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Stereoselective ester hydrolysis of monomeric and polymeric methacryl derivatives containing methionine by pig liver esterase (PLE)

Gabriele Korp and Helmut Ritter*

Bergische Universität-Gesamthochschule Wuppertal, Fachbereich 9, Gaußstrasse 20, D-5600 Wuppertal 1, Federal Republic of Germany

SUMMARY

In this study N-methacyloyl-D,L-methionyl-D,L-methioninmethyl-ester and the corresponding polymers were synthesized, and the PLE catalyzed hydrolysis of these products was then analayzed under stereochemical aspects. It is shown that PLE can stereoselectively hydrolyse L-ester functions bound to a polymer.

INTRODUCTION

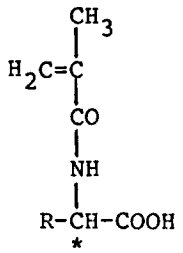
The enzymatic catalyzed hydrolysis of peptide functions in side groups of polymethacryl derivatives has been intensively investigated for several years.(1) Also the hydrolysis of a ^{14}C-marked polyethylen terephthalate by PLE in heterogeneous phase has been observed.(2) Though microorganisms and pure enzymes are gaining importantance, particularly for the entantioselective synthesis of natural products,(3) these stereochemical aspects have only rarely been applied to macromolecular chemistry. At an early stage of our study we were able to show the PLE catalyzed and kinetically prefered ester-hydrolysis of the L-components of the monomers N-methacryloyl-D,L-alaninethylester and N-methacryl-oyl-D,L-alanyl-D,L-alaninmethylester. Likewise, the corresponding acrylamide copolymers containing racemic aminoacids were turned into optically active polymers via stereoselective PLE hydrolysis of the ester function.(4)

Synthesis of Monomers and Polymers Containing Methionine

N-methacryloyl-D,L-methionine 1 was sythesized by reacting D,L-methionine with trimethyl-chlorsilane and methacrylchloride. The methylsilyl protection group was removed by hydrolysis. The product was then reacted with ethychloroformate, methionine- methyl-ester and asparagineaciddimethylester yielding the monomers: N-methacyloyl-D,L-methionyl-D,L-methioninemethylester 2 and N-methacryloyl-D,L-methionyl-D,L-asparagineaciddimethylester 3 . The addition of optically pure aminoacids to monomer 1 yielded the

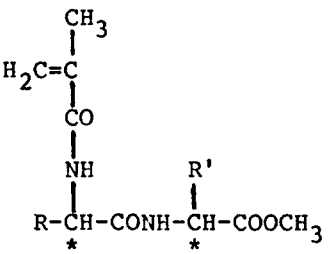
* To whom offprint requests should be sent

optically active model substances 2a, 2b, and 3a:



D,L

1:R= $CH_3SCH_2CH_2$-



2 :R'=R D,L - D,L

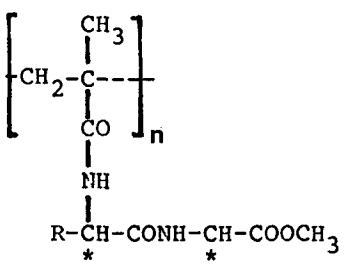
2a:R'=R D,L - L

2b:R'=R D,L - D

3 :R'=-CH_2COOCH_3 D,L - D,L

3a:R'=-CH_2COOCH_3 D,L - L

The monomers 2, 2a, and 2b were radically homopolymerized and copolymerized with acrylamid, yielding the products 4, 4a, 4b /5.

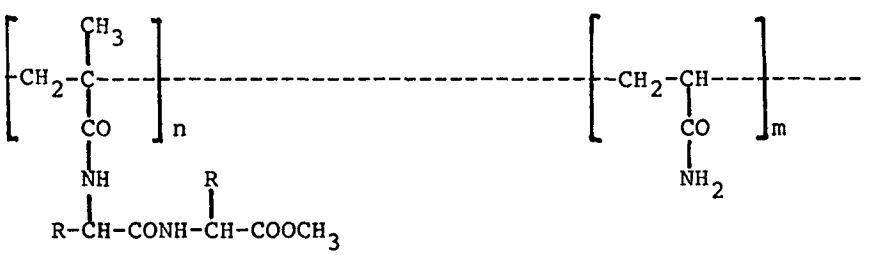


R=$CH_3SCH_2CH_2$

4 : D,L - D,L

4a: D,L - L

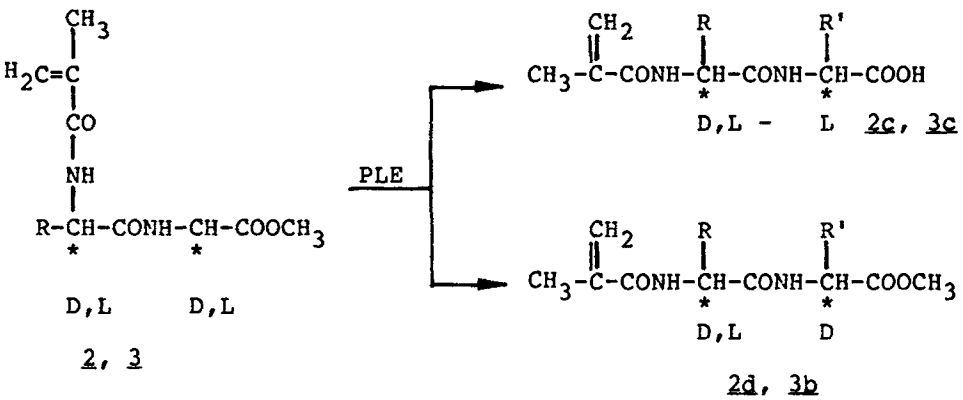
4b: D,L - D



5: R=$CH_3SCH_2CH_2$- (n:m = 1:10)

Enzymatic Hydrolysis of Monomer and Polymers Containing Aminoacidesters.

The monomers 2 and 3 were dispersed into a buffered aqueous solution (pH=7). After PLE was added at 33 °C the hydrolysis of the ester started immediately. The resulting change in pH-value was compensated with NaOH. After a 50% hydrolysis the products were extracted. From the alkalized solution an optically active ester was extracted while the extraction of the acidified solution yielded an acid component. These substances were identified with IR- and ^{1}H-NMR-spectroscopy. Polarimetrical measurements indicated the cleavage of the optical inactive educts 2 and 3 into an optically active acid 2c and 3c and into an optically active ester 2d and 3b. ^{1}H-NMR-spectroscopy showed that substance 3 was hydrolyzed to 70% at the α-ester function and to 30% at the β-ester function. Following reaction scheme may be drawn by comparing the optical rotation angles of the components 2c, 2d, 3b and 3c with the values of the optically pure model substances 2a and 3a:



The specific rotation angles $[\alpha]^{22°C}_{589nm}$ are listed in table 1. It can be infered that PLE preferably cleaves the naturally occuring L-aminoacid component. The optical purity p_i can be determined with the rotational angles in the following equation:(5)

$$p_i = \frac{\text{spec. rotation angle of enantiomer mixture}}{\text{spec. rotation angle of pure enantiomer}} \times 100$$

The optical purity of the unhydrolyzed ester component 2d is:
$p_i = 10.2/18 = 0.57$

The purity of the ester component 3b is:
$p_i = 12.8/30.8 = 0.42$

Table 1: Specific rotation angle of the optically active monomers (layer thickness: 10cm; concentration: c= 0.005g/ml in $CHCl_3$)
(C) ≙ chemically synthesized component
(E) = component produced by enzymatic hydrolysis

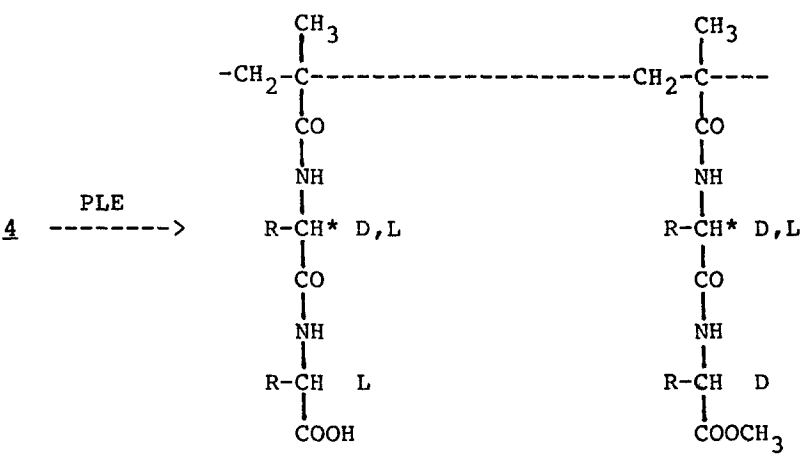
Monomer	L-ester 2a (C)	L-acid 2c (E)	D-ester 2d (E)	L-ester 3a (C)	L-acid 3c (E)	D-ester 3b (E)
$[\alpha]_{589nm}^{22°C}$	+18	+1.6	-10.2	+30.8	+7.4	-12.8

To find out if polymers 4 and 5 are also subject to stereoselective esterhydrolysis by PLE, the cleavage of the ester group in copolymer 5 was studied in a homogeneous aqueous phase. As was subsequently observed with copolymers containing alanine, (4) optically active products were also formed by the partial enzymatic ester hydrolyzation of the optically inactive methionine polymeres.

($[\alpha]_{589nm}^{22°C}$:-1.42 (c=0.01g/ml))

The same behavior is exhibited by the water-insoluble homopolymer 4 in a heterogeneous phase. PLE converted it into an optically active material

($[\alpha]_{589nm}^{22°C}$:-2.4 (c=0.005g/ml)) because of the partial hydrolysis of its ester functions. Verification of the stereospecific hydrolysation of the L-component in polymers cannot be achieved by the same methodes used for monomers. For this reason the pure model polymers D,L-L (4a) and D,L-D (4b) were synthesized to show the stereoselective property of the enzyme by their markedly different rates of hydrolysis. Both model polymers were exposed under the same conditions to PLE. Only the D,L-L model polymer (4a) yielded a water soluble fraction which was characterized through ^{1}H-NMR, which revealed a 30% acid portion. In contrast the D,L-D homopolymer (4b) was not hydrolyzed by the enzyme. The following reaction scheme was postulated:



In conclusion it may be stated that most work has been done in the field of small molecules to demonstrate the enantioselective hydrolysis via PLE. It was shown in this study for the first time that the enzyme PLE will also stereoselectively hydrolyse ester functions bound to a polymer.

EXPERIMENTAL

Monomers

N-methacryloylmethionine (1): To a suspension of 29.8g (0.2mol) D,L-methionine in 500ml absol. methylenchloride 55.8ml (0.4mol) triethylamine and 25.4ml (0.2mol) trimethylchlorsilane are added at 0°C. While stirring the solution 19.3ml (0.2mol) methacryloylchloride in 100ml absol. methylenchloride are added and the mixture is stirred at ambient temperature for 20h. The solvent is then evaporated, the residue stirred with 200ml 2N HCl, and the aminoacid is extracted twice with 500ml methylenchloride. The resulting organic phases are washed with 300ml 2N HCl followed by 500ml H_2O and dried over Na_2SO_4. The solvent is evaporated and the resulting oil is again solved in a small amount of methylenchloride and covered with an ether phase to induce crystallization. Yield: 42%, m.p.: 95°C,
I.R. (KBr): 1735 (C=O, acid), 1654 (C=O, amid), 1610cm^{-1} (C=C);
M.S. m/e = 217 (M+).

$C_9H_{15}NO_3S$ (217.2)	calc.	C 49.75	H 6.96	N 6.45
	found	C 49.71	H 8.88	N 6.33

N-methacryloyl-D,L-methionyl-D,L-methioninemethylester (2):
Solution I: 4.36g (0.02mol) N-methacryloyl-D,L-methionine 1 is solved in 30ml THF. 2.8ml (0.02mol) triethylamine and 0.1ml pyridine are added. At -15°C 1.9ml (0.02mol) ethylchloroformate are added dropwise.

Solution II: This solution is prepared 5 min before use by suspending 4g (0.02mol) D,L-methioneinmethylesterhydrochloride (7) in 30ml THF and adding 2.8ml (0.02) triethylamine. Water is added until the ester is fully dissolved. Before the two solutions are unified, solution I is stirred for two hours. After unification the reaction mixture is stirred vigorously for 8h at ambient temperature during which CO_2 evolves. THF is then destilled leaving a small amount of water and oil as residue. The oil is diluted with a small amount of ethyl acetate, washed with 1N HCl, H_2O, and a 5% aqueous solution of $NaHCO_3$ each three times. The residue base is removed by a small amount of water and the organic phase is dried over $MgSO_4$. The product is then isolated by destilling the the organic solvent and recrystallizing the residue in ethyl acetate/ petrolether.
Yield: 32%, m.p.: 90°C
I.R. (KBr): 1750 (C=O, ester), 1650 (C=O, amid), 1610 cm^{-1} (C=C)
M.S. m/e = 362 (M+)

$C_{15}H_{26}N_2O_4S_2$ (362.5)	calc.	C 49.70	H 7.23	N 7.72
	found	C 49.84	H 7.07	N 7.60

N-methacryloyl-D,L-methionyl-D,L-asparagineaciddimethylester (3):

The synthesis follows the same procedures as for 2.
Yield: 55%, m.p.: 75°C
I.R. (KBr): 1740 (C=O, ester) 1650 (C=O, amid), 1610 (C=C)
M.S. m/e = 360 (M+)

$C_{15}H_{24}N_2O_6S$ (360.2) calc. C 49.97 H 6.72 N 7.77
found C 50.12 H 6.67 N 7.61

Polymers

Homopolymer 4: 500 mg of monomer 2, 20 mol% AIBN as an initiator and 1mg dodecylmercaptane are dissolved in 1,5ml THF. The reaction mixture is heated to 60°C for 24h in nitrogen atmosphere. The product is diluted with 6ml THF and precipitated by pouring it into 100ml Ether. The polymer is filtered and dried.
Yield 83%,
(DMSO): $[\eta]$ =3.7 (dl/g, 24°C)

Homopolymer 4a and 4b: The polymerization follows the same procedures as for 4 using D,L-L-dipeptide (yielding 4a) and D,L-D-dipeptide (yielding 4b).
(0.005g/ml in CH_2Cl_2, 10cm) = +10.8 (4a)
(0.005g/ml in CH_2Cl_2, 10cm) = - 9,6 (4b)

Copolymer 5: 0.98g (14 mmol) acrylamide were radically polymerized with 0.5g (1.4mmol) of monomer 2 in 3ml THF at 60°C. The initiator was 20 mol% AIBN. After 1h the reaction mixture was diluted with 10ml of water and precipitated by pouring into acetone.
Yield: quantative
H_2O: $[\eta]$ =11.85 (dl/g, 24°C)

Acknowledgement

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