Reaction of Pepsin with ^{14}C-Labelled Substrates

By C. GODIN* and C. Y. YUAN
(*Department of Biology, University of Ottawa, Ottawa, Canada*)

Summary When pepsin is incubated with benzyloxycarbonyl-L-glu–[^{14}C]L-tyr ethyl ester, [^{14}C]tyr ethyl ester remains bound to the enzyme, but when benzyloxycarbonyl[^{14}C]-L-glu–L-tyr ethyl ester is used as a substrate, no radioactivity remains associated with the protein.

RECENTLY Akhtar and Al-Janabi have reported the formation of a covalent intermediate during the pepsin-catalysed hydrolysis of benzyloxycarbonyl-tyr-tyr.[1,2] This prompts us to publish results supporting their conclusion. Z-L-glu–[^{14}C]L-tyr ethyl ester (I) was prepared by the condensation of Z-glu anhydride and uniformly labelled [^{14}C]L-tyr ethyl ester.[3] Compound (I) was obtained in 38% yield, based on [^{14}C]L-tyr, and had an activity of 30,700 c.p.m./mg. Z-[^{14}C]L-glu–L-tyr ethyl ester (II) was obtained by the condensation of Z-[^{14}C]L-glu anhydride and L-tyr ethyl ester.[3] A 14% yield, based on [^{14}C]L-glu, was obtained; activity: 26,600 c.p.m./mg. The substrates (I, II, or [^{14}C]L-tyr ethyl ester) (4 μmoles) were dissolved in distilled water (0·25 ml). Twice-crystallized pepsin (Worthington Biochemical) (13 mg.) in 0·1M-acetate buffer pH 4·0 (0·5 ml.) was added and the mixture incubated at 37°. At the end of the incubation period, the mixture was transferred into a centrifuge tube, and 1 ml. of ethanol and 2 drops of concentrated HCl were added. The mixture was kept at 0° overnight. The precipitate formed was centrifuged down, washed twice with ethanol and twice with ether, suspended in acetone, filtered on a weighed paper disc,[4] dried, and counted to a $\pm 2\%$ standard error in a gas-flow end-window counter. The results were corrected for background and self-absorption.

When unlabelled substrate (I) was incubated with pepsin under the same conditions and the extent of hydrolysis determined colorimetrically with the Moore and Stein ninhydrin method,[5] 18, 48, and 58% of the peptide was hydrolysed after 8, 24, and 40 hr., respectively. When labelled substrate (I) was used the radioactivity in c.p.m./mg of the protein isolated after 0 and 24 hr. of incubation was 0 and 125. When labelled substrate (II) was used, no radioactivity was found associated with the protein even after 24 hr. of incubation. When [^{14}C]L-tyr ethyl ester, one of the products of the reaction, was incubated with pepsin some radioactivity was also incorporated into the protein (35 c.p.m./mg of protein after 24 hr.). From the radioactivity of the pepsin isolated after incubation with labelled substrate (I) and the specific activity of the substrate, it can be calculated that 0·3 moles of tyrosine ethyl ester was bound per mole of enzyme.

Akhtar and Al-Janabi have presented evidence that tyrosine was bound covalently to the enzyme.[1] In our experiment, when the radioactive pepsin isolated after incubation with labelled substrate (I), was incubated in a different medium, [^{14}C]tyr and [^{14}C]tyr ethyl ester were liberated in the medium and were identified by paper chromatography. If the radioactive protein was incubated for 24 hr. in distilled water at 37° about 20% of the radioactivity was set free as tyr and tyr ethyl ester. When it was incubated at 37° in acetic acid at pH4 for 2 hr. or at 22° in a mixture of acetic acid and HCl at pH2 for 20 hr., 95% of the radioactivity was lost in the medium as tyr and tyr ethyl ester.

This work was supported by a grant from the National Research Council of Canada.

(*Received, October 28th*, 1969; *Com.* 1638.)

[1] M. Akhtar and J. M. Al-Janabi, *Chem. Comm.*, 1969, 859.
[2] M. Akhtar and J. M. Al-Janabi, *Chem. Comm.*, 1969, 1002.
[3] M. Bergmann and L. Zervas, *Z. physiol. Chem.*, 1934, **224**, 11.
[4] F. C. Henriques, G. B. Kistiakowsky, C. Marguetti, and W. G. Schneider, *Ind. and Eng. Chem., Analyt. Edn.*, 1946, **18**, 349.
[5] S. Moore and W. H. Stein, *J. Biol. Chem.*, 1954, **211**, 907.