Ultraviolet Absorption Spectrum of Protyrosinase and Tyrosinase.* (20816)

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In a previous communication it was shown that no change in the sulfhydryl or disulfide groups of the protyrosinase molecule seems to occur upon activation and conversion to the active form, tyrosinase(1). In the present series of experiments the ultraviolet absorption spectrum of protyrosinase and tyrosinase was determined to ascertain whether a difference exists between the inactive and active forms of the enzyme. Manometric measurements of the enzymatic properties of these two substances show a definite difference with respect to the oxidation of tyramine HCl(2). The ultraviolet absorption spectrum should demonstrate a structural change if such exists.

In a study of the physical or chemical properties of a substance, a primary requirement is that one knows the "purity" of his preparation. During a series of extractions of the egg of the grasshopper, (*Melanoplus differentialis*), we obtained a protyrosinase which was considerably more concentrated than the original extractions. As we fractionated our preparation, the amount of non-dialysable substances decreased, the rate per mg (measured, of course, by activating this proenzyme) increased. The final preparation of the protyrosinase had 0.21% copper. Kubowitz(3) found 0.18% copper in his preparation of tyrosinase; Keilin and Mann (4), 0.3%, and Nelson and Dawson(5), 0.21%. The procedures followed in purifying and identifying the different samples of protyrosinase are similar to those previously described.‡ The preparation used in the present absorption experiment is that designated as B-18a. In the samples whose spectra are graphed for reproduction (Fig. 1) the method of treatment was as follows:

Left side of graph (Fig. 1). The B-18a was taken up in 0.1 M phosphate (pH 6.8) rather than 0.9% NaCl. Two cc of this were diluted to 32 cc with the final dialysate (0.1 M phosphate) and then filtered through a fritted glass filter. Of this, 14 cc were used as proenzyme; 10 cc were activated with 0.5 cc of sodium dodecyl sulfate or with 0.1 cc aerosol OT. The control cell for the proenzyme was the dialysate; for the enzyme the dialysate plus the activator used. These were mixed in the evening, the spectrum determined on the following morning and the activity in the afternoon of that day. The activity of proenzymes plus activator was determined for each protyrosinase solution used.

Right side of graph (Fig. 1). Treated in an identical manner save that the solvent was 0.9% NaCl.

A Hilger quartz spectrophotometer with a hydrogen arc light source was used.‡ An examination of Fig. 1 shows a distinct difference in the absorption spectra of the active and inactive form of the enzyme between 230 and 260 mμ. An absorption maximum at 310 mμ is common to both and has been attributed to the copper present by Dalton and Nelson(6). The data cannot be specifically interpreted. The increase is in the range of absorption of the ring structure amino acids. Theoretically, the absorption of the amino acids should be the same and in relation to the amounts and kinds present (this could not vary in these experiments). Lavin, Loring, and Stanley(7) have shown that structure may influence the absorption of the amino acids. Further work must be done on the absorption below 270 mμ to distinguish the various absorption bands of individual amino acids. Removal of the copper should change the absorption at 310 mμ if the assumption that this is due to copper is correct.

Summary. We have purified to some extent the proenzyme (protyrosinase) without activating it. There is a change in the absorption

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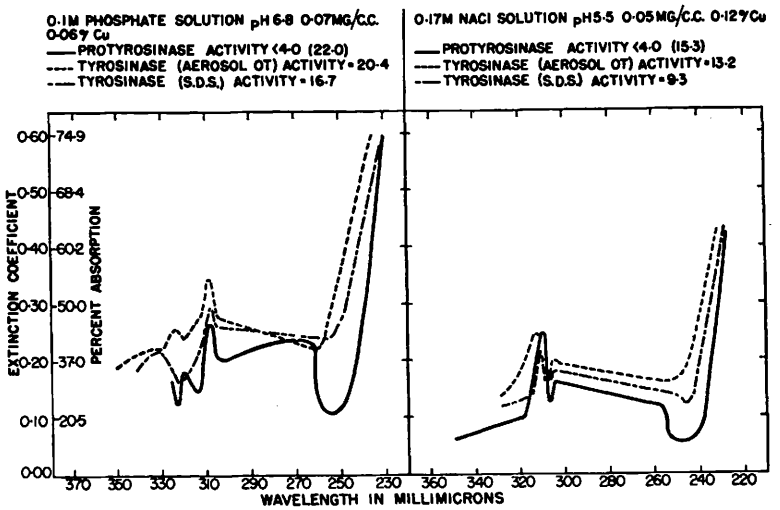

FIG. 1. Shows ultraviolet absorption curves for preparation, B-18a, both as protyrosinase and tyrosinase. Rates expressed as 4 for protyrosinase samples are impossible to accurately measure and can be assumed to represent zero activity. All other rates shown are for tyrosinase or activated protyrosinase. For further description see text.

spectrum in the ultraviolet accompanying the activation of the protyrosinase molecule to the active tyrosinase form.

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Effect of Iodine on Serological Specificity of Tobacco Mosaic Virus.* (20817)

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In complement fixation tests, Weaver and Price(14) found the Type strain of tobacco mosaic virus (TMV) to react optimally with its homologous antiserum, or with antiserum to closely related strains, when a specific weight of virus, designated as the optimum antigenic unit (OAU), was present; TMV strains were differentiated from one another by differences in their OAU as well as by differences in antiserum dilution end points (ADE) in homologous and heterologous reactions, thus indicating a high degree of specificity for the serological reaction used. In precipitin tests, iodinated globulin was found to react weakly or not at all with antiserum to native protein(6,11,16); serum globulin iodinated at pH 7.3 reacted with antiserum to native globulin whereas that iodinated at pH 8.5 did not(6). In the work here reported, an attempt was made to iodinate specific amino acid groups on the TMV particle at neutral (7.0) and alkaline (7.65) pH values and to correlate the changes thus pro-

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