding a final chloroform concentration of 2% (v/v) in the final 1 mL. The reaction in the capped eppendorf tube was again shaken at 1800 rpm until halted by the addition of 40 μL of citrate buffer, pH 3.0. While 1800 rpm was used, the reaction proceeds equally as well between 600 and 2200 rpm. However, below 600 rpm the reaction rate drops off. Quenched reactions were dried under reduced pressure, redissolved in 1 mL of a 50/50 DMSO/water mixture and assayed by HPLC as previously described.[7] In all cases no reaction was detected without antibody 21H3 being present.

(10) While this antibody was monoclonal, different classes/subclasses of antibodies may not necessarily exhibit the same properties.

(11) The water concentration was measured by the method of Langhals (Langhals, H. *Anal. Lett.* **1990**, *23*, 2243). The detection limit is 1 mg of H_2O in 1 mL of solvent.

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organic solvent, there is a consensus that some water is absolutely needed for the catalytic function of the enzyme.[18] We have investigated abzyme 21H3's water requirements in our octane assay medium (Figure 1B). In order to take full advantage of the immunoglobulin's optimal rates of reaction (using 0.6 mg of antibody), a water concentration of approximately 15% (v/v) was needed. *Nevertheless, excellent catalytic activity was still observed as low as 2% (v/v) water addition, and abzyme activity could still be detected in 0.12% (v/v) water.* Finally, it is interesting to note the hyperbolic curve obtained in figure 1B. From this plot it is reasonable to infer that water is affecting the kinetics by specific interactions with the catalytic antibody. In this case water acted as an activator; hence, a saturation curve was observed.

An investigation of catalytic antibodies in aqueous–organic biphasic and low water content media was undertaken. Hopefully, the experimental results garnered in this study will be conducive to further exploration of catalytic antibodies in organic solvents.[19]

Acknowledgment. This research was supported in part by a grant from the National Science Foundation (9116377).

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Synthesis of Carbocyclic Analogues of Guanosine 5′-(β-L-Fucopyranosyl diphosphate) (GDP-Fucose) as Potential Inhibitors of Fucosyltransferases

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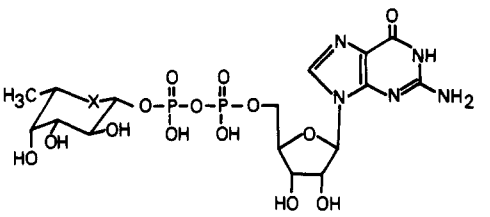
Received September 21, 1992

Summary: Two carbocyclic analogues of GDP-fucose consisting of 5a-carba-β-L-fucopyranose and its unsaturated counterpart have been synthesized as potential inhibitors of fucosyltransferases through the intramolecular Emmons–Horner–Wadsworth olefination of the 2,6-dioxophosphonate derivative, readily available from L-fucose, followed by chemo- and stereoselective reductions of the α,β-unsaturated inosose intermediate, which are the critical steps.

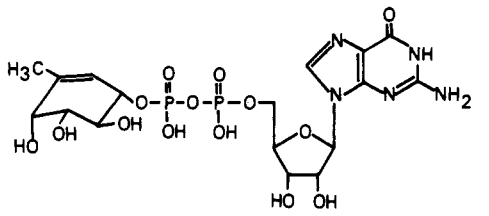
The fucosyltransferases (Fuc-T) are enzymes which catalyze transfer of L-fucopyranose from GDP-fucose (GDP-Fuc) to appropriate glycoconjugates. A number of studies have shown that invasiveness of tumor cells is correlated with an elevation of serum Fuc-T activity[1] or an increased fucose incorporation into cell surface glycoproteins.[2,3] Since fucose occurs at nonreducing termini in glycoconjugates,[4] it is conceivable that these phenomena are associated with structural changes in cell surface carbohydrates, in particular, changes involving glycoconjugate fucosylation.[5] Recently, the Lewis-type α(1→3/4)Fuc-T has gained special interest[6,7] due to its ability to synthesize

Scheme I



GDP-Fuc X = O

1 X = CH_2



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both sialosyl Le^X [SLe^X: Neu5Acα2→3Galβ1→4-(Fucα1→3)GlcNAc] and sialosyl Le^a [SLe^a: Neu5Acα2→3Galβ1→3(Fucα1→4)GlcNAc] determinants. Both determinants have been identified as ligands for endothelial–leukocyte adhesion molecule 1 (ELAM-1)[8] and for granule membrane protein 140 (GMP-140).[9] The interaction of SLe^X/SLe^a with ELAM-1 and GMP-140 seems to play an important role in an early stage of leukocyte extravasation and the inflammatory responses.[10] Inhib-

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