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PAPAIN CATALYZED ESTERIFICATION OF ALANINE BY ALCOHOLS AND DIOLS

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Summary: Papain catalyzed esterification of Boc-Ala-OH with various alcohols and diols under biphasic conditions was investigated. Long-chain diols $HO(CH_2)_nOH$ gave good yields of esterification up to n=10 whereas the homologous alcohols $C_nH_{2n+1}OH$ condensed well only for n = 2, 4, 6. Esterification with functionnalized primary alcohols is also described.

The ethyl esters of N-acetylphenylalanine, tryptophan and tyrosine have been prepared with α-chymotrypsin as a catalyst under biphasic conditions[1-3]. α-Chymotrypsin has also been used for the synthesis of dipeptides containing D-aminoacids[4]. However papain itself appears to be a more versatile catalyst for the synthesis of unusual peptides[5] since it is less specific than the serine proteases.

In a previous report[6] we showed that papain catalyzed the esterification of a wide range of N-Boc-aminoacids: ethanol but also benzyl alcohol have been used for these esterifications.

Long-chain alkyl esters of aminoacids are of interest and methods to prepare them are still being developped[7].

Investigation of the scope and limitations of the papain catalyzed esterification of aminoacids with various alcohols and diols was undertaken.

t-Butyloxycarbonyl (Boc) alanine was chosen as substrate. The reactions were run in a biphasic medium with a concentrated citrate-phosphate buffer (1 M) at pH 4.2 and methylene chloride as the organic solvent[3,6]. Only primary alcohols and diols have been selected since secondary alcohols such as 2-butanol gave only poor yields of esterification (10%).

We have found that only short-chain monoalcohols $C_nH_{2n+1}OH$ condensed well with Boc-Ala-OH to give good yields of esterification (n = 2, 4: 88%, 78% yield respectively). With 1-octanol poor yields were obtained (21%) whereas with 1-decanol (n = 10) and 1-dodecanol (n = 12) no condensation took place (see Table).
In the case of 1,n-diols $HO(CH_2)_nOH$ a monoesterification was observed.

$$\text{Boc-Ala-OH} + \text{HO}\ (\text{CH}_2)_n\text{OH} \xrightarrow{\text{papain}} \text{Boc-Ala-O}(\text{CH}_2)_n\ \text{OH}$$

Sometimes trace amounts of the diester Boc-Ala-$O(CH_2)_nO$-Ala-Boc were formed (<2%). With these diols good yields of esterification were found not only for short-chain alcohols (n = 2, 4) but also with longer chain diols (n = 8, 10: 87% and 66% yield respectively). Even 1,12 dodecanediol condensed with Boc-Alanine albeit in low yield (26%).

So papain is able to accept long-chain alcohols as substrates. The difference in yield between the two types of alcohols (monoalcohols and diols) may be due to differences in partition coefficient of these alcohols between water and the organic solvent.

The esters formed by papain catalyzed condensation have been identified by comparison with authentic samples synthetized by the DCC/DMAP method[8]. In the case of the monoalcohols $C_nH_{2n+1}OH$ the chemical esterification worked well (72% yield). However with the 1,n-diols $HO(CH_2)_nOH$ a mixture of mono and diesters was obtained.

$$\text{Boc-Ala-OH} + \text{HO}(\text{CH}_2)_n\text{OH} \xrightarrow[\text{DMAP}]{\text{DCC}} \text{Boc-Ala-O}(\text{CH}_2)_n\text{OH} + \text{Boc-Ala-O}(\text{CH}_2)_n\text{O-Ala-Boc}$$

Consequently the chemical yield of the desired monoester was poor (40% for n = 2 to 8; 25% for n = 10,12). Furthermore difficulties in separation between the two esters were encountered for long chain diols (n = 10, 12).

The interest of the papain catalyzed esterification is that only the monoester is formed and good yields are obtained for n = 2 to 10. However the alcohol is used in excess (see Table) and the method might not be advantageous for expensive long-chain alcohols.

The papain catalyzed esterification has been extended to some primary alcohols bearing functionnal groups. Allyl alcohol and cyanoethanol, for example, were condensed with success (79% and 65% yield respectively).

With ethanolamine itself it has not been possible to isolate the expected ester. However with N-t-butyloxycarbonyl (Boc) ethanolamine and N-benzyloxycarbonyl (Z) ethanolamine the esters have been obtained in reasonable yields (50%).

Lipases are known to act as catalyst in the esterification of acids[9]. However attempts to esterify aminoacids failed[10]. Recently transesterification between an ester and 1,6-hexanediol have been found to be catalyzed by lipases[11]. The esterification of aminoacids by papain with long chain 1,n-diols could be complementary to the existing methods of enzymatic esterification of acids.

Table: Esterification of Boc-Ala-OH by papain in a 1 M citrate-phosphate buffer pH 4,2[a]

alcohols				diols			
ROH	conditions[b]	Boc-Ala-OR[c,d] yield (%)	[α]D[e] deg	$HO(CH_2)_nOH$	conditions[b]	Boc-Ala-OR[c,d] yield (%)	[α]D[e] deg
C_2H_5OH	3/5/1	88	-42	$HO(CH_2)_2OH$	3/50/1.5	74[h]	-36
C_4H_9OH	3/5/1	78	-41	$HO(CH_2)_4OH$	3/50/1.5	96	-35
$C_6H_{13}OH$	3/1/1	63	-36	$HO(CH_2)_6OH$	3/5/1	90	-35
$C_8H_{17}OH$	3/1/1	21	-33	$HO(CH_2)_8OH$	3/5/1	87[i]	-32
$C_{10}H_{21}OH$	-			$HO(CH_1)_{10}OH$	3/2/1	66	-28
$C_{12}H_{25}OH$	-			$HO(CH_2)_{12}OH$	3/2/1	26	-21
$CH_2=CH-CH_2OH$	3/5/1	79	-46				
$CN-CH_2CH_2OH$	3/50/1.5	65[f]	-41				
$BocNHCH_2CH_2OH$	3/5/1	50	-25				
$ZNHCH_2CH_2OH$	3/5/1	50[g]	-22				

a) the esterifications were performed on 0.5 mmole Boc-Ala-OH and 100 mg of papain, 37°, 24 h; b) amounts of buffer/CH_2Cl_2/ROH in ml or g if the alcohol is solid; c) after 24 hrs the water phase was extracted with methylene chloride; the combined organic phases were washed with 10% $NaHCO_3$ and dried; purification was performed on LH 20 (Pharmacia, THF as eluant); d) NMR, IR, mass spectra are in agreement with the theory: e) in methanol, c 1 g/100 ml; f) m.p.: 71°C; g) m.p.: 80°C; h) m.p.: 83°C; i) m.p.: 28°C.

REFERENCES

[1] K. Martinek, A.N. Semenov, I.V. Berezin, Biochim. Biophys. Acta, **658**, 76 (1981)

[2] A.M. Klibanov, G.P. Samokhin, K. Martinek, I.V. Berezin, Biotechnol. Bioeng., **19**, 1351 (1977)

[3] a) D. Tarquis, P. Monsan, G. Durand, Bull. Soc. Chim. Fr, II-76 (1980).
b) J.L. Vidaluc, M. Baboulene, V. Speziale, A. Lattes, P. Monsan, Tetrahedron, **39**, 269 (1983)

[4] J.B. West, C.H. Wong, J. Chem. Soc., Chem. Commun., 417 (1986)

[5] C.F. Barbas, C.H. Wong, J. Chem. Soc., Chem. Commun., 533 (1987)

[6] D. Cantacuzène, F. Pascal, C. Guerreiro, Tetrahedron, **43**, 1823 (1987)

[7] C.L. Penney, P. Shah, S. Landi, J. Org. Chem., **50**, 1457 (1985)

[8] B. Neises, W. Steglich, Angew. Chem. Int. Ed. Engl., **17**, 522 (1978)

[9] a) L. Velluz, Bull. Soc. Chim. Biol., **16**, 909 (1934)
b) G. Langrand, J. Baretti, G. Buono, C. Triantaphylides, Tetrahedron Lett., **27**, 29 (1986)

[10] G. Kirchner, M. Scollar, A. Klibanov, J. Am. Chem. Soc., **107**, 7072 (1985)

[11] A.L. Margolin, J.Y. Crenne, A. Klibanov, Tetrahedron Lett., **28**, 1607 (1987)

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