Ферменты в неводных средах

REPORT

Colloidal Solution of Water in Organic Solvents: a Microheterogeneous Medium for Enzymatic Reactions

KAREL MARTINEK, A. V. LEVASHOV, YU. L. KHMELNITSKY, N. L. KLYACHKO, AND , I. V. BEREZIN Authors Info & Affiliations

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Abstract

To simulate in vitro the conditions under which enzymes act in vivo, enzyme molecules have been entrapped in hydrated reverse micelles of a surfactant in organic solvents. In this system the catalytic activity of one of the enzymes studied (peroxidase) became much higher than in water, and the specificity of the other enzyme (alcohol dehydrogenase) was dramatically altered.

bodies against antigens available only in femtomole quantities, even in the early steps of the purification of mixtures. Although immunization of spleen cells in vitro is an effective way of defining the maximum repertoire of potential antibody responses in a given animal or strain (*12*), practical limitations, including the poor survival capacity of clones generated in vitro, have largely prevented the use of this technique for construction of antibody "libraries." The addition of factors produced by thymic cells (in this study provided as conditioned medium from heterologous thymus cell suspensions) aids in the generation of antibody-secreting lymphoblasts in vitro (*4*) and in the survival of specific hybridomas derived from such cultures (*2*). For antigens available in extremely short supply, fusion frequencies must be maximized so that the relatively small number of clones responding to a given antigen will have a sufficient probability of survival to be recovered in the form of viable hybridomas. The latter consideration led to the choice of the UCR-M3 cell line in our studies because these cells have an excellent fusion rate and produce stable hybrids, although they have the disadvantage of being secretors of the MPC-11 myeloma IgG.

Many biologically active antigens would probably be amenable to studies similar to those we described. Together with other high-efficiency methodology such as HPLC and gas-phase protein sequencing (*13*), this would appear to provide a new approach to the isolation and chemical characterization of peptides present in only picomole quantities per gram of living tissues. The approach would be to prepare a relatively purified preparation of the antigen (having unambiguously detectable biological activity in a specific bioassay), to produce highly specific monoclonal antibodies against that antigen as shown by the bioassay, and then to purify the antigen by large-scale affinity chromatography; this would be followed by HPLC, ascertaining the amino acid composition on less than 100 pmole of the material (*14*), and determining the amino acid sequence on 1 nmole or less by gas-phase sequencing.

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References and Notes

1. One hypothalamus fragment (rat or pig) yields, by our extraction methods, 150 to 500 fmole of GRF. These numbers as well as others in the text referring to molar quantities of pure GRF are based on quantitative amino acid analyses of GRF recently isolated from human tumors. On the basis of physicochemical and biological evidence, materials from all three sources are assumed to be similar in structure and specific activity. One GRF unit is defined as the weight of an in-house preparation of purified hypothalamic rGRF (GRF reference standard) known to produce half-maximal stimulation of secretion of growth hormone in the bioassay (*3*).
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9. Immunoglobulins were isolated from hybridomas as follows. Actively growing cultures in MEM with 20 percent serum were shifted into medium containing 0.2 percent serum and glucose (4.5 g/liter), with glutamine, pyruvate, and mercaptoethanol as described above, at an initial cell density of 10^6 per milliliter. Culture was continued for 48 to 72 hours, during which the cells continued to multiply. The resulting medium was precipitated with 50 percent saturated ammonium sulfate at 4°C to isolate the immunoglobulin fraction.
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15. This work was supported by grants AD 09690-07 and AM 18811-07 from the National Institutes of Health and by the Robert J. Kleberg, Jr., and Helen C. Kleberg Foundation and the Saraimne Andrews Foundation. R.A.L. is recipient of NIH research career development award, DE 00057. We thank D. Martineau, M. K. Culkin, F. Castillo, R. Schroeder, and G. Kleeman for technical assistance.

7 June 1982; 30 August 1982

Colloidal Solution of Water in Organic Solvents: A Microheterogeneous Medium for Enzymatic Reactions

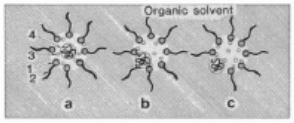
Abstract. *To simulate in vitro the conditions under which enzymes act in vivo, enzyme molecules have been entrapped in hydrated reverse micelles of a surfactant in organic solvents. In this system the catalytic activity of one of the enzymes studied (peroxidase) became much higher than in water, and the specificity of the other enzyme (alcohol dehydrogenase) was dramatically altered.*

In vitro studies of enzymes are usually conducted in water. In the living cell, however, enzymes mostly act on the surface of biological membranes or inside them. Even the diffusion-free enzymes of plasma have a medium whose physical parameters (dielectric permeability, polarity, viscosity, and so on) and chemical composition only resemble those of the aqueous solutions used in most in vitro studies. Moreover, properties of water near an interface are vastly different from those of "bulk" water (*1*). For these reasons it is considered by many that traditional enzymologic studies of the behavior of enzymes in aqueous solutions provide an imperfect picture of biological reality (*2*).

To simulate in vitro the conditions of enzyme action in vivo, enzymatic reactions are often performed in mixtures of water and organic solvents with a high concentration of a nonaqueous component (*3*). However, such homogeneous media are also far from ideal. To provide a more realistic compromise between classical enzymology and the enzymology of complex biological systems, immobilized (covalently bound, adsorbed, or matrix-entrapped) enzymes have been developed (*4*).

Recently, a new mode of studying protein function has been introduced. It has been proposed that enzymes be dissolved not in water but in a colloidal solution of water in an organic solvent. In such systems many enzymes retain their catalytic activity; examples are chymotrypsin, trypsin, lysozyme, ribonuclease, pyrophosphatase, peroxidase, alcohol dehydrogenase, lactate dehydrogenase, pyruvate kinase, cytochrome c, catalase, some forms of cytochrome P-450, and so forth (*5–9*). For lipolytic (interfacial) enzymes, heterogeneous media of this kind have been used for some time (*10*).

Research on "micellar enzymology" is still in its infancy, but there are some methodological aspects that will, in our opinion, stimulate such studies in the future. First, it is easy to prepare a colloidal system containing an enzyme; the enzyme (lyophilized or as a concentrated aqueous solution) is added, with stirring or shaking, to a solution of a surfactant in an organic solvent, where it is incorporated in an appropriate way into reverse micelles (*11, 12*). For example, hydrophilic protein molecules (such as many plasma enzymes) can avoid direct contact with the organic medium, as shown in (a) in the insert. Interfacial



enzymes (such as lipases) can interact with the surface layer of the micelle (b) or penetrate it, while typical membrane enzymes may come into contact with the organic solvent (c) (*13*). Second, the composition of such a microheterogeneous medium can be varied over a wide range with respect to the lipid surfactant, the organic solvent, and the degree of hydration of the reverse micelle. As the degree of hydration increases, the aggregation number and size of the reverse micelles grow (*11*). This provides a great many possibilities for choosing the optimal conditions for a particular enzyme (*14*). Third, the structure of micelle-entrapped protein and the kinetics of enzymatic reactions can be followed by routine spectroscopic techniques, since colloidal solutions of enzymes transmit light (*5–9*). Fourth, enzymes in reverse micelles can be studied at sub-zero temperatures without freezing (*7*).

It is already known that enzymes can be solubilized in organic solvents by use of surfactants without the loss of their catalytic activity (*5–9*). We now report that a micelle-entrapped enzyme may have a dramatically altered specificity. As an example, consider the oxidation of aliphatic alcohols to the respective aldehydes—$RCH_2OH + NAD^+ \rightarrow RCHO + NADH + H^+$—catalyzed by alcohol dehydrogenase from horse liver (*15*). The apparent second-order rate constant ($k_{cat}/K_{m,app}$) for substrates of the type $H(CH_2)_nOH$ is maximal for octanol ($n = 8$) in aqueous solution and for butanol ($n = 4$) in colloidal solution (Fig. 1).

Moreover, enzymatic catalysis may be more effective in a micellar medium than in water. For example, the value of k_{cat} for peroxidase oxidation of pyrogallol in reverse micelles of Aerosol OT in octane

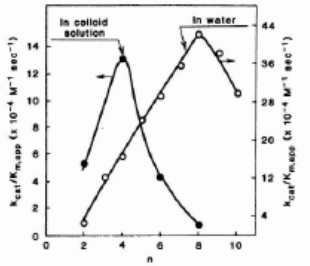


Fig. 1. Dependence of the second-order rate constant for oxidation of aliphatic alcohols of the $H(CH_2)_nOH$ type, catalyzed by alcohol dehydrogenase from horse liver, on the length of the hydrocarbon fragment in a molecule of the substrate (n). Experimental conditions: 25°C; (●) colloid system consisting of 0.1*M* Aerosol OT plus 8.5 percent (by volume) aqueous buffer (0.02*M* phosphate, *p*H 8.8) plus octane; (○) water (0.02*M* phosphate, *p*H 8.8). Measurements were made with the enzyme saturated with NAD^+. The initial steady-state rate of formation of NADH was followed spectrophotometrically (340 nm). The kinetic assay was carried out as described in (*15*).

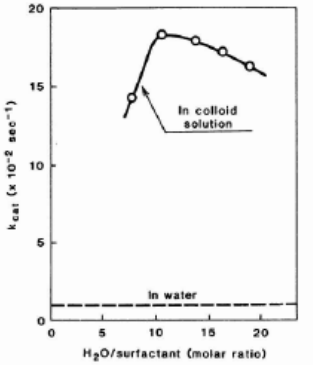


Fig. 2. Dependence of the first-order rate constant for peroxidase oxidation of pyrogallol on the content of water solubilized in 0.1*M* Aerosol OT plus aqueous buffer (0.02*M* phosphate-borate-acetate, *p*H 7.0) plus octane at 26°C. For comparison, the value of k_{cat} in the same buffer (dashed line) is shown. The initial steady-state rate of formation of purpurogallin was followed spectrophotometrically (420 nm). The kinetic assay was carried out as described in (*5, 16*).

is about 20 times higher than in water (*16*) (Fig. 2).

Such micellar effects on the catalytic activity and specificity of enzymes may be due to (i) microenvironmental effects on the reactivity of enzyme groups or substrate molecules (*17*), (ii) conformational alterations of the protein structure (*18*), and (iii) partition of the substrate or other molecules involved in the reaction between the aqueous and organic phases as well as the surface layer of the micelle (*19*). Assessment of the contribution of each of these mechanisms will require further study. Even at this stage, however, our data demonstrate the distorted (alcohol dehydrogenase) or "anemic" (peroxidase) behavior of an enzyme in water compared to that in a colloidal solution.

In addition, solubilization of enzymes in organic solvents has potential applications outside enzymology. For example, micellar solutions and water-in-oil microemulsions may be useful as reaction media in organic synthesis (to shift chemical equilibria compared to those in aqueous solution), in analyses for water-insoluble reagent, and as a means of modifying proteins with water-insoluble compounds (*20*).

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Ферменты в неводных средах

- Зачем и почему?

Ферменты в неводных средах

- Неводное окружение – оптимальная среда для конформации с максимальной каталитической активностью
- Плохая растворимость субстратов в воде
- Плохая термодинамика процесса
- Повышение активности за счет катализа средой
- Снижение скорости побочных реакций

Ферменты в неводных средах Оптимальная конформация для активности и стабильности

Science. 1984 Jun 15;224(4654):1249-51.

Enzymatic catalysis in organic media at 100 degrees C

A Zaks, A M Klibanov

PMID: 6729453
DOI: 10.1126/science.6729453

Abstract

Porcine pancreatic lipase catalyzes the transesterification reaction between tributyrin and various primary and secondary alcohols in a 99 percent organic medium. Upon further dehydration, the enzyme becomes extremely thermostable. Not only can the dry lipase withstand heating at 100 degrees C for many hours, but it exhibits a high catalytic activity at that temperature. Reduction in water content also alters the substrate specificity of the lipase: in contrast to its wet counterpart, the dry enzyme does not react with bulky tertiary alcohols.

Ферменты в неводных средах Оптимальная конформация для активности и стабильности

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Proceedings of the National Academy of Sciences of the United States of America

Proc Natl Acad Sci U S A. 1985 May; 82(10): 3192–3196.

doi: 10.1073/pnas.82.10.3192

Enzyme-catalyzed processes in organic solvents.

A Zaks and A M Klibanov

Abstract

Three different lipases (porcine pancreatic, yeast, and mold) can vigorously act as catalysts in a number of nearly anhydrous organic solvents. Various transesterification reactions catalyzed by porcine pancreatic lipase in hexane obey Michaelis-Menten kinetics. The dependence of the catalytic activity of the enzyme in organic media on the pH of the aqueous solution from which it was recovered is bell-shaped, with the maximum coinciding with the pH optimum of the enzymatic activity in water. The catalytic power exhibited by the lipases in organic solvents is comparable to that displayed in water. In addition to transesterification, lipases can catalyze several other processes in organic media including esterification, aminolysis, acyl exchange, thiotransesterification, and oximolysis; some of these reactions proceed to an appreciable extent only in nonaqueous solvents.

Ферменты в неводных средах. Катализ средой

Biochimica et Biophysica Acta, 1039 (1990) 290–296
Elsevier

BBAPRO 33652

The solvent effects on the kinetics of bacterial formate dehydrogenase reaction

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[1] *A.V. Palladin Institute of Biochemistry, Academy of Sciences of the Ukrainian SSR, Kiev,* [2] *Department of Chemistry, M.V. Lomonosov Moscow State University, and* [3] *A.N. Bach Institute of Biochemistry, Academy of Sciences of the U.S.S.R., Moscow (U.S.S.R.)*

$$NAD^+ + HCOO^- \rightarrow NADH + CO_2\uparrow$$

Ферменты в неводных средах. Катализ средой

Влияние растворителя на константу Михаэлиса формиат-дегидрогеназы по формиату

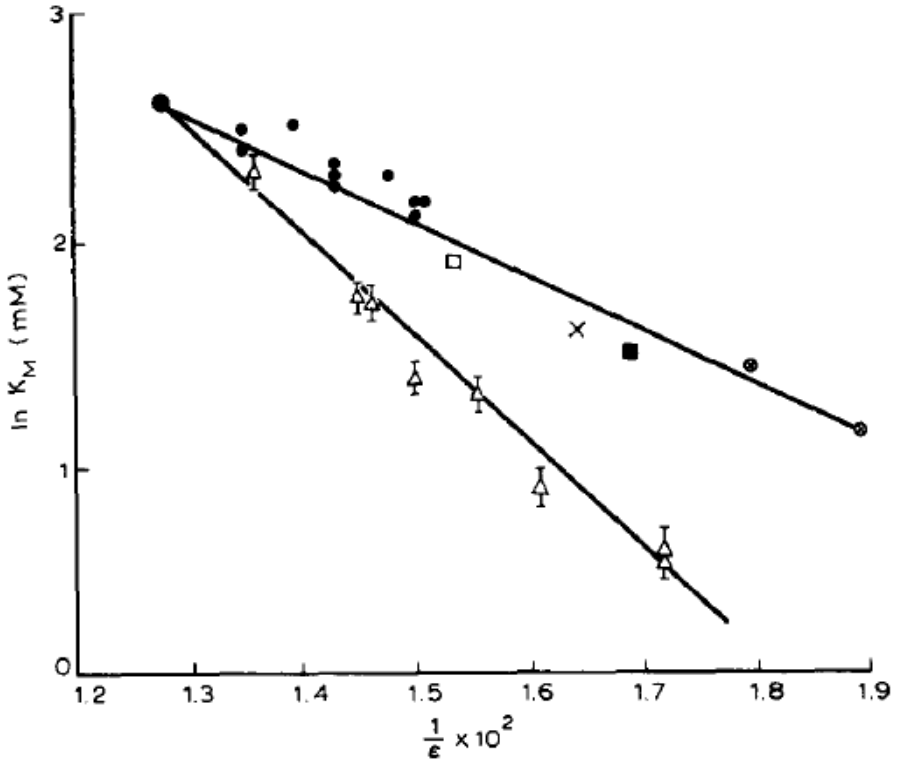


Fig. 1. Dependence of formate dehydrogenase K_m for formate on dielectric permittivity of water/organic solvents at different concentrations (pH 7.0; 25 °C): sucrose (●), 0–60% (w/v); methanol (□), 27% (v/v); ethanol (X), 27% (v/v); isopropanol (■), 27% (v/v); dioxane (⊗), 27 and 30% (v/v); glycerol (Δ), 0–55% (v/v). [NAD^+] = 1.5 mM, formate 1 ÷ 300 mM.

Ферменты в неводных средах. Катализ средой

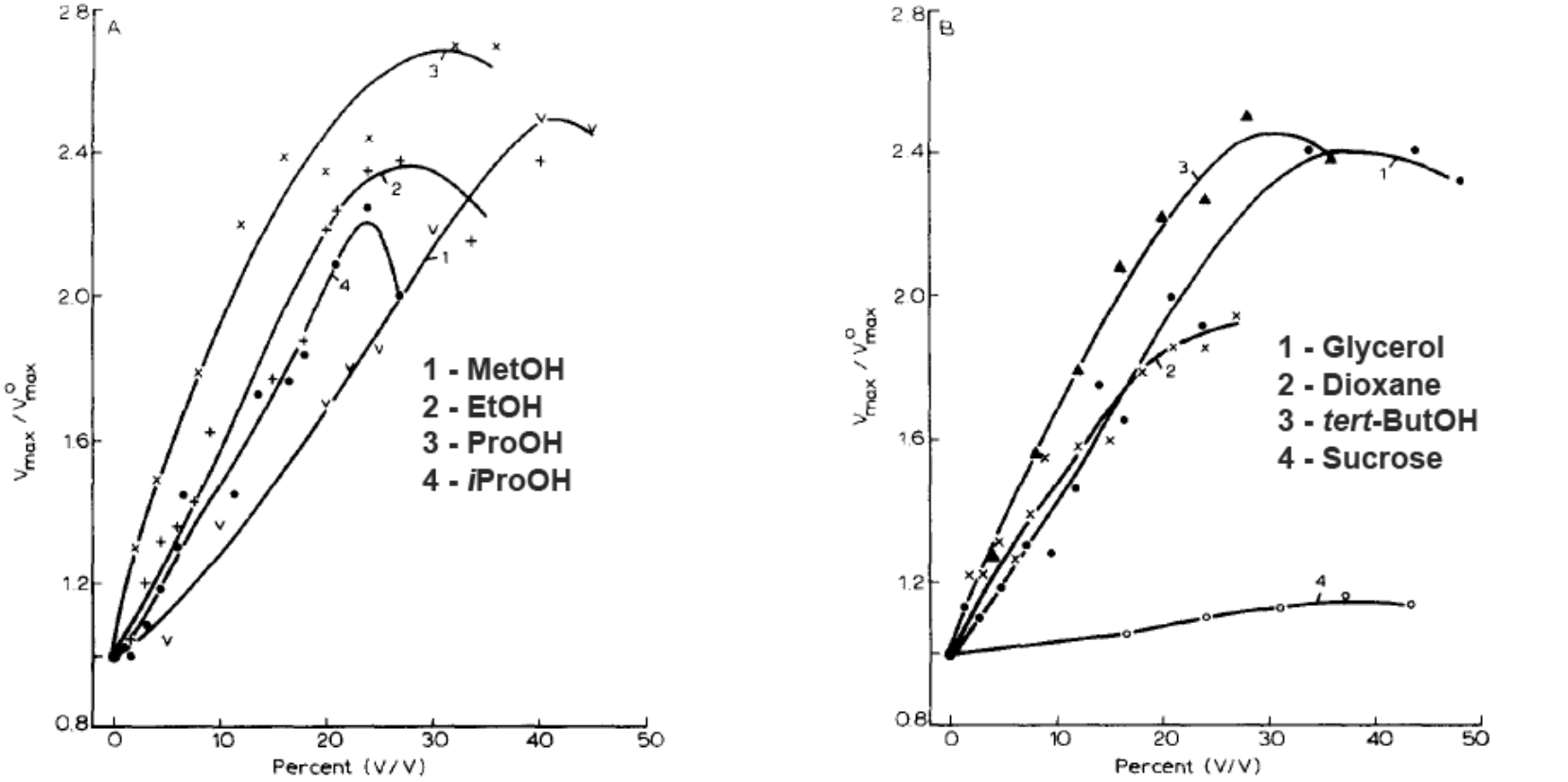


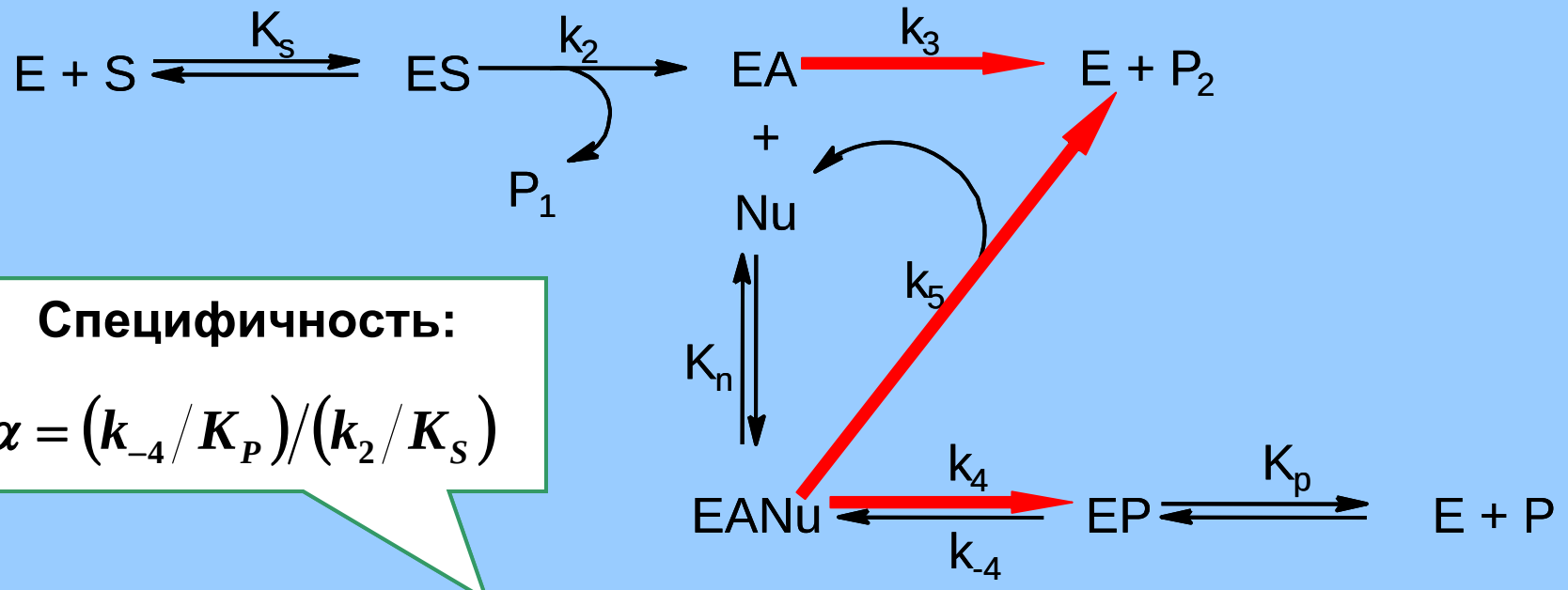
Fig. 2. Dependence of relative increase of maximal rate (V_{max}/V^0_{max}) on the volume concentration of organic solvent (pH 7.0, 25 °C). (A) Methanol (1), ethanol (2), propanol (3) and isopropanol (4). (B) Glycerol (1), dioxane (2), *tert*-butanol (3) and sucrose (4). [NAD^+] = 1.5 mM; formate 0.3 M.

Влияние растворителя на активность формиатдегидрогеназы

Ферменты в неводных средах.

Снижение скорости побочных реакций

Кинетическая схема ферментативного переноса ацильной группы через образование комплекса ацилфермент-нуклеофил



Специфичность:

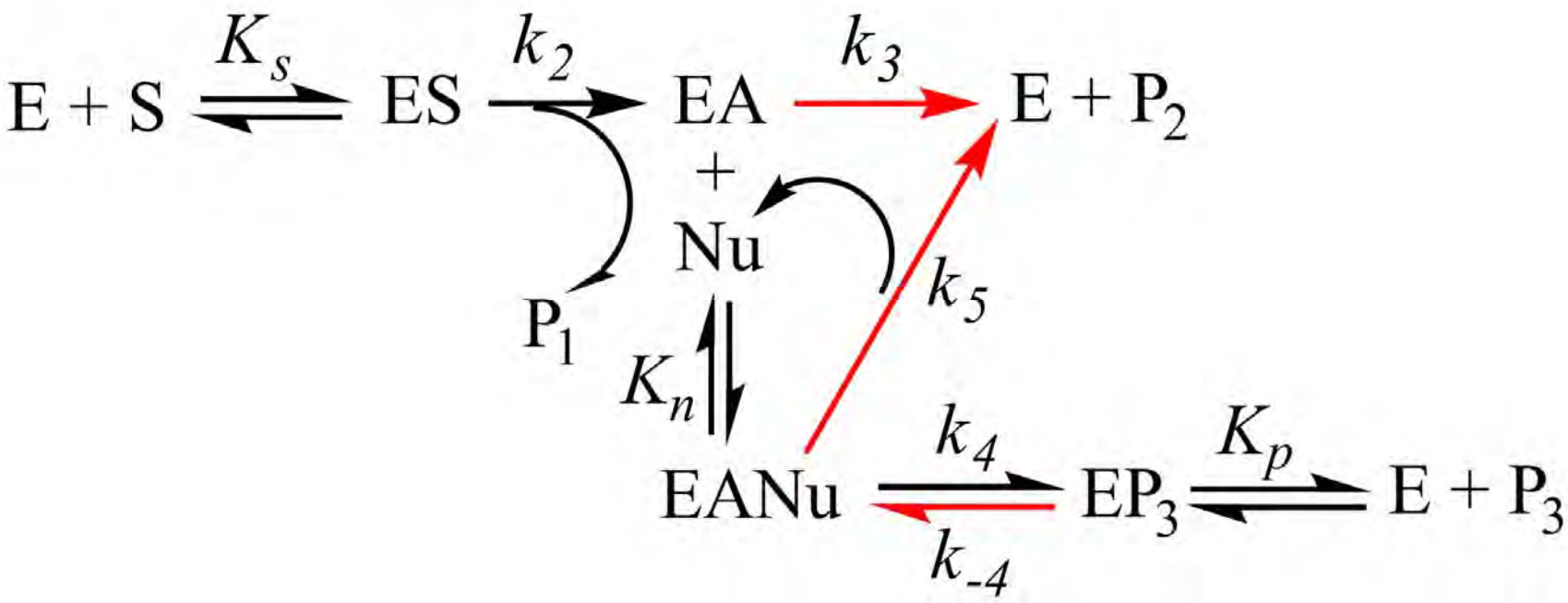
$$\alpha = \left(k_{-4}/K_P\right)/\left(k_2/K_S\right)$$

$$\begin{cases} \dfrac{dp}{dt} = e\,\dfrac{k_2}{K_S}\,\dfrac{\beta\cdot n\cdot s - \alpha\cdot p\cdot(1+\beta\cdot\gamma\cdot n)}{1+\beta\cdot n+\beta\cdot\gamma\cdot n} \\ \dfrac{dp_2}{dt} = e\,\dfrac{k_2}{K_S}\,\dfrac{(1+\beta\cdot\gamma\cdot n)\cdot(s+\alpha\cdot p)}{1+\beta\cdot n+\beta\cdot\gamma\cdot n} \\ e = e_0\Big/\left(1+\dfrac{s}{K_S}+\dfrac{p}{K_P}+\dfrac{k_2}{K_S}\left(\dfrac{(s+\alpha\cdot p)\cdot(n+K_n)\cdot\beta}{k_4\cdot(1+\beta\cdot n+\beta\cdot\gamma\cdot n)}\right)\right) \\ n_0 = n+p \qquad s_0 = s+p+p_2 \end{cases}$$

Нуклеофильность:

$$\left(\frac{V_{Syn}}{V_{Hydr}}\right)_{Init} = \frac{\beta\cdot[n]_0}{1+\beta\cdot\gamma\cdot[n]_0}$$

ОБЩАЯ КИНЕТИЧЕСКАЯ СХЕМА СИНТЕЗА β–ЛАКТАМНЫХ АНТИБИОТИКОВ



Гидрола: Пенициллинацилаза слот (АЕН)

Recombinant Alpha-Amino Ester Acid Hydrolase from *Xanthomonas rubrilineans* VKPM B-9915 is a Highly Efficient Biocatalyst of Cephalexin Synthesis

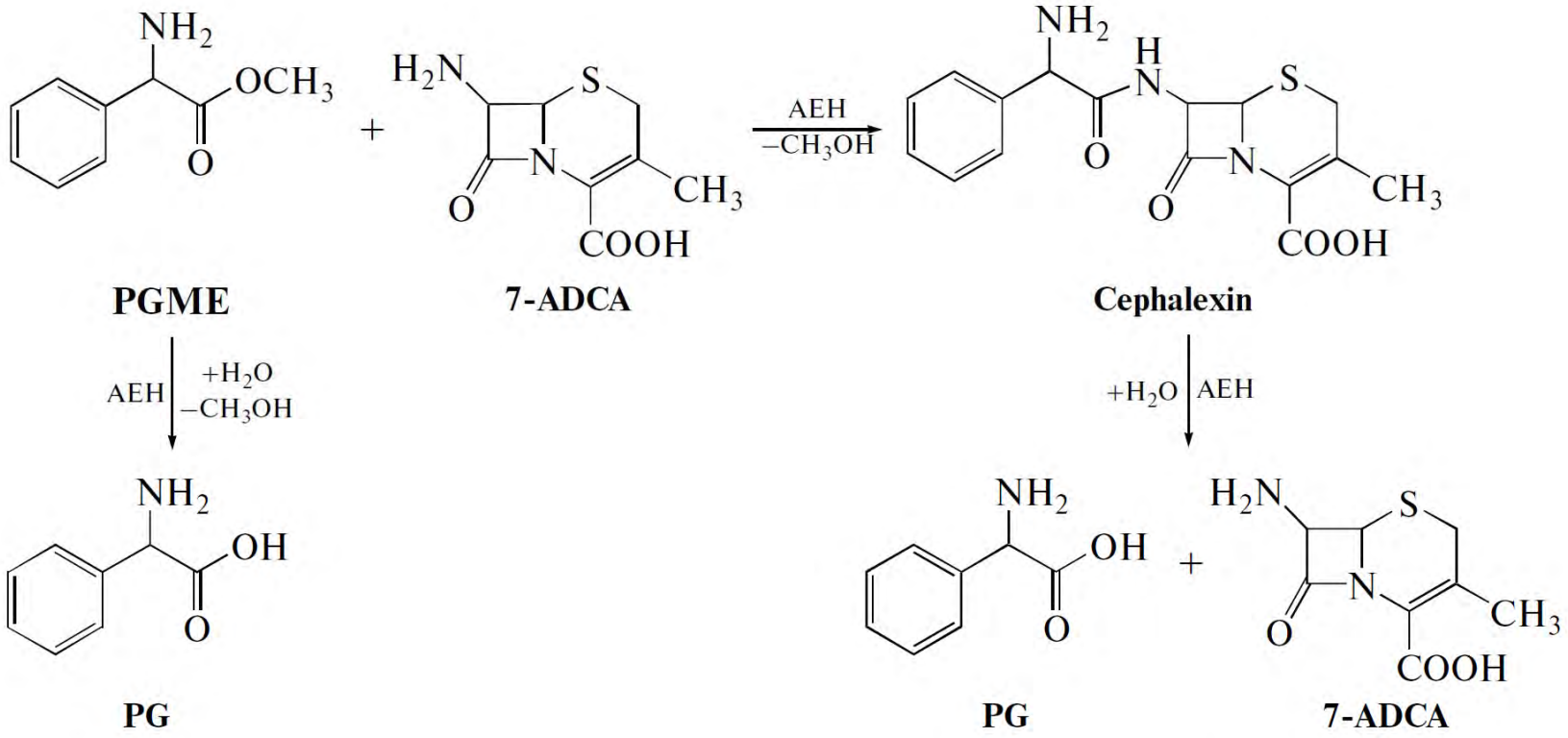
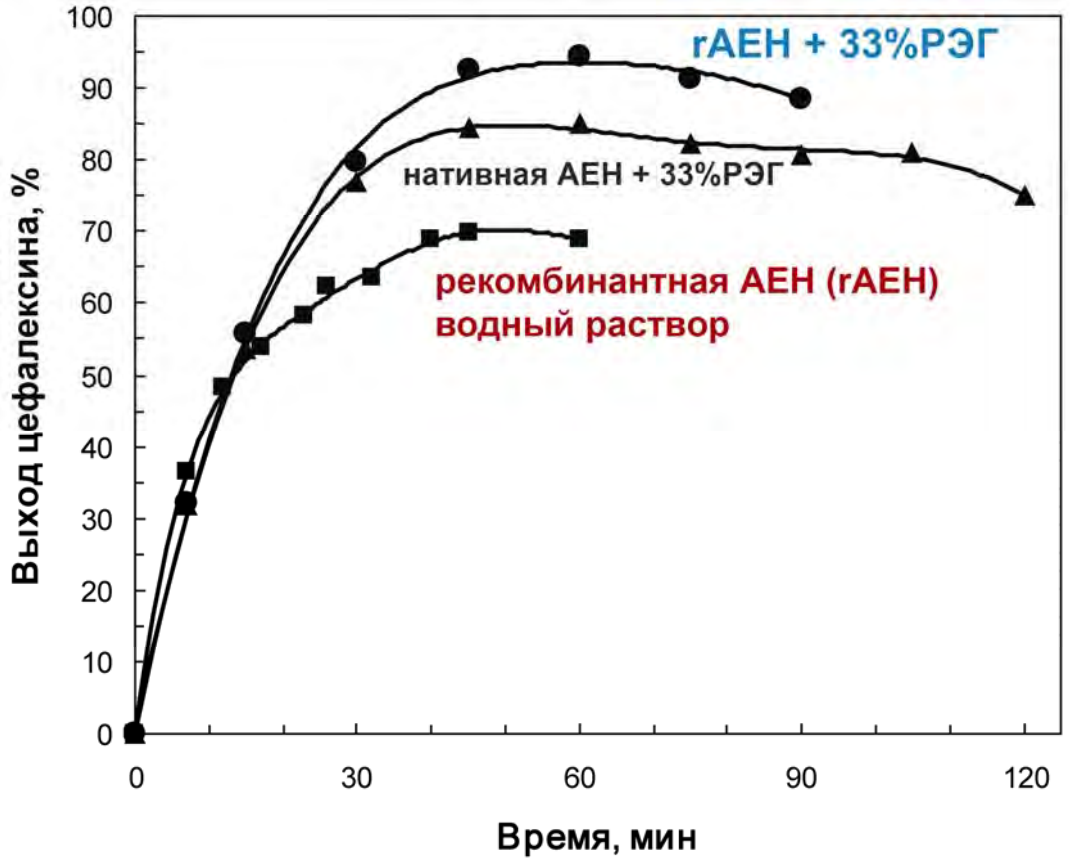


Fig. 1. Scheme of enzymatic synthesis of cephalexin from 7-ADCA and PGME catalyzed by AEH.

Recombinant Alpha-Amino Ester Acid Hydrolase from *Xanthomonas rubrilineans* VKPM B-9915 is a Highly Efficient Biocatalyst of Cephalexin Synthesis



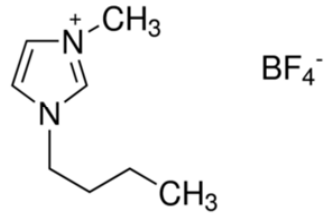
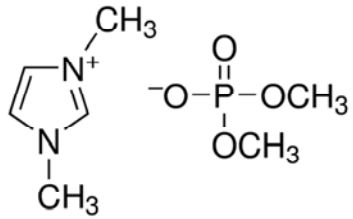
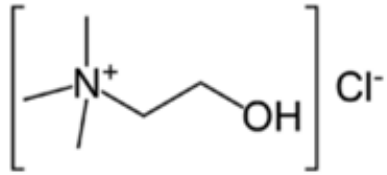
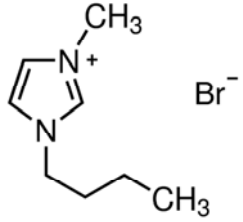
Влияние добавления полиэтиленгликоля и источника АЕН на выход цефалексина

ФОРМИАТДЕГИДРОГЕНАЗЫ В ИОННЫХ ЖИДКОСТЯХ

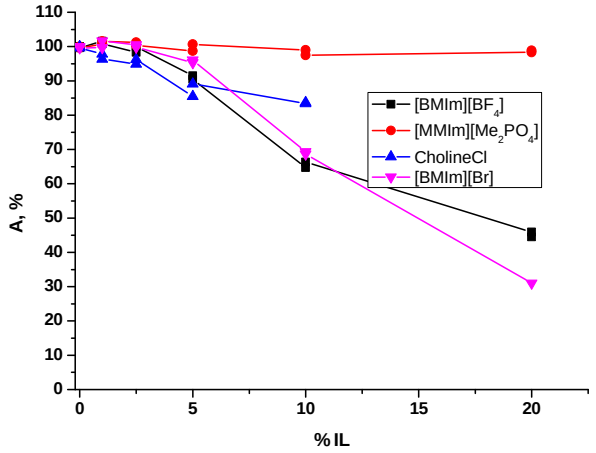
- Ионные жидкости – перспективная среда для проведения биотрансформации

- Высокая растворимость органических соединений в ИЖ

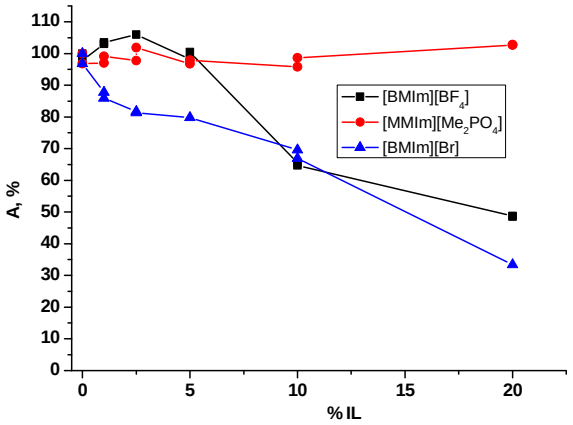
Популярные ионные жидкости

Ionic liquid	Structure
[BMIm][BF_4]	
[MMIm][Me_2PO_4]	
CholineCl	
[BMIm][Br]	

Активносить ФДГ из разных источников в ИЖ



PseFDH



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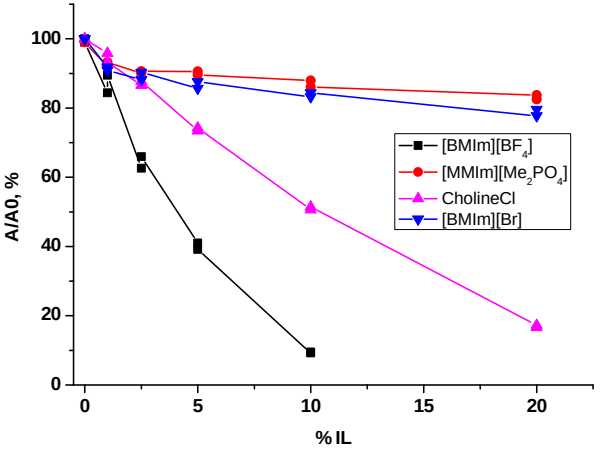
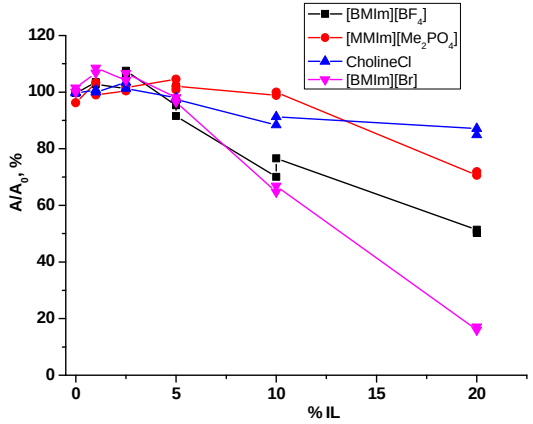
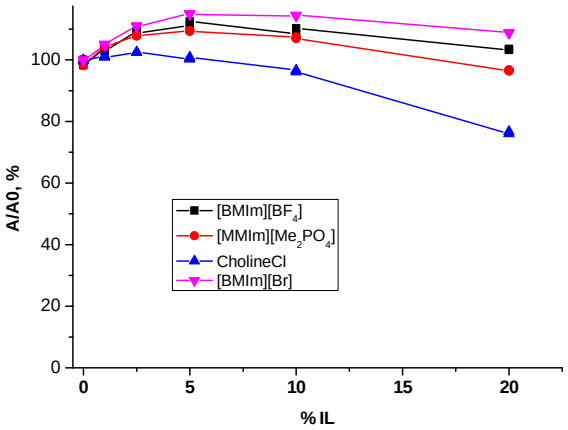


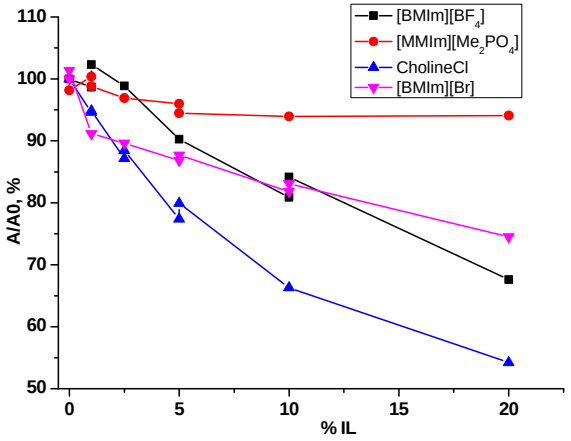
SauFDH



OpaFDH

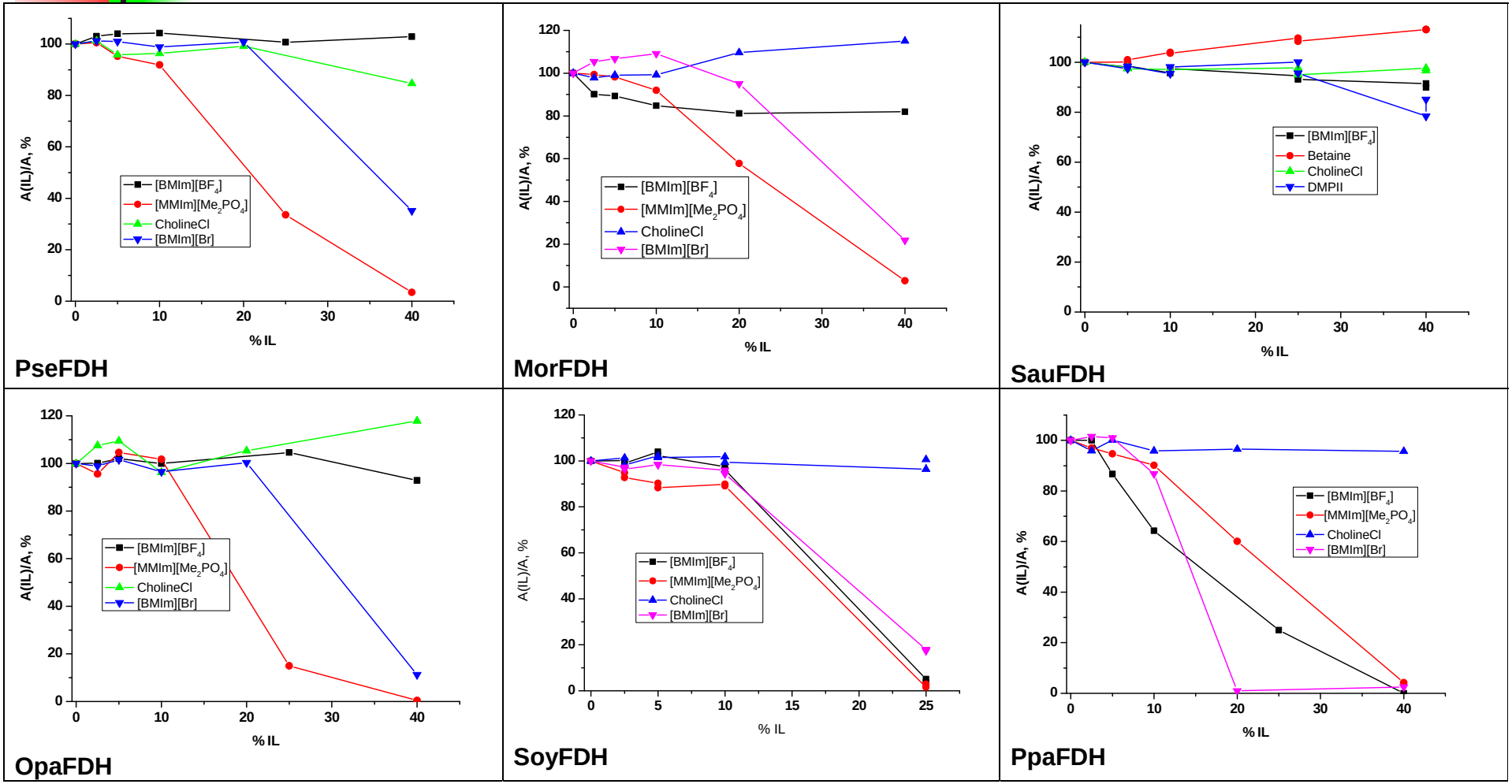


SoyFDH



PpaFDH

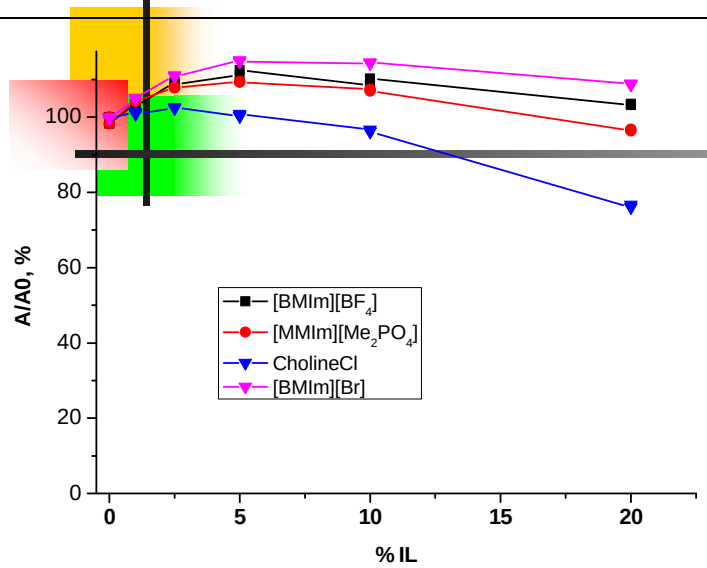
Стабильность ФДГ в ИЖ



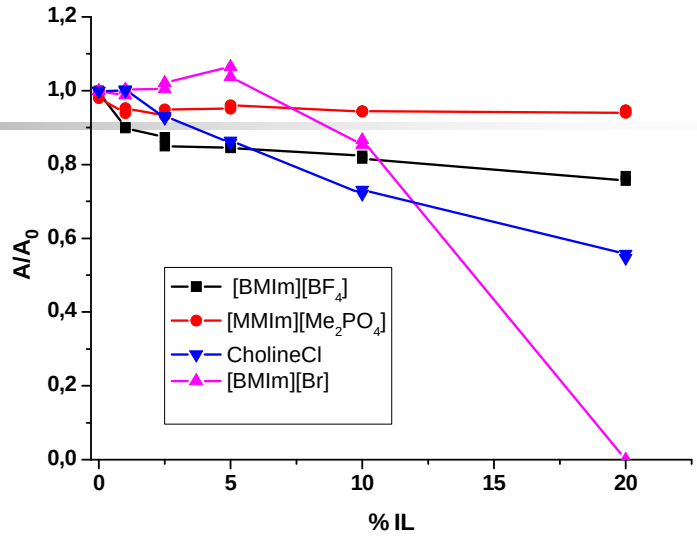
Свойства мутантных ФДГ в ионных жидкостях

InnoTech MSU

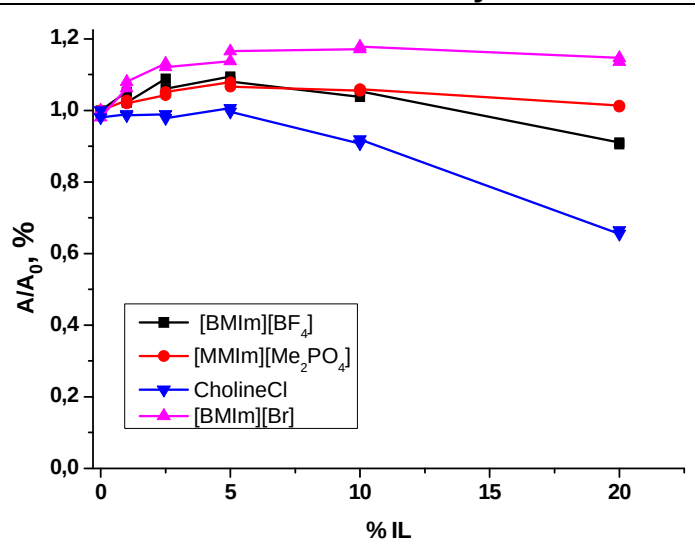
Влияние концентрации ИЖ на активность различных SoyFDH



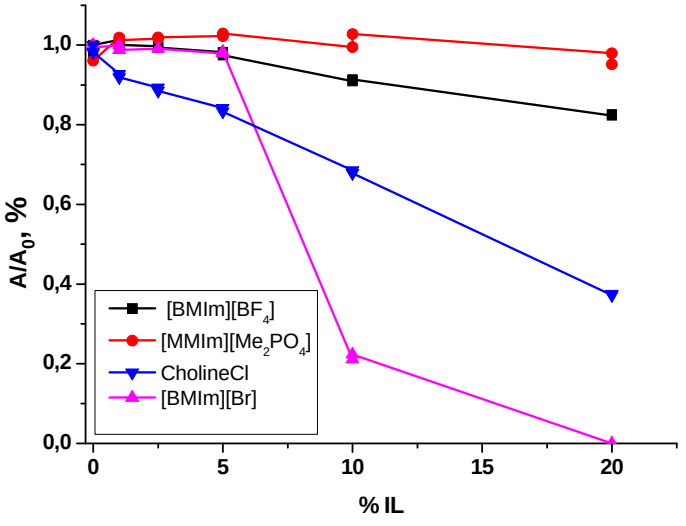
wt_soyFDH



SoyFDH Phe290Asn

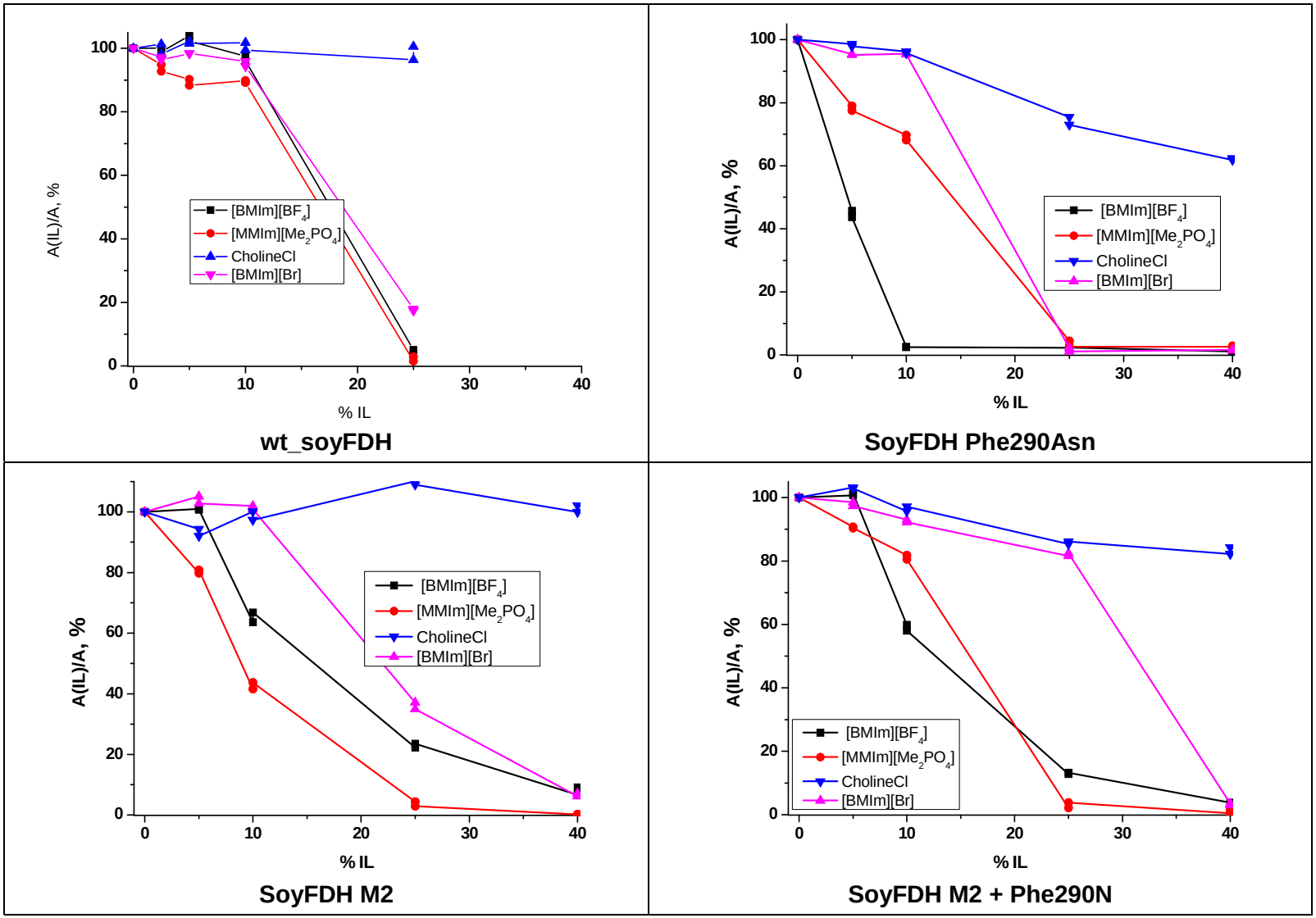


SoyFDH M2



SoyFDH M2 + Phe290Asn

Влияние концентрации ИЖ на стабильность различных SoyFDH



Короткоцепочечная NADP-зависимая алкогольдегидрогеназа из гипертермофильной археи *Thermococcus sibiricus*

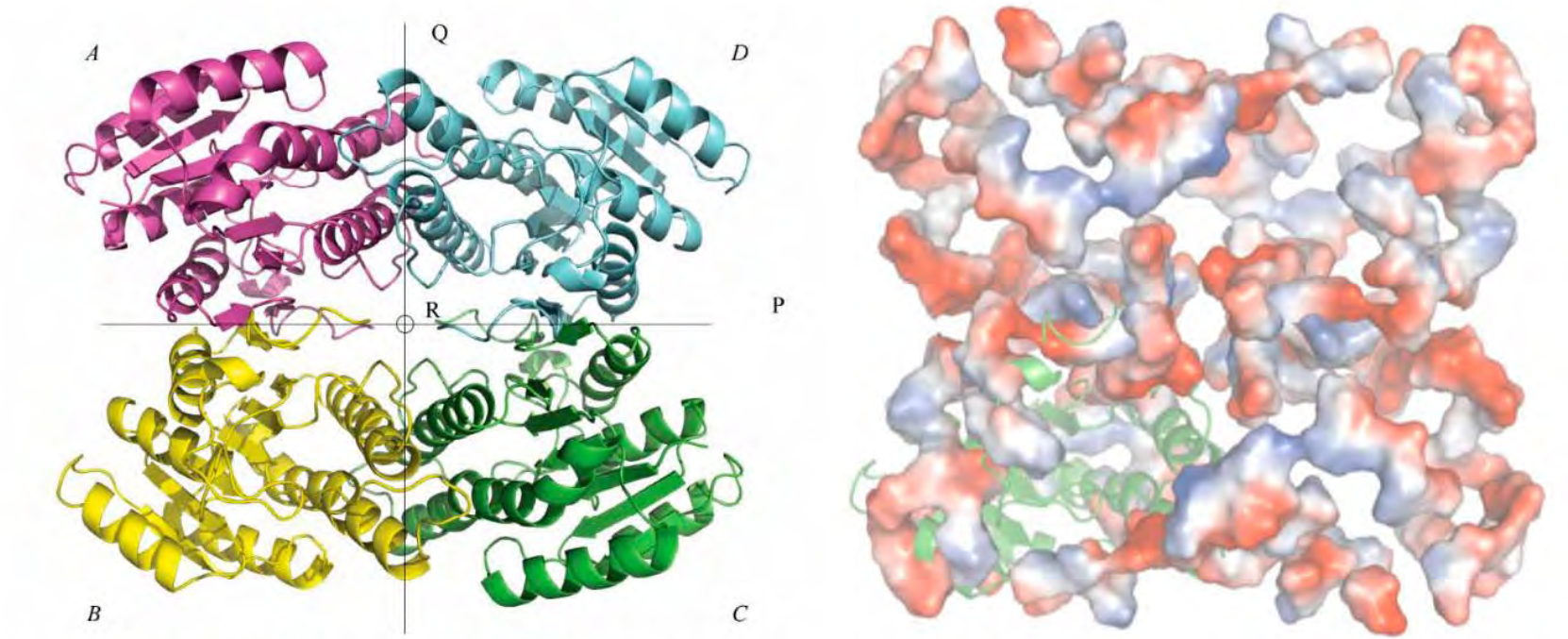
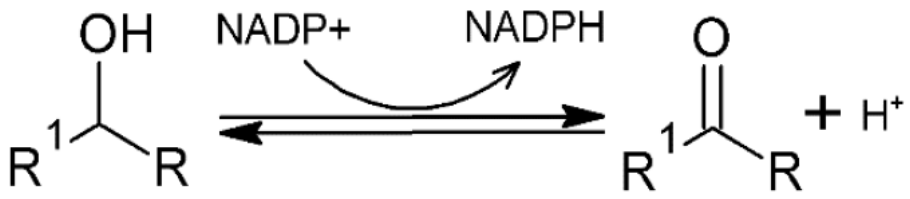
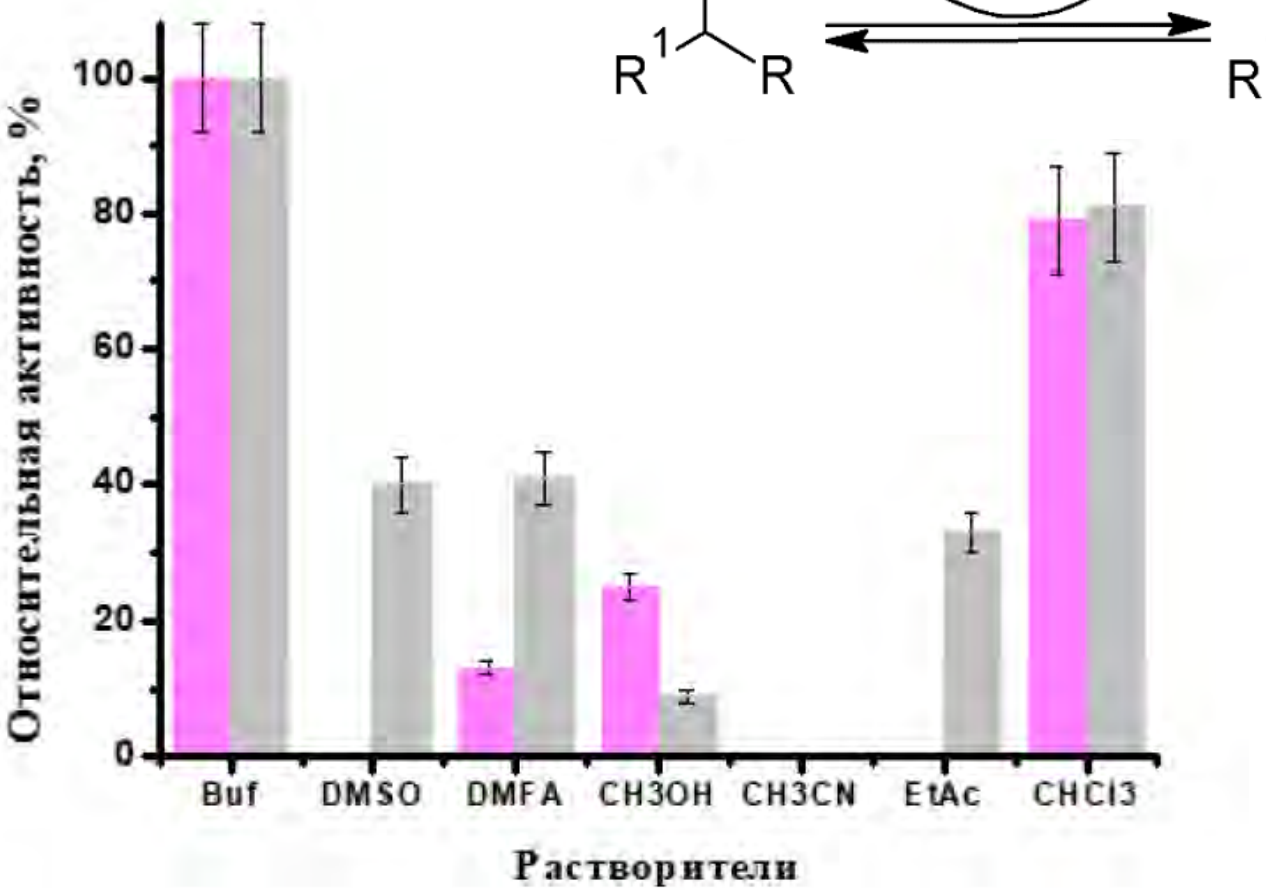


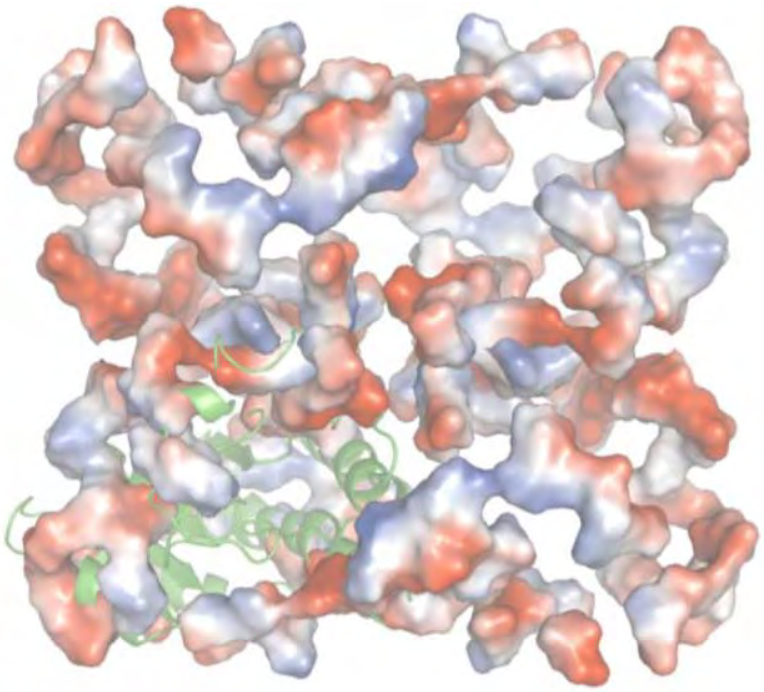
Рисунок 3. (**A**) **Тетрамер TsAdh319**. Субъединица A в фиолетовом цвете, B – в желтом, C – в зеленом, D – в серо-голубом. Субъединицы симметричны относительно осей P, Q, R, ось R-перпендикулярна плоскости листа. (**B**) Поверхность тетрамера TsAdh319, образованная скомпенсированными заряженными остатками (ионные пары и солевые мостики): ленточная модель субъединицы представлена зеленым цветом.

Короткоцепочечная NADP-зависимая алкогольдегидрогеназа из гипертермофильной археи *Thermococcus sibiricus*





Короткоцепочечная NADP-зависимая алкогольдегидрогеназа из гипертермофильной археи *Thermococcus sibiricus*



Высокая стабильность фермента при повышенных температурах и в органических растворителях обусловлена большим количеством ионных пар на поверхности белковой глобулы (показаны красно-розовым цветом)