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Selectivity in Carbonic Anhydrase Catalyzed Hydrolysis of Standard N-Acetyl-DL-amino Acid Methyl Esters

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Abstract: *Carbonic anhydrase-catalyzed hydrolysis of some standard N-acetyl-DL-amino acid methyl esters proceeds with high enantioselectivity. This enzyme hydrolyses selectively D amino acid derivatives in contrast to proteases which have a L stereoselectivity.*

Carbonic anhydrase (carbonate hydrolyase, EC 4.2.2.1) is a zinc containing enzyme whose essential function is to catalyze the reversible hydration of carbon dioxide to bicarbonate[1-6](eq. 1). Carbonic anhydrase

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+ \qquad (1)$$

has also been shown to catalyze in vitro the reversible hydration of some carbonyl compounds[7] and the irreversible hydrolysis of a number of reactive functions[7-13] such as activated esters, carbonates, acid chlorides and related systems. Recently, we reported the enantioselective hydrolysis of methyl mandelates catalyzed by carbonic anhydrase.[14] We found that R enantiomers are better substrates but enantiomeric excess values were moderate (40-51%). We report here the enantioselective hydrolysis of some standard N-acetyl-DL-amino acid methyl esters catalyzed by bovine carbonic anhydrase (BCA).

The racemic substrates **1-9** (Scheme 1) were prepared in straightforward manner by esterification of the commercially available corresponding N-acetyl amino acids or by acetylation of amino acid esters. Preparative-scale enzyme catalyzed hydrolyses were performed on 2 mmoles of the substrates with 40 mg of BCA[15] in a phosphate buffer at pH 7.5. The reaction was indicated by the decrease of pH, which was maintained at its initial value by the addition of a dilute NaOH solution. The reactions were monitored from

Scheme 1

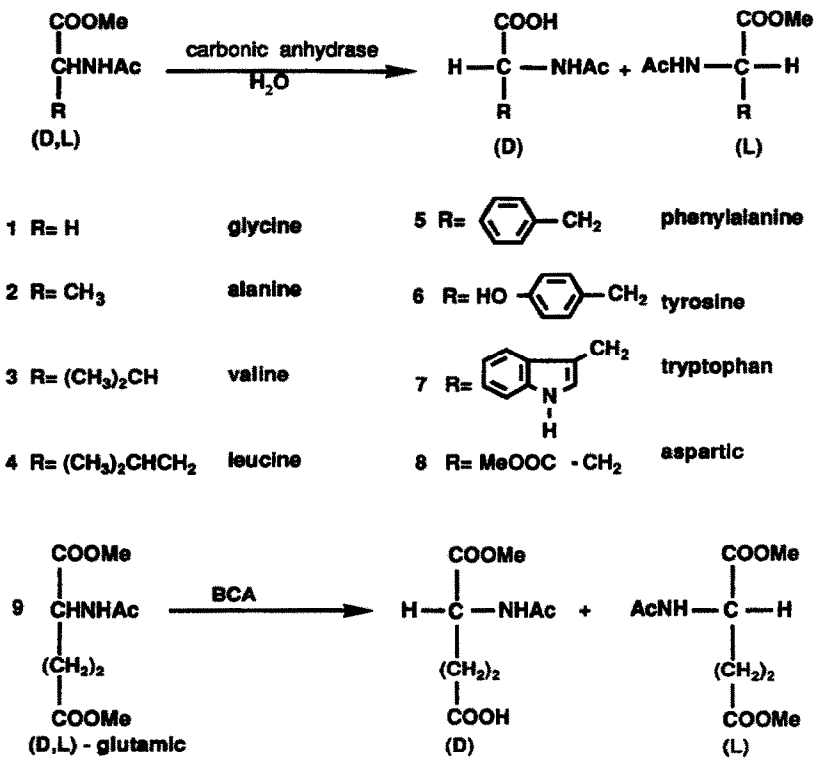


Table 1. Carbonic Anhydrase Catalyzed Hydrolysis of Amino Acid Derivatives.

Compound	Time (h)	Recovered ester			Acid product		
		Yield (%)	*ee* (%)	Abs. conf.	Yield (%)	*ee* (%)	Abs. conf.
1	5	23	–	–	20	–	–
2				not a substrate			
3				not a substrate			
4	118	77	70	L	70	68	D
5	36	83	≥95	L	81	≥95	D
6	14	75	0	–	73	0	–
7				not a substrate			
8	63	80	≥95	L	77	≥95	D
9	34	79	≥95	L	72	≥95	D

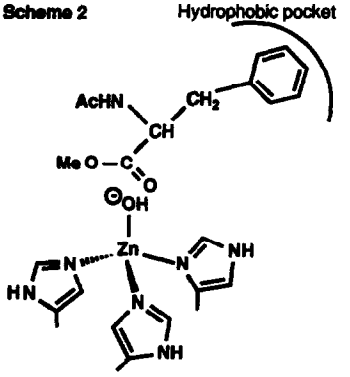
the consumption of the base and terminated when 50% of the substrate was hydrolyzed. The unreacted esters were recovered from the reaction mixtures by extraction with diethyl ether at pH 8.0. The product acids present in the aqueous phase as sodium salts were extracted with ethyl acetate after acidification to pH 2.0. The results are summarized in Table 1. Recovery yields ranged from 70-83%. Optical purities and absolute configurations were determined by comparison with the optical rotations reported in the literature. Enantiomeric purities (enantiomeric excess values) of acid products were further confirmed by reaction with (S)-(−)-1-(1-naphthyl)ethyl amine in the presence of 1-(2-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride followed by ^{1}H NMR (200 MHz) analysis of the resulting diastereomeric amides.

We first examined the hydrolysis of the achiral glycine derivative **1** and found that this compound is a good substrate for BCA. The low recuperation yields of glycine derivatives are due to the high solubility of these compounds in water. Derivatives of alanine (**2**), valine (**3**), and tryptophan (**7**) are not substrates for BCA whereas the tyrosine derivative (**6**) is hydrolyzed without enantioselectivity. Hydrolysis of the leucine compound **4** proceeded very slowly and with fair enantioselectivity (*ee* ~ 70%). Hydrolysis of N-acetyl-DL-phenylalanine methyl ester **5** and N-acetyl aspartic acid dimethyl ester **8** proceeded rather smoothly and the products were of high enantiomeric purity (*ee* ≥ 95%). The hydrolysis of diester **8** is regiospecific for the α–ester group. The BCA-catalyzed hydrolysis of the glutamic derivative **9** gave unexpected results: the reaction is regioselective for the ester group on the side chain. The hydrolyses stop at the mono-acid stage and the corresponding diacids are not detected in the reaction. Of special interest is the fact that the absolute configuration of the preferentially hydrolyzed enantiomer is D. The enantioselectivity of BCA is complementary to the L–stereoselectivity pattern of proteases.

In order to check that the enzymatic activity was not caused by the presence of hydrolases as impurities, the hydrolysis of **5** was repeated and found strongly inhibited in the presence of acetazolamide (5–acetylamino-1,3,4–thiadiazole–2–sulfonamide). It is well documented that esterase and hydrase functions of carbonic anhydrase are subject to the powerful and highly selective inhibitory action of aromatic sulfonamides.[1] Furthermore, BCA consist of a mixture of two isozymes (A and B isomers). The two resolved isozymes purified by electrophoresis are commercially available and were found to exhibit the same hydrolytic activities on the substrate **5**.

Several distinct but structurally homologous isozymes of CA are known to occur in higher vertebrates. Nevertheless, the basic features of the catalytic mechanism appear to be common to all forms.[16-18] Several X-ray crystallographic analysis of native, mutant and inhibitor-bound (arylsulfonamides) CA have been reported. The zinc ion is coordinated to three histidines and to a hydroxide ion at high pH. The catalytic zinc is located near the center of the enzyme molecule close to the bottom of a 15 Å-

Scheme 2 Hydrophobic pocket



deep cavity (Scheme 2) large enough to accommodate aromatic compounds such as phenylalanine or arylsulfonamides. One part of the cavity is dominated by hydrophobic amino acid side chains, whereas another part has a hydrophilic character.

CA is a stable enzyme commercially available in a pure lyophilized form. The substrate specificity and the stereoselectivity of CA is not yet well known but this enzyme could be a useful catalyst in organic synthesis. Molecular modeling studies and search of highly enantioselective hydrolyses are in progress.

Acknowledgments

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