A NEW SOLUBILIZING GROUP FOR SYNTHETIC PEPSIN SUBSTRATES

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Received August 26, 1968

Lack of substrate solubility is a major problem in studies of the hydrolysis of synthetic peptide substrates by pepsin. This stems from the need to fulfill the specificity requirements for hydrophobic, preferably aromatic, residues in the substrate at the site of peptic hydrolysis. Such substrates, esterified at the C-terminus, are preferable to their non-esterified counterparts but have even lower solubilities (Inouye, et al., 1966; Jackson, et al., 1968).

This difficulty has lead some investigators (Denburg, et al., 1968; Lutsenko, et al., 1967) to use incubation media which contain organic solvents, e.g. alcohols, to increase the solubility of the substrates. This practice is questionable in kinetic studies of peptic action in view of the known inhibitory effect of alcohols on pepsin (Tang, 1965; Zeffren, et al., 1967). An alternate approach is to incorporate a solubilizing group into the synthetic substrate. This communication reports on the use of the gluconyl group as a potential solubilizing agent by comparing solubility and kinetic data for N-gluconyl-L-phenylalanyl-L-tyrosine methyl ester (Gluc-Phe-Tyr-OMe) with N-acetyl-L-phenylalanyl-L-tyrosine methyl ester (Ac-Phe-Tyr-OMe).

MATERIALS AND METHODS

The substrates were obtained from Cyclo Chemical Corp., who reported the following properties: Ac-Phe-Tyr-OMe (lot 3396/R), mp 125-130°, lit.

mp 115-116° (Clement and Synder, 1966), 7.23% nitrogen (theory, 7.19%), homogeneous by TLC in three solvent systems, Gluc-Phe-Tyr-OMe (lot D-1008), mp 160-162°, 5.4% nitrogen (theory, 5.38%), homogeneous by TLC in three solvent systems. The pepsin used in this investigation was the three times crystallized preparation (Pentex, Inc., Kankakee, Illinois, Lot D-3709) previously described here (Jackson, et al., 1965).

The Michaelis constant, K_m, and the molecular activity coefficient, k_3, at pH 2 and at pH 4.5, were calculated from plots of S/v versus S, where v is the initial reaction velocity. Hydrolysis was measured by the ninhydrin procedure previously described (Schlamowitz, et al., 1968). The solubility of the two substrates at pH 4.5 and their molecular extinction coefficients at 280 mμ were also measured.

RESULTS AND DISCUSSION

Table 1 compares Ac-Phe-Tyr-OMe to Gluc-Phe-Tyr-OMe with respect to

TABLE 1

SOLUBILITY AND KINETIC DATA FOR TWO SYNTHETIC PEPSIN SUBSTRATES

Substrates	pH	Solubility (mM), 26°C	k_3[a] (min^{-1})	K_m[a] (mM)	$\frac{k_3}{K_m}$ (min^{-1} mM^{-1})	ε_M^{280}
Ac-Phe-Tyr-OMe[b]	2.0		4.4	1.75	2.5	
	4.5	3.6	2.9	2.77	1.045	1083
Gluc-Phe-Tyr-OMe	2.0		1.57	3.25	0.484	
	4.5	11.0	0.855	4.05	0.211	1083

[a]The data reliable to ± 5%. All values were obtained at 37°. The pepsin concentration used was 6 μM; ionic strength, 0.1.

[b]Jackson, et al. (1968).

solubility and kinetic constants. The solubility of the gluconyl peptide is about three-fold greater than the solubility of the acetyl peptide. The accuracy of measurement of a given degree of hydrolysis is thus considerably enhanced using the gluconyl derivative. From the data for K_m and k_3 it may be seen that the ratio $\frac{\frac{k_3}{K_m}\ (\text{pH } 4.5)}{\frac{k_3}{K_m}\ (\text{pH } 2)}$ for Gluc-Phe-Tyr-OMe equals 0.437 and for Ac-Phe-Tyr-OMe this ratio is 0.419. The consistency of these values indicates that the decrease in enzyme activity with pH reflects changes on the enzyme and implies that the substitution of a gluconyl for an acetyl group in the substrate is not responsible for the change in the response of pepsin toward the substrate as a function of pH.

The study of other pepsin substrates containing the gluconyl group is currently underway and will be reported at a later date.

ACKNOWLEDGEMENT

This research was supported by Grant AM 09322 from the National Institutes of Health, United States Public Health Service.

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