MICELLAR ENZYMOLOGY:

FUNDAMENTALS AND APPLICATIONS

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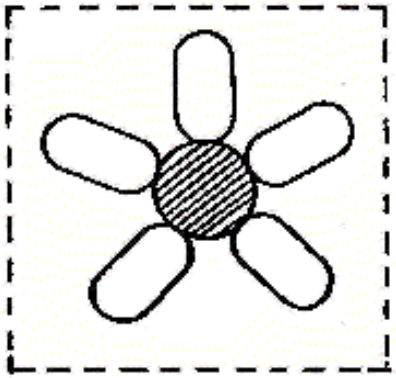
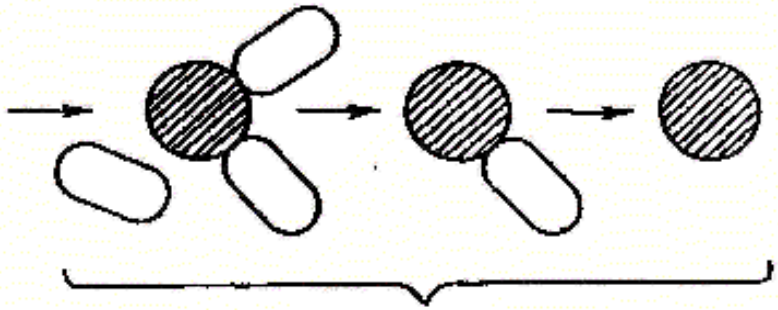
STRATEGY AND TACTICS IN ENZYMOLOGY

DESINTEGRATION



homogenization, extraction, purification



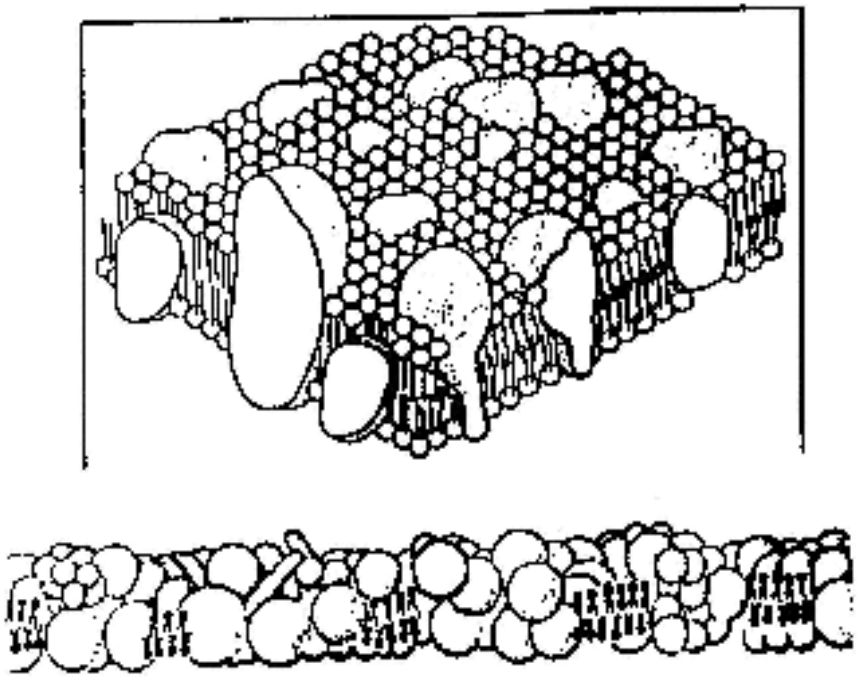
ENZYME PREPARATIONS

BIOCATALYST

- PROTEINS
- LIPIDS
- CARBOHYDRATES
- VITAMINS (COFACTORS)
- Low Molecular Weight
- Compounds and WATER

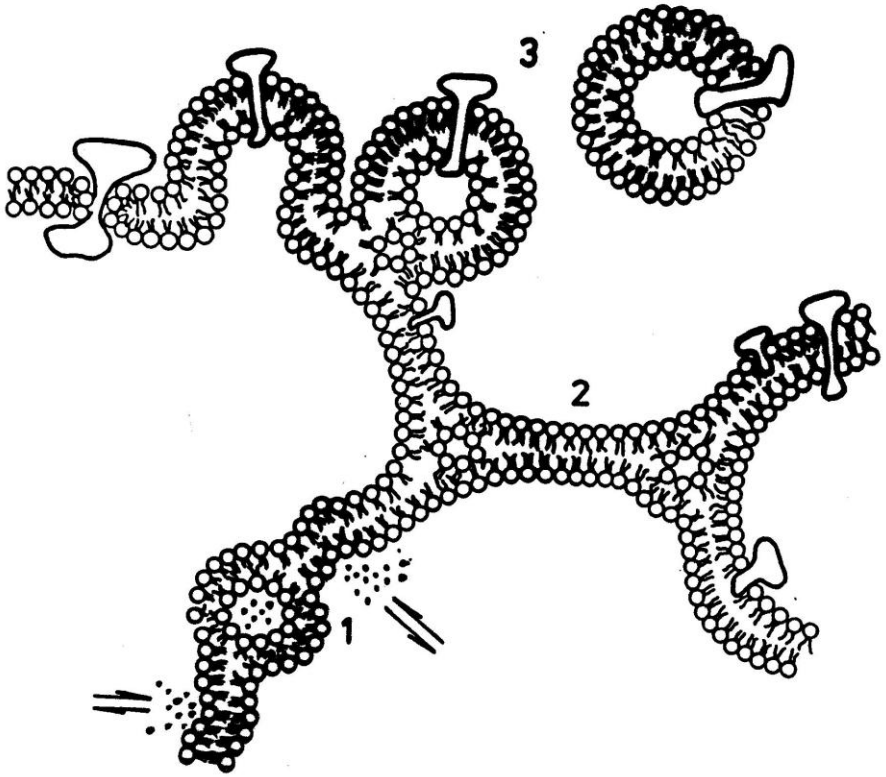
RECONSTRUCTION

MOLECULAR ORGANIZATION OF BIOMEMBRANES ("FLUID MOSAIC" MODEL)



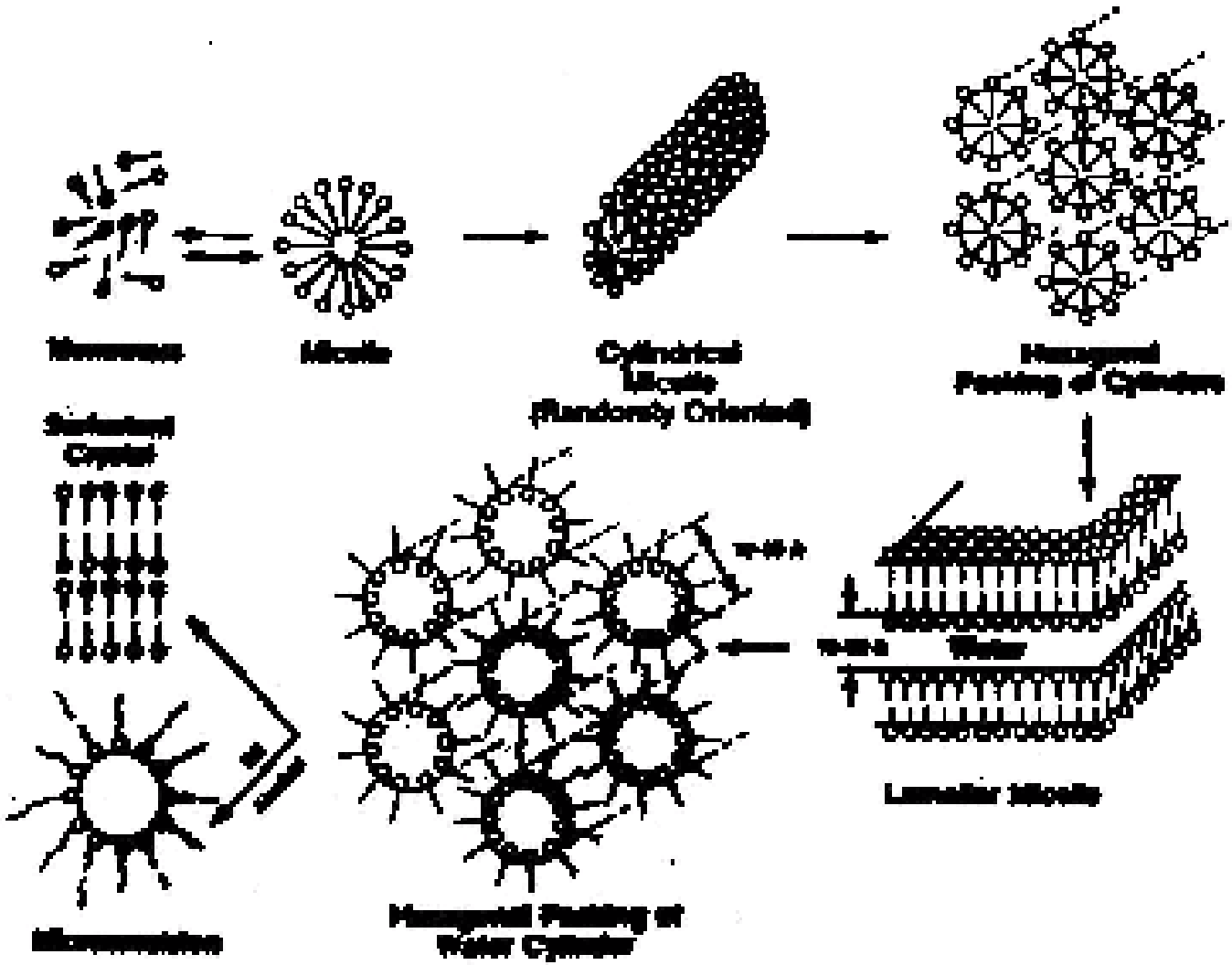
After S.J. Singer & G.L. Nicolson (1972) Science 175, 720

A METAMORPHIC MOSAIC MODEL OF BIOLOGICAL MEMBRANES



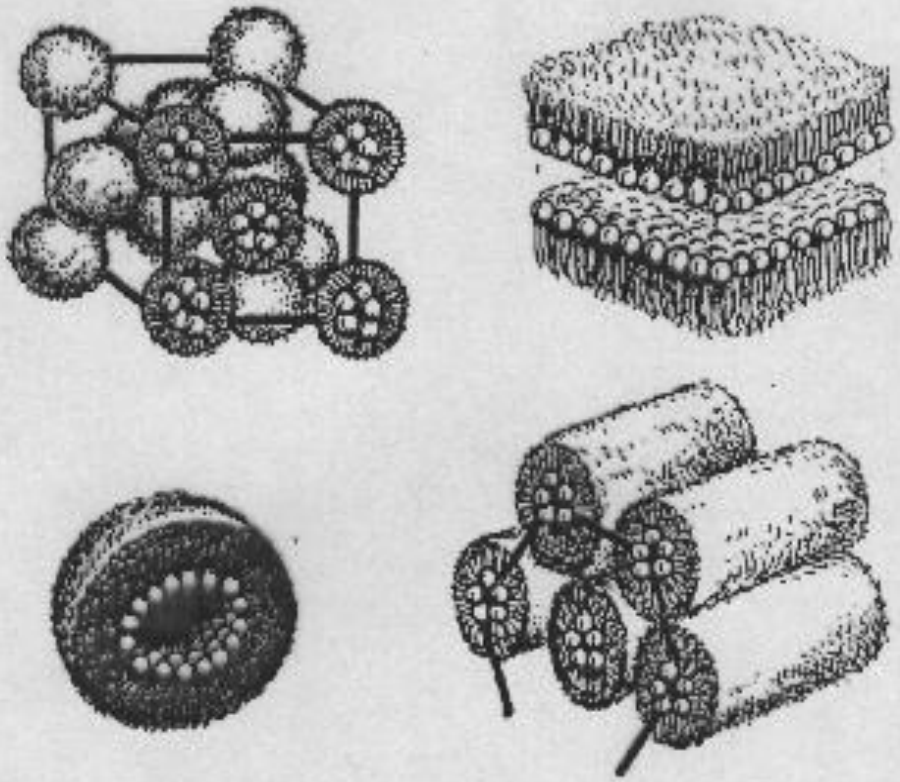
1 - transbilayer transport of polar molecules;
2 - membrane continuity between membrane bound compartments;
3 - budding off of a membrane bound vesicle.

P.R. Cullis, B. de Kruijff et al. (1980) Can.J.Biochem. 58, 1091

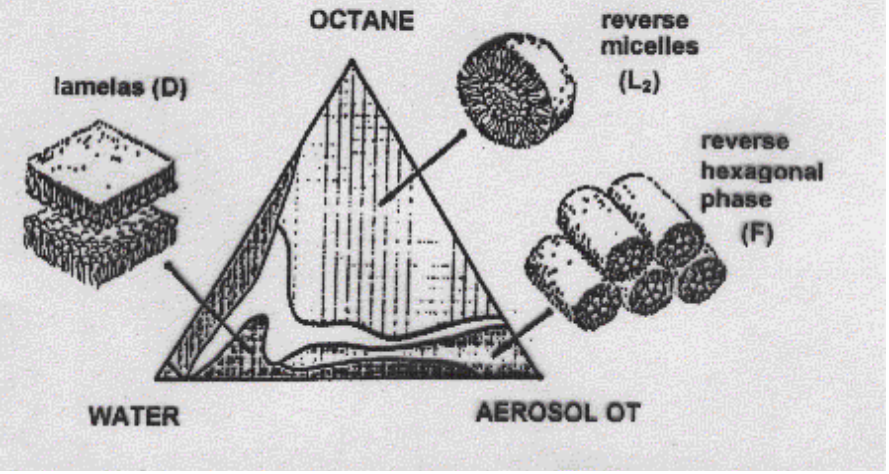


Different structures of micelles and mesophase .

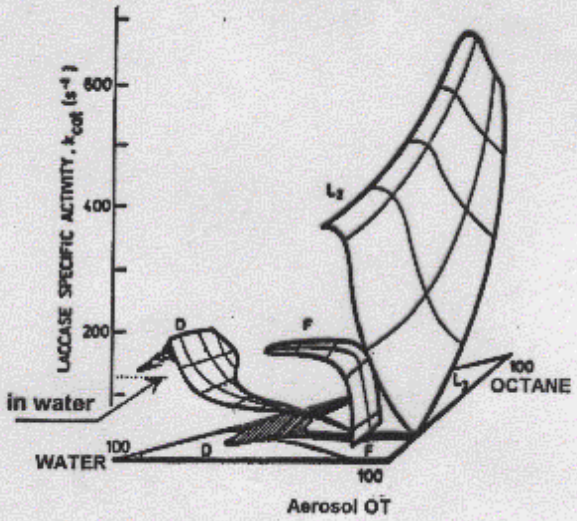
TYPES OF SURFACTANT AGGREGATES (MICELLES)



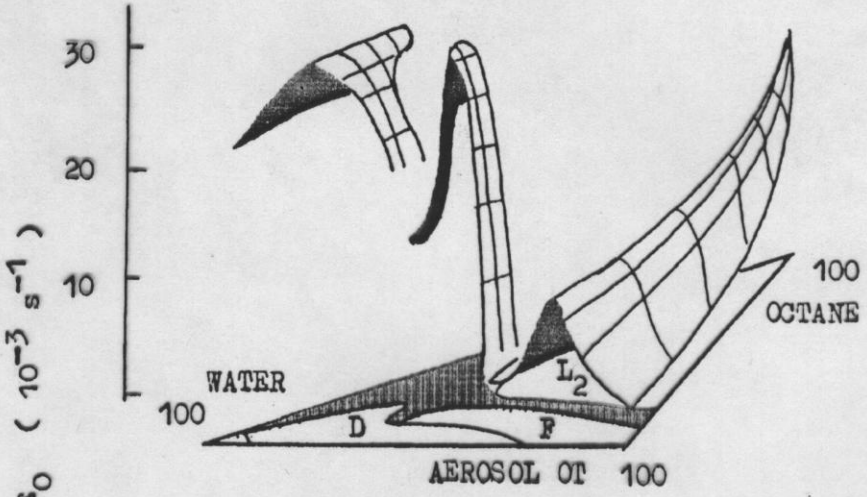
PHASE DIAGRAM OF THE "AEROSOL OT – WATER – OCTANE" SYSTEM



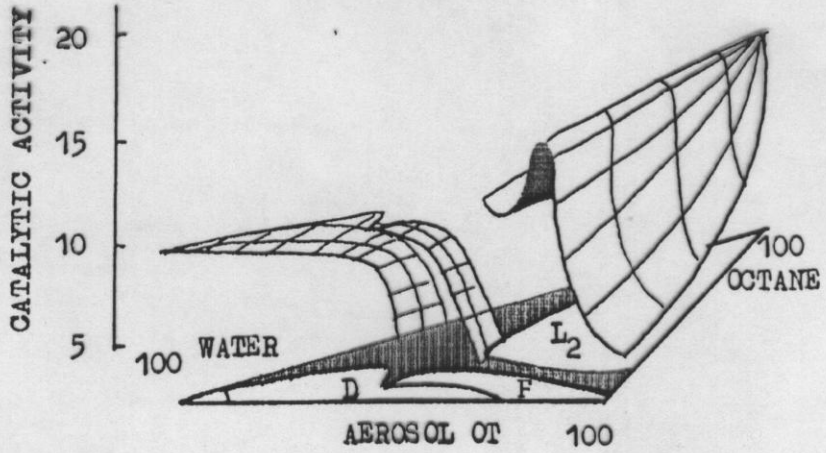
Laccase in surfactant-water-organic solvent system



STEAROYLATED α-CHYMOTRYPSIN



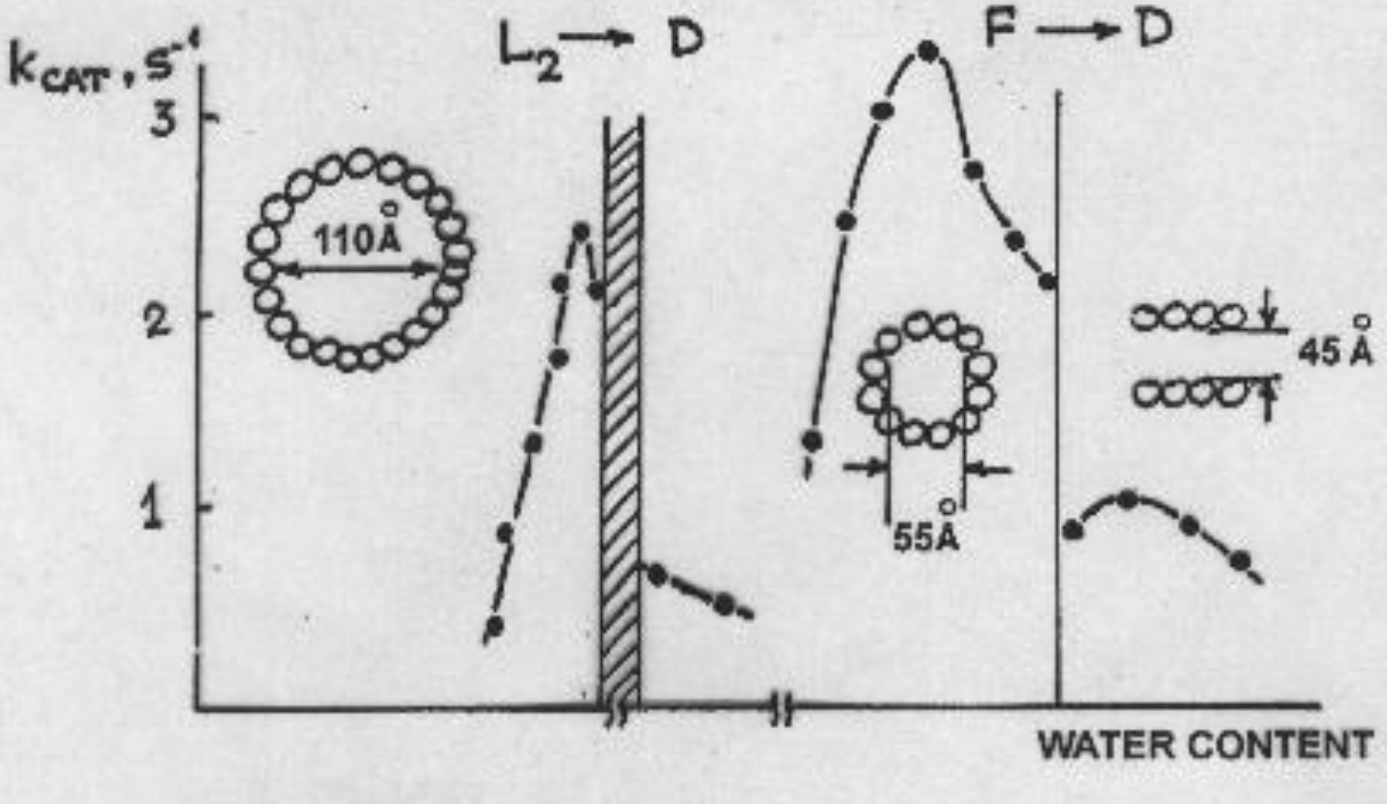
NATIVE α-CHYMOTRYPSIN



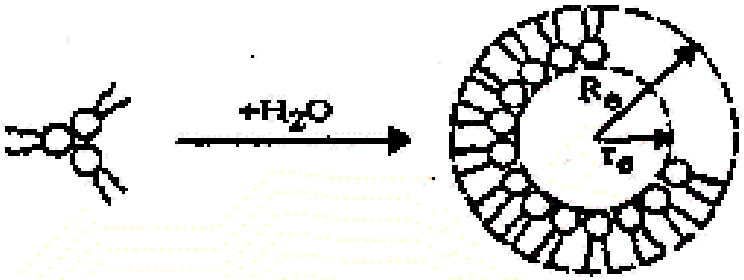
trans-CINNAMOYLIMIDAZOLE HYDROLYSIS

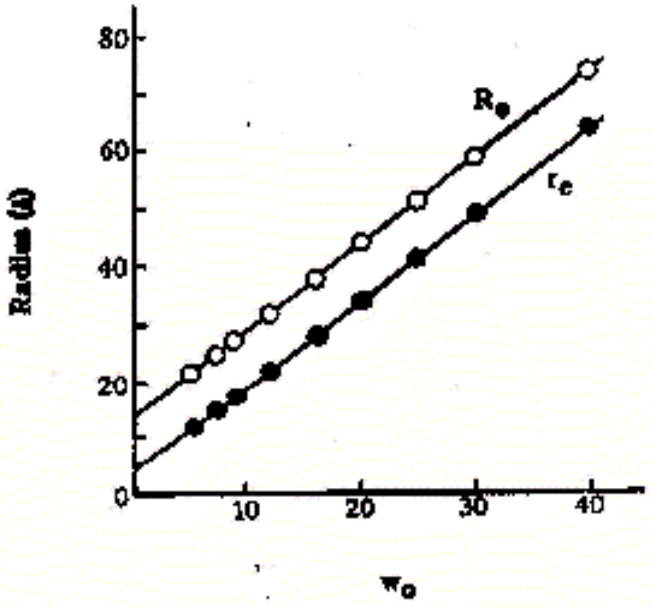
CHANGES IN CATALYTIC ACTIVITY UPON PHASE TRANSITIONS IN THE SYSTEM

HORSE LIVER ALCOHOL DEHYDROGENASE

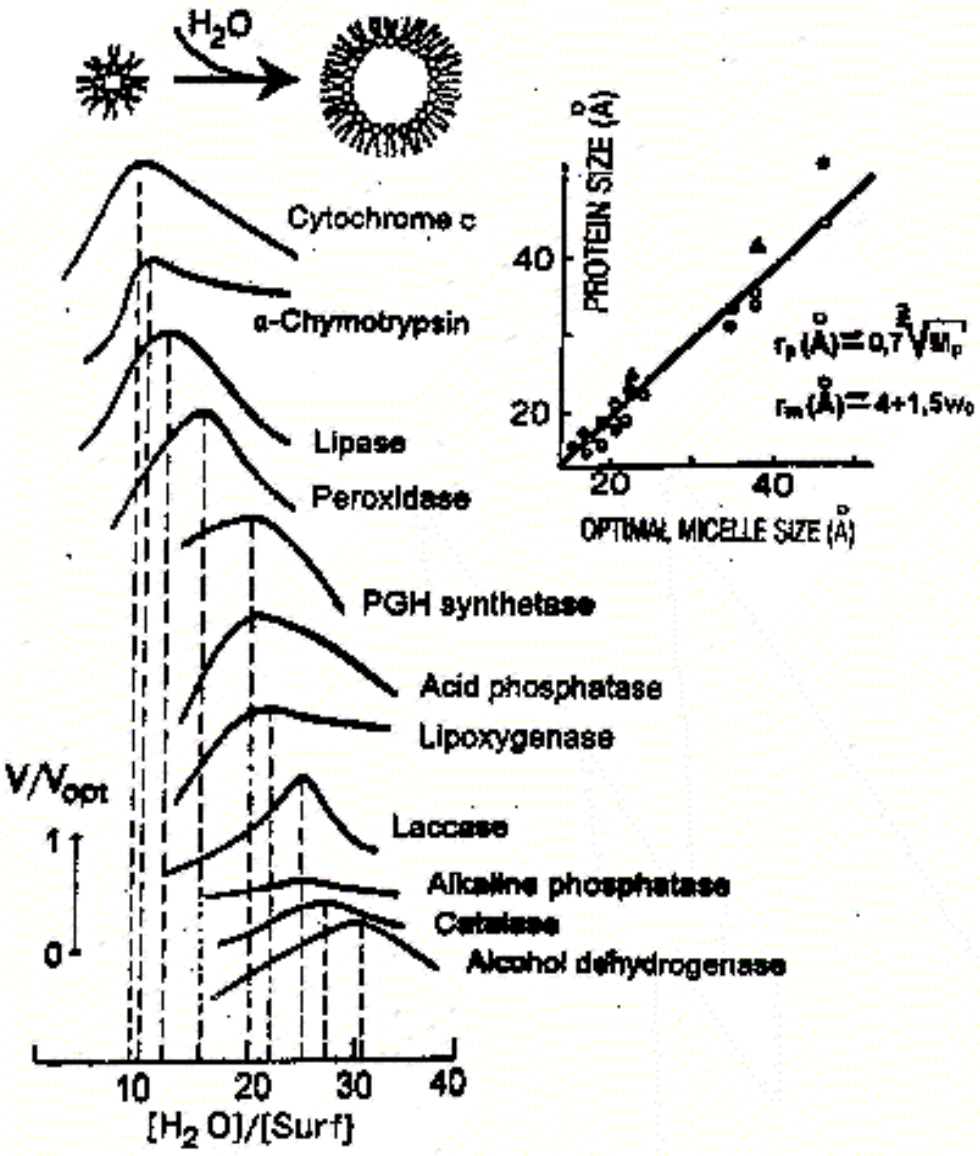


DEPENDENCE OF THE HYDRODYNAMIC (STOCKS) RADIUS (R_e) AND RADIUS OF INNER WATER CAVITY (r_e) OF AEROSOL OT REVERSED MICELLES IN OCTANE ON THE HYDRATION DEGREE (w_o)

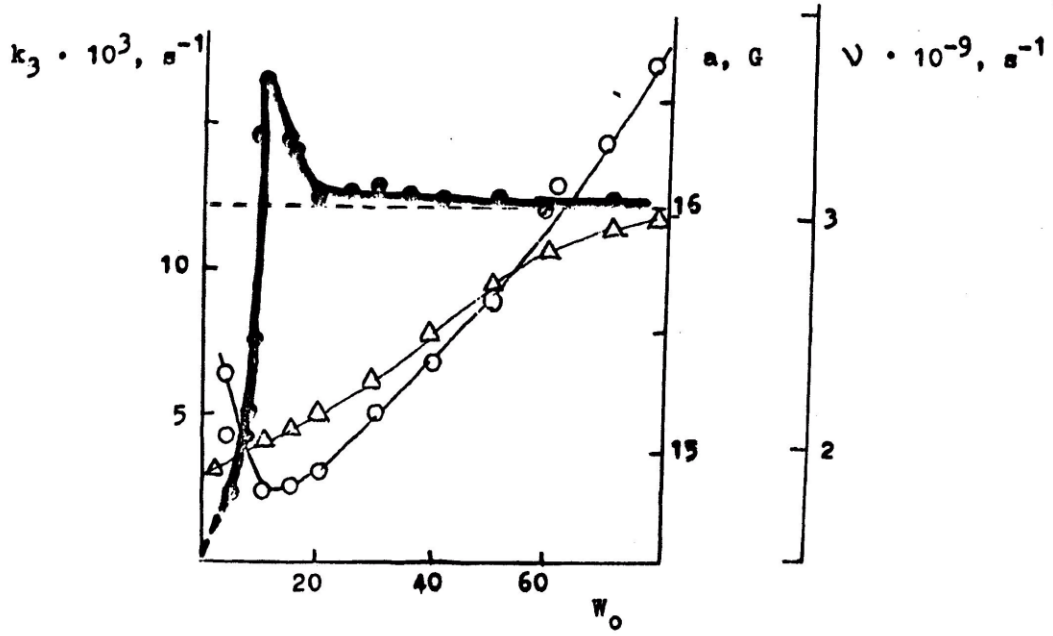




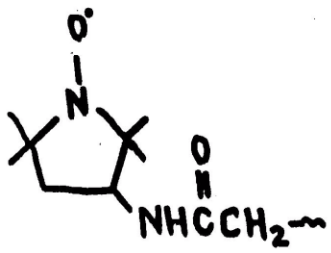
REGULATION OF ENZYME BY SURFACTANT HYDRATION



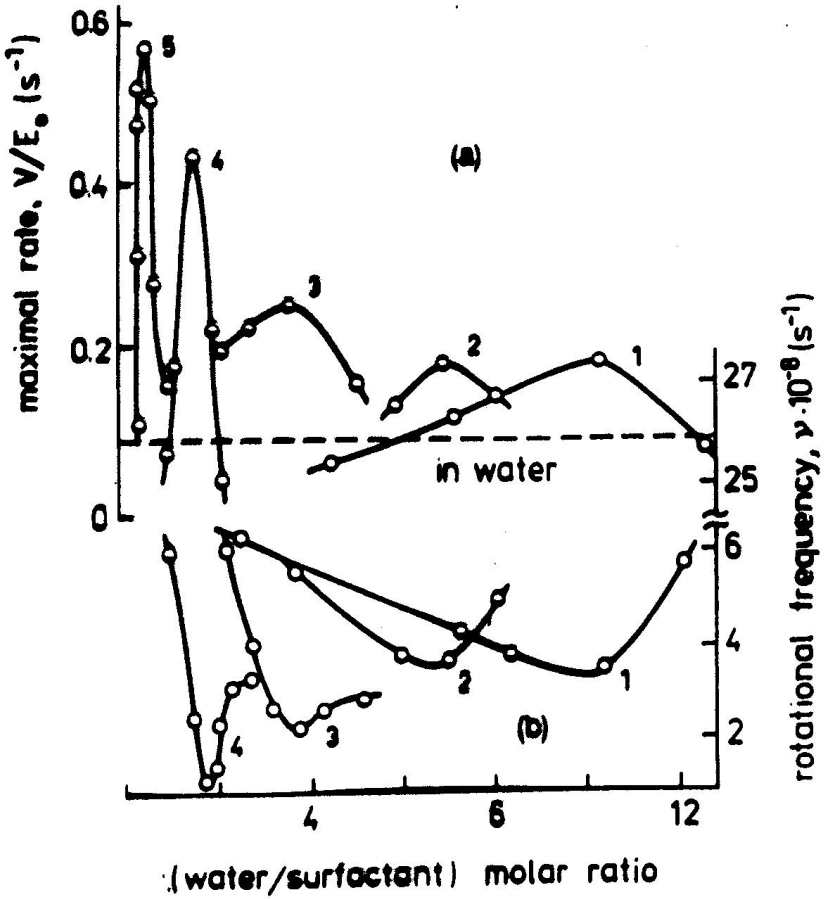
FIRST-ORDER RATE CONSTANT, k_3 (●), FOR DEACYLATION OF TRANS-CYNNAMOYL-CHYMOTRYPSIN, ROTATION FREQUENCY, ν (○), AND HYPERFINE SPLITTING CONSTANT, a (△), FOR SPIN-LABELLED CHYMOTRYPSIN IN THE "AOT - WATER - OCTANE" SYSTEM



Spin label:



O.V.Belonogova, G.I.Likhtenshtein et al. (1983) Biokhimia, 48, 379



Dependence on water/surfactant molar ratio of (a) the maximal rate $V/[E]_0$ of α-chymotrypsin-catalyzed hydrolysis of N-benzoyl-L-tyrosine p-nitroanilide, and (b) the rotational frequency ν of the spin label in the active site of the enzyme in the system Aerosol OT-water-glycerol-octane. Water/glycerol volume ratios are: 1, 100:0; 2, 80:20; 3, 50:50; 4, 20:80; 5, 6:94. Dashed line shows the values of $V/[E]_0$ and ν in aqueous solution.

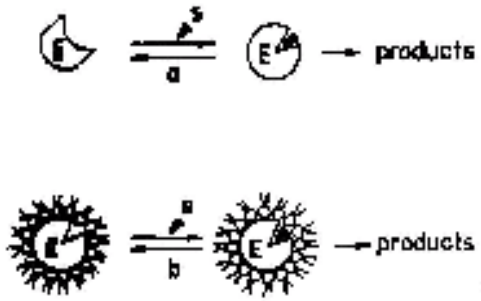


FIG. 23 Schematic representation of conformational transitions in α-chymotrypsin molecule (E) induced by substrate (S) binding (route a) and by entrapment of the enzyme into the reverse micelle (route b).

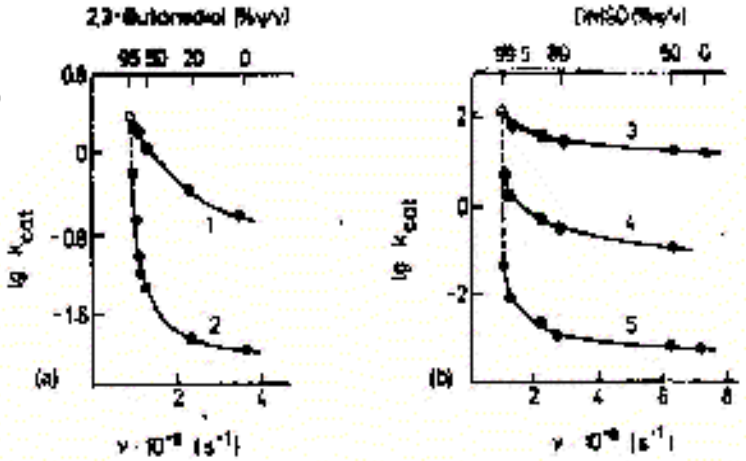
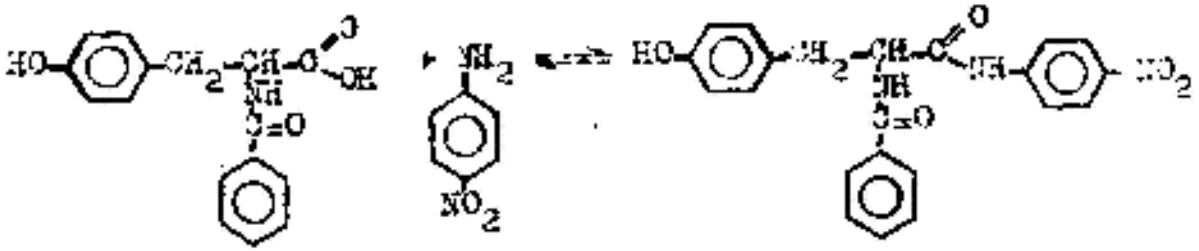
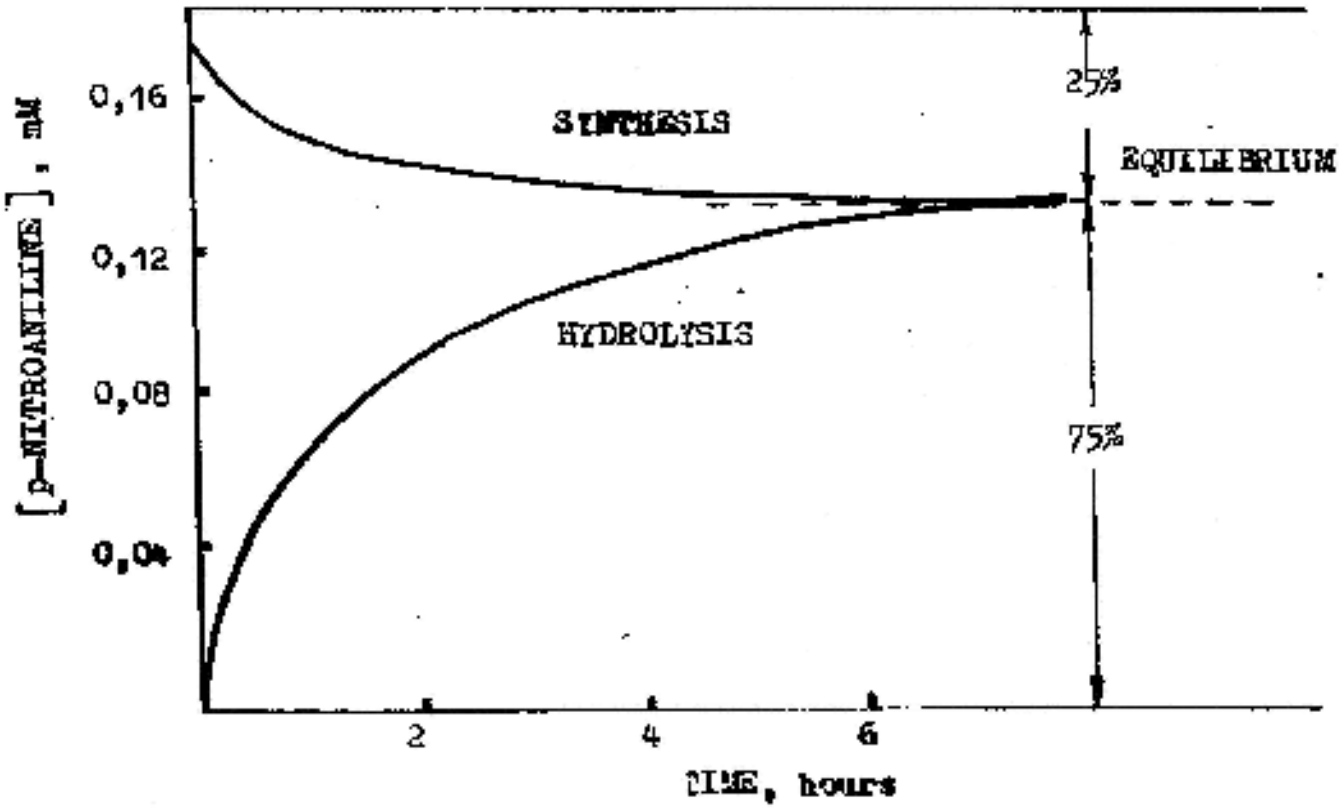


FIG. 24 Relation between the catalytic activity $V/[E]_0$ of α-chymotrypsin determined under optimal conditions and the rotational frequency ν of the spin label in the active site of the enzyme in the systems Aerosol OT-water-2,3-butanediol-octane (a), and CTAB-water-dimethyl sulfoxide-octane-chloroform (b). Substrates: 1, N-benzoyl-L-tyrosine p-nitroanilide; 2, N-succinyl-L-phenylalanine p-nitroanilide; 3, N-benzyloxycarbonyl-L-tyrosine p-nitrophenyl ester; 4, N-acetyl-L-tryptophan p-nitrophenyl ester; 5, N-acetyl-L-tyrosine p-nitrophenyl ester. Concentrations of cosolvents (upper axes) are referred to the volume of the aqueous phase. Unfilled circles are limiting values obtained according to Eq. (4). (From Ref. 114.)

KINETICS OF SYNTHESIS AND HYDROLYSIS OF N-BENZOYL-L-TYROSINE p-NITROANILIDE BY α-CHYMOTRYPSIN IN AEROSOL OT - 2,3-BUTANEDIOL/WATER - OCTANE SYSTEM



$[AOT] = 0,1$ M
$w_0 = 0,46$
$BD/H_2O = 99/1$ v/v %
$[CT] = 0,2$ M
pH = 9



APPLIED AREAS OF MICELLAR ENZYMOLOGY

BIOCHEMISTRY AND PROTEIN CHEMISTRY

1. METHODS AND TOOLS
2. SUPRAMOLECULAR DESIGN

(FINE) ORGANIC SYNTHESIS

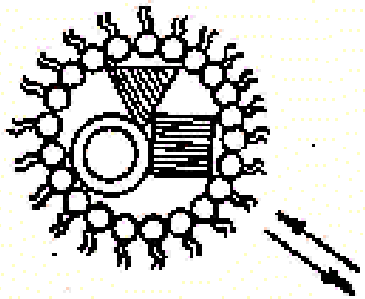
1. CONVERSION OF WATER-INSOLUBLE COMPOUNDS
2. SHIFT OF EQUILIBRIUM

CHEMICAL AND CLINICAL ANALYSIS

1. DETERMINATION OF WATER-INSOLUBLE COMPOUNDS
2. ENZYME IMMUNOASSAY
3. SIGNAL/MINESCENT ASSAY (USING FIREFLY LUCIFERASE)

MEDICINE (THERAPY)

1. MEDICINES FOR OUTWARD APPLICATIONS
2. DRUG CARRIERS, NANOPARTICULES
3. MEMBRANOTROPICS (FOR CELL PENETRATION)



Applied areas of Micellar Enzymology

1. **Fine organic synthesis**

   - **Equilibrium** shift
   - **Conversion** of water-insoluble compounds

2. **Chemical and biochemical analysis**

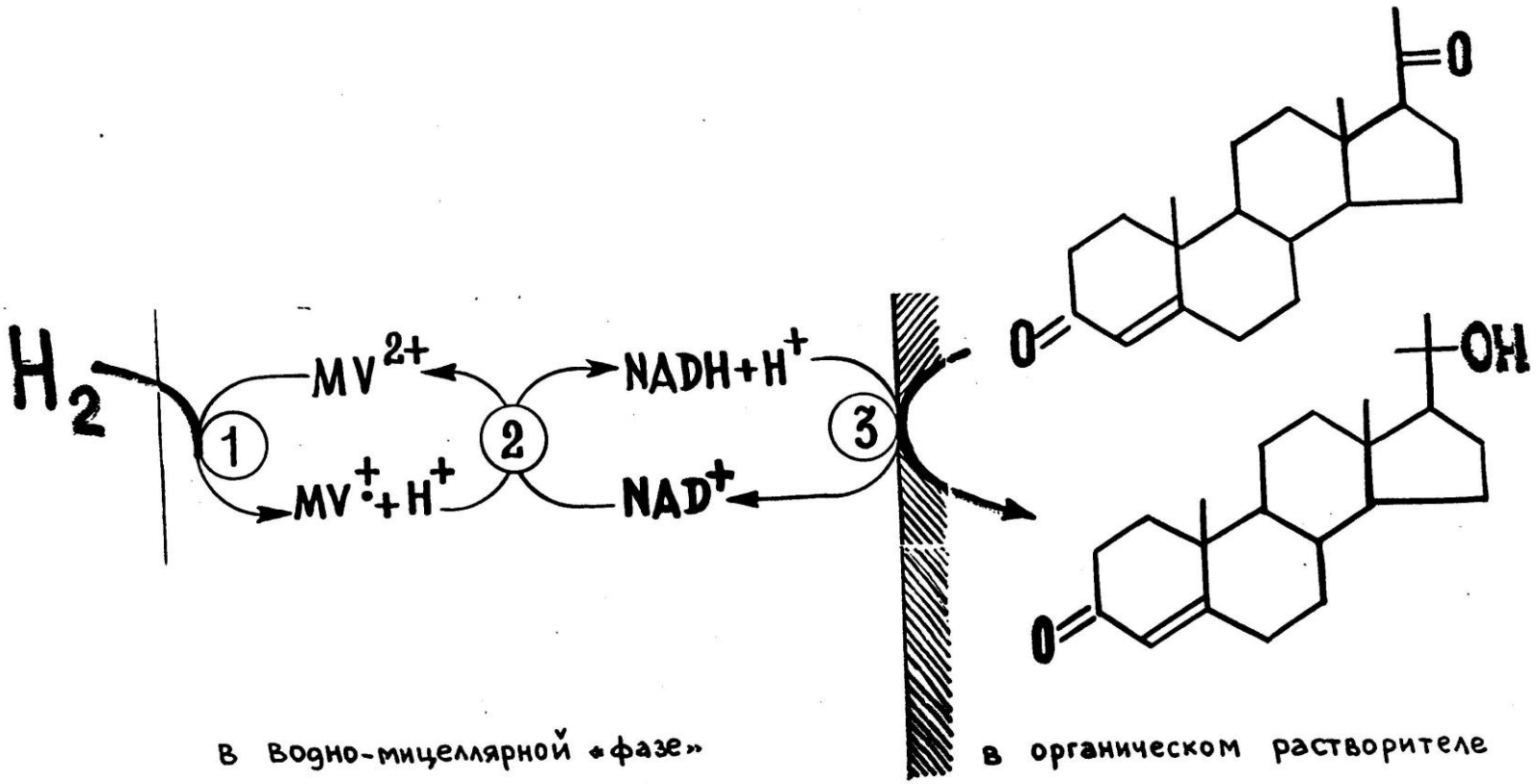
   - **Improved** traditional systems
   - **Detection** of hydrophobic compounds
   - **Enzyme** immunoassay

3. **Protein (enzyme) chemistry**

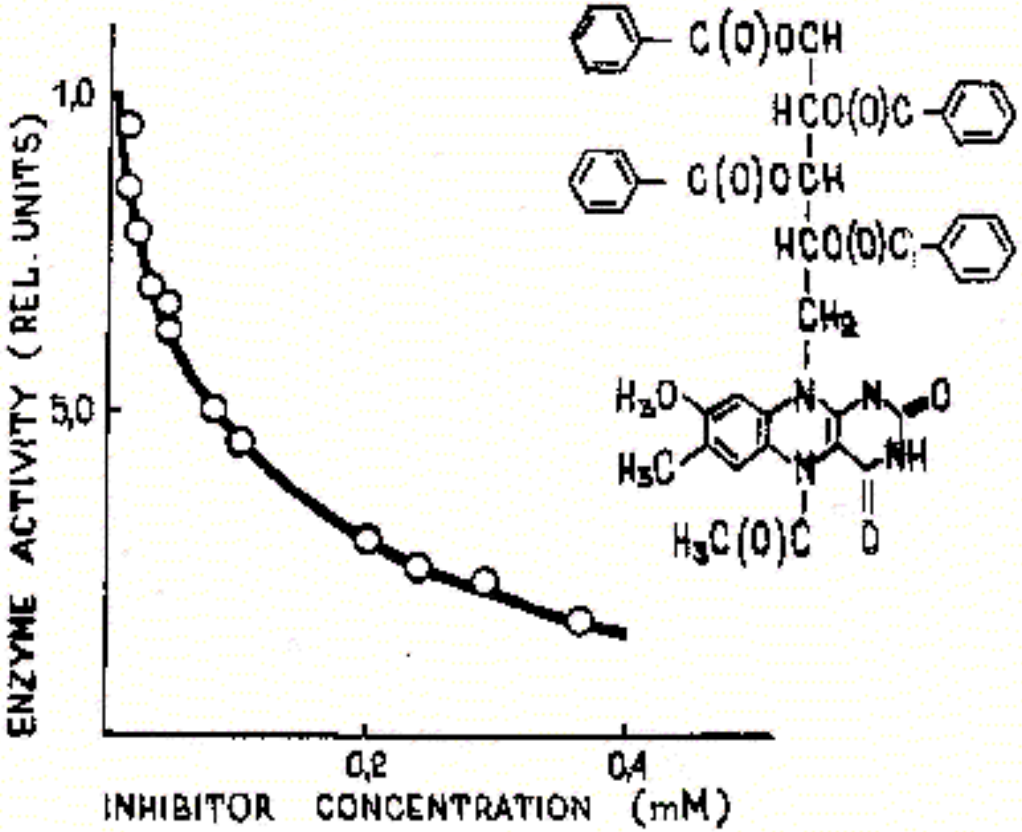
   - **Protein (enzyme) isolation** and purification
   - **Chemical** modification (hydrophobization & hydrophilization)
   - **Supramolecular** (enzyme) design
   - **Formation** of protein(enzyme) -containing nanoparticles

AREAS OF INFLUENCE IN MICELLAR SYSTEMS:

- Micellar matrix
- Protein (enzyme) molecule
- External (bulk) water- immiscible solvent
- Internal water-miscible solvent


H2
MV2+
MV+·+H+
NADH+H+
NAD+
1
2
3
O
O
OH
в водно-мицеллярной «фазе»
в органическом растворителе

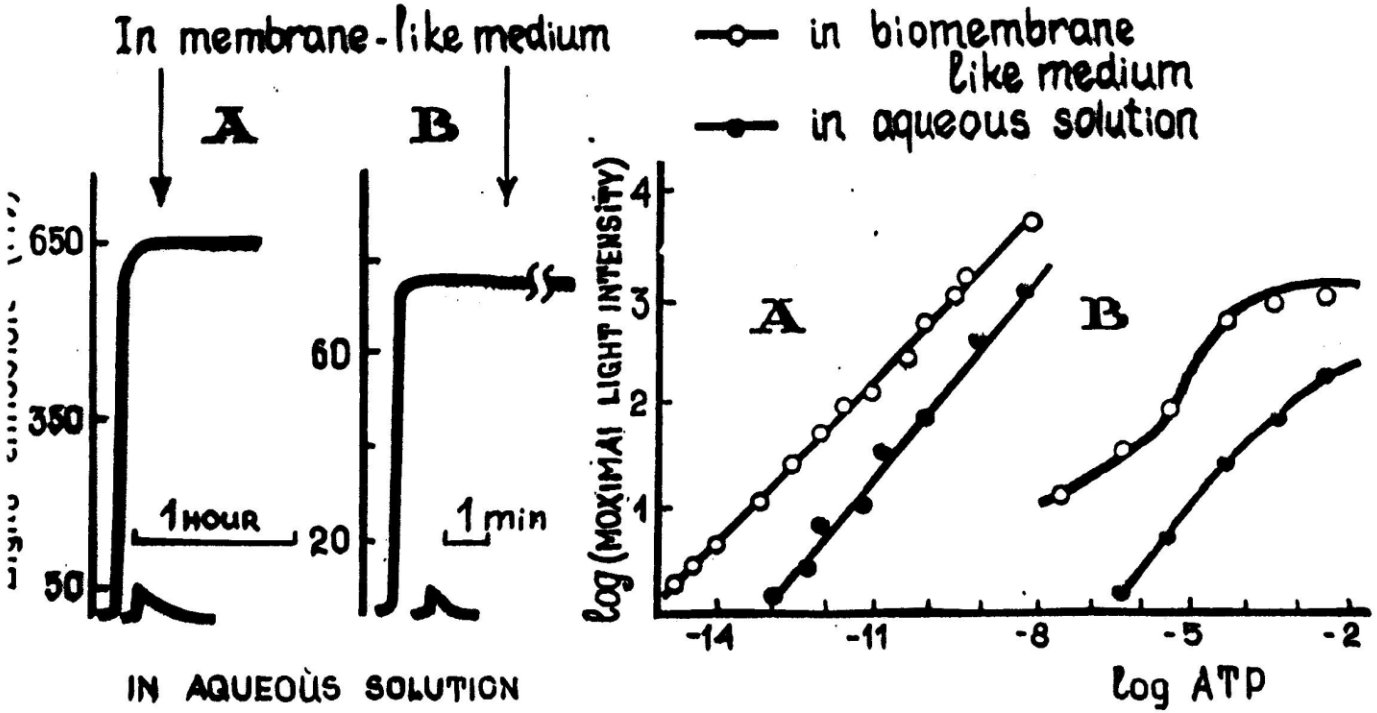
LIPOXYGENASE INHIBITION WITH RIBOFLAVIN ANALOG IN THE REVERSED MICELLES



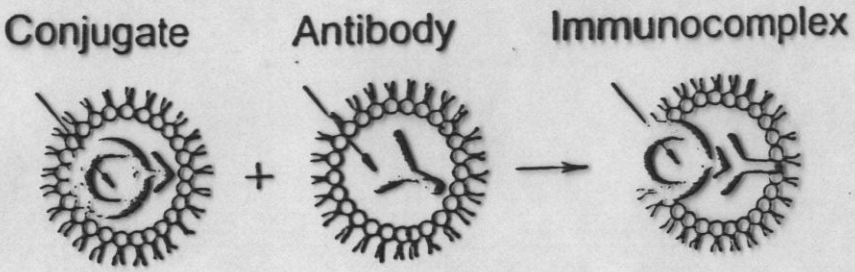
Kurganov et. al (1985) J. Biochem. Biophys. Methods

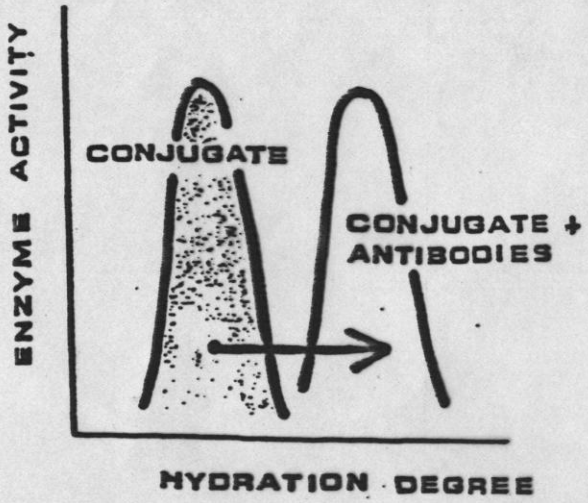
BIOLUMINESCENT ASSAY
with firefly *Luciola mingrelica* luciferase

$$ATP + luceferin + O_2 \xrightarrow{luciferase} AMP + PP + CO_2 + oxyluciferin + h\nu$$

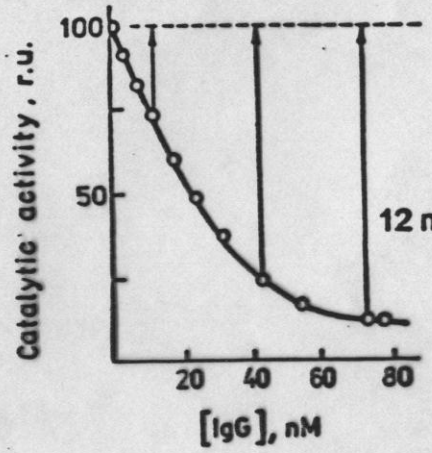


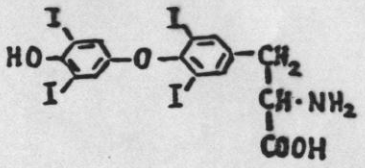
HOMOGENEOUS IMMUNOASSAY IN REVERSED MICELLAR SYSTEMS



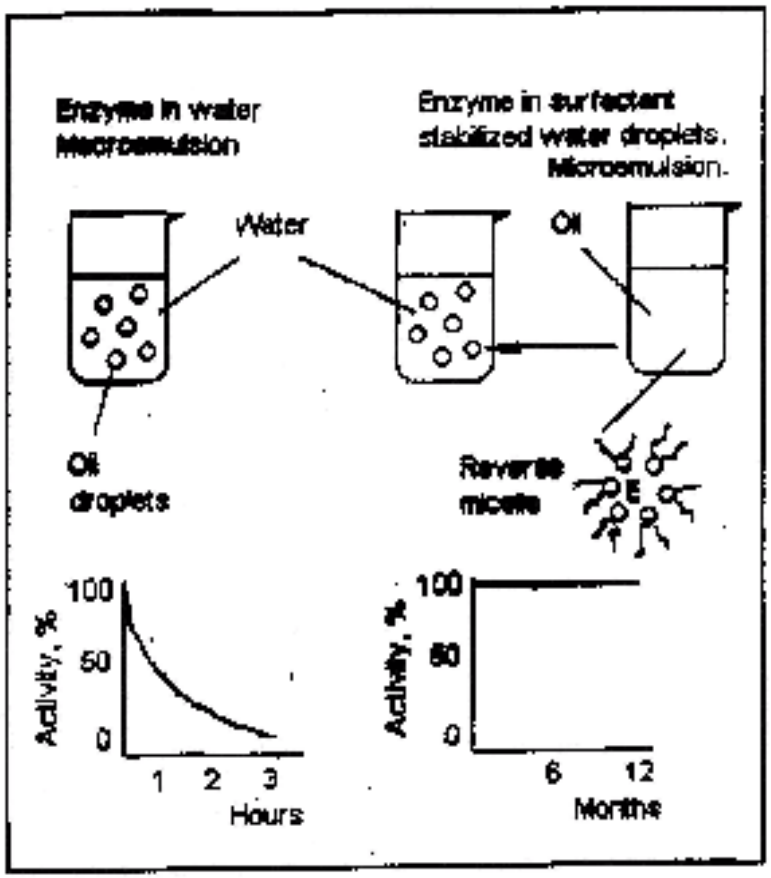


0.1 M AOT, [HRP-Thyroxine]=0.55 nM, wo=15





When thyroxin-specific antibodies are added to a peroxidase-thyroxine conjugate solubilized in reversed micelles, the immunocomplex is formed (differs in size from initial enzyme-antigen conjugate) which leads to an alteration of the enzymatic activity profile (the importance of geometric fit of protein molecule and inner cavity of micelle). Measuring the catalytic activity of peroxidase (in conjugate with antigen) at fixed optimal for conjugate hydration degree one can see the catalytic activity decreasing. Addition of free thyroxine into the system causes the dissociation of the complex with restoration of the catalytic activity. The sensitivity of the assay procedure can be optimized by adjusting the size of reversed micelles.

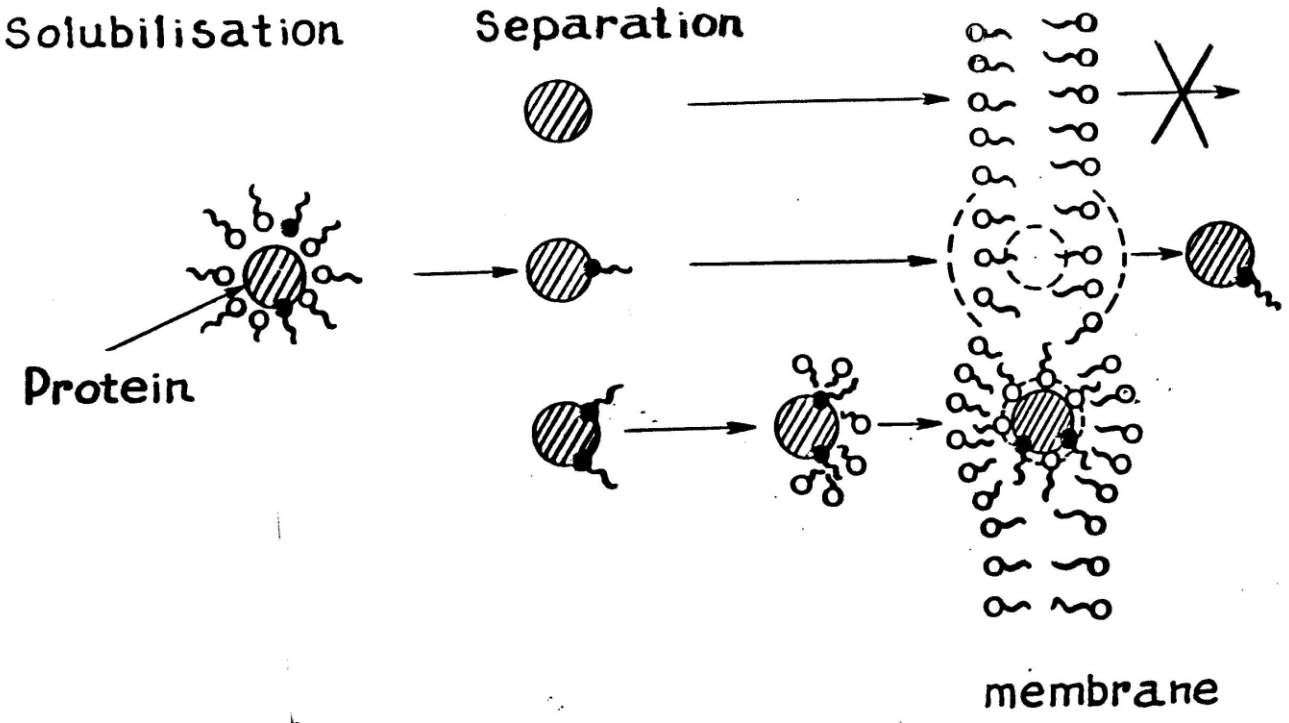


COLLAGENASE STABILITY DEPENDING ON THE WAY OF THE ENZYME ENTRAPMENT INTO MEDICAL FORMULATIONS FOR OUTWARD APPLICATIONS:

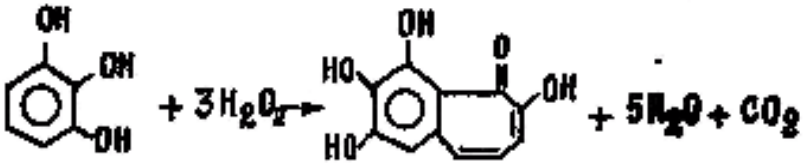
A – Macroemulsion (aqueous solution of enzyme and oil droplets);

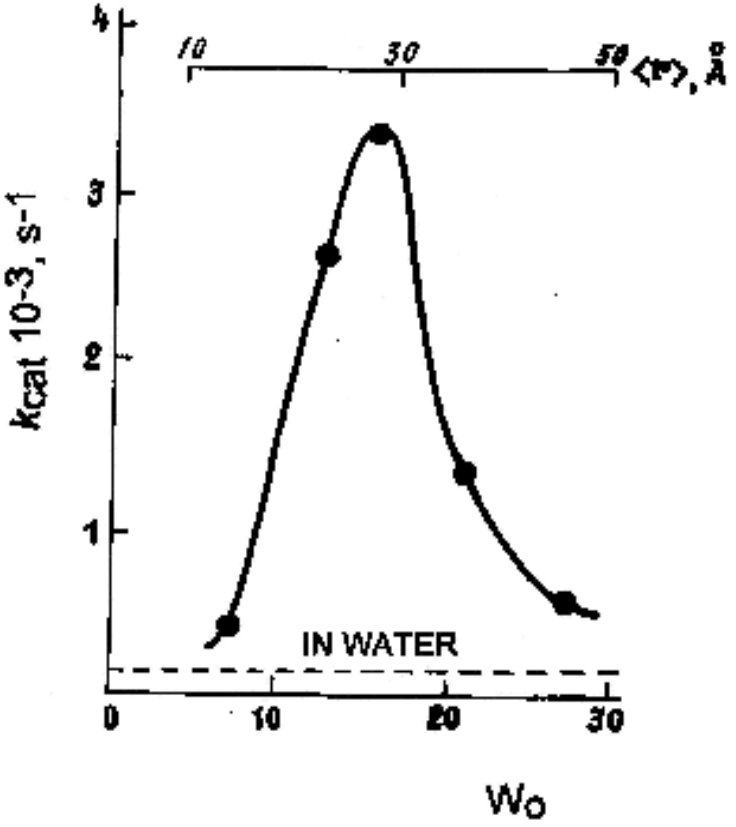
B – Microemulsion (enzyme-containing reverse micelles in oil macrophase) followed by macroemulsion.

Hydrophobization of protein by Fatty acid in the reversed micelles



REGULATION OF THE CATALYTIC ACTIVITY OF HORSERADISH PEROXIDASE IN REVERSED MICELLES OF AOT IN OCTANE BY HYDRATION DEGREE

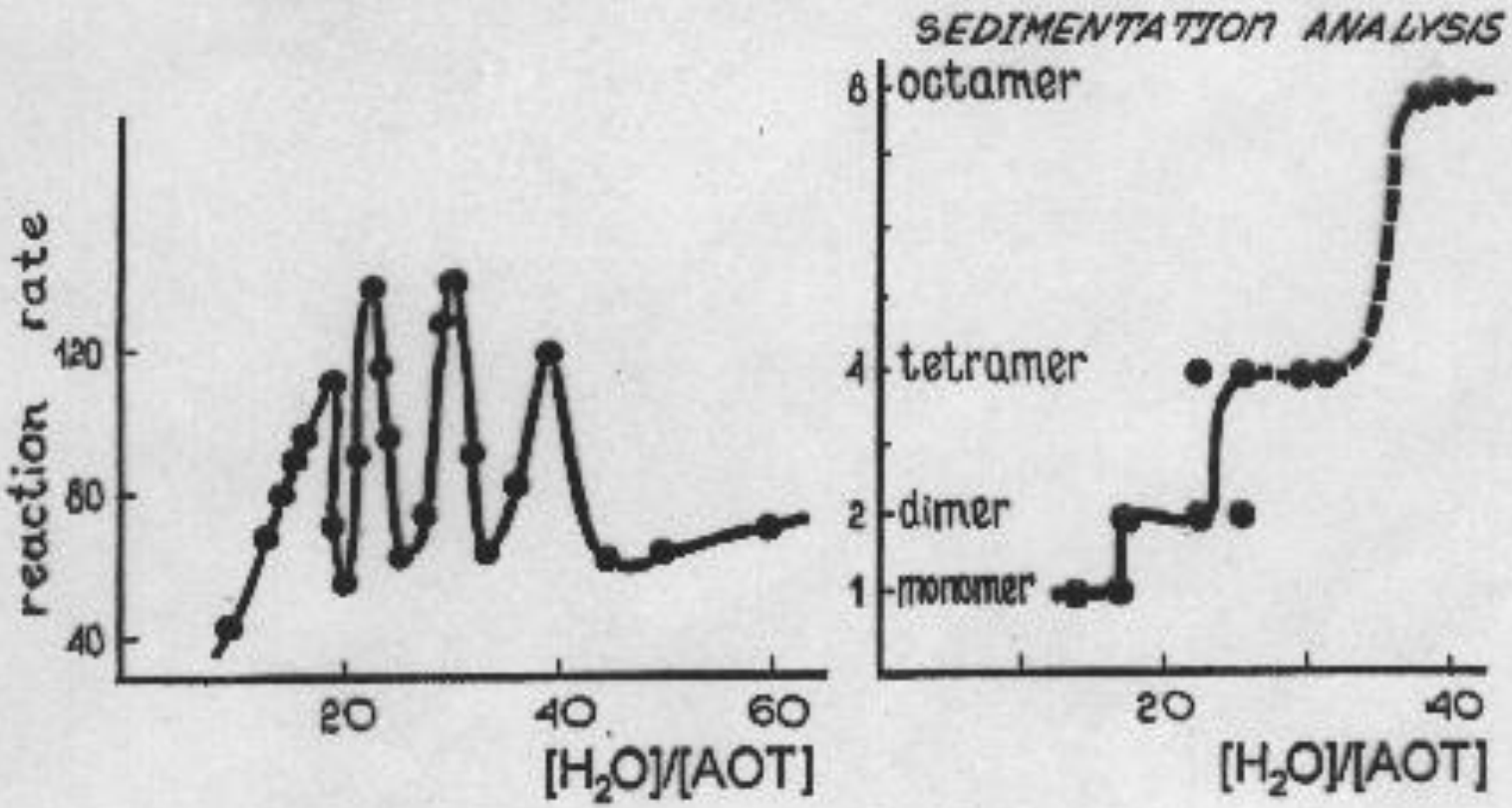




N.L.Klyachko, A.V.Levashov, K.Martinek (1984) Mol. Biol. (Russ.) 18, 1019

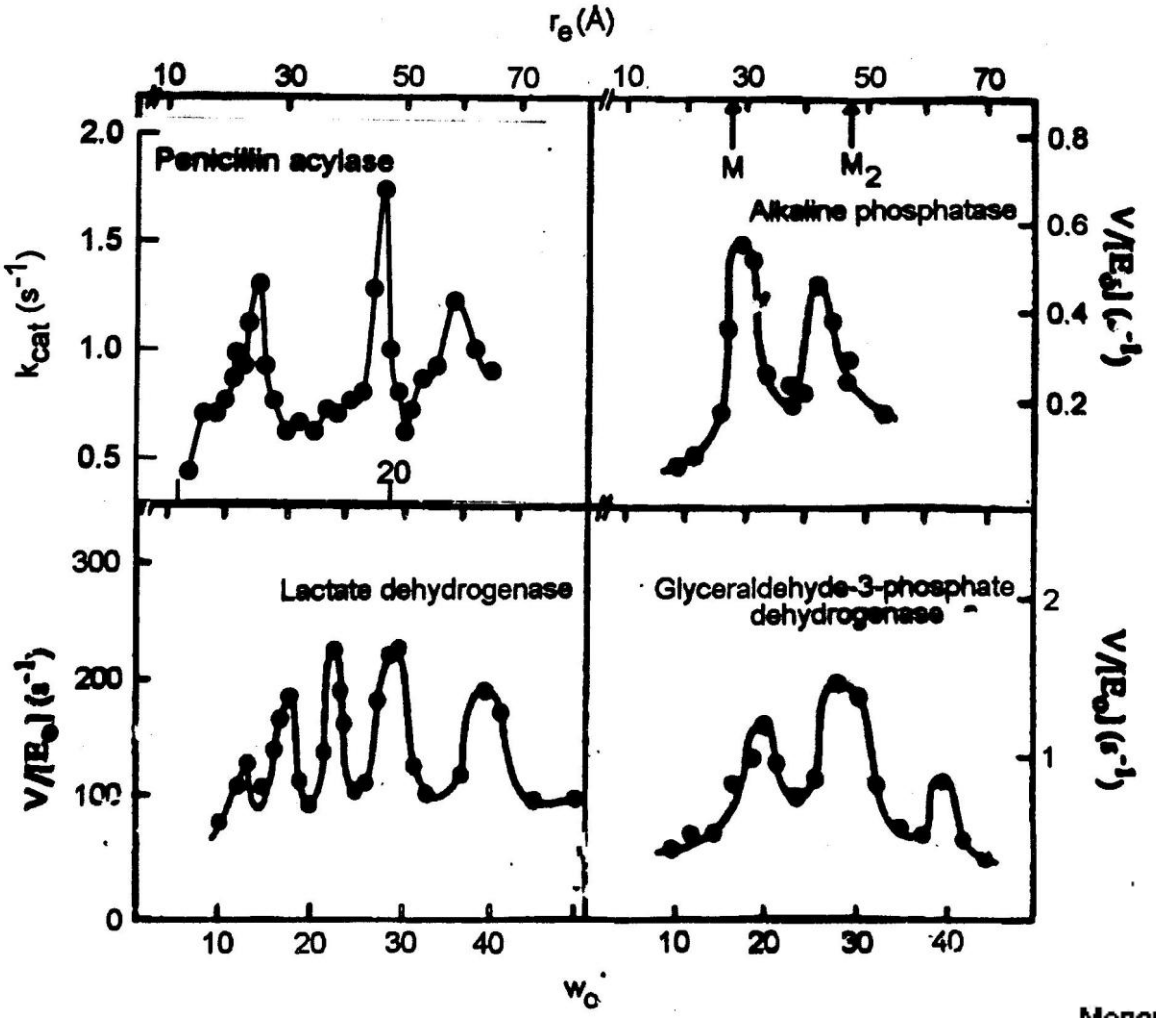
REGULATION OF THE OLIGOMERIC ENZYME

Lactatedehydrogenase
consists of four equal subunits



OLIGOMERIC ENZYMES IN REVERSED MICELLES OF AEROSOL OT IN OCTANE.

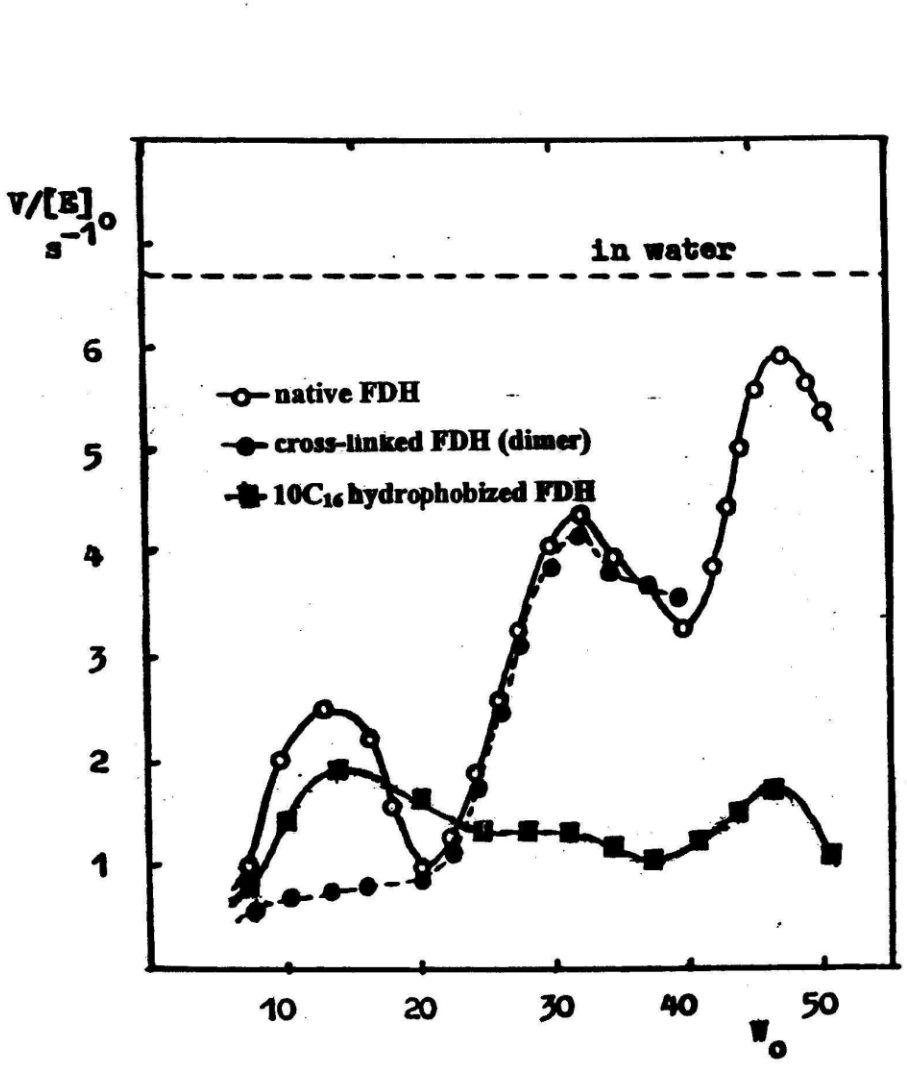
CATALYTIC ACTIVITY PROFILES VS HYDRATION DEGREE (INNER CAVITY RADIUS).

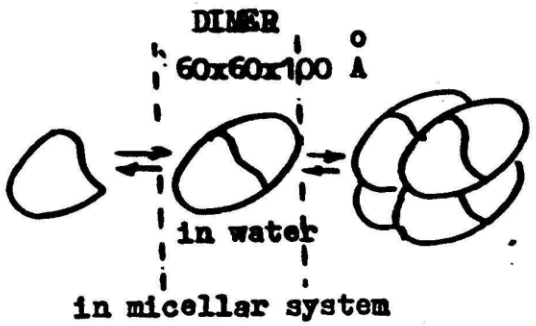


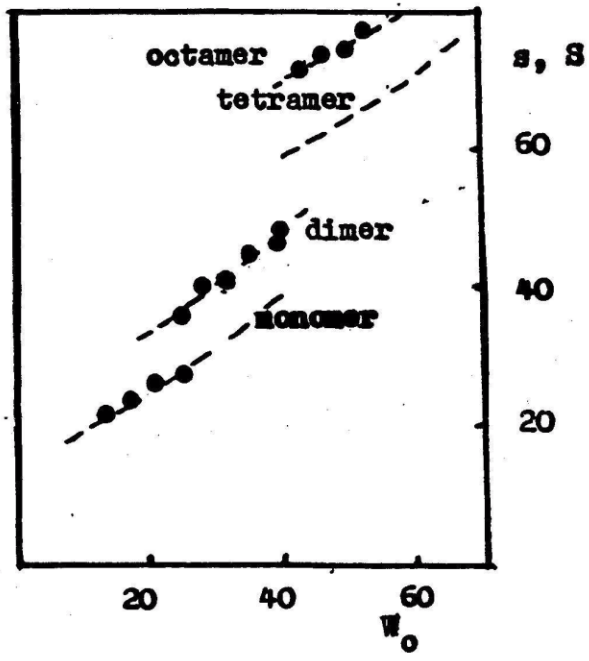
First optima for GAPDH and LDH

	GAPDH			LDH		
	M, kDa	w_o	r, A	M, kDa	w_o	r, A
Monomer	34	21	35.5	37	14	25

Catalytic activity and supramolecular structure of formate dehydrogenase from *Pseudomonas sp.101* in the system of reverse micelles

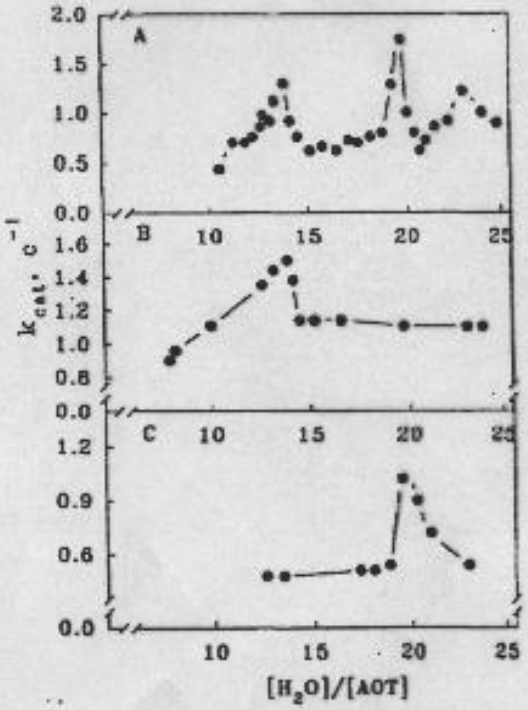




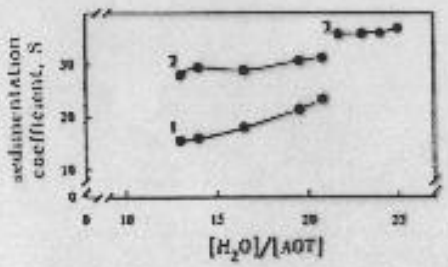


REGULATION OF THE SUPRAMOLECULAR STRUCTURE AND THE CATALYTIC ACTIVITY OF PENICILLIN ACYLASE FROM E.COLI IN THE SYSTEM OF REVERSED MICELLES OF AEROSOL OT IN OCTANE

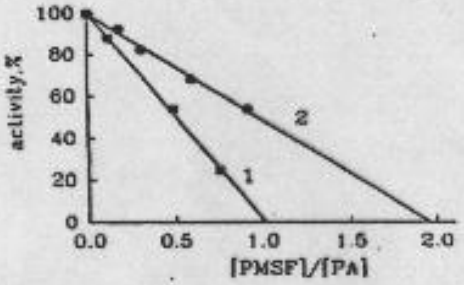
V.E. Kabakov et al. FEBS Letters (1992) v.311, No 3, 209-212



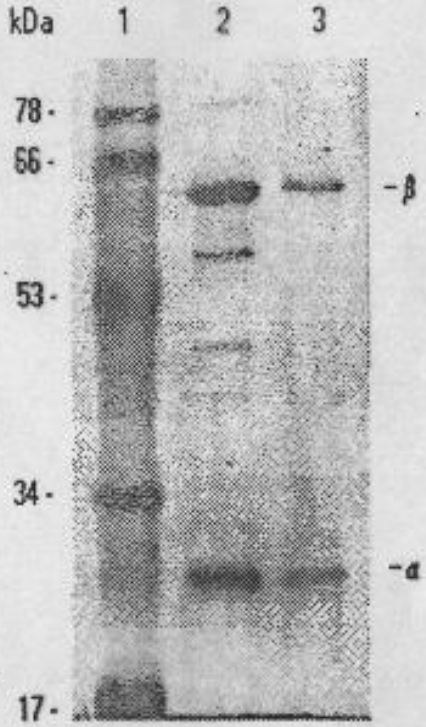
The dependence of the catalytic activity (k_{cat}) of PA on the hydration degree, w_0, in the AOT RM system in octane. (A) Intact preparation of PA. (B) α-subunit preparation. (C) β-subunit preparation.



The dependence of the sedimentation coefficients (S) of RM containing PA on the hydration degree. (1) α-subunit, (2) β-subunit, (3) $\alpha\beta$-dimer.



PMSF titration of PA active sites in the system of AOT RM. (1) w_0=23, (2) w_0=14. Before activity measurments were carried out the enzyme was incubated for 10 min in a range of volumes (0-10 μl) of 10 μM PMSF solution.



SDS-PAGEof PA preparations. Lanes: (1) molecular mass standards (kit LKB 1860-102), (2) the intact preparation of the enzyme, (3) sediment after 60 min centrifugation at 100,000 x g.

KINETIC AND STABILITY STUDIES OF PENICILLIN ACYLASE IN REVERSED MICELLES

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Centro de Engenharia Biológica e Química, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

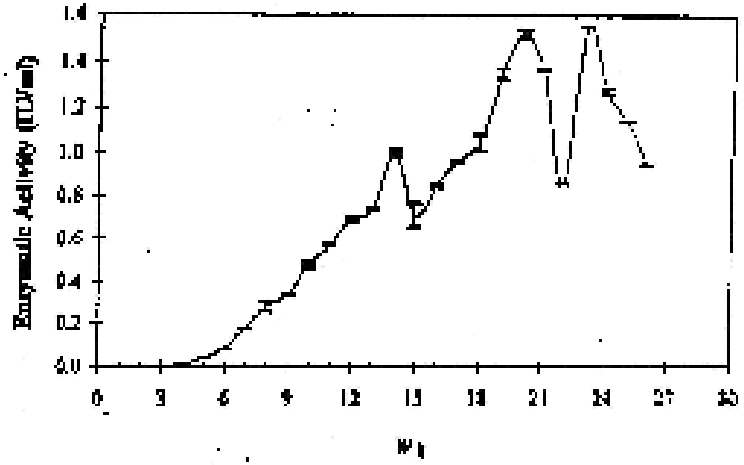


FIGURE 3 Enzymatic activity profile of penicillin acylase encapsulated in AOT reversed micelles in isooctane varying W_0 ($T = 30°C$, $[AOT] = 150mM$, $[Enzyme]_{aq} = 0.125 mg/ml$).

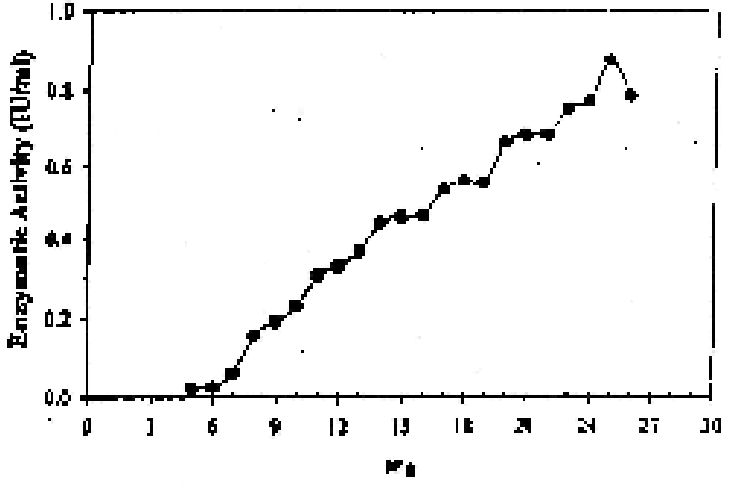
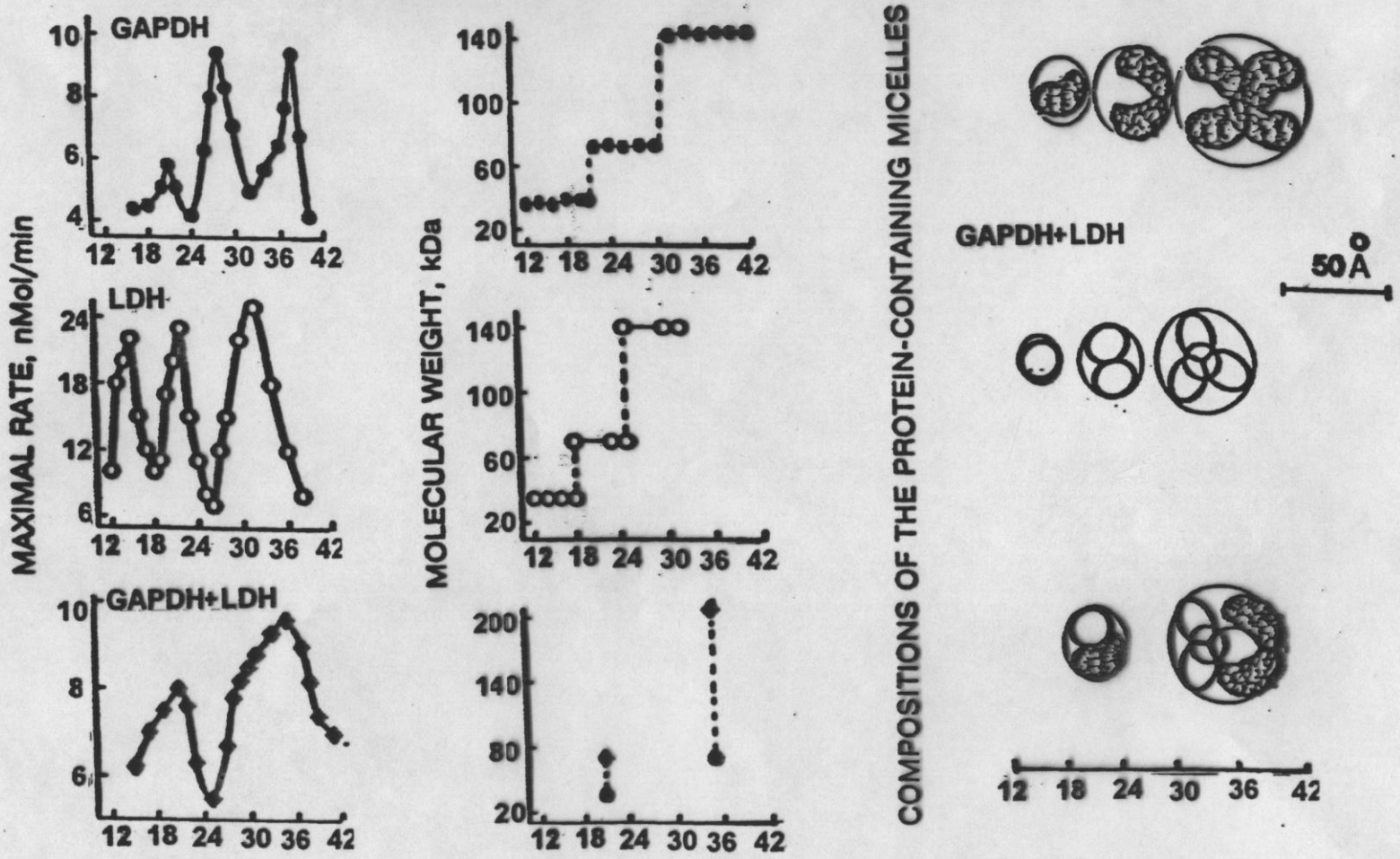
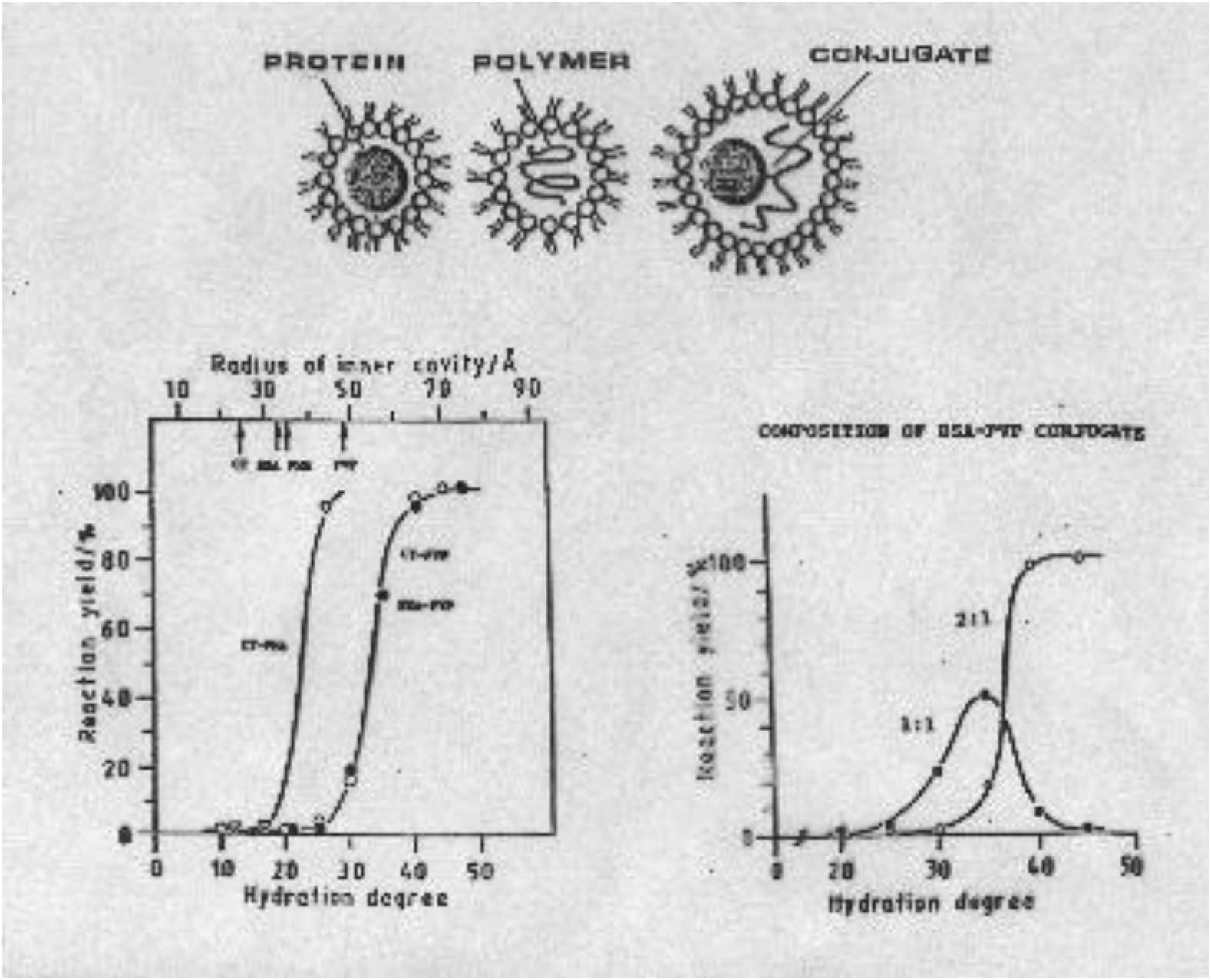


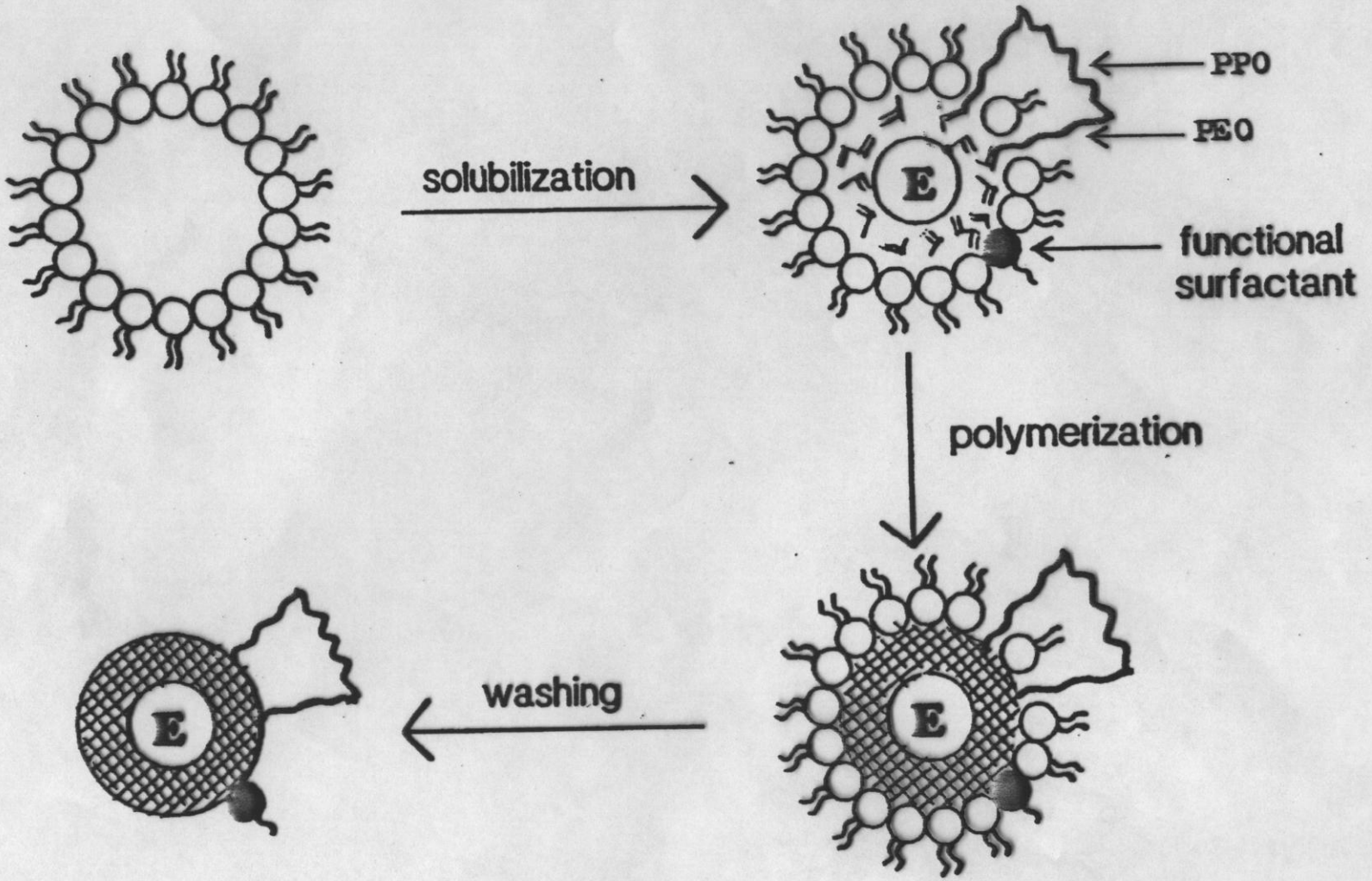
FIGURE 4 Enzymatic activity profile of partially denatured penicillin acylase encapsulated in AOT reversed micelles in isooctane versus W_0 ($T = 30°C$, $[AOT] = 150mM$, $[Enzyme]_{aq} = 0.332mg/ml$, % Deactivation = 66%).

GLYCERALDEHYDE-3-PHOSPATE DEHYDROGENASE AND LACTATE DEHYDROGENASE IN REVERSE MICELLES: HOMO AND HETEROOLIGOMERIC COMPLEXES

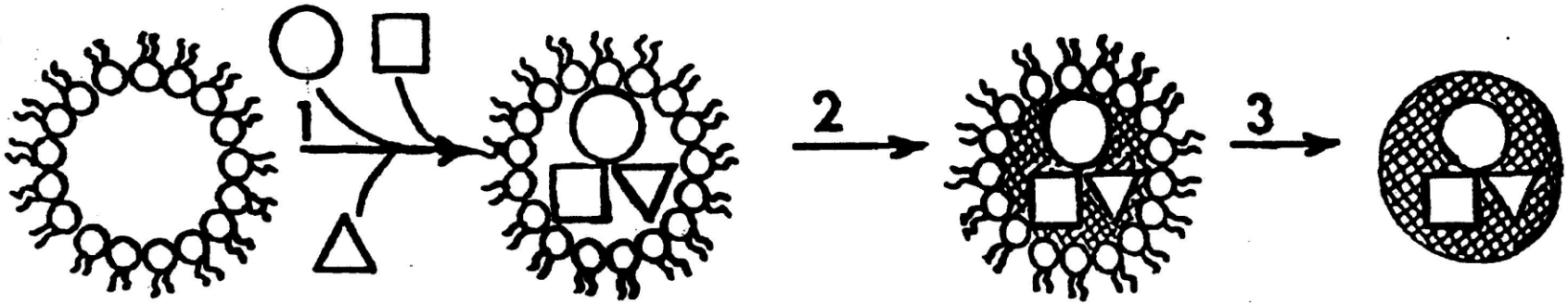



PROTEIN
POLYMER
CONJUGATE
Radius of inner cavity/Å
10
30
50
70
90
Reaction yield/%
100
80
60
40
20
0
0
10
20
30
40
50
Hydration degree
COMPOSITION OF BSA-PVP CONJUGATE
Reaction yield/%
100
50
0
2:1
1:1
0
20
30
40
50
Hydration degree

PREPARATION OF ENZYME-CONTAINING NANOPARTICLES



SCHEME OF PREPARATION OF NANOPARTICLES AND MACROMOLECULAR CONJUGATES BY USING REVERSED MICELLES OF SURFACTANTS

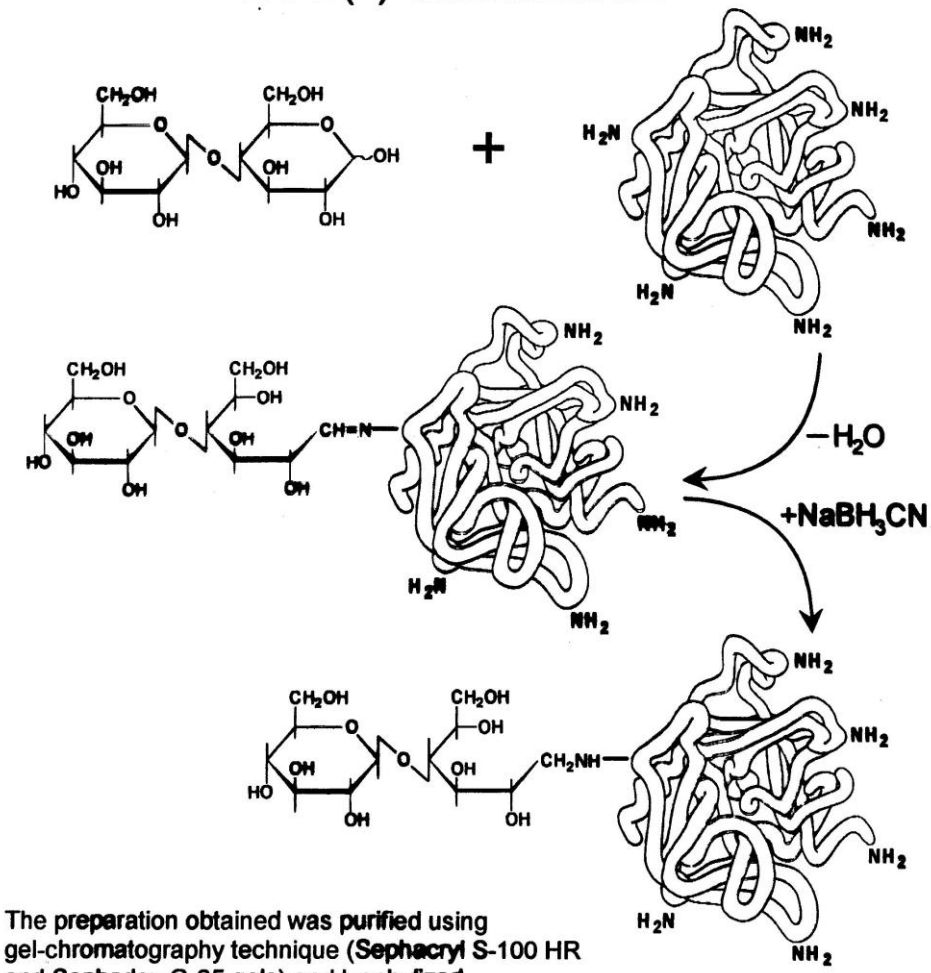


1 – solubilization–construction,

2 – chemical fixation (cross–linking by bifunctional reagents or polymerization)

3 – washing from surfactant.

MODIFICATION OF α–CHYMOTRYPSIN BY D(+)-CELLOBIOSE

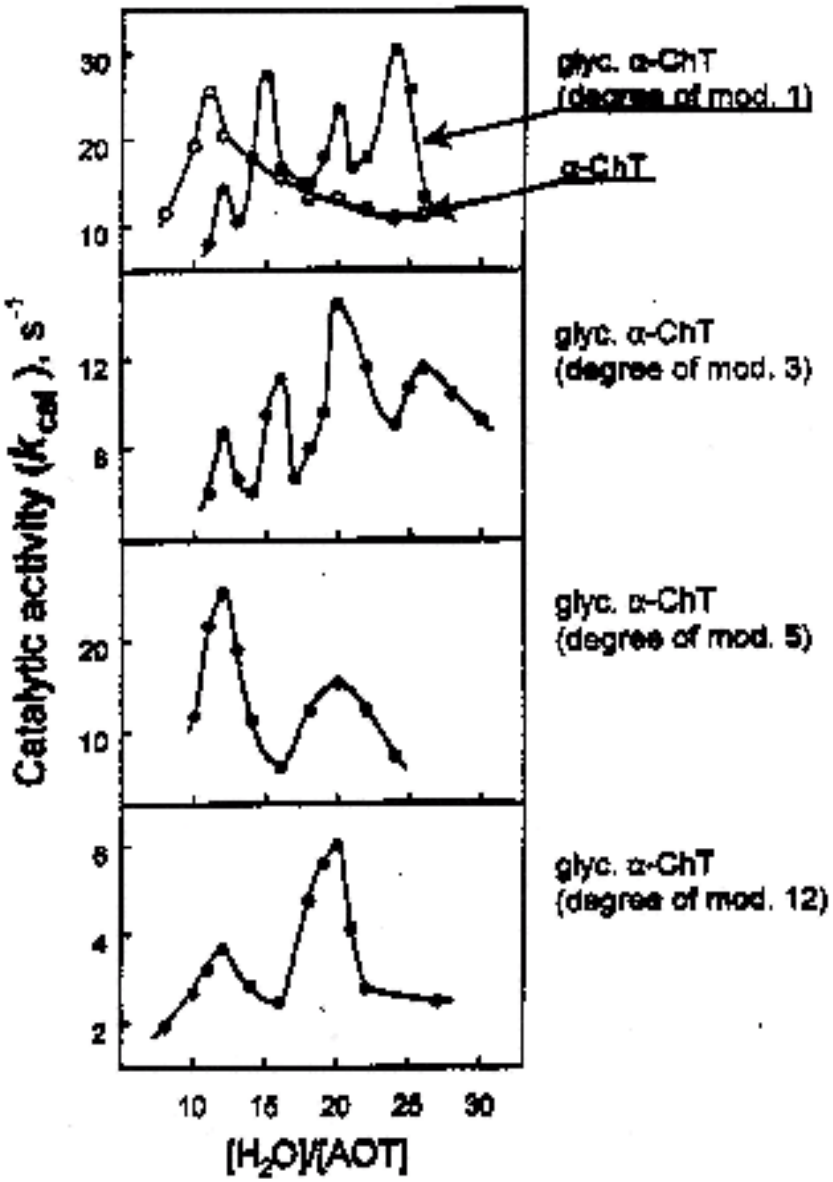


The preparation obtained was purified using gel-chromatography technique (Sephacryl S-100 HR and Sephadex G-25 gels) and lyophylized.

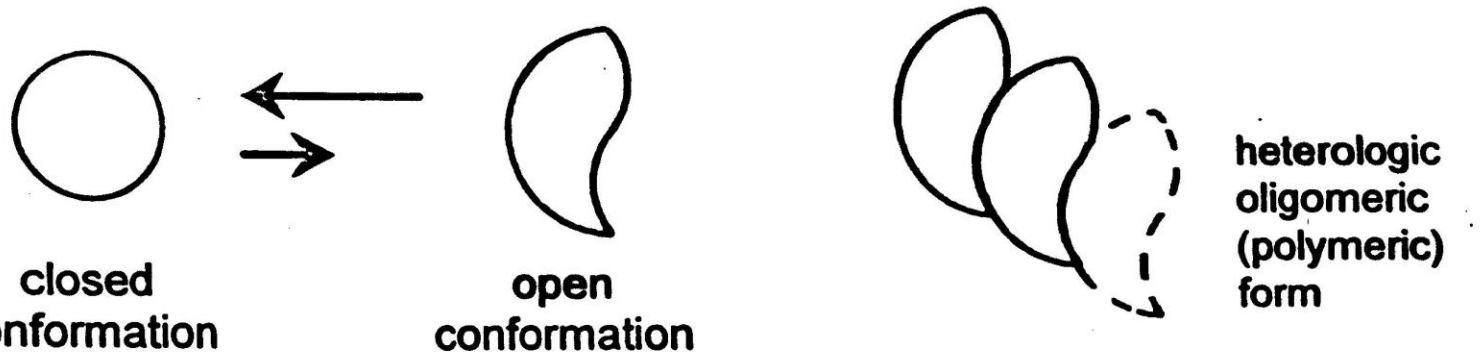
Physico-chemical properties of glycosylated α-chymotrypsin:

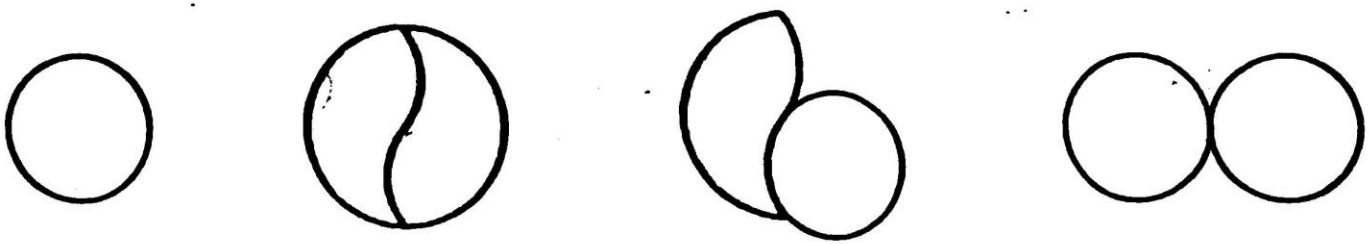
1. A number of preparations with degrees of modification from 1 to 12 was obtained.
2. According to SDS PAAG-electroforesis the preparation had one narrow band close to 25 kDa.
3. The catalytic activity of modified enzymes was not less than 50% from native one according to standard active site titration procedure.

REGULATION OF THE CATALYTIC ACTIVITY OF NATIVE AND ARTIFICIALLY GLYCOSYLATED α-CHYMOTRYPSIN WITH DIFFERENT DEGREE OF MODIFICATION IN REVERSED MICELLES OF AEROSOL OT IN OCTANE



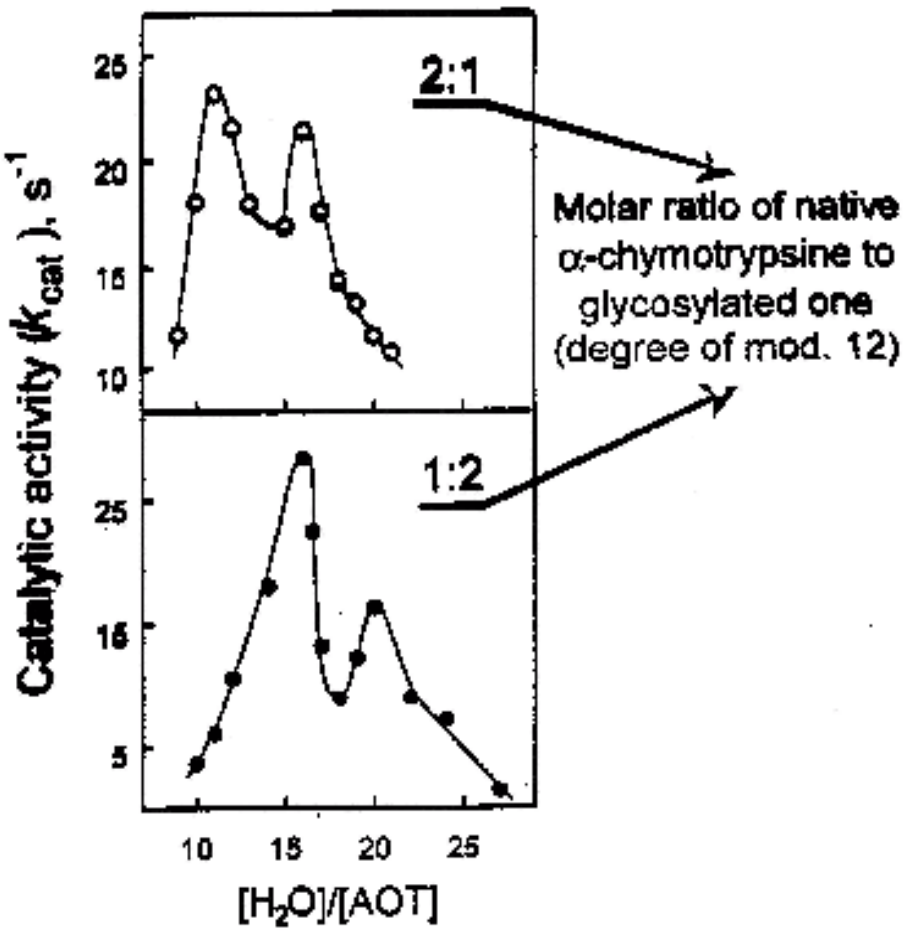
DIFFERENT TYPES OF α-CHYMOTRYPSIN DIMERS AND POSSIBLE MECHANISM OF THEIR FORMATION





r, Å		20.5	25.8	33.4	41.0
$\frac{[H_2O]}{[AOT]}$	calc.	11.0	14.5	19.5	25.0
	exp.	11.0	16.0	20.0	

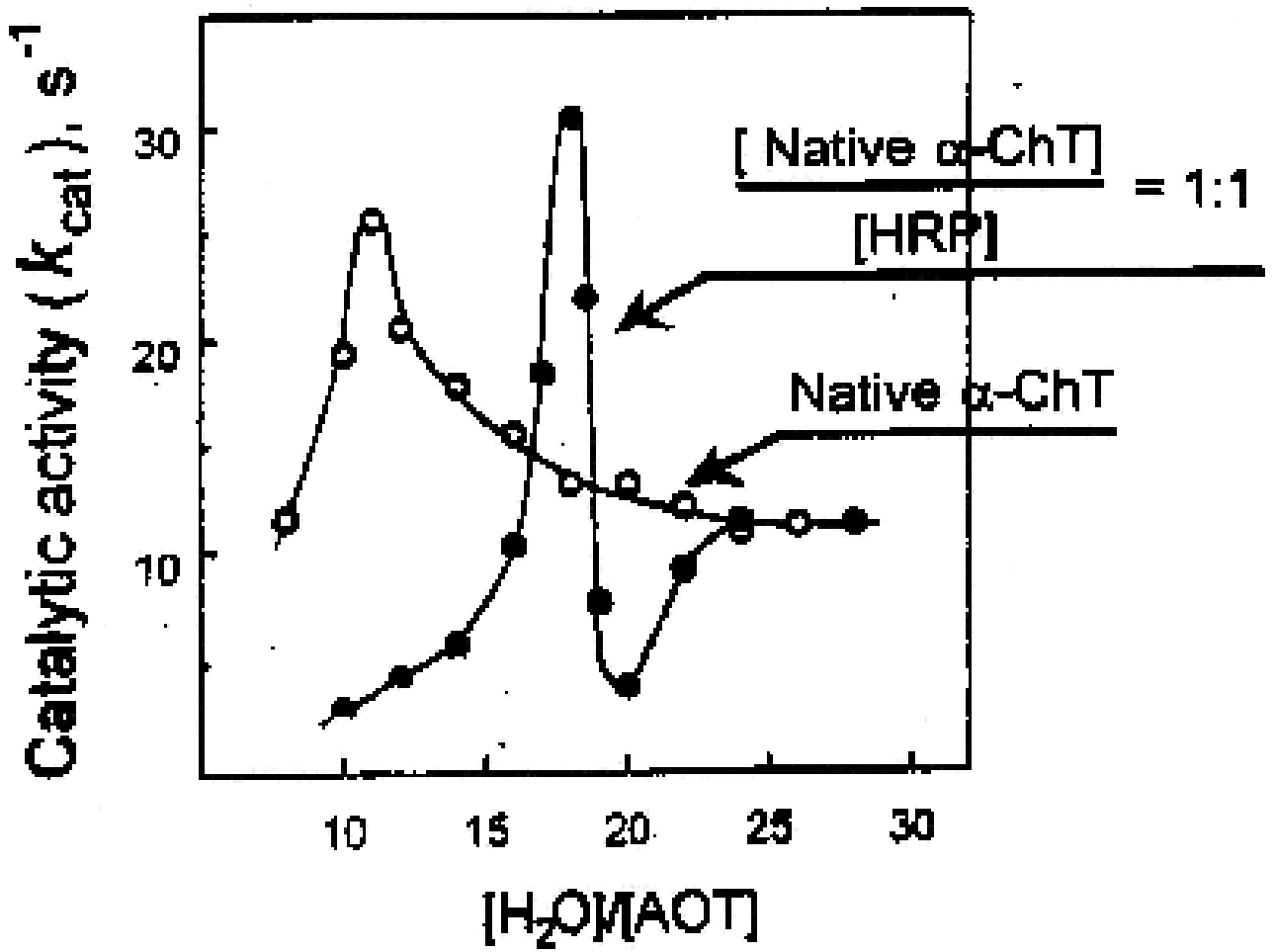
ABILITY OF NATIVE α-CHYMOTRYPSIN TO FORM NON-COVALENT COMPLEXES WITH ARTIFICIAL GLYCOPROTEIN (GLYCOSYLATED α-CHYMOTRYPSYN)



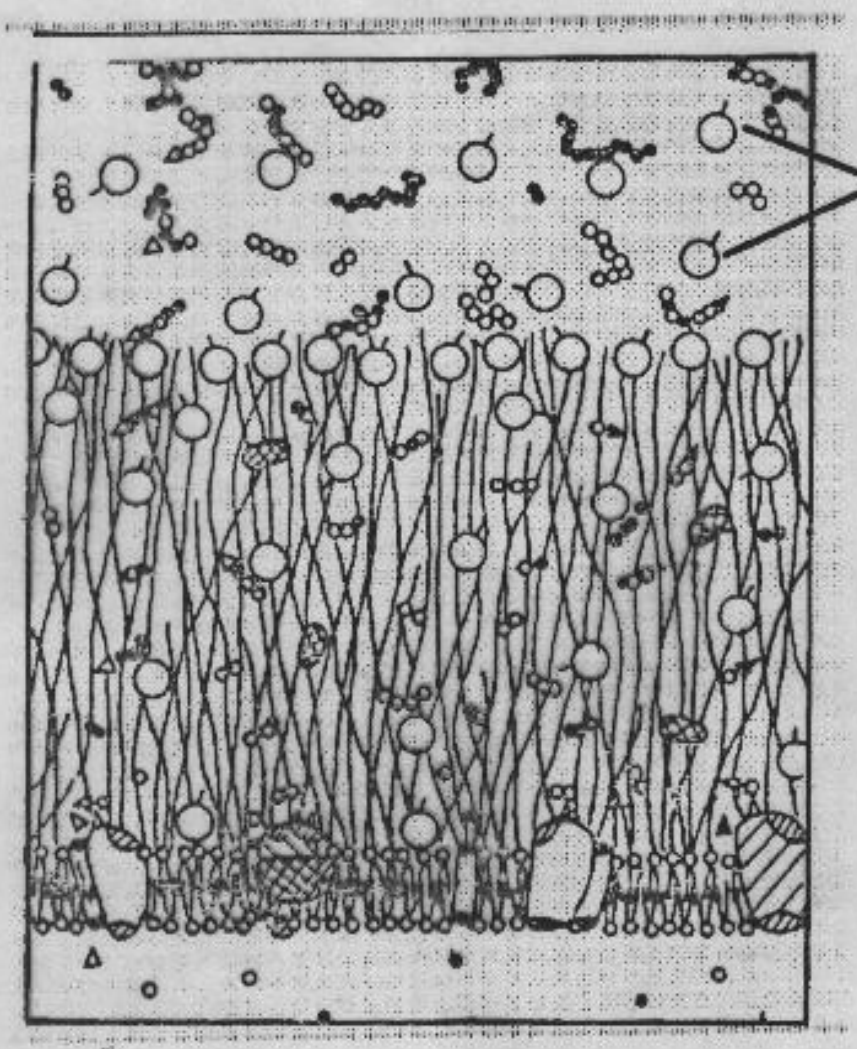
Experimental conditions:

0.1M AOT, 50 mM TRIS, pH 8.5
α-Chymotrypsin concentration in the system was 22nM.
α-ChT catalytic activity was measured with specific substrate *Succinyl-Ala-Ala-Pro-Phe p-nitroanilide.*

ABILITY OF NATIVE α-CHYMOTRYPSIN TO FORM NON-COVALENT COMPLEXES WITH NATURAL GLYCOPROTEIN (HORSERADISH PEROXIDASE)



SCHEMATIC REPRESENTATION OF INTESTINAL MEMBRANE DIGESTION



A.M. Ugolev, Membrane digestion, L.:"Nauka", 1972

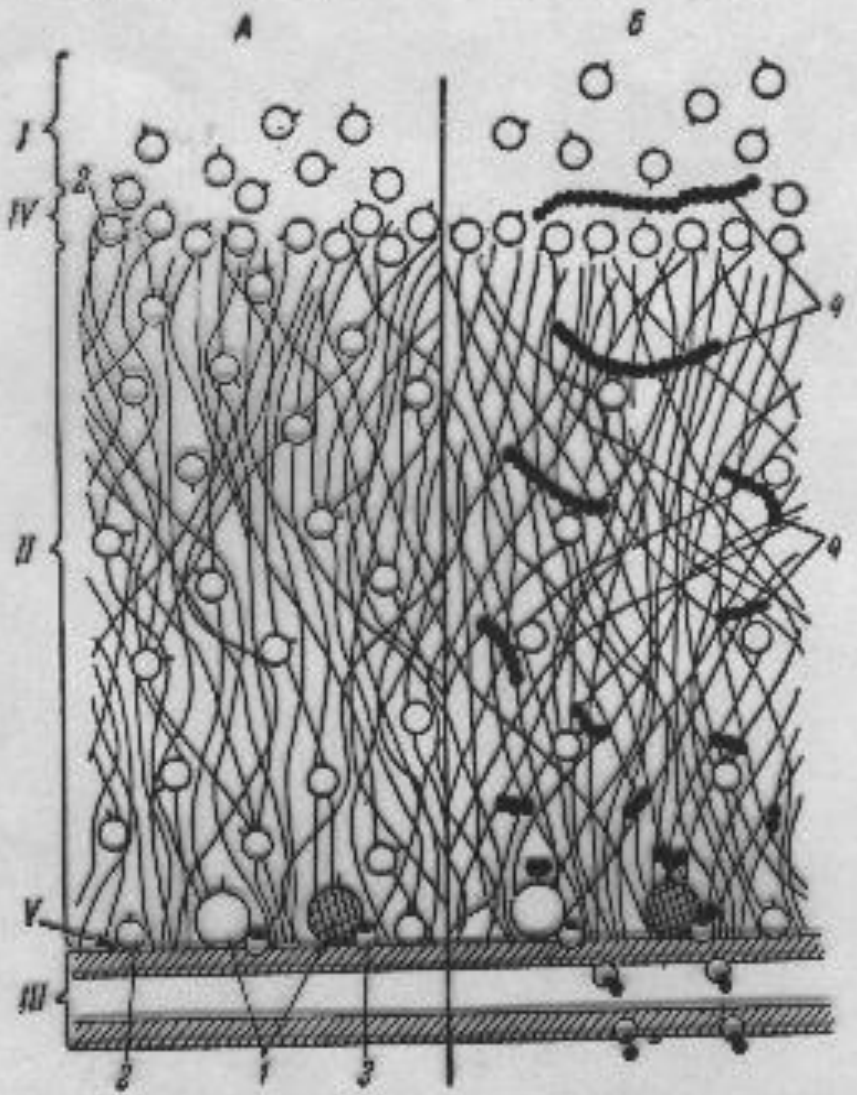
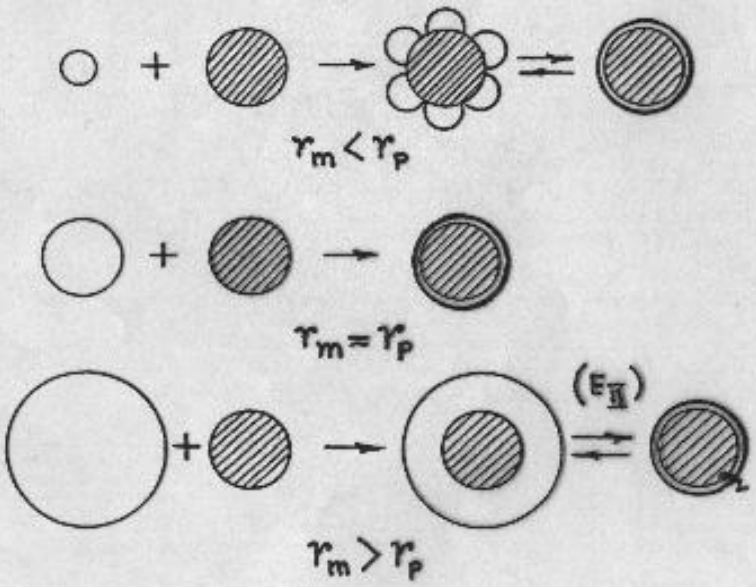
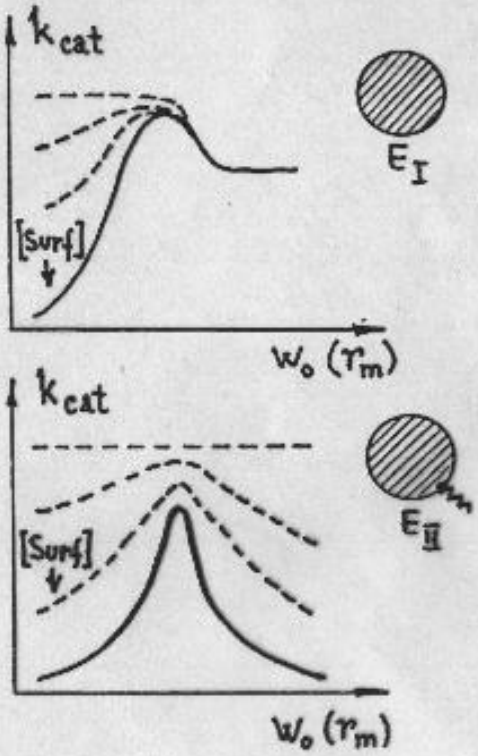


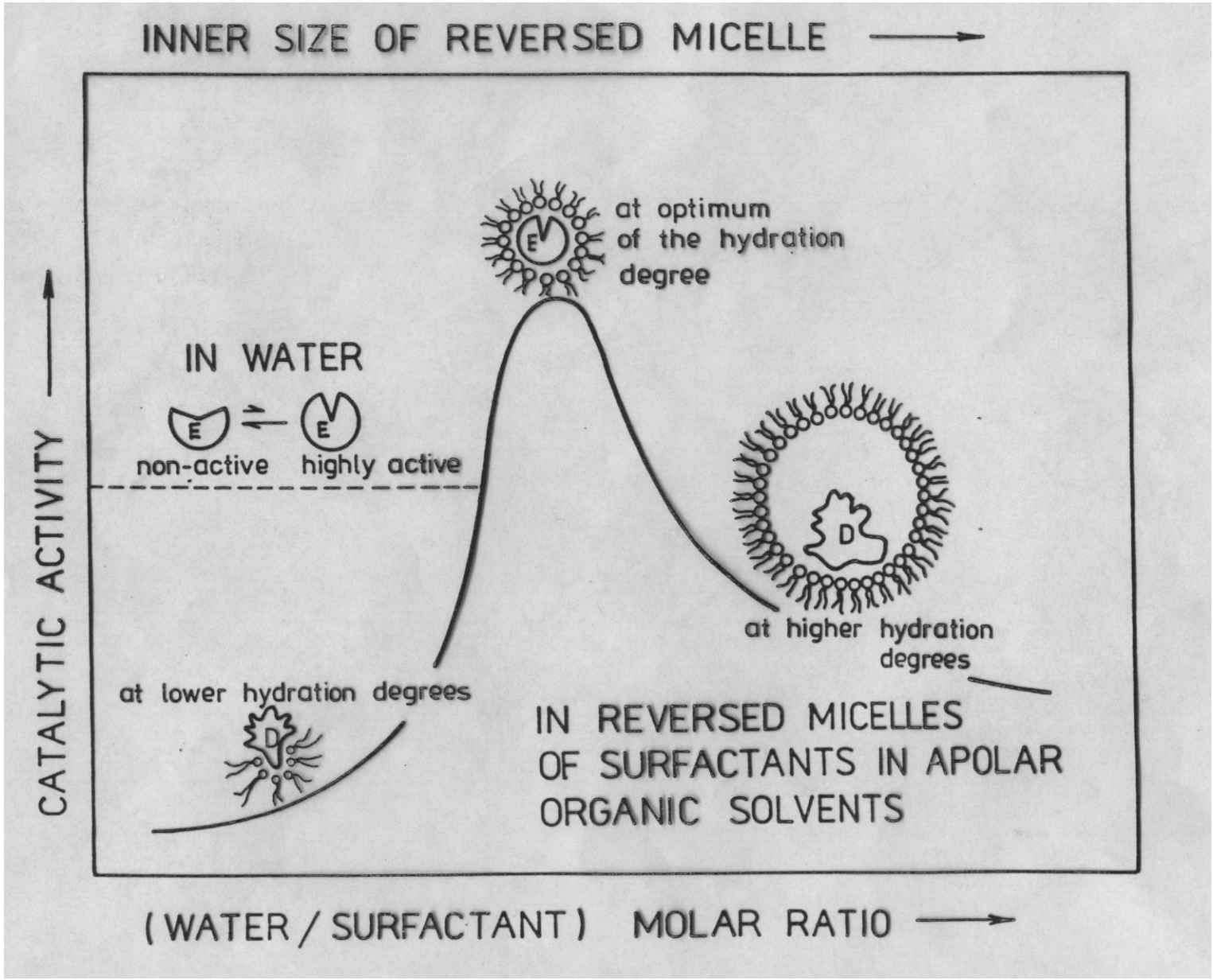
Рис. 5. Эпигеральные и адсорбированные ферменты при мембранном пищеварении (схематическое изображение фрагмента люминальной поверхности микроворсинки).

А — распределение ферментов; Б — взаимоотношение ферментов, переносчиков и субстратов. I — полость; II — гликокаликс; III — трехслойная мембрана; IV — люминальная поверхность гликокаликса; V — люминальная поверхность трехслойной мембраны. 1 — собственно кишечные ферменты; 2 — адсорбированные ферменты; 3 — переносчики; 4 — субстраты.

MODES OF PROTEIN-CONTAINING MICELLES FORMATION AND THEIR KINETIC MANIFESTATION






INNER SIZE OF REVERSED MICELLE
CATALYTIC ACTIVITY
(WATER / SURFACTANT) MOLAR RATIO
at optimum of the hydration degree
IN WATER
non-active
highly active
at higher hydration degrees
at lower hydration degrees
IN REVERSED MICELLES OF SURFACTANTS IN APOLAR ORGANIC SOLVENTS

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