Modification of Blue Light Photoresponses by Riboflavin Analogs in *Neurospora crassa*

JOHN PAIETTA and MALCOLM L. SARGENT

Department of Genetics and Development, University of Illinois, Urbana, Illinois 61801

ABSTRACT

The effect of riboflavin analogs on blue light responses in a riboflavin mutant of *Neurospora crassa* was studied. The analogs 1-deazariboflavin and roseoflavin, which have red-shifted absorption, acted as photoreceptors for the photosuppression and phase shifting of circadian conidiation by 540 nm light, but were ineffective as photoreceptors for the induction of carotenoid synthesis. These results provide additional evidence implicating a flavin photoreceptor for at least two blue light responses of *Neurospora*.

In a number of organisms, blue light responses appear to be mediated by a flavin or flavoprotein photoreceptor (22, 23). For the fungus *Neurospora crassa*, spectrophotometric studies have implicated a flavin-mediated photoreduction of a b-type Cyt in such responses (3, 14). Recent studies with mutant strains of *Neurospora* have also indicated flavin-Cyt b involvement because a Cyt b (2, 4) or flavin (17, 18) deficiency correlates with a decreased sensitivity to light. The specific flavoprotein(s) involved has not yet been identified, but it does not appear to be nitrate reductase (19), which has been suggested as a potential blue light receptor for *Neurospora* (12).

Another approach that has proven useful in photobiology is the replacement of natural chromophores by analogs. Examples include the use of retinal analogs in the study of *Halobacterium* membranes (25) and mammalian vision (6). Only a few studies, however, have utilized analogs in the study of blue light responses. Page (16) used L-lyxoflavin, a riboflavin analog, in studies which indicated a flavin photoreceptor for trophocyst formation in *Physcomyces*, roseoflavin acted as a photoreceptor for phototropism (15), while in *Neurospora* it was ineffective as a photoreceptor for carotenogenesis (24).

The present work examines the effect of riboflavin analogs on three *Neurospora* blue light responses: the suppression and phase shifting of circadian conidiation, and the induction of carotenoid synthesis. Two analogs, 1-deazariboflavin and roseoflavin, which have red-shifted absorption (26) as compared to riboflavin (535, 505, and 445 nm absorption maxima, respectively) were chosen for study following preliminary experiments with 17 different analogs. *Neurospora* will not normally respond to wavelengths above 520 nm for the three photoreponses described above (8, 9, 21). A response, therefore, of an analog-supplemented culture to longer wavelengths of light (e.g. 540 nm) can be unambiguously interpreted to have been poteniated by the analog.

MATERIALS AND METHODS

Fungal Strains. The bd (41-4, FGSC No. 1859) and rib-2 (Yso534r, FGSC No. 1873) strains were obtained from the Fungal Genetics Stock Center (Humboldt State University, Arcata, CA). The rib-2 strain is a nonleaky auxotroph which requires riboflavin (11). A double mutant of genotype *bd rib-2* was constructed according to standard procedures (7).

Flavin Analogs. 1-Deazariboflavin and roseoflavin were generously provided by Drs. D. L. Graham and E. F. Rogers (Merck). The structural formulae of the analogs are shown in Figure 1. Absorption spectra of aqueous solutions of the analogs were determined in a Cary 14 spectrophotometer at room temperature.

**Photoresponse Assays.** (a) Photo-induced Circadian Conidiation. Inoculated growth tubes containing glucose-arginine medium (20) were incubated in darkness for 1 to 2 d and then transferred to 6°C for 12 h (dark) in order to synchronize the conidiation rhythm. Following this cold pulse, the growth tubes were shifted to 25°C and kept under constant 540 nm light (Unitron Koehler research illuminator, model LKR; heat filter [15% CuSO₄ 1.5-cm path length]; and Farrand interference filter [half-band width 5 nm]). The growth tubes were subsequently scored as to whether or not suppression of circadian conidiation had occurred, i.e. whether conidiation was continuous, or periodic as in the controls. Growth tubes were aerated in all cases (20 ml/min, 100% RH) to promote optimal banding patterns. A Li-Cor LI-185 photometer with a LI-200S radiometric sensor was used to measure light intensities.

(b) Photo-induced Phase Shifting. Growth tubes were inoculated and placed in constant darkness; after 24 h they were transferred to 6°C for 12 h (dark) in order to synchronize the conidiation rhythm. The growth tubes were then shifted to 25°C and, after about 80 h of growth, were exposed to a pulse of 540 nm light. Light pulses were given in all cases at CT² 2200, which is the CT at which the center of a conidial band occurs (10). The amount of phase shift was determined by a method previously reported (10). Aeration was used for growth tubes (17) in order to stimulate conidiation so that conidial banding could be clearly observed.

(c) Photoinduced Carotenoid Synthesis. The assay for this response is the same as previously reported (17). Mycelial pads were incubated under various experimental conditions and the carotenoids extracted with acetone and methanol. The A of the extracts at 473 nm was used as the measure of photoinduced carotenoid synthesis. Values are expressed in A units/100 mg of mycelial dry weight.

1 Present address: Department of Biochemistry, Ohio State University, 484 West 12th Avenue, Columbus, OH 43210.

2 Abbreviation: CT, circadian time.
strain. The concentration of riboflavin used (1 μM) was the minimum sufficient to promote near optimal growth when used alone.

**Photoinduced Phase Shifting.** Phase shifts can also be induced by 540 nm light in cultures supplemented with riboflavin plus 1-deazariboflavin or roseoflavin (Table I). With both analogs, phase shifts were observed from exposures to light of 30 to 60 min given at CT 2200. The phase shifts induced in the presence of 1-deazariboflavin were approximately twice those with roseoflavin. The maximum phase shifts induced with analog supplementation were about one-third or less than those that can be induced in riboflavin grown bd rib-2 by a brief pulse (15 s, 0.5 w/m²) of white light (17). In the controls (riboflavin only), no or only slight phase shifts were induced by the 540 nm light. The ratios of analog to riboflavin were the same as in the photosuppression assays.

**Photoinduced Carotenogenesis.** For this response, no or extremely weak photoreceptor activity was observed for 1-deazariboflavin or roseoflavin. The levels of induced carotenoids in analog-grown cultures were only slightly above the background for the riboflavin supplemented control (Table II). The maximum levels induced here were about one-fifth or less than those that occur after a brief pulse (30 s, 0.63 w/m²) of white light in the bd rib-2 strain grown with riboflavin supplementation (17). The ratios of analog to riboflavin were the same as in the assays for the other responses.

**DISCUSSION**

The results reported here provide additional evidence indicating that a flavin is a photoreceptor for some of the blue light responses of *Neurospora*. The experiments have shown that the riboflavin analogs 1-deazariboflavin and roseoflavin can act as photoreceptors. The analogs clearly functioned as photoreceptors inasmuch as the responses were induced by a wavelength of light (540 nm) that is beyond the normal range of sensitivity for *Neurospora*. This is the second report, therefore, showing that a flavin analog can act as a photoreceptor for blue light responses, as Otto et al. (15) have found that roseoflavin will function as a photoreceptor for phototropism in *Phycomyces*.

Phase shifting and photosuppression clearly occurred in the presence of roseoflavin and 1-deazariboflavin, but carotenogenesis was not induced to a significant extent. Song et al. (24) also found

### Table I. Phase Shifting at 540 nm for Cultures Grown on Flavin Supplements

<table>
<thead>
<tr>
<th>Flavin Supplementation</th>
<th>30 minᵇ</th>
<th>60 minᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.3 ± 0.4</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Riboflavin + 1-deazariboflavin</td>
<td>2.0 ± 0.5</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>Riboflavin + roseoflavin</td>
<td>0.8 ± 0.6</td>
<td>1.2 ± 0.7</td>
</tr>
</tbody>
</table>

ᵃ Means of five replicates ± se.
ᵇ Irradiation time at 0.05 w/m².

### Table II. Carotenoid Synthesis Induced by 540 nm Light for Cultures Grown on Flavin Supplements

<table>
<thead>
<tr>
<th>Flavin Supplementation</th>
<th>Induced Carotenoidsᵃ</th>
<th>30 minᵇ</th>
<th>60 minᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.02 ± 0.02</td>
<td>0.03 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Riboflavin + 1-deazariboflavin</td>
<td>0.04 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Riboflavin + roseoflavin</td>
<td>0.03 ± 0.02</td>
<td>0.05 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ A units/100 mg dry weight; means of five replicates ± se.
ᵇ Irradiation time at 0.05 w/m².
roseoflavin to be ineffective as a photoreceptor for carotenogenesis in Neurospora. These results are consistent with the idea that a different photoreceptor may be involved for carotenogenesis as compared to phase shifting and photosuppression (4, 17). Carotenogenesis may be induced by singlet oxygen (1) and the analogs’ photochemical properties may make them less suitable as photoreceptors for this response.

The fact that roseoflavin was relatively ineffective as a photoreceptor for all of the photoresponses is consistent with two other studies. Song et al. (24) have determined that roseoflavin is kinetically inefficient as a photoreceptor based on its spectrophotometric properties, and Otto et al. (15) found that roseoflavin’s efficiency as a photoreceptor for Phycomyces phototropism is 0.1% that of the normal photoreceptor.

It should be noted that at least two other factors may be influencing the results obtained here. First, the degree of replacement of riboflavin by the analogs in the photoreceptor is unknown. The analogs do cause a concentration-dependent inhibition of growth. The inhibition of growth results from incorporation of the analogs into cellular flavoproteins, rendering many of them inactive (26). The level of substitution may therefore be proportional to the input ratios (8:1 for roseoflavin and 15:1 for 1-deazariboflavin), but we do not have any measurements to determine the exact level for the photoreceptor. For Phycomyces, the degree of replacement of roseoflavin into the photoreceptor was estimated to be similar to the input ratio (15). As a second factor, roseoflavin’s absorption at 540 nm is only two-thirds of that at its absorption maximum (i.e. 505 nm), while the 540 nm absorption of 1-deazariboflavin is greater since its maximum is at 535 nm. A definitive, quantitative comparison of the analogs’ activity as photoreceptors is therefore not possible since a number of factors may be responsible for the greater activity of 1-deazariboflavin.

Although these experiments demonstrate that flavins can potentiate some photoresponses of Neurospora, the nature of the interactions involved cannot be completely interpreted at this time. The simplest assumption consistent with the flavin deficiency phenomenon (17) would be that the flavin analog is taken up and incorporated into the flavoprotein which is the photoreceptor, thereby modifying its properties in comparison to a riboflavin-containing photoreceptor. It is clear that 1-deazariboflavin and roseoflavin are taken up since they are inhibitory to growth, presumably because they are nonfunctional in flavoprotein enzymes of various types (26). Whether or not they replace a natural flavin in the photoreceptor and function similarly is, however, not yet proven. Experiments (18) with 7-ethyliboflavin, 8-ethyliboflavin, and 7,8-diethylriboflavin support the general notion that at least some riboflavin analogs can enter the cell, replace riboflavin in flavoproteins, and function with normal efficiency as photoreceptors. Another interpretation which must be considered is that the added flavins may be acting like photosensitizing dyes. This interpretation is relevant inasmuch as Briggs (4) has found that methylene blue and red light can be used to cause photosuppression and phase shifting of circadian conidiation in Neurospora. Additionally, in Fusarium, methylene blue, toluidine blue, and neutral red can be used as artificial photoreceptors for carotenogenesis (13). It has not yet been possible to rule out this alternative interpretation, a problem made more difficult because in some cases photosensitizing dyes (e.g. methylene blue) can photoreduce a Cyt b in corn (5) and Neurospora (unpublished results cited in [5]) that may be involved in photoresponses.

LITERATURE CITED