The Effect of Vitamin K₁ on Oxidative Phosphorylation of Rat Liver Mitochondria Irradiated with Ultraviolet Light*

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Almost 20 years have elapsed since the isolation of vitamin K₁ by Doisy et al. (1) and Dam et al. (2); yet, there is still no adequate explanation for its action in animals. It has been recognized for some time that vitamin K is required for the synthesis of prothrombin (3, 4) but it has never been established whether this is a specific or nonspecific effect. It has been found that vitamin K₁ is an essential part of the photosynthetic phosphorylation process in green plants (5). Martius and Nitz-Litzow (6) have postulated that vitamin K₁ plays a similar part in the animal world; i.e., that it is a necessary cofactor for oxidative phosphorylation.

In the work to be presented, the authors have taken advantage of the sensitivity of vitamin K to ultraviolet light (8) to substantiate and extend previous reports by Martius and Nitz-Litzow (6). Preliminary accounts of this work have appeared (9, 10).

EXPERIMENTAL

Mitochondria from livers of mature male Sprague-Dawley rats (300 to 350 gm.) were prepared in 0.44 μ sucrose-0.1 mM EDTA¹ by a modification of the method of Schneider and Hogeboom (11). All procedures and preparations were carried out at 0°C unless otherwise stated. The mitochondrial pellet obtained from 12 gm. of liver was resuspended in 24 ml. of 0.1 M Tris buffer, pH 7.4.

Irradiation of the mitochondrial suspension was accomplished with a Mineral light lamp² which emits light at 2537 A. A Petri dish containing the mitochondrial suspension was placed on a magnetic stirrer about 2 inches beneath the light source and irradiated for 30 minutes. Preliminary results showed that mitochondria were affected in the same manner whether the irradiation was conducted aerobically or anaerobically, although in the present work all operations were carried out under aerobic conditions. Control mitochondria were stirred simultaneously with the experimental mitochondria but were shielded from the light source. When vitamin K₁³ was added to normal or irradiated mitochondria, the same amount of 0.1 M Tris buffer, pH 7.4, was added to the control and the mixtures were stirred again for 15 to 23 minutes. Irradiated vitamin K₁ was prepared by irradiating the vitamin for 3 hours with the same light source mentioned above.

Oxygen consumption was determined at 30°C with the Warburg manometric technique, and inorganic phosphate disappearance was followed by the method of Lowry and Lopez (12). The conditions and components of the various reaction mixtures are indicated in the figures.

RESULTS

The results in Table I show that mitochondrial P:O ratios resulting from β-hydroxybutyrate oxidation are sensitive to 2537 A irradiation. The average decrease of the P:O ratios was about 66 per cent. The low P:O ratios of the controls were probably attributable to stirring and consequent aging of the mitochondrial suspensions since when mitochondria were used immediately after preparation, P:O ratios of 2.6 to 2.8 were obtained with the same system.

When vitamin K₁ was added to the irradiated preparation, almost complete restoration of the P:O ratio was observed (Table II). Addition of vitamin K₁ produced a slight lowering of oxygen consumption but also a rise in esterified phosphate and thus a considerable restoration of the P:O ratio.

Addition of vitamin K₁ which had been exposed to ultraviolet irradiation was found to have no effect on the P:O ratio of irradiated mitochondria (Table III). Table IV shows that ultraviolet irradiation had no effect on the P:O ratio resulting from mitochondrial oxidation of reduced cytochrome c, and Table V shows that added vitamin K₁ had no influence on either oxidation or phosphorylation of normal mitochondria. No significant difference in ATPase activity was observed between normal and irradiated mitochondrial preparations and both possessed low activity.

DISCUSSION

The decrease in the P:O ratios of mitochondria after irradiation with 2537 A of ultraviolet light is a completely reproducible phenomenon. Although the observed decreases ranged from 50 to 75 per cent, the average was about 66 per cent regardless of the magnitude of the P:O ratio before irradiation. This can be interpreted to indicate that the factor being destroyed or inactivated by the ultraviolet light, presumably vitamin K₁, acts in two of the postulated three phosphorylation steps between DPN and oxygen. Martius and Nitz-Litzow (6) found...
TABLE I
Effect of ultraviolet irradiation on oxidative phosphorylation of rat liver mitochondria

The system contained 0.01 M \( \beta \)-hydroxybutyrate, 0.001 M DPN\(^+\), 1.5 \( \times \) 10\(^{-6}\) M cytochrome \( c \), 0.005 M ADP, 0.0125 M inorganic phosphate, 0.1 M KF, 0.01 M MgCl\(_2\), and mitochondria. Total volume, 3.2 ml.; pH 7.4; temperature, 30\(^\circ\)C.

<table>
<thead>
<tr>
<th>Ultraviolet irradiation</th>
<th>( \Delta P_i )</th>
<th>Oxygen</th>
<th>P:O</th>
<th>Change</th>
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<tbody>
<tr>
<td></td>
<td>( \mu ) moles</td>
<td>( \mu ) atoms</td>
<td></td>
<td>%</td>
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<tr>
<td>-</td>
<td>3.17</td>
<td>2.50</td>
<td>1.22</td>
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<td>0.63</td>
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<td>6.45</td>
<td>4.51</td>
<td>1.40</td>
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* \( P_i \), inorganic phosphate.

TABLE II
Effect of added vitamin \( K_1 \) on oxidative phosphorylation by ultraviolet-irradiated mitochondria

When vitamin \( K_1 \) was added to irradiated mitochondria, it was observed that the P:O ratio of this system was increased almost to control levels. In most of the experiments this increase was accomplished by an increase in phosphorylation with some concomitant decrease in oxidation. The fact that the P:O ratio was increased leads us to interpret these results as indicating that vitamin \( K_1 \) is a cofactor in mitochondrial phosphorylating reactions. Supporting evidence for this interpretation was provided by the use of irradiated vitamin \( K_1 \), which was incapable of reviving the irradiated mitochondrial system. Because vitamin \( K_1 \) did, but irradiated vitamin \( K_1 \) did not revive irradiated mitochondria, it would appear that the substance in mitochondria that is being destroyed by irradiation is probably similar or identical to vitamin \( K_1 \).

The results of Martius and Nitz-Litzow (6) and those presented here indicate that vitamin \( K_1 \) is not concerned with all of the phosphorylations resulting from the oxidation of \( \beta \)-hydroxybutyrate. In an effort to assign a specific phosphorylative site to vitamin \( K_1 \), reduced cytochrome \( c \) was used as subst-
strate, and the P:O ratios of irradiated and control mitochondria were compared. Since no difference was observed in the P:O ratios of the irradiated or control mitochondria we conclude that vitamin K is concerned with either one or both of the phosphorylations occurring between DPN and cytochrome c. (This interpretation is, of course, subject to the previous conclusion that ultraviolet light destroys mitochondrial "vitamin K.") The oxidation-reduction potential of vitamin K, $-0.06$ volts at pH 7.0 (13), is also consistent with this conclusion.

Exposure of mitochondria to ultraviolet light did not affect the oxidation of $\beta$-hydroxybutyrate, whereas the accompanying phosphorylation was decreased. This indicates that vitamin K is part of an alternate pathway between DPN and cytochrome c and that it is not acting in the direct oxidation pathway. A similar proposal has also been advanced by another group (14).

**SUMMARY**

Rat liver mitochondria were exposed to ultraviolet light at 2537 Å and a decrease in P:O ratio with the use of $\beta$-hydroxybutyrate was observed. When vitamin K was added to irradiated mitochondria, P:O ratios were restored almost to control levels. Vitamin K had no effect on control mitochondria, and irradiated vitamin K had no effect in restoring P:O ratios of irradiated mitochondria. Ultraviolet irradiation did not affect the P:O ratio obtained with the use of cytochrome c as substrate. The implications of these findings are discussed.

**REFERENCES**

The Effect of Vitamin K₁ on Oxidative Phosphorylation of Rat Liver Mitochondria Irradiated with Ultraviolet Light
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