THE EFFECT OF MODIFIERS ON THE HYDROLYSIS OF ESTERS AND PEPTIDES BY CARBOXYPEPTIDASE A*

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Received April 22, 1968

In the course of kinetic investigations of carboxypeptidase A we have found that N-substituted products of hydrolysis of certain synthetic peptide and ester substrates activate peptidase but inhibit esterase activity. Similar effects have been observed with a number of compounds, referred to collectively as modifiers, which have properties analogous to those of such N-terminal blocking groups (2,10-12). The present communication extends our previous reports concerning these phenomena and relates them to the kinetic data currently available on carboxypeptidase catalysis.

Results and Discussion

Unless otherwise indicated, the materials, methods and conditions for assay employed in the present study were identical to those described previously (2). In one series of experiments the effect of a single concentration of modifiers, .05 M, was examined with 10^{-3} M carbobenzoxyglycyl-L-phenylalanine (CGP) and hippuryl-d,l-β-phenyllactate (HPLA) (Table I). In another series, modifier concentration was varied while that of the substrate, benzoylglycyl-L-phenylalanine (BGP) and HPLA, were constant (Figs. 1 and 2).

* Supported by Grant-in-Aid HE-07297 from the National Institutes of Health of the Department of Health, Education and Welfare.

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TABLE I

EFFECT OF MODIFIERS ON PEPTIDASE (CGP) AND ESTERASE (HPLA) ACTIVITY

Modifier	Peptidase Activity[a] %	Esterase Activity[a] %
Carbobenzoxyglycine	230	33.6
Carbobenzoxyglycylglycine	346	38
Benzoylglycine	290	32
Benzoylglycylglycine	291	33
Benzamide[b]	252	53.2
Benzyl alcohol	263	35
Phenol	208	47.5
Cyclohexanol	302	25
Benzene[c]	152	76
Cyclohexanone	207	44
Cinnamic acid	513	12
Ethanol	90	91
n-Propanol	108	87
2-Chloroethanol	97	84
2,2,2-Trichloroethanol	206	30
2,2-Dimethylpropanol	260	24
3,3-Dimethylbutanol	409	14

[a] Peptidase activity determined at 1×10^{-3} M CGP, pH 7.5, 25°, 1.0 M NaCl, .02 M Veronal. Esterase activity determined at 1×10^{-3} M HPLA, pH 7.5, .005 M Tris, 25° C. In each case the modifier concentration was 0.05 M.

[b] .025 M benzamide.

[c] ~.01 M benzene.

The hydrolysis of the peptide BGP by native carboxypeptidase displays substrate activation (2). In contrast, the hydrolysis of the ester analogue HPLA exhibits substrate inhibition (5). A model postulating multiple binding

loci has been proposed to account for these and other kinetic anomalies of carboxypeptidase (10-12). This model suggests that a product common to the hydrolysis of a peptide and an ester pair ought to affect the rate of their hydrolysis in opposite directions, i.e. activating one while inhibiting the other activity. Indeed, benzoylglycine (BG), a product both of BGP and HPLA markedly increases the hydrolysis of BGP, but decreases that of HPLA (Fig. 1). This effect is even more pronounced when carbobenzoxyglycine (CG) is employed as the modifier. Product activation of CGP hydrolysis by CG* had been noted previously (13).

These effects are not restricted to N-substituted dipeptide hydrolysis products. They have also been observed with di- and tripeptides, e.g. CGG or CGGG, and with a number of compounds with characteristics similar to those of the blocking groups of such products (Table I).

For the majority of compounds examined the activation of peptidase activity is proportional to the inhibition of esterase activity. Such effects can be quite marked. Thus, at 1×10^{-3} M substrate, cinnamic acid increases the rate of CGP hydrolysis five-fold while it decreases that of HPLA eight-fold (Table I).

With a given modifier and substrate pair the quantitative details of activation or inhibition vary as a function of substrate concentration, as would be expected. Thus, the hydrolysis of BGP is known to exhibit substrate activation. Hence, if a modifier competes with this substrate, its effect should decrease with increasing substrate concentration, as demonstrated for the cyclohexanol activation of BGP hydrolysis (Fig. 2). Similarly, the hydrolysis of HPLA is known to exhibit substrate inhibition, and increasing HPLA concentration decreases the inhibition by modifiers.

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*CG has also been found to activate the hydrolysis of one ester, o-hippuryl glycolate (4) but to inhibit the hydrolysis of another, i.e. cinnamoylphenyllactate (1).

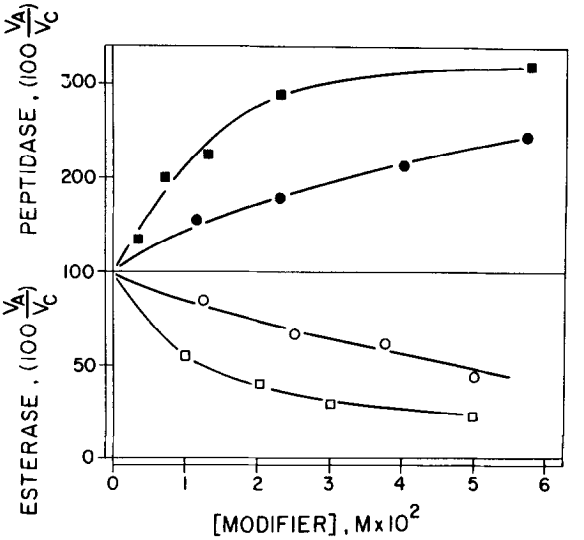

Figure 1 - Activation of BGP hydrolysis (1×10^{-3} M) and inhibition of HPLA hydrolysis (1×10^{-3} M) as a function of the concentration of BG (●,○) and CG (■,□). Peptidase assays were performed in 1.0 M NaCl, .05 M Tris, pH 7.5 and 25°, esterase assays in .2 M NaCl, .005 M Tris, pH 7.5 and 25°.

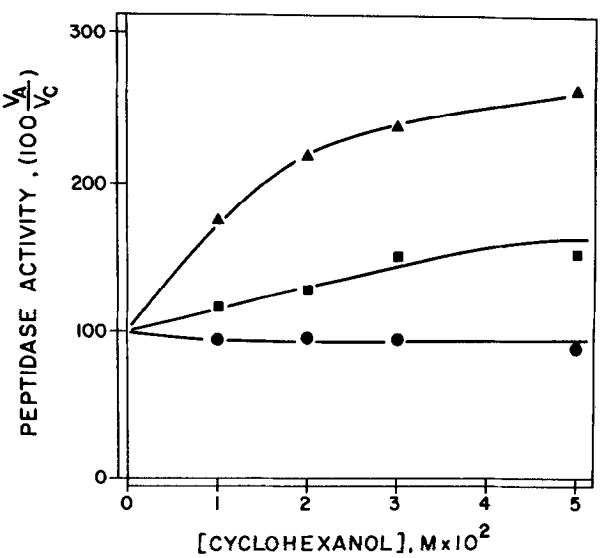

Figure 2 - Cyclohexanol activation of BGP hydrolysis at concentrations of 1×10^{-3} M (▲), 4×10^{-3} M (■), and 2×10^{-2} M (●) BGP. Assays performed in 1.0 M NaCl, .05 M Tris, pH 7.5 and 25°.

The chemical and physical properties of the agents which act in this manner are of obvious interest. The hydrophobic nature of the modifiers and their effects on activation are reminiscent of the inhibition of chymotrypsin by analogous compounds (3,6,7,14) and suggest hydrophobic interactions with the enzyme. Table I reveals that while all of these agents have hydrophobic character, a carboxyl group of an aromatic acid is not required for such reciprocal activation and inhibition since amides and alcohols also produce similar effects at comparable concentrations. The aromaticity of the blocking groups does not seem to be a specific requirement either; cyclohexanol also produces very marked and similar changes. The bulky aliphatic alcohols, 3,3-dimethylbutanol, 2,2-dimethylpropanol and 2,2,2-trichloroethanol act in analogous fashion and are more effective than straight-chain alcohols. Regardless of the chemical and physical features which may govern the effects of the modifiers, the activation of BGP and inhibition of HPLA hydrolysis by the same agent further emphasize the capacity of carboxypeptidase to discriminate between substrates, which in this instance differ only in the bond susceptible to hydrolysis.

While a number of mechanisms could explain such results, one of them is based on the multiple binding loci model (2,10-12). The activation of peptide hydrolysis can be regarded as a release of peptide inhibition. This could occur if a modifier competes with the peptide for a non-productive peptide binding locus, but not for the productive peptide binding locus. Similarly, the inhibition of esterase activities could be understood if modifiers were to bind to loci required for ester catalysis. This interpretation suggests overlapping loci for ester catalysis and for non-productive peptide and modifier binding, as has been suggested (10).

These effects could be brought about directly through modifier and substrate competition for specific binding loci, or indirectly through substrate or modifier (product) induced changes in the conformation of the protein. The extent of conformational change could depend on the exact

structural features of substrates or modifiers and could further effect both productive binding and catalysis. Such a postulate for the mode of action of products and other modifiers would seem reasonable since conformational changes of the enzyme have been observed in the crystalline state on binding of at least one peptide substrate, i.e. glycyl-L-tyrosine (8,9). Studies of the effects of modifiers on the carboxypeptidase catalyzed hydrolysis of esters and peptides are presently being extended to examine these hypotheses.

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