Ultraviolet Radiation Effects on Low Molecular Weight Inorganic Phosphates of Yeast

Abstract. *Ultraviolet radiation causes the loss of ortho-, pyro-, tripoly-, tetrapoly- and trimetaphosphate from yeast cells to the medium. Analyses of combined cell and medium phosphates show a two-and-a-half-fold increase in pyrophosphate after irradiation while inorganic and total phosphate remain constant.*

Ultraviolet (UV) radiation causes large amounts of phosphate-containing compounds to be lost from yeast cells to the medium over a 24-hour period (*1–3*). Time-course studies (*1*) showed that relatively small doses of radiation cause a sustained slow loss over a 24-hour period while large doses cause a high rate of loss, reaching a maximum total loss within 4 hours. Actively metabolizing cells which are irradiated lose phosphate at a rate five times greater than irradiated cells without exogenous substrate. Ultraviolet irradiation has also been shown to prevent the uptake of inorganic phosphate by yeast (*4*) showing that this phosphate loss is not caused solely by a breakdown of the structure of the cell membrane.

Most previous studies on ultraviolet-induced phosphate loss from cells have dealt with physical and chemical factors influencing this loss. Some work has been done on the identification of the organic phosphates which are affected by irradiation (*1, 3*) but little is known about the effects of these radiations on the inorganic phosphates which are present. Many microorganisms, including yeast, are known to contain a variety of inorganic phosphates —orthophosphate, pyrophosphate, tripolyphosphate, tetrapolyphosphate (the last three are low molecular weight straight-chain polyphosphates), a high molecular weight polyphosphate, and trimetaphosphate (a cyclic phosphate) (*5*).

The experiments reported here were designed to answer the following questions: (i) What low molecular weight inorganic phosphates are present in *Saccharomyces cerevisiae* and at what concentration? (ii) What are the effects of UV radiation on the loss of the low molecular weight inorganic phosphates from yeast cells? (iii) What are the effects of UV radiation on the distribution of the various low molecular weight inorganic phosphates in yeast?

Maintenance and growth of the yeast, a strain of *Saccharomyces cerevisiae*, and general experimental procedures have been previously described (*1*). The washed yeast, 2×10^8 cells per milliliter (4.375 mg dry weight per ml of cells), was suspended in water. Twenty milliliters of yeast were irradiated while being stirred in a Pyrex crystallizing dish (77 × 38 mm). The suspension was irradiated for 8 minutes with two General Electric 15-watt germicidal lamps placed 63 mm above the surface of the suspension. Each lamp could deliver 95 percent of its energy at 2537 Å. The intensity of radiation at the surface of the stirred cell suspension was 29.67 ergs/mm^2 sec^{-1}. Inorganic phosphates were extracted according to the procedure of Schmidt *et al.* (*6*) and separated by two-dimensional ascending chromatography (*7*). The phosphates were identified by autoradiography (*8*). Quantitative analyses of the various phosphates were performed by the methods of Karl-Kroupa (*7*) and Martin and Doty (*9*). The results of all experiments were statistically analyzed by comparing the means for small samples (*10*).

Figure 1 shows how two different doses of UV radiation, corresponding to 8 and 10 minutes of exposure, affects the rate of loss of P^{32}-labeled phosphate from yeast cells. From this graph, the conditions were selected for the chromatographic studies and the quantitative analysis of the various inorganic phosphates in the cells and in the suspending medium. All samples were irradiated 8 minutes and incubated for 4 hours.

Chromatographic study showed ortho-, pyro-, tripoly-, tetrapoly-, and trimetaphosphate in our strain of yeast and after UV irradiation all molecular species were in the suspending medium. Figure 2 is a radioautograph material lost to the medium after irradiation; the inorganic phosphates in irradiated and nonirradiated yeast cells gave qualitatively similar chromatograms. From Fig. 2, identification of the various phosphates and elution for quantitative

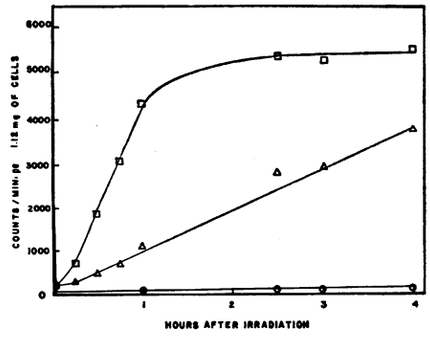

Fig. 1. The effect of ultraviolet radiation on the loss of phosphates from cells as measured in cell-free medium. Squares 10-minute irradiation; triangles, 8-minute irradiation; circles, no irradiation.

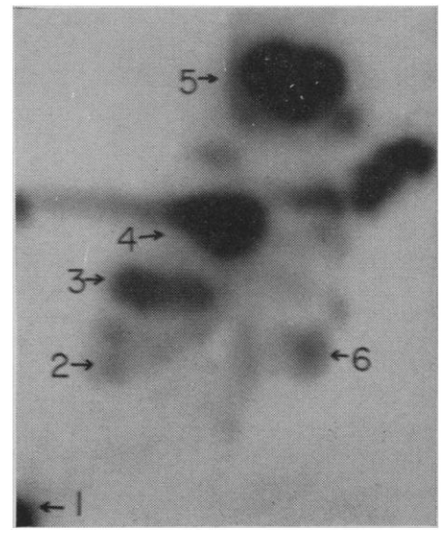

Fig. 2. Radioautograph produced by chromatography of irradiated yeast incubation medium. *1*, Origin; *2*, tetrapolyphosphate; *3*, tripolyphosphate; *4*, pyrophosphate; *5*, orthophosphate; *6*, trimetaphosphate.

Table 1. The effect of ultraviolet radiation on the distribution of low molecular weight inorganic phosphates in yeast. Analysis made 4 hours after irradiation. Values given as milligrams of phosphorus per gram of dried yeast ± standard error from the mean. Numbers in parentheses represent the number of experiments per group.

Phosphate	Phosphorus (mg ± S.E.)				*t-test* ($P \leq$)
	Non-irradiated (6)	Irradiated (3)			
	Cells + medium	Cells	Medium	Cells + medium	
Total	6.91 ± 0.66	6.48 ± 0.07	0.90 ± 0.27	7.38 ± 0.21	0.50
Ortho	3.57 ± 0.54	3.31 ± 0.94	.47 ± 0.01	3.78 ± 0.25	.50
Pyro	0.31 ± 0.07	0.70 ± 0.06	.14 ± 0.04	0.84 ± 0.03	.001
Tripoly	.42 ± 0.04	.28 ± 0.01	.05 ± 0.00$_2$	.33 ± 0.01	.50
Tetrapoly	.42 ± 0.06	.39 ± 0.03	.04 ± 0.004	.43 ± 0.03	.50
Trimeta	.39 ± 0.52	.27 ± 0.13	.05 ± 0.01	.32 ± 0.13	.50

studies presents no special problems owing to the clean separation and sharp resolution of the molecular species.

Table 1 gives the results of the quantitative analysis of the various inorganic phosphate compounds inside and outside the cells after UV irradiation. The amounts may be compared to those in nonirradiated cells before irradiation where the phosphates under discussion represent 74 percent of the total phosphate extracted and 69.8 percent of these is orthophosphate. Figure 1 shows that phosphate leakage is negligible in nonirradiated cells.

The total inorganic phosphate in the system does not differ significantly from that of the nonirradiated cells although irradiation causes loss of all inorganic phosphate species into the medium to the extent of 9.3 to 16.7 percent. This is in contrast to the situation for organic phosphates where certain ones "leaked" selectively from irradiated cells (*1*). Pyrophosphate is the only individual phosphate which shows a significant change in the total amount present in the system after irradiation. The amount of pyrophosphate in the cells plus the suspending medium is approximately 2.5 times that of the control cells. Normally, pyrophosphate represents 4.5 percent of the total inorganic phosphate in the yeast cell. After irradiation, this value is increased to about 11 percent. Katchman and Fetty (*5*) reported that 0.4 mg of phosphorus of high molecular weight polyphosphate was found in *S. cerevisiae* during the logarithmic growth phase and approximately 0.2 mg during the plateau phase. Thus, sufficient polyphosphate is present so that degradation of high molecular weight polyphosphate could account for the increase in pyrophosphate without causing changes in measured total inorganic phosphate.

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Microstructure of Polymers by Tritium Autoradiography

Abstract. *Polystyrene, polypropylene, and polyethylene, containing tritium-labeled additives and crystallized in thin films, were examined with autoradiographic stripping film. In the structures formed by isotactic polystyrene, tritiated atactic polymer concentrated in specific patterns in the outer regions. Polypropylene spherulites showed marked differences in the distribution of the tritiated additive, dilauryl thiodipropionate, whenever their optical properties also differed. Autoradiographs of polyethylene spherulites containing low molecular weight tritiated polyethylene exhibited concentric ring patterns similar to those observed on viewing the polymer film in a polarizing microscope.*

The microstructure of polymers, especially their crystalline forms, has been investigated by a variety of techniques (*1*). We now report the application of an additional tool, tritium autoradiography, which has unique potential for studying polymer microstructure. Special attention is given to spherulite structures, often found on crystallizing such polymers as polystyrene, polyethylene, and polypropylene.

When an impure material is crystallized, impurities are excluded from the crystalline network. We have utilized this principle in our work. The "impurity," a small amount of a tritium-labeled additive, is deliberately incorporated in the polymer. On annealing from the melt, the additive is rejected from certain areas of the crystallizing polymer and deposited in others. Distribution and localization of the labeled additive is determined with autoradiographic stripping film. The resultant film, examined microscopically, reveals information on microstructure and polymer crystal morphology.

The main advantage of tritium in autoradiography is the high resolution obtainable. Due to the low energy of its beta radiation, the effective range in photographic emulsion is less than 1 μ (*2*). Therefore, radioactive regions can be easily distinguished in a specimen at separations of less than 2 μ.

Three polymer systems have been studied: polystyrene, polypropylene, and polyethylene. Appropriate tritium-labeled additives were synthesized for each system by conventional chemical methods. Tritium concentrations were made sufficiently high to give good autoradiograms in 3 to 20 days, depending on the material and its concentration in the samples.

For studies with isotactic polystyrene, the stereoisomeric modification was prepared with a tritium label. Acetophenone was reduced with tritium-labeled lithium borohydride (*3*) to give tritiated α-methyl benzyl alcohol. Catalytic dehydration of this alcohol with sulfosalicylic acid at 150°C produced styrene. The labeled styrene was thermally polymerized to tritiated atactic polystyrene. The product, having a specific radioactivity of 180 μc/mg, was used as the additive for isotactic polystyrene.

For polyproplyene, tritium-labeled dilauryl thiodipropionate was used as the additive. This compound, in nonradioactive form, is often used as an antioxidant in polypropylene. Synthesis was achieved by reducing methyl laurate with tritiated lithium borohydride to give lauryl alcohol-H^3. The alcohol was then esterified with thiodipropionic acid to yield the desired additive, tritiated dilauryl thiodipropionate. The product had a specific activity of 140 μc/mg.

For the work with polyethylene, a fraction of low molecular weight was tritiated by the catalytic reduction of its unsaturated groups with tritium gas. The labeled polymer had a molecular weight (number average) of 320, and a specific activity of 1440 μc/mg. It differed from the host polymer only in molecular weight and in the absence of unsaturated groups.

Thin films were prepared for autoradiographic examination by depositing small amounts of the polymer-additive mixtures on microscope slides. In some instances, films were deposited by evaporation of solvent from decalin solutions; in others the dry materials were spread between two slides and melted. The deposits were re-melted be-