THE HYDROLYSIS OF O-HIPPURYLGLYCOLATE CATALYZED BY CARBOXYPEPTIDASE A. EVIDENCE FOR POSSIBLE ALLOSTERIC EFFECTS

E. T. Kaiser, S. Awazu and F. W. Carson

Department of Chemistry, University of Chicago

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In recent studies we have analyzed the kinetics observed in the carboxypeptidase A-catalyzed hydrolysis of O-acetyl-L-mandelate and O-hippuryl-L-mandelate.[1,2] The hydrolytic behavior of the former ester fits a relatively simple Michaelis-Menten scheme, the only complicating factor being competitive inhibition by one of the products, L-mandelate, and that of the latter can be interpreted in terms of a combination of both noncompetitive substrate inhibition and competitive product inhibition.[2,3] To the two kinds of behavior found for these substrates, we now add a third, substrate activation, which we have observed in the carboxypeptidase A-catalyzed hydrolysis of O-hippurylglycolate. O-Hippurylglycolic acid, m.p. 165-66° (uncorr.), was synthesized in a manner analogous to that used for the preparation of O-hippuryl-L-mandelic acid.[2] The rate data determined at pH 7.5 and 25.0° for this compound which is the first reported ester substrate for carboxypeptidase A lacking an asymmetric carbon atom are represented in Fig. 1. As can be readily seen, the plot of $v_o/(E)_o$ vs. $(S)_o$ is indicative of substrate activation. The empirical equation $v_o/(E)_o = a(S)^2 + b(S)$, where $a = 1.39 \times 10^7$ l.^{2}/moles^2/min. and $b = 7.8 \times 10^4$ l./mole/min.,

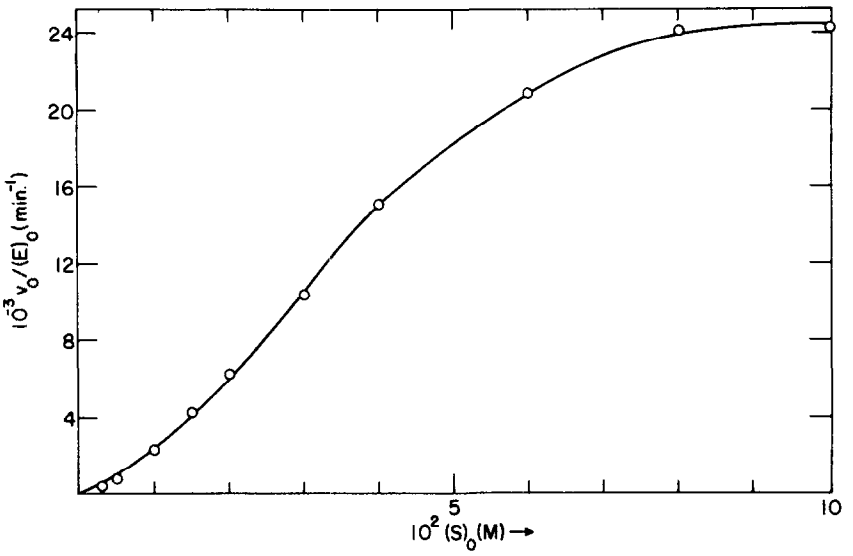

Fig. 1. $v_o/(E)_o$ vs. $(S)_o$ for the Hydrolysis of O-Hippuryl-glycolate Catalyzed by Carboxypeptidase A at pH 7.5, 25°.

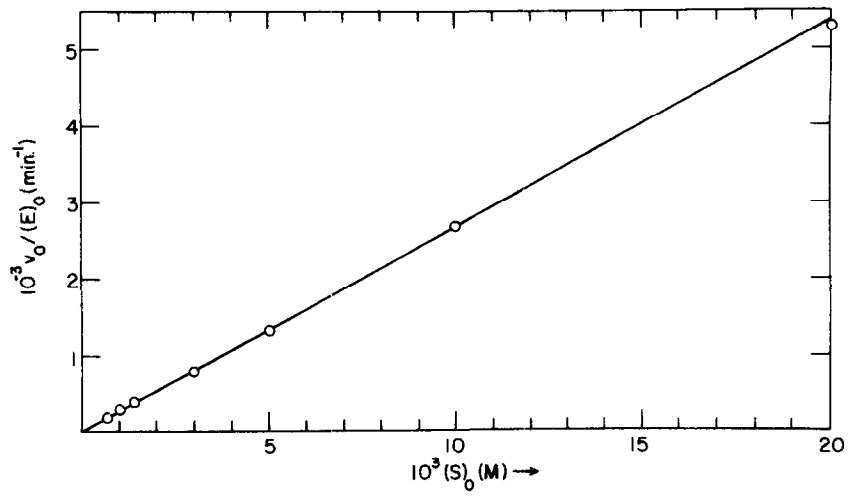

Fig. 2. $v_o/(E)_o$ vs. $(S)_o$ for the Hydrolysis of O-Hippuryl-glycolate Catalyzed by Carboxypeptidase A at pH 7.5, 25° in the Presence of 0.05 M N-Carbobenzyloxyglycine.

holds over the substrate concentration range from 3×10^{-4} to more than 1×10^{-2} M.

It is tempting to suggest that the substrate activation seen in the hydrolysis of O-hippurylglycolate means that carboxypeptidase A may have allosteric character. In support of this possibility we have found that the substrate activation shown in Fig. 1 is no longer apparent after the addition of an activating modifier, 0.05 M N-carbobenzyloxyglycine, as indicated in Fig. 2. In the presence of 0.05 M N-carbobenzyloxyglycine the rate of the carboxypeptidase A-catalyzed hydrolysis of O-hippurylglycolate obeys the equation $v_o/(E)_o = c(S)$ where $c = 2.7 \times 10^5$ l./mole/min. at substrate concentrations from 7×10^{-4} to 2×10^{-2} M.

These kinetic observations are similar to effects of substrates and modifiers found with enzymes recognized to be allosteric.[4] Of course, since the relationship between the sites involved in the formation of enzyme-activator or enzyme-substrate complexes is not known at present it is not possible to say conclusively whether the activation phenomena found in the carboxypeptidase A-catalyzed hydrolysis of O-hippurylglycolate result because of conformational changes in the enzyme or because of other factors.

If carboxypeptidase A is indeed an allosteric enzyme, then a number of intriguing questions can be raised. Is the allosterism associated with the monomeric state of the enzyme or with polymeric forms? Also, are the cooperative effects which have been reported[5] for some other proteolytic enzymes of moderate molecular weight indicative of their allosteric character? Physical and chemical experiments designed to provide further information concerning these questions are in progress in our laboratory.

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