Pergamon

Tetrahedron Letters, Vol. 35, No. 48, pp. 9051-9054, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01926-6

Esters of Cross-linked Polyvinyl Alcohol with Fatty Acids: New Stationary Phases for the Separation of Lipolytic Enzymes

Enrico Cernia, *Giancarlo Ortaggi, Simonetta Soro

Dipartimento di Chimica, Università degli Studi di Roma "La Sapienza", Piazzale Aldo Moro 5, 00185 Roma (Italy)

Massimo Castagnola

Dipartimento di Chimica, Università Cattolica di Roma "Sacro Cuore", Largo Francesco Vito 1, 00100 Roma (Italy)

Abstract: A new class of functionalized synthetic polymers with a modular affinity toward lipidic molecules was prepared and tested in the successful separation of lipases by a single step chromatography. The polymers were characterized by infrared spectroscopy, electron microscopy and enzymes adsorption.

Increasing interest is currently devoted to the separation and purification processes for the sophisticated isolation of even small amounts of compounds. This is particularly true in the field of biotechnologies where one of the most outstanding and promising aspect is the separation of isoenzymes which generally characterize the commercial preparations and possess different catalytic properties especially as to regio- and stereoselectivity [1].

We have now prepared a new class of polymers with an high modular structure which are particularly indicated for the purification of biomolecules by hydrophobic chromatography. These polymers are formally esters of cross-linked polyvinyl alcohol (CL-PVA) with linear fatty acids of different length and are prepared by cross-linking PVA with the appropriate excess of epichlorohydrin to obtain the maximum degree of reticulation (CL-PVA-X-40); then the cross-linked polyvinyl alcohol (CL-PVA) was refluxed in the appropriate acyl chloride ($CH_3(CH_2)_nCOCl$; n= 6, 8, 10, 12, 16) containing pyridine to neutralize the hydrogen chloride formed and the corresponding esters of CL-PVA were isolated (scheme 1.).

All these esters are white powders which were characterized by scanning electron microscopy and infrared spectroscopy; they are insoluble in water and in the common organic solvents. The esters differ in many respects from linear PVA. They undergo swelling in water and are stable towards the thermal decomposition, mainly dehydratation. Scanning electron microscopy pointed out the beads possess a wide superficial distribution, are sufficiently compact and uniform, so that appear to be idoneous as stationary phase in chromatographic separations.

The infrared spectra of the esters show a sharp absorption at 1750 cm^{-1} which disappeared after base-hydrolysis. This technique allowed to calculate exactly the relative amount of esterification of the resins: these values are collected in table 1 and show that the percentages of esterification are quite high and decrease only with C16 fatty acid, probably for the steric repulsions between the carbon chains.

Scheme 1. The two steps for the preparation of esters of cross-linked PVA with fatty acids (PVA-CL-Cn)

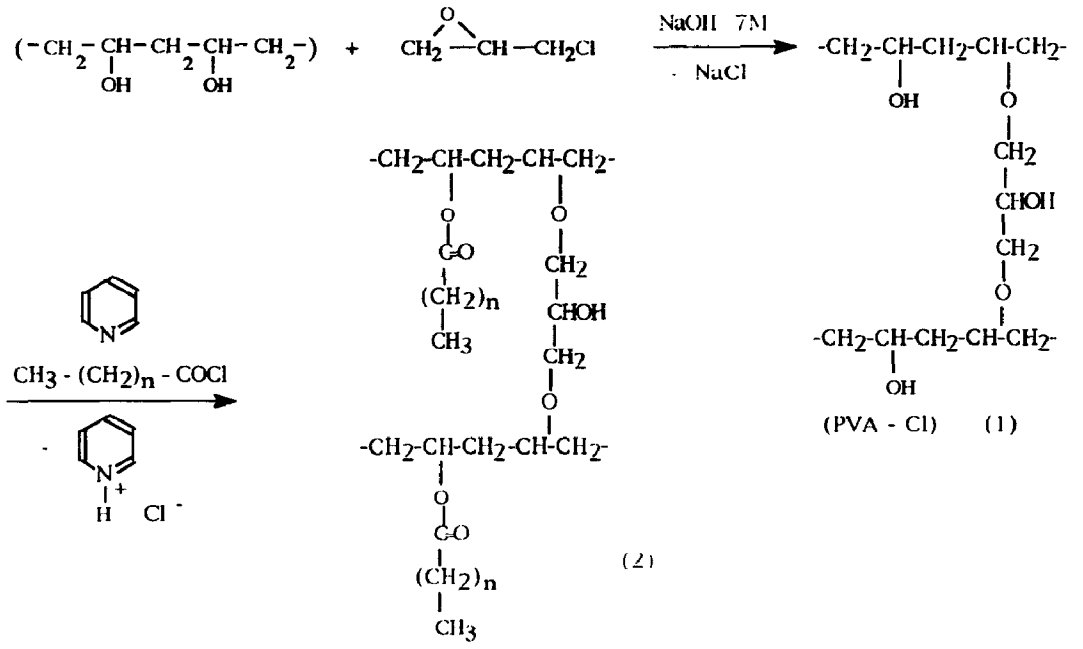


Table 1. Esterification grade of CL-PVA

n° carbon atoms	C6	C8	C10	C12	C16
mmol acid/ g CL-PVA-Cn	6.05	5.50	4.64	4.18	3.03
esterification (%)	67	81	72	79	51

However, the most interesting feature of above resins is the modulation of their selectivity toward lipases simply by changing the fatty acid chain. Owing to our interest in the reactions catalyzed by lipase from *Candida rugosa* [2](fats, oil industry, ester synthesis, resolution of racemic esters, acids and alcohols)[3], we have successfully tested the above polymers both for the absorption toward the enzyme and in the column chromatography of commercial samples[4].

The butch experiments were performed by suspending the appropriate amount of CL-PVA-Cn in buffer of N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES) 20 mmol/L, ethylenediaminetetraacetic acid (EDTA) 2 mmol/L, pH 7.6 and adding a solution of *Candida rugosa* lipase in the same buffer. After two hours of vigorous stirring the suspension was filtered and washed with the buffer solution. The solid, the filtered and the washings were tested for lipolytic activity by titration of the acid formed by the hydrolysis of tricaprylin. The data are presented in table 2 and show that the C-10 ester is by far the most efficient matrix for lipase absorption.

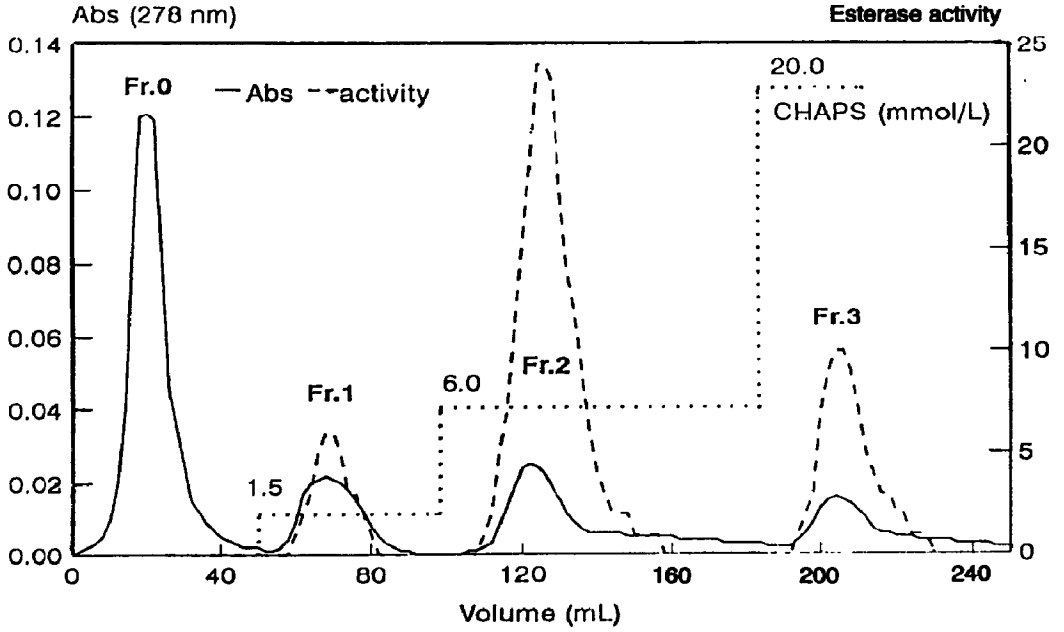
Table 2. Lipase adsorption data

	Adsorbed lipase on CL-PVA-Cn (mg)				
Lipase in initial solution (mg)	**C6**	**C8**	**C10**	**C12**	**C16**
100	96	95	97	96	96
200	192	188	193	192	146
300	232	283	291	261	218
400	308	331	384	324	264
500	3387	387	474	369	306
600	363	432	499	37365	360
1000	450	671	768	404	452

The CL-PVA resins esterified with C-10 and C-12 fatty acids were used for column chromatography of commercial lipase by eluting with HEPES-EDTA buffer, pH 7 6 containing 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) at increasing concentrations (1.5, 6.0, 20 mmol/L) (fig.1). Three fractions were separated (1, 2 and 3); the lipolytic and esterase activity and the electrophoretic analysis showed that fraction 1, 2 and 3 contain one, three and two isoforms, respectively.

Thus the polymers appear to be efficient for the chromatographic separation and purification of isoforms of commercial lipases, a process which often requires exhausting steps [5,6] Further work is in progress to investigate the versatility and selectivity of the above polymers towards lipidic substrates

Figure 1. Affinity chromatographic separation of a commercial preparation of *Candida rugosa* lipase by CL-PVA-C12



References and notes

1. Alberghina, L.; Schmid, R.D.; Verger, R. *Lipase: Structure, Mechanism and Genetic Engineering*, VCH: New York, GBF Monography, Vol. 16, 1991, 369-372.
2. (a) Theil, F.; Schick, H.; Winter, G.; Reck,, G.; *Tetrahedron* **1991**, 47, 7569. (b) Matsuda, H.; Minami, N.;Asai, M.; Nakanishi, H. *Biotechnol. Bioeng.* **1991**, 38, 1218. (c) Goldberg, M.; Thomas, D.; Legoy, M.D. *Eur. J. Biochem.* **1990**, 190, 603. (d) Tsai, S.-W.; Chiang, C.-L. *Biotechnol. Bioeng.* **1991**, 38, 206.
3. Pieroni, G.; Gargouri, Y.; Sarda, L.; Verger, R. *Advances in Colloid and -Interface Science* **1990**, 32, 341
4. Lipase (EC 3.1.1.3.) from *Candida rugosa* was obtained from Sigma Chemical Co.
5. Branchi-Horn, M.C.; Guglielmina, M.L.; Elling, L.; Sparrow, L.G. *Biochim. Biophys. Acta* **1990**, 1042, 51-54
6. Wu, S.H.; Guo, Z.W.; Sih, C.J. *J. Am. Chem. Soc.* **1990**, 112, 1990

(*Received in UK* 23 *May* 1994; *revised* 26 *September* 1994; *accepted* 30 *September* 1994)