

Aus dem Institut für Technologie und Biosystemtechnik

**Emine Capan
Ulrich Jahnz
Klaus-Dieter Vorlop**

**Pre-activated LentiKat -hydrogels for covalent binding
of enzymes**

Manuskript, zu finden in www.fal.de

Published in: Landbauforschung Völkenrode Sonderheft 241,
pp. 151-153

**Braunschweig
Bundesforschungsanstalt für Landwirtschaft (FAL)
2002**

Pre-activated LentiKat[®]-hydrogels for covalent binding of enzymes

Emine Capan¹, Ulrich Jahnz² and Klaus-Dieter Vorlop¹

Abstract

A mild new enzyme immobilisation technique using pre-activated poly (vinylalcohol) hydrogel (LentiKats[®]) as an immobilisation matrix was developed. This new technique is based on the following procedure: First chitosan is entrapped in LentiKats[®] as non-dissolved small particles according the standard method (Jekel et al. 1998). Subsequently, the amino-groups of chitosan are activated with glutardialdehyde. After removing non reacted glutardialdehyde in a washing step, LentiKats[®] are incubated with the enzyme solution. The enzyme molecules diffuse into the hydrogel matrix and are covalently bound. As model reaction we use the enantioselective synthesis of optically active (R)-cyanohydrins.

Keywords: cyanohydrin, entrapment, enzyme immobilisation, LentiKats[®], (R)-oxynitrilase, pre-activated polyvinylalcohol hydrogel (PVA)

1 Introduction

PVA hydrogels as an immobilisation matrix are widely used because these hydrogels are very elastic and stable. In addition, PVA is an inexpensive material for entrapment. PVA hydrogels are mainly generated by the freeze thawing method or by cross-linking with boric acid (Muscat et al. 1996, Lozinsky and Plieva 1998). The problem with these methods is the complicated generation of the hydrogels. To overcome this shortcoming, a new and mild gelation method was developed. This gelation method, based on partial drying of LentiKat[®]Liquid (a polyvinylalcohol-containing aqueous solution which is commercially available from geniaLab, see also geniaLab 2001) at room temperature (Jekel et al. 1998), results in lens-shaped hydrogels (LentiKats[®]) and offers the following advantages: low costs for matrix and production, easy preparation, excellent mechanical stability, easy separation from reaction mixture (diameter 3-4 mm) and low diffusion limitation (thickness 200-400µm). LentiKats[®] were successfully used for the immobilisation of cells (Durieux et al. 2000, Wittlich et al. 1999a, Wittlich et al. 1999b, Welter et al. 1999). Due to the enzymes' small size and their subsequent

diffusion out of a gel matrix, an entrapment of pure enzymes in polyvinylalcohol hydrogels (LentiKats[®]) often fails. A possibility to keep enzymes in LentiKats[®] is to cross-link the enzyme with chitosane and glutardialdehyde to obtain a supra-molecular structure prior to entrapment in LentiKats[®] as shown in Figure 1 (geniaLab 2001, Gröger et al. 2001).

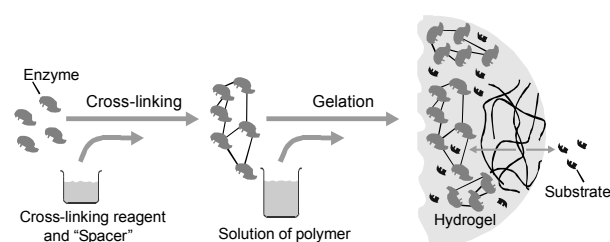


Figure 1:
Entrapment of cross-linked enzyme in LentiKats[®]

Instead of first cross-linking the enzyme and entrapping the resulting complex, we pursue the following alternative procedure: First chitosan is entrapped in LentiKats[®] as non-dissolved small particles. Subsequently, the amino-groups of chitosan are activated with glutardialdehyde.

After removing non reacted glutardialdehyde in a washing step, the LentiKats[®] are incubated with (R)-oxynitrilase. The enzyme molecules diffuse into the hydrogel matrix and are covalently bound (Figure 2).

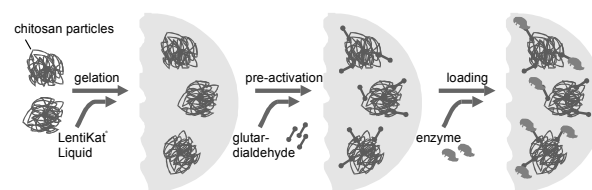


Figure 2:
Entrapment of enzyme in pre-activated LentiKats[®]

The (R)-oxynitrilase from almonds, E.C.4.1.2.10, which catalyses the reversible condensation of hydrogen cyanide with aldehydes, is a useful and promising enzyme for biotransformation processes (Figure 3).

¹ Emine Capan and Klaus-Dieter Vorlop, Institute of Technology and Biosystems Engineering, Federal Agricultural Research Centre (FAL), Bundesallee 50, 38116 Braunschweig, Germany

² Ulrich Jahnz, geniaLab Biotechnologie GmbH, Bundesallee 50, 38116 Braunschweig, Germany

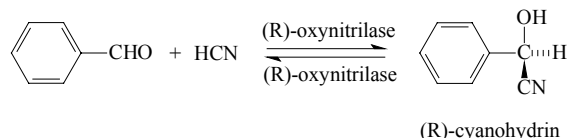


Figure 3:
Synthesis of (R)-cyanohydrin.

The resulting optically active cyanhydrins are expedient starting materials for the preparation of several important classes of compounds such as α -hydroxyketones, α -hydroxyacids, β -aminoalcohols as well as aminonitriles and aziridines.

2 Materials and methods

2.1 Preparation of LentiKats[®]

LentiKat[®]Liquid (geniaLab, Braunschweig) was liquified according to manufacturer's directions (geniaLab 2001). 5 g chitosan was shaken in 5 ml of phosphate buffer (20 mM, pH 7). The chitosan solution and 10 ml water were added under thorough mixing to 80 g LentiKat[®]Liquid to give a final volume of 100 ml. Using a printing device with over 400 identical pins, small droplets of approximately 4 μ l were floored on a plastic dish and exposed to drying. After drying down to 30 % of the initial mass the resulting lenses were submerged in a stabilising solution (geniaLab) to reswell for 30 minutes.

Subsequently, the amino-groups of chitosan were activated with 30 ml glutardialdehyde (50 % (v/v)) for 16 h at 4 °C.

After removing non reacted glutardialdehyde by a washing step LentiKats[®] were incubated with (R)-oxynitrilase (purchased from ASA Spezialenzym GmbH, Braunschweig; specific activity: 51 U/mg protein; amount of protein: 14 mg/mL). At first different amounts of enzyme (3.14-15.69 mg protein) were dissolved in 8 ml of phosphate buffer (200 mM, pH 7). In a subsequent step, 2 g LentiKats[®] were added at the current enzyme solution. In this step, the enzyme molecules diffused into the hydrogel matrix and were covalently bound. The concept of this immobilisation method is shown in Figure 2.

2.1 Activity of the immobilised enzyme

To 400 ml of a solution of 0.1 mM benzaldehyde in citrate buffer (50 mM pH 3.75), 0.5 g KCN were added. After removing non covalently bound (R)-oxynitrilase in a washing step 1, LentiKats[®] were added to the reaction mixture. The reaction mixture was stirred for 30 minutes at 20°C.

The conversion of benzaldehyde was analysed by photometer ($\lambda = 250$ nm) and the formation of (R)-cyanohydrin was analysed by HPLC using a chiral column (chiralpak[®] AD, Merk-Eurolab, Germany). Analysis was done at 20°C (column) with hexan/2-propanol (90/10 v/v) as the eluent at a flow rate of 1.0 ml/min.

3 Results and discussion

To check whether the enzyme had really diffused into the hydrogel matrix and was covalently bound inside the pre-activated LentiKats[®] we measured the activity of washed LentiKats[®] for different concentrations of the enzyme as well as of the supernatants in relation to the activity of the free enzyme. The results are collected in Table 1. As can be seen from Table 1, the amount of bound activity was rather poor.

Table 1:
Activity of pre-activated LentiKats[®] incubated with different concentrations of enzyme. LK: LentiKats[®], pro: protein, chi: chitosan, U: units)

Deployed activity (4 mL+1g LK), U	Activity of 1g LK, U	Covalently bonded protein, μ g _{prot} /g _{LK}	Covalently bonded protein, μ g _{prot} /mg _{chi}
80	1.37	27	0.54
100	1.39	27	0.54
133	1.42	28	0.56
200	1.59	31	0.62
400	1.59	31	0.62

Even a five-fold increase in enzyme concentration (80 to 400 U in total) caused an enhancement of only 15% (27 to 31 U). To investigate whether this was due to an insufficient diffusion of the enzyme into the hydrogel, the activity of soaked LentiKats[®] was measured as depicted in Figure 4.

The graphs clearly show that a major amount of the employed enzyme indeed diffuses into the hydrogel and that the poor results after washing are based on an inadequate binding capacity of the activated chitosan particles.

4 Conclusion and Outlook

This study proves that the idea of pre-activated LentiKats[®] is a viable method for enzyme immobilisation. However, further work has to focus on increasing the binding capacity inside the hydrogel. Entrapment of materials other than chitosan and other reactive compounds other than the aldehyde groups, espe-

cially nanoparticles with oxirane groups, will possibly show better results.

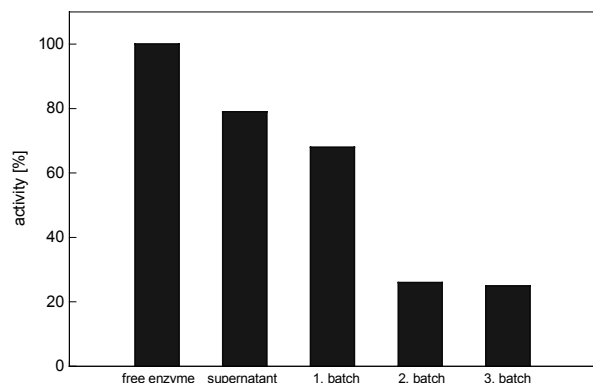


Figure 3:

Activity of free enzyme, supernatant and LentiKats[®]. 1. batch: without washing the LentiKats[®] before testing on activity, 2. and 3. batch: with washing the LentiKats[®] before testing on activity

References

- Durieux A, Nicolay X, Simon J-P (2000), Continuous malolactic fermentation by *Oenococcus Oeni* entrapped in LentiKats[®], *Biotechnol Lett* 22:1679-1684
- genialLab Biotechnologie GmbH (2001), <http://www.genialab.com>
- Gröger H, Capan E, Barthuber A, Vorlop K-D (2001) Asymmetric synthesis of an (R)-cyanohydrin using enzymes entrapped in lens-shaped gels. *Org Lett* 3:1969-1972
- Jekel M, Buhr A, Vorlop K-D (1998) Immobilisation of biocatalysts in LentiKats. *Chem Eng Technol* 21:275-278
- Lozinsky VI and Plieva FM (1998) Poly(vinylalcohol) cryogels employed as matrices for cell immobilisation. 3. Overview of recent research and developments. *Enzyme Microb Technol* 23:227-242
- Muscat A, Prüße U, Vorlop K-D (1996) Stable support materials for the immobilization of viable cells. In: Wijffels R H, Buitelaar R M, Bucke C, and Tramper J (eds): *Immobilized Cells: Basics and Applications*, Elsevier Amsterdam, pp 55-61
- Welter K, Willke T, Vorlop K D (1999): Production of itaconic acid by LentiKats[®]. *SchrR Nachwachsende Rohstoffe* 14:520-521
- Wittlich P, Schlieker M, Jahnz U, Willke T, Vorlop KD (1999a): Bioconversion of raw glycerol to 1,3-propanediol by immobilized bacteria. *Proceedings 9th European Congress on Biotechnology* (July 11-15, Brussels) no. P2762, ISBN 805215-1-5
- Wittlich P, Schlieker M, Lutz J, Reimann C, Willke T, Vorlop KD (1999b): Bioconversion of glycerol to 1,3-propanediol by LentiKats[®]. *SchrR Nachwachsende Rohstoffe* 14:524-532