Highly efficient immobilization of laminarinases from marine mollusks in novel hybrid polysaccharide-silica nanocomposites

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The sol-gel technology is presently believed to be one of the most promising approaches for the immobilization of enzymes. Its main advantage lies in the fact that the entrapment of proteins proceeds without formation of covalent linkages between biomolecules and matrix. As a results, the enzymes are in their intact state after the immobilization. This is the reason why they hold functionality that is supplemented by a substantial increase in their long-term and thermal stability.

The aim of this study was to extend our method for the immobilization of a highly labile enzymes, laminarinases.

1,3-β-D-glucanases – laminarinases (EC 3.2.1.6) **from marine mollusks** *Spisula sachalinensis* and *Chlamys albidus* belonging to *O*-glycoside hydrolyses (EC 3.2.1)

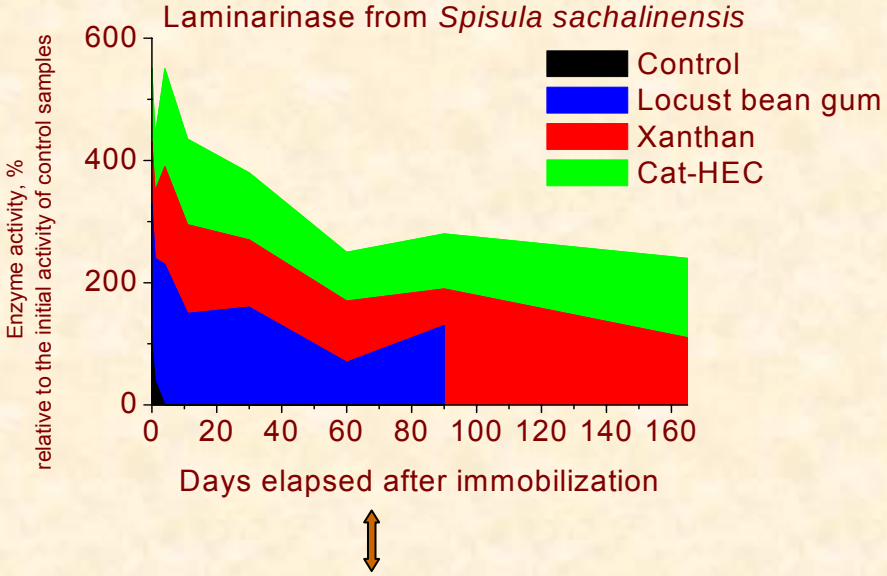
Tetrakis(2-hydroxyethyl) orthosilicate (THEOS) was taken as a precursor. Silica nanocomposites were synthesized in aqueous solutions containing xanthan, locust bean gum or cationic derivative of hydroxyethylcellulose (KAT-HEC)

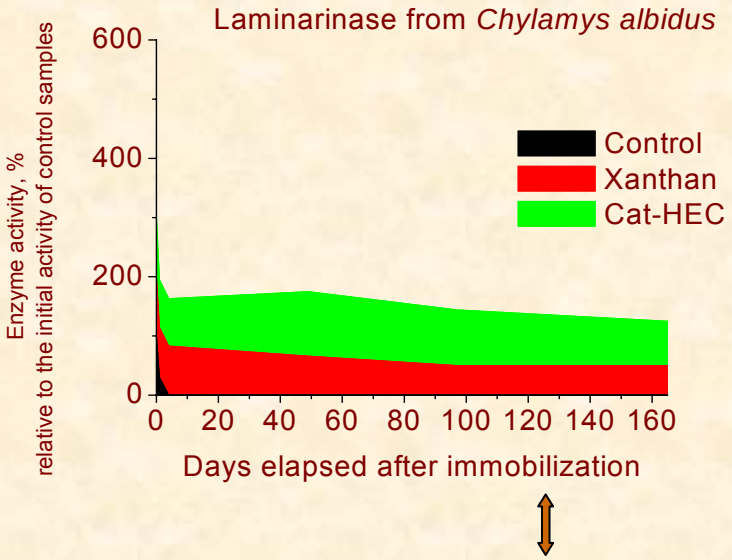
The successful immobilization

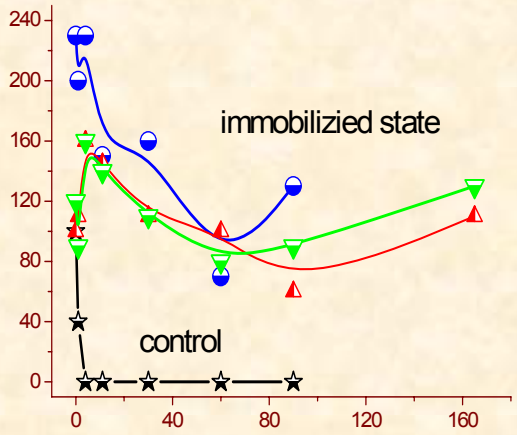
is due to advantages of the new precursor and synthesized biocatalysts

- ***The entrapment conditions are dictated by the enzyme, but not the sol-gel processes;***
- ***It can be performed at pH and temperature suitable for the enzyme functioning.***
- ***The organic solvents are not used to solubilize the precursor;***
- ***Catalysts for the promotion of the sol-gel process are not added because of the catalytic action of the polysaccharides inside the matrix;***
- ***A biocatalyst can be prepared at reduced concentrations of THEOS that reduces the heat release in the course of the precursor hydrolysis;***
- ***The porous structure of hybrid nanocomposite provides the accessibility of immobilized enzyme by the enzymatic substrate and proper functioning, whereas the protein molecules are not easily washed out of the matrix;***
- ***The immobilized enzymes demonstrated activity at low concentration comparable to their content in the living cells.***

The control experiment is the activity of laminarinases in the aqueous solution and immobilized state in matrices with various polysaccharides (0.3 wt. %) is experiment. The biocatalysts were prepared in the initial solution with 10-20 wt. % of THEOS

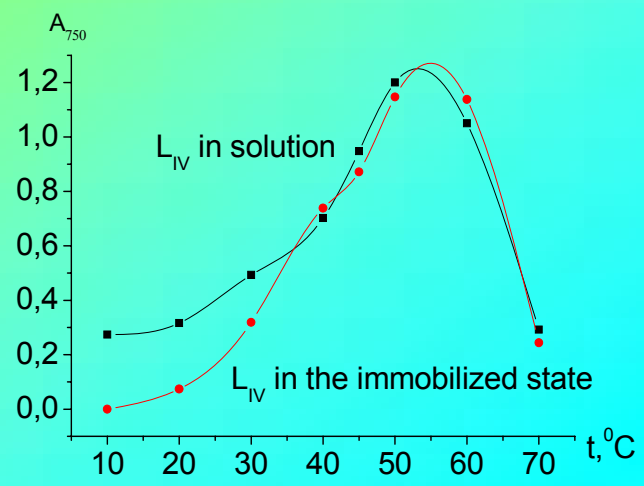


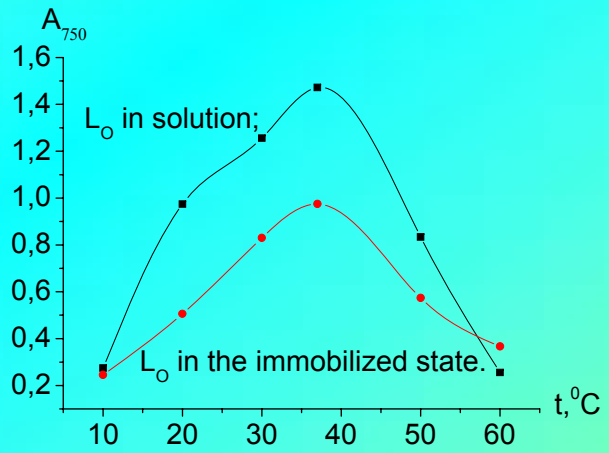


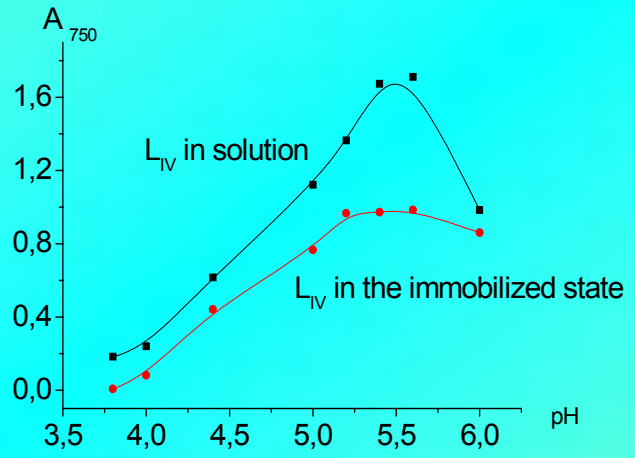


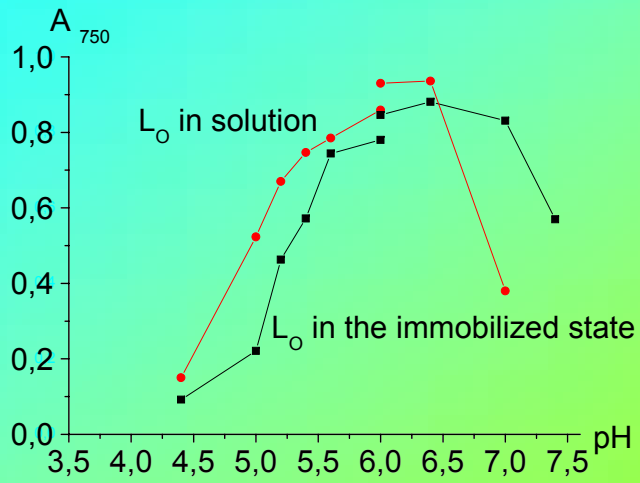
The comparison of both laminarinases functioning in the immobilized state makes it obvious that they exhibit various sensitivity to the composition of hybrid nanocomposites

A further study of glucanases was performed to characterize some details of their functioning in the immobilized state. When comparing them with similar parameters determined for the free enzymes in solution, one does not find notable differences. It is only necessary to add that there was extending of temperature and optimal region pH.

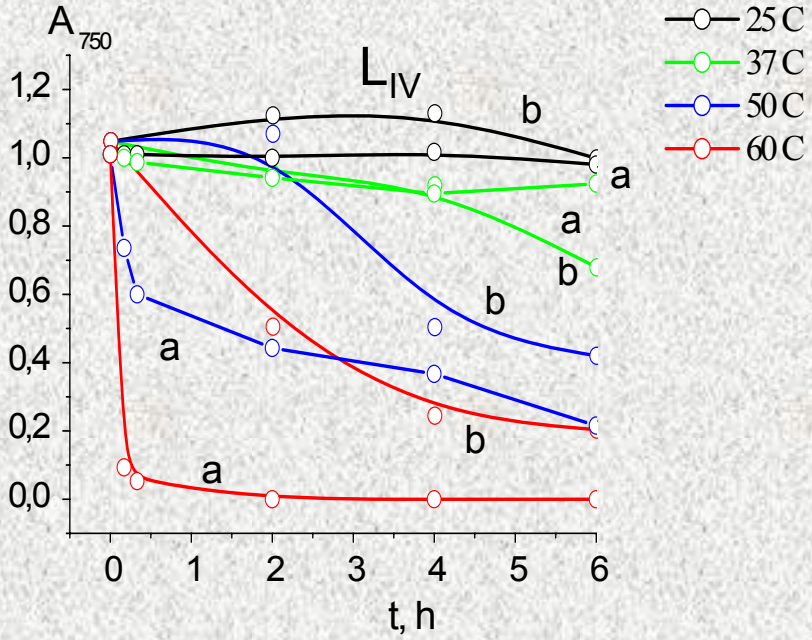




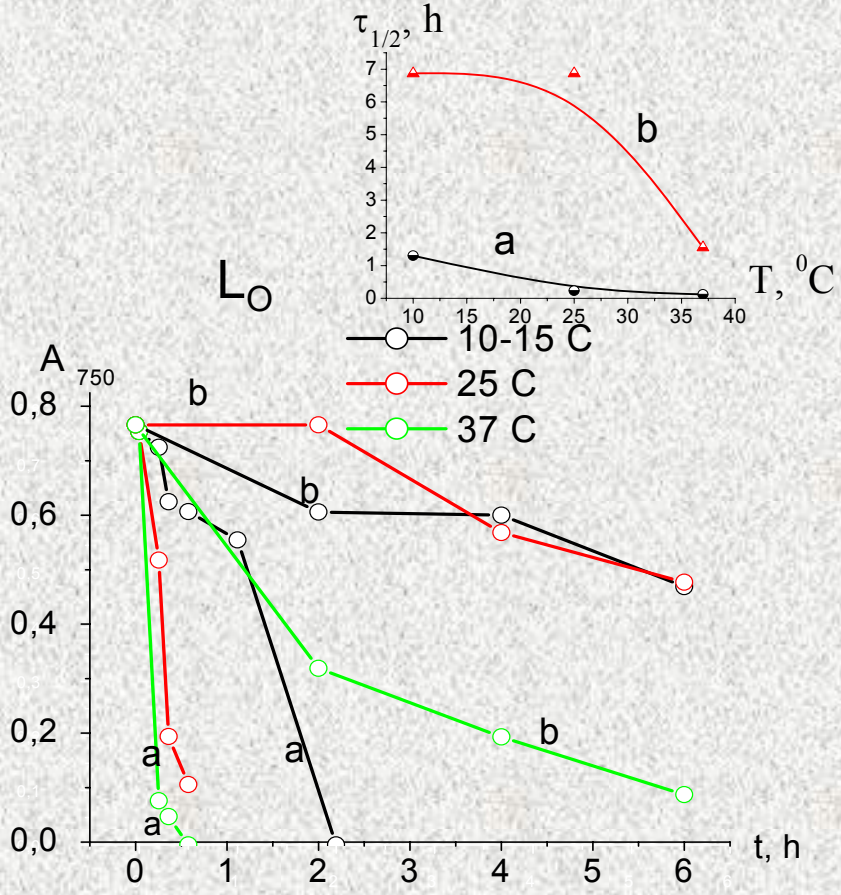




The immobilization gave prominent rise to the temperature stability of glucanase Lo in comparison with that in the solution. The former demonstrates a time dependence of the concentration of sugars released in the course of an enzymatic hydrolysis at 37°C. The latter represents the half-life times of enzyme at various temperatures. It was determined on the example of enzymatic reaction performed at 37°C.



a – in solution
b – in the immobilized state

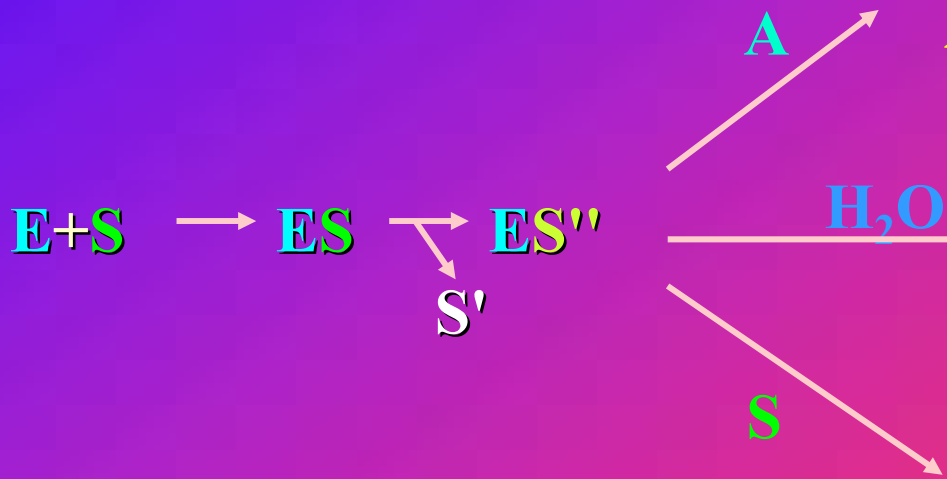


Characteristics of laminarinases in solution and immobilized state

Enzyme, source	Type of hydrolysing bond	Mw (kDa)	Location	K_m (mg/ml)	Optimum conditions		
					pH	T °C	NaCl, M
L_O, *Chlamys albidus*	Glc -1→3	20[a]/38[b]	Solution	0.7	6.0	36	0.1-0.25
			Immobilized state	4.0	6.0	37	
L_{IV}, *Spisula sachalinensis*	Glc -1→3	22-39	Solution	0.25	5.6	50	0.01-0.3
			Immobilized state	3.0	5.6	55	

[a] determined by the gel filtration -
[b] determined by the electrophoresis (SDS-PAGE)

Endo-1,3-β-D-glucanases of marine organisms, found at first as hydrolases, catalyze three reactions running simultaneously and practically with almost equal efficiency.



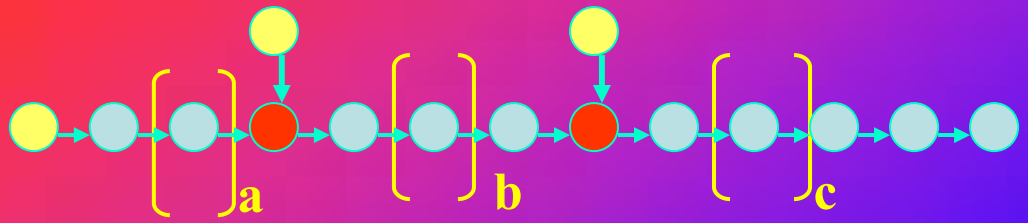
S''-A
(reaction of transglycosylation)

S''-OH
(reaction of hydrolysis)

S''-S
(glucanosyl transferase activity)

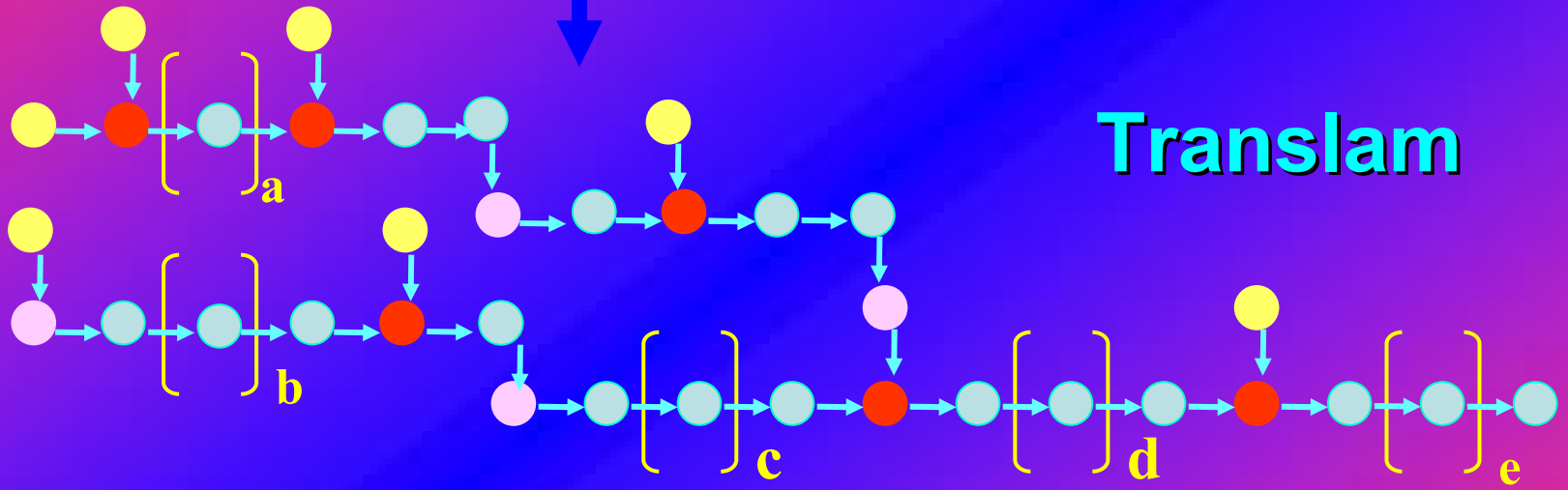
Biochemistry (Moscow) 1997. 62: 1300.

The hypothetical mechanism of the receiption of translam



Laminaran from *Laminaria cichorioides*

1,3-β-D-glucanase from *Chlamys albidus*



Translam

- 3,6-O-di-substituted Glc residue
- 3-O-substituted Glc residue
- 6-O-substituted Glc residue
- nonsubstituted Glc residue

Zvyagintseva, T.N., Elyakova, L.A., Isakov, V.V., 1995. Enzymatic conversion of laminarans into 1->3;1->6-β-D-glucans, possessing immunostimulating activity (in Russian). Bioorg. Khim. 21(2), 218-225.

Products of Enzymatic Transformation of laminaran from *Laminaria cichorioides* and Their Properties

Laminaran → **E (Lo)**

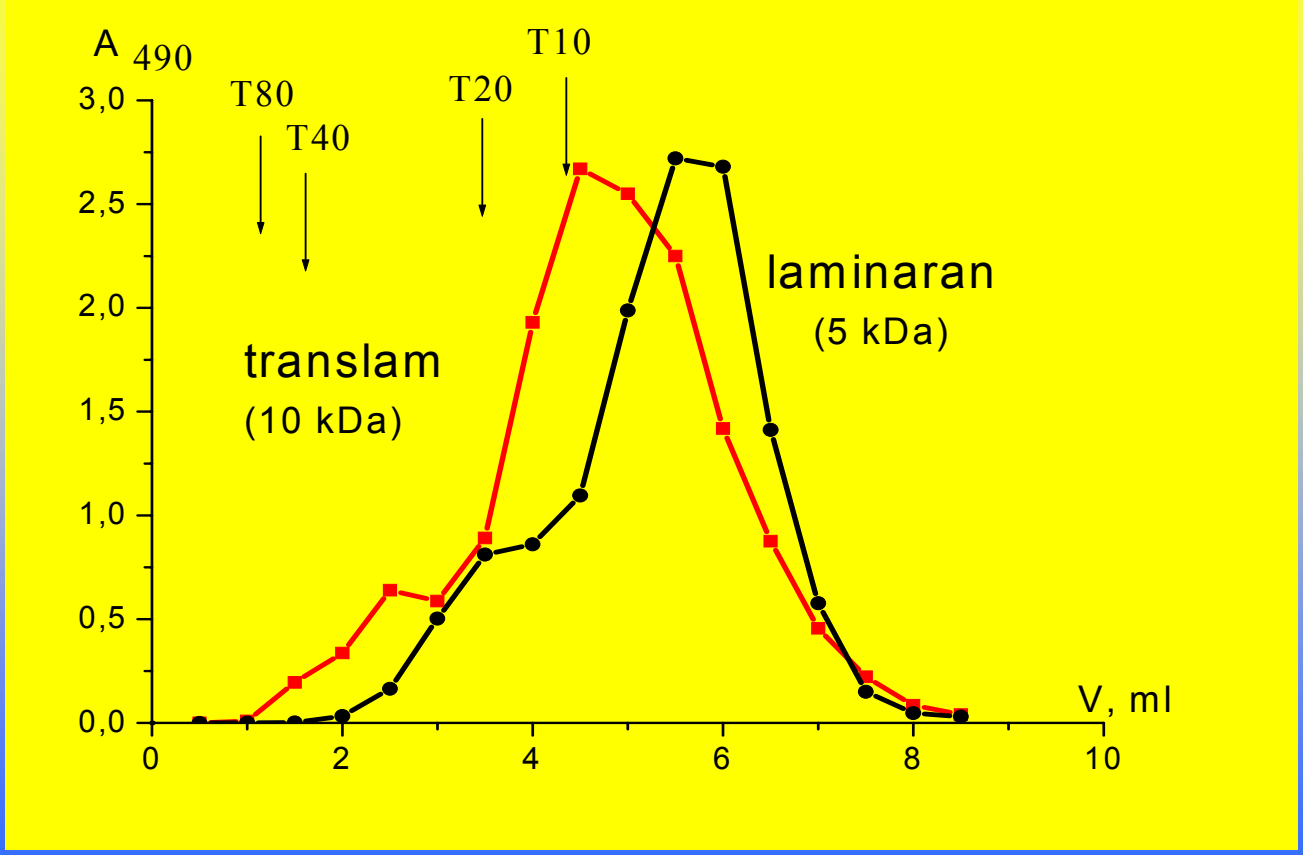
TRANSLAM
immunostimulator, radioprotector, crioprotector and antitumor agent

ANTIVIR
Phytoimmunostimulator: potato, tobacco, tomato, soybean

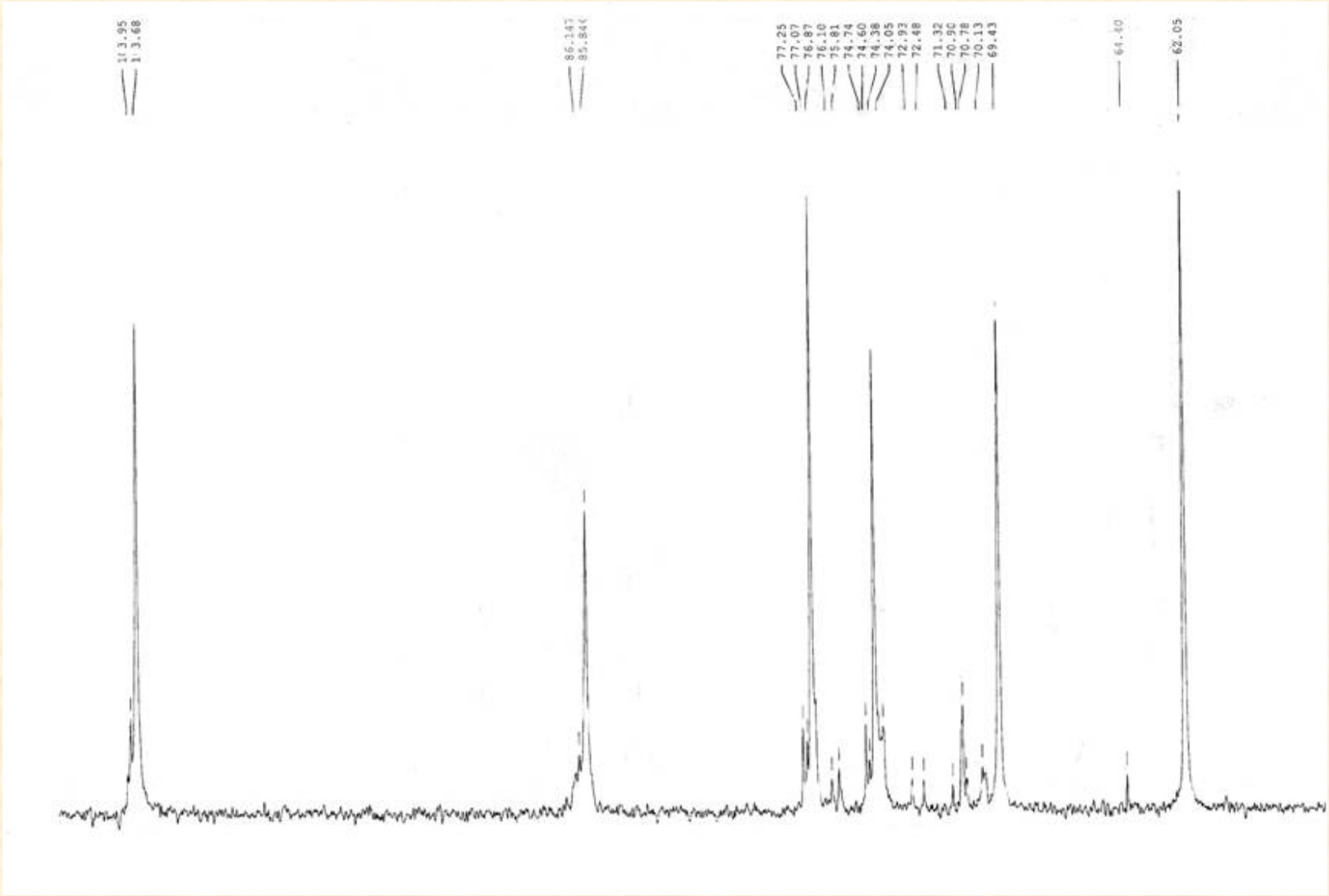
Glc + Glc_n
Glc1
↓
6
X = 1,2,3 *are the main components*

Glucan	1→3:1→6	Yield, %	M.m., kDa
Laminaran	90:10		3-5
Translam	75:25	25	8-10
Antivir	80:20	20	6-8

Gel permeation chromotography on Superdex 75 HR 10/30 of laminaran from *L. cichorioides* and translam obtained by action laminarinase L_O in the immobilized state.

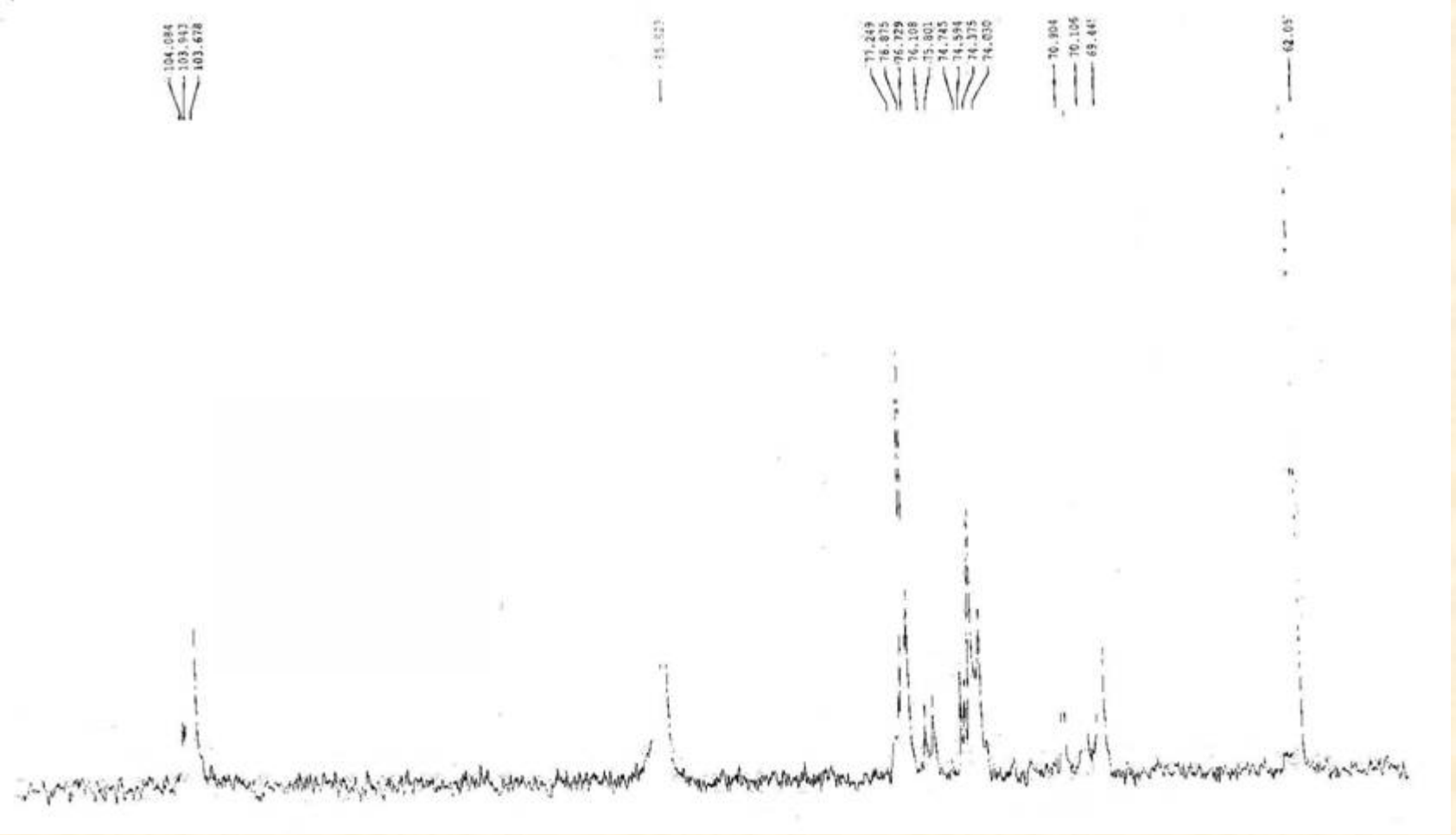


^{13}C-NMR-spectra of initial laminaran



A 1,3;1,6-β-D-glucan (laminaran) contains β-1,6-bound glucose residues (10%) and mannitol

^{13}C-NMR-spectra of translam



A new 1,3;1,6-β-D-glucan (translam) contains β-1,6-bound glucose residues (20-25%), but no mannitol

Conclusions

The results presented in the article demonstrated that the 1,3-β-D-glucanases were successfully immobilized in the novel hybrid polysaccharide-silica nanocomposites.

1,3-β-D-Glucanases

✓had the maximal activity at conditions (pH, temperature and ionic strength) that were optimal for them in solutions before the entrapment;

✓provided the synthesis biologically active, branched 1,3;1,6-β-D-glucan (translam);

✓retained or even had sometimes an increased activity, became more thermally stable and demonstrated prolonged long-term stability.

These facts give evidence that the suggested immobilizing method is ideally suited for the entrapment of enzymes and development of biocatalyst for biotechnological applications.

Our articles

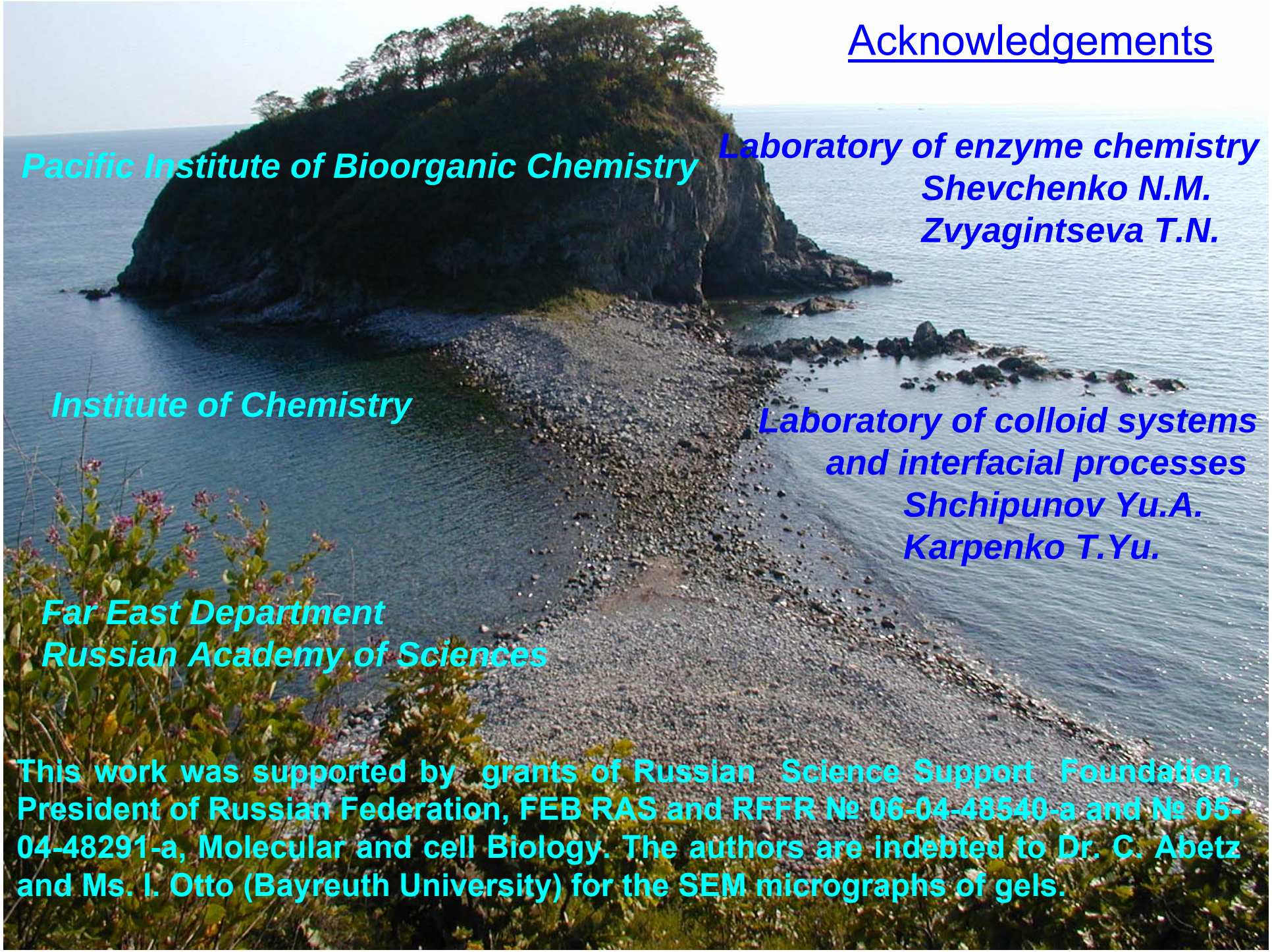
1. Shchipunov Yu. A., Mukhaneva O.G., Zvyagintseva T. N., Shevchenko N. M. Polyelectrolyte complexes of naturally occurring fucoidans with cationically and hydrophobically modified hydroxyethyl cellulose. //Visokomolecular. Soedin.(Russian) Ser. A, 2003. V. 45. P. 295-303.

2. Karpenko T.Yu., Shchipunov Yu.A., Bacunina I.Yu., Burtseva Yu.V., Zvyagintseva T.N. Biocatalysts Prepared by the Immobilization of O-glycoside Hydrolases inside Polysaccharide-Silica Nanocomposites. In Proceedings of 18-th European Conference on Biomaterials including Third Young Scientists' Forum. 2003. T099. October. Stuttgart, Germany.

3. Shchipunov Yu. A., Karpenko T. Yu., Bakunina, I. Yu., Burtseva, Yu. V., Zvaygintseva T. N. A new precursor for the immobilization of enzymes inside sol–gel-derived hybrid silica nanocomposites containing polysaccharides. J. Biochem. Biophys. Meth. 2004, V. 58. P. 25-39.

4. Burtseva Yu. V., Karpenko T. Yu., Shevchenko N. M., Zvyagintseva T. N., Shchipunov Yu. A. Properties and specificity endo-1,3-β-D-glucanases of marine mollusks immobilized in hybrid nanocomposites. Regional Science Conference. 2004. P. 75. November. Vladivostok, Russia.

5. Shchipunov Yu. A., Burtseva Yu. V., Karpenko T. Yu.,Shevchenko N. M., Zvyagintseva T. N. Immobilization and characterization of laminarinases (endo-1,3-B-D-glucanases) from marine mollusks in novel hybrid polysaccharidesilica nanocomposites. Journal of Molecular Catalysis B: Enzymatic. 2006.



Acknowledgements

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Thank you
for your attention