fied catalase (*Kat. f.* 5400) or pyruvic acid to reduce the apparent uptake of O_2 by destruction of H_2O_2. No peroxidase activity could be detected in the filtrate by measurement of purpurogallin formed from pyrogallol in the presence and absence of H_2O_2, and a purified horseradish peroxidase (*P.Z.* 270) did not significantly alter the course of oxidation of IAA. The failure of the filtrate to oxidize cadaverine or putrescine indicated that no true diamine oxidase was present. An oxidation of *p*-phenylenediamine did not produce H_2O_2 and probably proceeded by means of hydrolytic deamination.

Up to 48-percent stimulation of the maximum oxidative rate on 10 μM IAA occurred with 0.001*M* $MnCl_2$, and various compounds produced the following percentage inhibitions: 0.01*M* azide, 100; 0.01*M* cyanide, 100; 0.1*M* semicarbazide, 100; 0.01*M* phenylhydrazine, 95; 0.01*M* fluoride, 87; 0.001*M* methylene blue, 86; 0.005*M* 2,4-dinitrophenol, 65; 0.01*M* 8-hydroxyquinoline, 47; 0.00015*M* $CuSO_4$, 46; 0.001*M* bisulfite, 40; 0.00005*M* 2,4-dichlorophenol, 36; and 0.005*M* iodoacetate, 31. This enzyme is similar to the enzyme from peas but not that from pineapples in inhibition by 8-hydroxyquinoline, and differs from both in being inhibited by 2,4-dichlorophenol instead of either no effect (*5*) or stimulation (*7*), respectively. It resembles the enzyme from pineapples (*5*) but not that from peas (*8*) in lack of stimulation by 0.0018*M* maleic hydrazide. Activation by Mn^{++} is common to all the IAA oxidases, although Mn^{++} does not appear to be an absolute requirement for the enzyme from *P. versicolor*. A large number of other compounds tested were without significant effect.

The end-product of the reaction has not been identified but is a tan-colored precipitate of carbonyl nature and may be a polymerized form of indole-3-aldehyde on the basis of theoretical considerations and various tests for higher aldehydes. The presence of bisulfite prevents the formation of this precipitate.

Sodium nitrite at 0.001*M* concentration greatly accelerated the oxidation of IAA enzymatically. It was soon found that nitrite alone, but not nitrate, could produce this oxidation. This reaction was similar to one of Salkowski's (*9*) color tests for IAA; however, HNO_3 was not employed in our situation. A straight-line response in rate to increasing concentrations of nitrite up to 0.002*M* and an uptake of 1 mole O_2 regardless of nitrite level suggests that this salt acts as a homogeneous catalyst in solution. The activity of 0.001*M* $NaNO_2$ was about equal to that of 1 ml of culture filtrate. Optimal oxidation occurred at the same *p*H as with enzyme. The R.Q. of the system was also 1. The influence of inhibitors was generally similar to that mentioned for the enzymatic reaction, except for failure of NaF to produce any appreciable inhibition. Heating did not inactivate nitrite alone in solution or added to filtrate. No nitrite could be detected in the filtrate by means of the sulfanilic acid-α-naphthylamine test (*10*), which was found to be sensitive to $1.65 \times 10^{-5}M$ NO_2, a level far too low to induce observable oxidation of IAA. The use of up to 80 μM of ammonium sulfamate to destroy NO_2 in the Warburg vessels had no effect on enzymatic oxidation but inhibited the catalytic oxidation by nitrite nearly 40 percent. No dehydrogenase activity of the enzyme could be demonstrated, but it was observed that IAA alone could slowly reduce methylene blue and that this reduction took place rapidly in the presence of nitrite. A cyclic shift from

$$HNO_2 \rightarrow HNO + H_2O \rightarrow H_3NO_2 \rightarrow HNO_2$$

may be responsible for such catalytic action.

Additional and more detailed data regarding the IAA oxidase from this new source will be published elsewhere.

References and Notes

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11 March 1954.

Adsorbability of Bacteriophage T_1 after Ultraviolet Irradiation in the Dry State

Ruth F. Hill and Harald H. Rossi

Radiological Research Laboratory, Columbia University, New York 32

It has been reported previously that phage T_1, after ultraviolet irradiation in the dry state, cannot be photoreactivated (*1*). Since photoreactivation requires that the phage be adsorbed to its host, *Escherichia coli* strain B, it is possible that the simultaneous combination of dryness and ultraviolet impairs the adsorbability of the phage (*2*). Therefore, this property was investigated (*3*).

A stock of T_1 labeled with P^{32} was prepared (*4*) in the following manner: *E. coli* strain B from an overnight slant culture and phage from a broth stock were added to peptone-glucose medium (*5*) in final concentrations of 1×10^8 bacteria and 1.5×10^4 phages/ml; P^{32} was added so that the final concentration was 144 μc/ml, and the suspension was aerated at 37°C. After preliminary centrifugation at 3200 rev/min, to eliminate bacterial debris, two ultracentrifugations were carried out. The first pellet was resuspended in 2-percent ammonium acetate and the second in broth. Radioactive assays were made of (i) the original P^{32} solution, (ii) the acetate suspension after the first ultracentrifugation, (iii) the acetate supernatant, and (iv) the final broth suspension. This was done by drying 1-ml samples in planchettes and counting with an end-window type of Geiger counter.

Table 1. Radioactivity at various stages in purification of P^{32}-labeled T_1.*

Material	Volume (ml)	Counting rate† (counts/min ml)	Counting rate corrected to 10 ml volume (counts/min ml)
Original aeration tube	24	3.32×10^7	7.94×10^7
Ammonium acetate suspension after first ultracentrifugation	15	2.00×10^6	3.00×10^6
Ammonium acetate supernatant after second ultracentrifugation (later decanted)	15	5.56×10^5	8.35×10^5
Final broth suspension	10	1.90×10^6	1.90×10^6
Expected final counting rate = $(3.00 \times 10^6) - (8.35 \times 10^5)$ = 2.17×10^6 counts/min ml.			

* Phage titer in final broth suspension = 8.73×10^{10} T_1 particles/ml; maximum counting rate per phage = $1.90 \times 10^6 / 8.73 \times 10^{10} = 2.18 \times 10^{-5}$ counts/min; maximum original activity incorporated into phage = 2.4 percent.
† One microcurie gave 2.3×10^5 counts/min.

Results are summarized in Table 1. The final concentrations of P^{32} activity per phage and per milliliter of broth were calculated to be 1×10^{-10} μc/phage and 8.2 μc/ml. These activities were not high enough to give significant killing as was shown by the fact that a reassay of the phage in the final suspension, 1 mo after its preparation, showed no change.

The possibility that a significant fraction of the P^{32} activity was still in the medium outside of the phage must be considered. If it is assumed that, at the worst, only 80 percent of the activity in the ammonium acetate supernatant was discarded and 20 percent left behind after decantation, then calculation utilizing the values in Table 1 shows that no more than 8 percent of the total final activity would be in the medium. Adsorption of activity in excess of this would be due to adsorption of radioactive phage rather than to removal by bacteria of radioactive phosphate from the outside medium (*6*).

The final broth suspension was used to prepare four samples of spray-dried phage on cover glasses (*1*). Two cover glasses were dropped into 5-ml broth without previous ultraviolet irradiation and two were irradiated for 120 sec each. All four broth suspensions were then assayed for dark and light survival.

"Wet" radioactive controls were then prepared as follows. The labeled stock was diluted 1 : 24 in phosphate buffer (*7*). A sample of this suspension was exposed to ultraviolet radiation for 120 sec. Nonirradiated and irradiated phosphate buffer suspensions were diluted further into broth by a factor of 10 and assayed for dark and light survival. The two dilution factors were calculated to give equal concentrations of phage (active and inactive) in all broth samples.

The six broth suspensions were used to test adsorbability. Each sample was tested as follows: 1 ml of the broth suspension was added to 2 ml of a suspension of *E. coli* B in phosphate buffer at 37°C (final bacterial concentration, 1.6×10^9); at 5 min, 1 ml was removed for P^{32} assay; at 6 min, 0.05 ml was diluted into 5-ml broth for assay of dark and light survival; at 7 min, the remainder was centrifuged at 3200 rev/min for 4 min; 1 ml of the supernatant was then removed for P^{32} assay.

Table 2 shows the results. There is no photoreactivation for the two samples containing dry-irradiated phage, whereas the sample irradiated in phosphate buffer shows a light survival almost 20 times the dark survival. The radioactive counting rates are so high that statistical error is negligible. All six samples show an adsorption of radioactivity much more than 8 percent. They are all nearly the same, giving about the 40 percent to be expected. Hence, it appears that impairment of adsorbability is not responsible for the impairment of photoreactivability in dry-irradiated phage T_1.

Table 2. Relationship between photoreactivability and adsorbability for T_1 irradiated with ultraviolet light while "wet" and while "dry."

Sample	T_1 assays of adsorption tube		Dark survivors (percent)	Light / Dark	Counts/min ml of adsorption tube	Counts/min ml of supernatant	P^{32} adsorbed (percent)
	Dark	Light					
Dry-control	3.12×10^{8}*		100		2045	1255	39
Dry-control	2.64×10^{8}*				2255	1520	33
Dry-irradiated	3.06×10^6	3.12×10^6	1.1	1.0	2035	1235	39
Dry-irradiated	2.40×10^6	2.01×10^6	0.83	0.84	1915	1105	42
Wet-control	3.45×10^8		100		2735	1585	42
Wet-irradiated	2.13×10^6	4.02×10^7	0.62†	19	2665	1550	42

* The values of these controls average 2.88×10^8.
† This reduction in dark survival is not due to an increase in the ultraviolet inactivation cross section. The corresponding survival, as measured in the broth suspension before dilution into the adsorption tube, is 1.2. The observed reduction may be due to "abortive infection" (*8*).

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8 March 1954.

Preparation of Soluble Monoamine Oxidase

G. C. Cotzias, I. Serlin, and J. J. Greenough

Division of Physiology, Medical Department, Brookhaven National Laboratory, Upton, Long Island, New York

Since its discovery in 1928 (*1*), monoamine oxidase (*2*) has stubbornly resisted various attempts at purification (*1, 3, 4*). An explanation for this behavior emerged from the demonstration of the enzyme's intimate adherence to the cytoplasmic particulate elements (*5*). Various efforts were subsequently made to release this system into solution by means of disruption of these bodies. The result was either loss of activity or the production of smaller microscopic particles to which in turn the enzyme was attached (*6*). Such experiences strengthened the earlier opinion that "monoamine dehydrogenase" was part of a complex of several enzymes acting in unison or in sequence (*4, 7*), in a fashion parallel to that postulated in the case of succinic dehydrogenase (*8, 9*).

In order to achieve dissociation of this labile deaminase from the insoluble cytoplasmic residue in liver dispersions, some extraction procedures were reinvestigated (*10*). Morton's method (*11*) yielded an inactive aqueous phase with activity still bound to the tissue fragments that were drawn into the butanol. Extraction with ethyl ether merely forced the activity-rich particles to align themselves at the interphase, while mixtures of ether and alcohols caused irreversible inactivation, even in the cold. On screening a number of surface active agents, however, a surprising tolerance of this oxidase toward many commercial soaps and detergents became apparent. Three of these agents actually caused enhancement of the deamination rate (*12*) of tyramine by rat and rabbit liver homogenates in phosphate buffer. The increment of activity, in the presence of isooctylphenoxypolyethoxyethanol (*13*), polyoxyethylenelauryl alcohol (*14*), and Alconox® ranged in one experiment from 60 to 25 percent, in that order.

Isooctylphenoxypolyethoxyethanol, in addition to increasing the average reaction velocity more effectively than the others, also produced a striking translucency of the homogenates. Maximal clearing occurred as rapidly as 60 sec after addition of the alcohol. The performance and the appearance of the homogenates remained thereafter virtually unchanged, even after they had been stored for 3 days at room temperature. This long-chain alcohol was therefore used in more detailed investigation.

Variation of detergent concentration from 1 to 70 percent changed very slightly either the degree of clearing or the activity of a 20-percent rabbit liver homogenate. Routine use of a 5-percent (V/V) concentration was arbitrarily adopted for all subsequent tissue preparations.

Centrifugation of the treated homogenates at 2000 *g* for 30 min yielded a small volume of sediment that was poor in monoamine oxidase activity, while brisk activity was demonstrable in the supernatant. The untreated homogenates presented, as was expected (*5*), the opposite distribution, with the voluminous sediment containing almost all the activity. The picture was not significantly changed when centrifugation at 144,000 *g* (calculated for the bottom of the tubes) was allowed to proceed for 180 min. This higher centrifugal force did cause the accumulation of a waxy material on top of the sparkling clear supernatant. This waxy layer was poor in activity and was discarded in subsequent experiments.

Clear supernatants were centrifuged at 144,000 *g* in some experiments for several hours at about +4°C (*15*), followed by lowering of the temperature, without changing the centrifugal force. The contents of the tubes became solidified and were cut into several segments. Assay of fractions secured in this manner showed that a slow sedimentation of the enzyme had taken place. After removal of a firm, pasty pellicle of heavy, inactive material, the lowest segments had 25 percent higher activity than the uppermost.

The specific gravity of nonfractionated, active supernatants was of the order of 1.016 to 1.018. Sucrose solution, the medium widely employed in 0.88*M* concentrations for centrifugal isolation of intracellular components, had a specific gravity of 1.120. It has already been established (*11*) that particles of microsome size are sedimented out of this medium following centrifugation at 41,000 *g* for 2 hr. It would seem doubtful, therefore, whether any such submicroscopic granules could have remained in suspension following our protracted centrifugation at higher speeds, in a medium of lower specific gravity, and in the presence of phosphate buffer, which is known to cause these particles to aggregate. Furthermore, it was also demonstrated that the liver microsomes disintegrate on exposure to the detergent: the silky appearance of suspensions of isolated microsomes (*16*) was instantly lost on exposure to this agent, and an orange-red color appeared, which was due possibly to the release into solution of cytochromes. Recentrifugation showed little discernible sediment. Similar results were obtained in the case of washed rat liver mitochondria.

Other tissue enzymes, as well, were brought into solution by this procedure. Assays for acid and alkaline phosphatase, diamine oxidase, D-amino acid oxidase, and histaminase showed high activity in liver extracts of this type. Our experience with succinic oxidase, on the other hand, was in striking contrast.