LIPASE-CATALYZED ESTERIFICATIONS IN MICROEMULSIONS

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Water-in-oil microemulsions or reverse micellar systems, are thermodynamically stable and optically isotropic solutions with water constituting the dispersed phase separated from the continuous organic phase by surfactant molecules (1, 2). The dispersed water-pools act like a microreactor, with a favorable aqueous microenvironment for enzyme and an enormous interface through which hydrophobic substrates can be cleaved (3, 4).

Lipase is the enzyme which catalyzes the hydrolysis of long-chain aliphatic esters to glycerol and free fatty acids. The reaction is reversible and the enzyme can be shown to catalyze the synthesis and the transesterification of esters.

In a previous work (5) we examined the catalytic behavior of lipase from *Rhizopus delemar* in different types of microemulsion systems. In addition we studied the esterification of a aliphatic alcohol, such as hexanol, with free fatty acids of different chain lenghts in a nonionic microemulsion system formulated with tetraethyleneglycoldodecylether ($C_{12}E_4$) in decane.

In present study we tested the esterification of various alcohols with fatty acids, in anionic microemulsion systems formulated with AOT in isooctane. We used lipases from various species with different specificities, such as, *Rhizopus delemar*, 1, 3 regio specific, *Rhizopus arrhizus*, 1, 3 regio specific *Geotrichum candidum*, specific for cis-D9-unsaturated fatty acids, and *Penicillium simplicissimum* non specific.

In another set of experiments we examined the esterification of an aliphatic alcohol (hexanol) and a natural fatty acids such as oleic, lauric, octanoic and butyric acid catalyzed by lipase from

Rhizopus delemar. We examined the effect of the chain-lenght of fatty acids and the effect fo the structure of alcohols. Different fatty acids such as oleic, lauric, octanoic and butyric acid and various alcohols such as hexanol cyclohexanol and cholesterol were tested. The rate of esterification was determined by tracing the depletion of the fatty acid, using the spectrophotometric assay of Lowry and Tinsley (6) as modified by Han and Rhee (7).

Different parameters such as pH, temperature and water content were studied. We found that the initial velocity of the reaction depends on the amount of water present in the microemulsion system. Fig. 1A shows the relationship between the degree of synthesis vs. time. From the slopes of these curves we can determine the relationship between the initial velocity and the water content, as expressed by R, where R= $[H_2O]/[AOT]$ (Fig. 1B).

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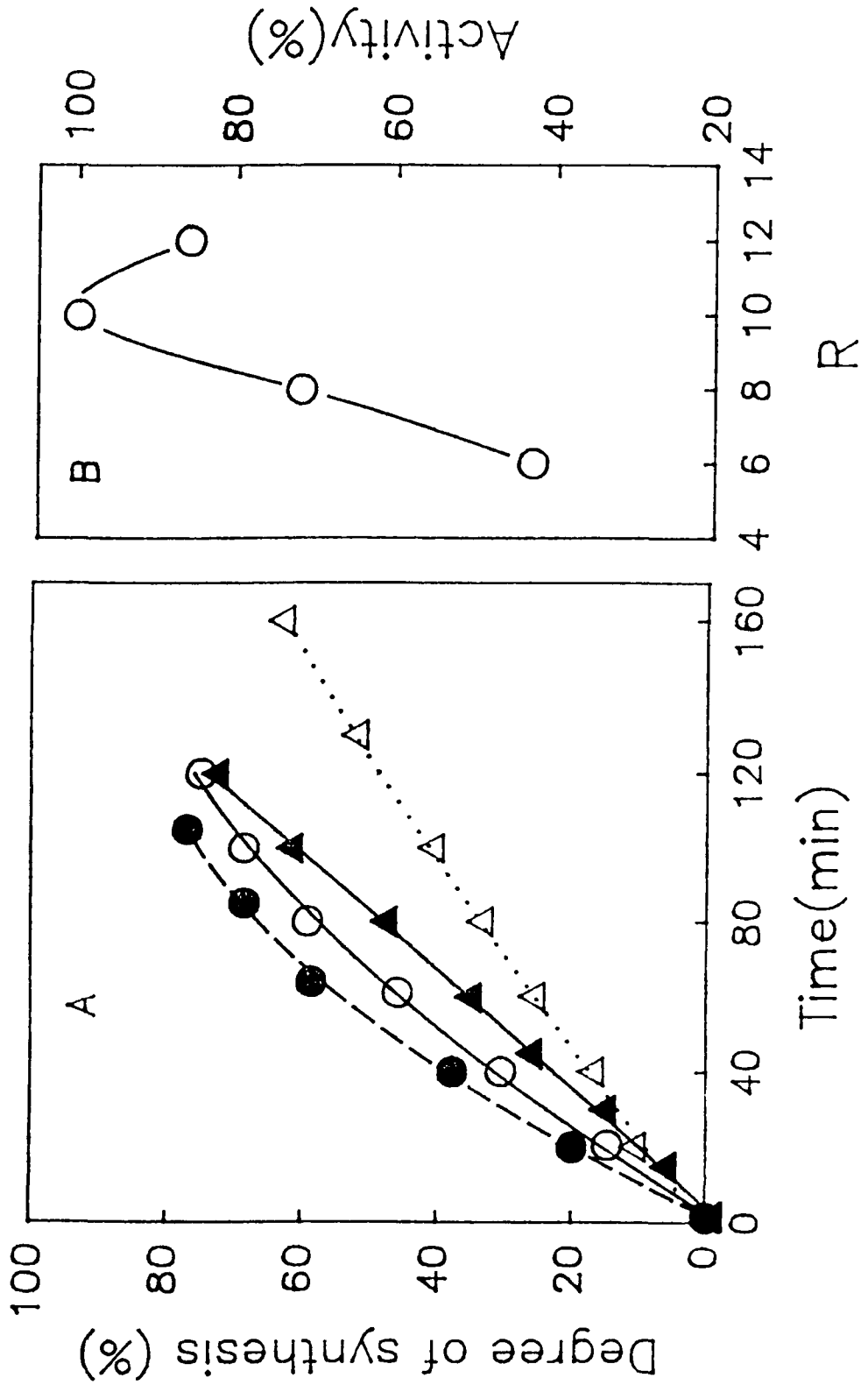

Fig. 1. Esterification of hexanol with oleic acid catalyzed by lipase from *Rhizopus delemar*, in AOT/isooctane microemulsion systems. (A) Time dependence at the following R values: (▲) 6, (▲) 8, (●) 10, (●) 12. (B) Dependence of enzyme activity on R value.