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Tyrosinase and Phenols. Action of Diversely Activated Tyrosinase on Monohydric and O-Dihydric Phenols.*

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Since the separation and naming of the enzyme, tyrosinase, by Bertrand[1] much confusion has arisen as to its fundamental nature and mode of action. At the present day, it is customary to assume that this enzyme possesses the ability to catalyze the aerobic oxidation of both monohydric and o-dihydric phenols. The ratio of the two activities, however, seems to have become a question of some importance to those especially interested in the isolation and purification of the enzyme. That the two activities belong to the same enzyme complex seems reasonably well established as the result of the work of Nelson *et al.*[2] An inactive form of tyrosinase, called protyrosinase has been found in the diapause egg of the grasshopper, *Melanoplus differentialis*.[3] This has been shown to be converted into the active enzyme, tyrosinase, by a variety of chemical and physico-chemical reagents.[4] Tyrosinase, thus produced, shows similar catalytic characteristics but at the same time exhibits rather different physical properties such as resistance to temperature,[5] degree of molecular fragmentation, etc.[6]

The present communication presents data to show the extent to which variously activated samples of tyrosinase from the egg of the grasshopper are able to catalyze the aerobic oxidation of both monohydric (tyramine-HCl, p-cresol) and o-dihydric phenols (catechol).

Material and Method. Protyrosinase was obtained from the diapause egg of the grasshopper, *Melanoplus differentialis*, as previously indicated.[7] Different lots were standardized uniformly by activation with the anionic detergent, sodium di-octyl sulfosuccinate (aerosol OT), so as to catalyze the uptake of 100 mm^3 of oxygen by 0.3 ml of 0.4% tyramine-HCl at 25°C in 10 minutes. This is referred to as 100% activation of the protyrosinase.[8] Oxygen determinations were carried out in a Warburg manometer as already noted.[8] Various activators were employed in producing the tyrosinase added to the different substrates, *e. g.*, aerosol OT, sodium dodecyl sulfate, $HgCl_2$, heat (70°C-10′), and urea. All activators were used with each lot of protyrosinase and at the same time so that possible differences due to the age of the enzyme sample would be at a minimum. Substrates, made up and used in 0.4% solution or in equivalent concentrations, included tyramine-HCl, p-cresol, and catechol. Total volumes of enzyme, buffer, activator and substrate in manometer cups equalled 3 ml. Comparisons in activity were also made with the relative volumes of reagents as employed by Adams and Nelson.[9] Mixtures with and without the addition of gelatin were also used.[9] Three complete and separate lots of data have been recorded for comparison.

Results. Protyrosinase, when activated by the reagents above mentioned, produces a tyrosinase which, in general, shows marked activity for both monohydric (tyramine-HCl, p-cresol) and o-dihydric phenols (catechol). Such an enzyme, according to the nomencla-

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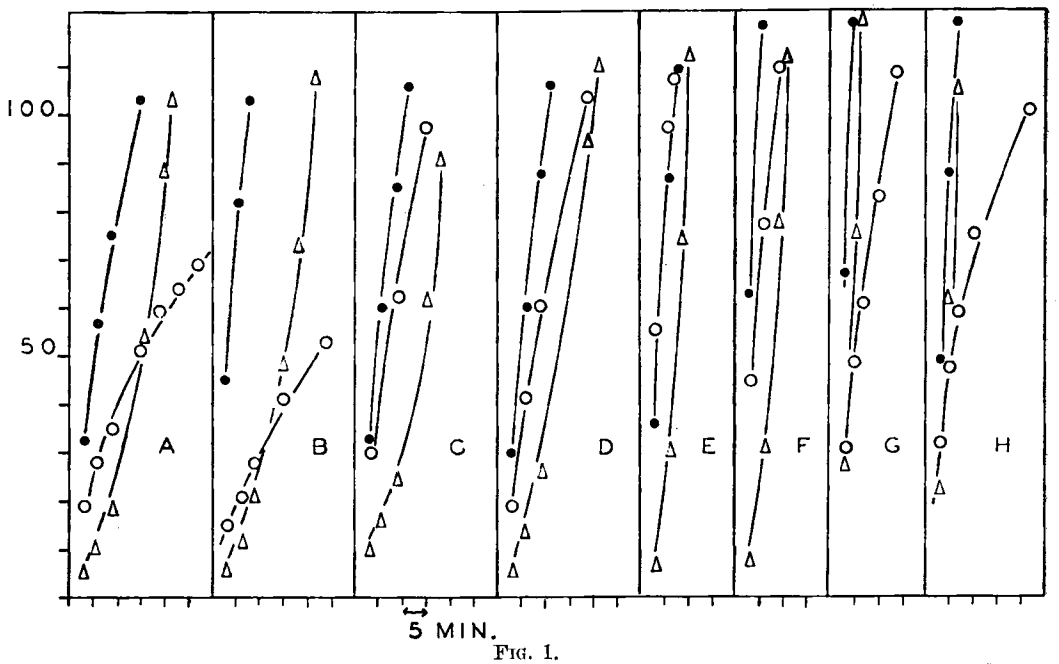

FIG. 1.

Shows action of aerosol OT activated tyrosinase samples upon substrates, tyramine-HCl, p-cresol and catechol. Abscissa, time in minutes; ordinate, O_2 consumption in mm^3. Solid dots = tyramine-HCl. Open circles = catechol. Open triangles = p-cresol. A, B, and C are 3 different lots of enzyme similarly prepared. D, same as C but with gelatin added. E, F, G, and H are results obtained by Adams and Nelson method. E = enzyme + aerosol OT + gelatin + substrate. H = enzyme + aerosol OT + gelatin + substrate. E and F for same sample of enzyme. G and H for same sample of enzyme. For further description, see text.

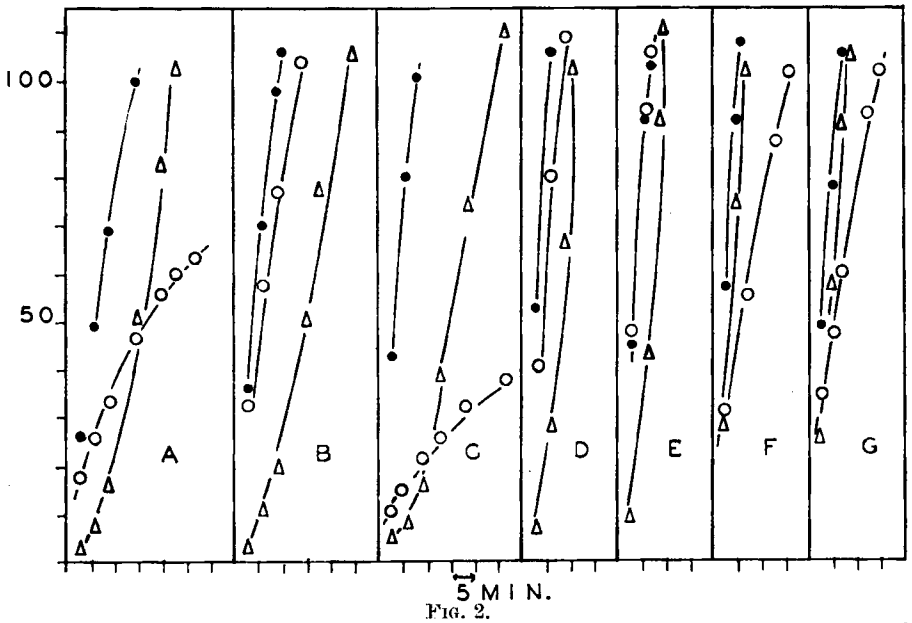

FIG. 2.

Same as Fig. 1. except for sodium dodecyl sulfate. A, B, and C are 3 different lots of enzyme. D, E, F, and G are results obtained by Adams and Nelson method. D = enzyme + NaDS + substrate. E = enzyme + NaDS + gelatin + substrate. F = enzyme + NaDS + substrate. G = enzyme + NaDS + gelatin + substrate.

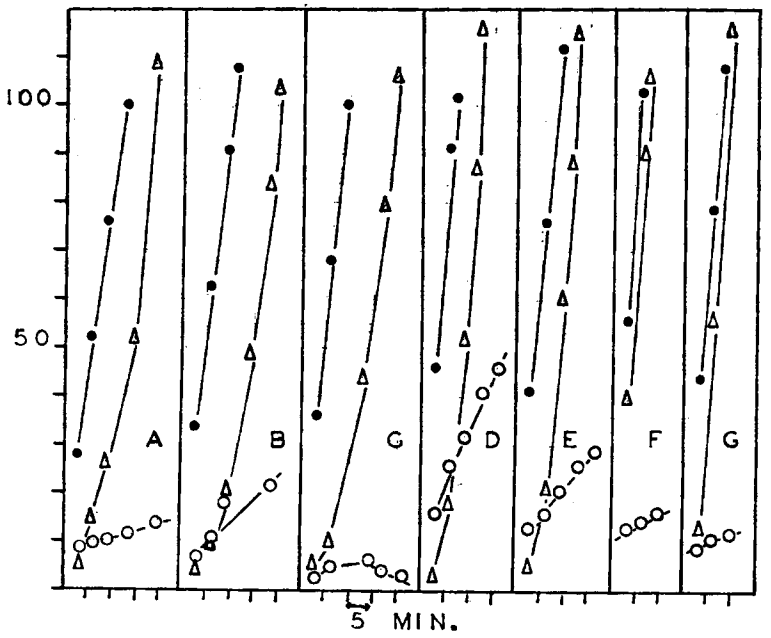

FIG. 3.

Same as Fig. 1 except for $HgCl_2$ ($3. \times 10^{-5}$M). A, B, and C are 3 different lots of enzyme. D, E, F, and G are results obtained by Adams and Nelson method. D = enzyme + $HgCl_2$ + substrate. E = enzyme + $HgCl_2$ + gelatin + substrate. F = enzyme + $HgCl_2$ + substrate. G = enzyme + $HgCl_2$ + gelatin + substrate.

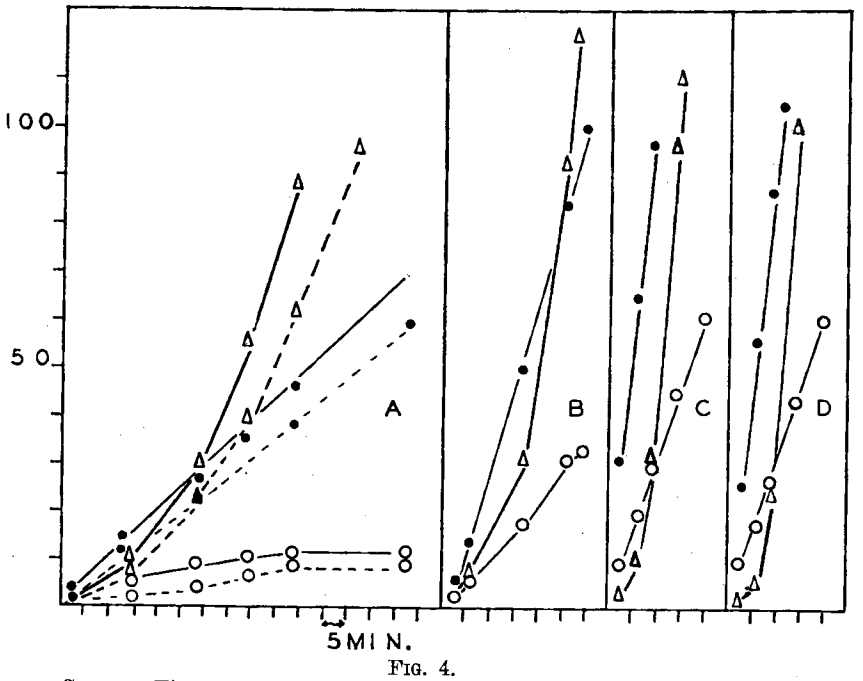

FIG. 4.

Same as Fig. 1 except for urea. Solid lines for 3. M urea; broken lines for 4. M urea. A and B are for different lots of enzyme. C and D are results obtained by Adams and Nelson method. C = enzyme + urea + substrate. D = enzyme + urea + gelatin + substrate.

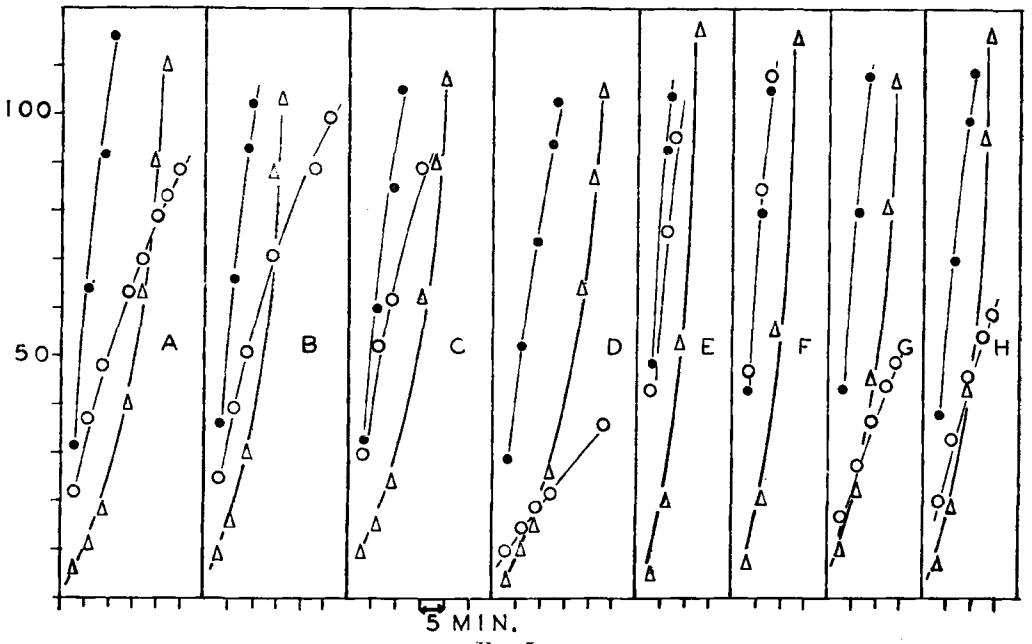

FIG. 5.

Same as Fig. 1 except for exposure to 70°C for 10 minutes. A, B, C, and D are for different lots of enzyme. E, F, G, and H are results obtained by Adams and Nelson method. E = enzyme + heat + substrate. F = enzyme + heat + gelatin + substrate. G = enzyme + heat + substrate. H = enzyme + heat + gelatin + substrate.

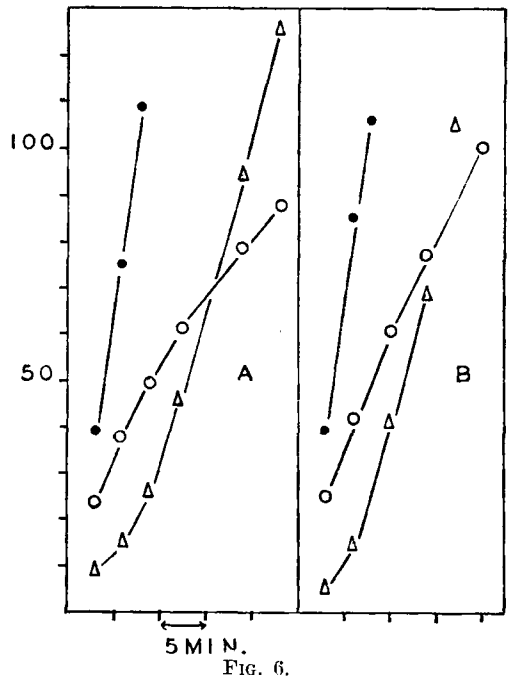

FIG. 6.

Shows effect of different amounts of gelatin on aerosol OT activated enzyme. Otherwise same as Fig. 1. A = enzyme + gelatin + substrate in proportion 1:1:2. B = enzyme + gelatin + substrate in proportion 1:3 1/3:2.

ture of Nelson and Dawson[2] would, therefore, possess both cresolase and catecholase activity. Variations in the degree to which these substrates are oxidized are evident in the different samples of protyrosinase even when activated by the same reagent (Fig. 1 to 6). The general reactions shown will be discussed under the results for the various activators employed.

Sodium dioctyl sulfosuccinate (aerosol OT), the anionic detergent most extensively employed in work on the activation of protyrosinase, produces a tyrosinase strikingly active for tyramine-HCl and about equally active toward p-cresol and catechol (Fig. 1). Experiments carried out using the relative proportions of reagents as suggested by Adams and Nelson[9] show little difference in results from those produced by the author's methods (Fig. 1). Addition of gelatin to the reaction mixture has little effect upon the enzyme's activity (Fig. 1, 6). For aerosol OT activated tyrosinase one might reasonably conclude that no marked differences in its activity for monohydric and o-dihydric phenols exist.

Sodium dodecyl sulfate activation produces a tyrosinase with properties closely resembling

those for aerosol OT (Fig. 2). All 3 substrates are oxidized but with no marked or significant differences in favor of any of the three. Results are practically identical regardless of the method used in carrying out the experiments (Fig. 2).

$HgCl_2$ activated enzyme shows a marked monohydric phenol activity and a correspondingly low value for a o-dihydric substrate. This perhaps might be termed an enzyme high in cresolase activity and low in catecholase activity. Results obtained by the Adam and Nelson[9] method give similar values (Fig. 3).

Urea activated tyrosinase closely resembles that produced by $HgCl_2$ in showing low o-dihydric activity (Fig. 4). Recently, marked similarities in the resistance of the tyrosinase produced by $HgCl_2$ and urea to temperature and $HgCl_2$ have also been pointed out.[5, 10]

Activation of protyrosinase by chemical means is more or less open to the objection that the chemical reagents themselves produce, in addition to activation, other secondary chemical changes which in turn lead to erroneous conclusions.

Activation by heat (70°C-10′) affords a rather unique method for comparing the effects of chemical and physical reagents upon protyrosinase. Fig. 5 shows graphically the action of heat activated tyrosinase on the three substrates. A rather wide range in rates of reaction occurs. No marked differences in behavior of heat activated samples of tyrosinase over those produced by purely chemical means seem evident.

Adams and Nelson[9] observed rather marked effects of inert proteins like gelatin upon the inactivation of their enzyme. When added to tyrosinase from the grasshopper egg no significant results were noted (Fig. 6).

[10] Bodine, J. H., and Tahmisian, T. N., *Arch. Biochem.*, 1943, **2**, 403.

Discussion. That tyrosinase from either plant or animal sources varies to a great degree in its activity towards monohydric and o-dihydric phenols seems well established (Nelson and Dawson[2]). Tyrosinase from different species of plants has been shown by the work of Nelson and his students[2] to be greatly modified in its final catalytic properties by the methods employed in its isolation and purification. In the present experiments, tyrosinase from the one species of grasshopper, when produced through activation by different reagents, also shows marked variations in its catalytic responses to different substrates. Some evidence of these changes has recently been shown in results of experiments dealing with the effects of synthetic detergents on the protyrosinase of grasshopper eggs.[6] The degree to which differences in activity of plant tyrosinase are produced by chemical means appears greater than that found for the enzyme from the grasshopper egg.

Summary. 1. Protyrosinase from the egg of the grasshopper, *Melanoplus differentialis* was activated to form tyrosinase by the following reagents: sodium dioctyl sulfosuccinate (aerosol OT), sodium dodecyl sulfate, $HgCl_2$, heat (70°-10′), and urea. 2. Tyrosinase, produced by different activators, was allowed to act upon monohydric (tyramine-HCl, p-cresol) and o-dihydric (catechol) phenols as substrates and relative rates of activity studied. 3. All enzymes tested showed activity for both monohydric and o-dihydric phenols. 4. Urea and $HgCl_2$ activated tyrosinase showed high monophenolase activity and low catecholase activity. All other enzyme samples exhibited both monophenolase and catecholase activity in varying degrees. 5. Animal and plant tyrosinases seem similar in that both possess enzymatic activities more or less conditioned by chemical treatments.