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Enzymic Formation of Glycolate in *Chromatium.* Role of Superoxide Radical in a Transketolase-Type Mechanism†

Sumio Asami and Takashi Akazawa*

ABSTRACT: Chromatophores prepared from *Chromatium* exhibit a light-dependent O_2 uptake in the presence of reduced 2,6-dichlorophenolindophenol, the maximum rate observed being 10.8 μmol (mg of Bchl)$^{-1}$ h^{-1} (air-saturated condition). As it was found that the uptake of O_2 was markedly inhibited by superoxide dismutase, it is suggested that molecular oxygen is subject to light-dependent monovalent reduction, resulting in the formation of the superoxide anion radical (O_2^-). By coupling baker's yeast transketolase with illuminated chromatophore preparations, it was demonstrated that [U-^{14}C]-fructose 6-phosphate (6-P) is oxidatively split to produce glycolate, and that the reaction was markedly inhibited by superoxide dismutase and less strongly by catalase. A coupled system containing yeast transketolase and xanthine plus xanthine oxidase showed a similar oxidative formation of glycolate from [U-^{14}C]fructose 6-P. It is thus suggested that photogenerated O_2^- serves as an oxidant in the transketolase-catalyzed formation of glycolate from the α,β-dihydroxyethyl (C_2) thiamine pyrophosphate complex, whereas H_2O_2 is not an efficient oxidant. The rate of glycolate formation in vitro utilizing O_2^- does not account for the in vivo rate of glycolate photosynthesis in *Chromatium* cells exposed to an O_2 atmosphere (10 μmol (mg of Bchl)$^{-1}$ h^{-1}). However, the enhancement of glycolate formation by the autoxidizable electron acceptor methyl viologen in *Chromatium* cells in O_2, as well as the strong suppression by 1,2-dihydroxybenzene-3,5-disulfonic acid (Tiron), an O_2^- scavenger, suggest that O_2^- is involved in the light-dependent formation of glycolate in vivo.

It has long been recognized that higher plants and green algae evolve O_2 photosynthetically, but at the same time they fix atmospheric O_2 in light. The mechanism of the latter phenomenon or photorespiration is one of the central problems of modern plant biology (Goldsworthy, 1970; Jackson and Volk, 1970; Chollet and Ogren, 1975). The light-induced O_2 uptake is partly explained by the RuP_2[1] oxygenase reaction which results in the formation of glycolate and the subsequent oxidation of glycolate catalyzed by glycolate oxidase (Tolbert, 1973; Glidewell and Raven, 1976). However, this enzymic mechanism alone cannot account for the overall mechanism of O_2 uptake in light (Radmer and Kok, 1976). Indeed a sizable amount of O_2 absorbed by plants appears to be ascribed to a Mehler-type reaction, and several investigators have suggested that ATP formation is coupled to this reaction (Egneus et al., 1975). It has been known that O_2 generated in the Mehler reaction is subject to a monovalent reduction process, producing O_2^- in photosystem I of chloroplast preparations (Allen and Hall, 1974; Asada et al., 1974). However, O_2^- and other reduced oxygen derivatives are known to be toxic in biological systems and it is often argued that the ubiquitous distribution of superoxide dismutase from strict anaerobic organisms to aerobes presumably reflects the detoxification of such harmful agents (Fridovich, 1975; Hewitt and Morris, 1975).

Current biochemical studies on the enzymic mechanism of glycolate formation during photosynthesis have focused on the role of O_2. A recent fashionable concept is that the oxygenase reaction catalyzed by chloroplastic RuP_2 carboxylase is responsible for the photorespiratory production of glycolate

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[1] Abbreviations used are: Bchl, bacteriochlorophyll; DCPIP, 2,6-dichlorophenolindophenol; Fd, ferredoxin; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; RuP_2, ribulose 1,5-bisphosphate; Tiron, 1,2-dihydroxybenzene-3,5-disulfonic acid; C_2, α,β-dihydroxyethyl; TPP, thiamine pyrophosphate; Tricine, *N*-tris(hydroxymethyl)methylglycine.

(Tolbert, 1973; Chollet and Ogren, 1975; Lorimer et al., 1976). A second important mechanism is based on the classical experiment by Bradbeer and Racker (1961) involving the oxidative cleavage of α,β-dihydroxyethyl (C_2) TPP derived from the intermediates of the Calvin–Benson cycle catalyzed by transketolase. This hypothesis was extended by Shain and Gibbs (1971) who demonstrated that broken chloroplast preparations supplemented with transketolase catalyzed the light-dependent formation of glycolate from fructose 6-P. Since the reaction was inhibited by catalase, these authors theorized that H_2O_2 generated in photosystem I oxidizes the C_2-TPP complex produced in the transketolase reaction. Still other mechanisms of glycolate biosynthesis have been proposed (Zelitch, 1975), and some investigators have suggested that multiple pathways exist for the formation of glycolate during photorespiration (Eickenbusch et al., 1975; Zelitch, 1975). Previously we reported that cell-free extracts from *Chromatium* synthesize glycolate from either fructose 6-P or xylulose 5-P in the presence of ferricyanide (Asami and Akazawa, 1975b). However, it has yet to be determined what is the natural oxidant in this system, although H_2O_2 was found to be ineffective in producing glycolate in the *Chromatium* system.

In the work reported in this paper, we demonstrate the effectiveness of O_2^- in the light-dependent formation of glycolate catalyzed by a transketolase system in *Chromatium*.

Materials and Methods

Bacterial Culture and Preparation of Chromatophores and Crude Transketolase. The experimental procedure for culturing cells of *Chromatium vinosum* under photoautotrophic conditions (2.5 klx) at 25 °C and subsequent harvesting at the late-logarithmic growth phase were reported previously (Akazawa et al., 1972). The collected bacterial cells were washed once with ice-chilled H_2O and 25 mM Tris-HCl buffer (pH 7.5) containing 10% glycerol and 0.1 mM EDTA, and finally suspended in the latter buffer solution. They were then ruptured at 4 °C in a Kubota Insonator (Model 200M) at maximum input power. The resulting sonicates were centrifuged at 12 000*g* for 10 min at 4 °C, and the supernatant fraction was applied to a column of Sephadex G-25, which has been equilibrated with the buffer solution described above. The fraction which passed through the column was centrifuged at 105 000*g* for 90 min at 4 °C, and the pellet was washed once with the above buffer solution and finally suspended in the same solution; this served as the chromatophore preparation. The supernatant fraction obtained by ultracentrifugation was made to $(NH_4)_2SO_4$ saturation (50–80%) and the precipitate dissolved in the above buffer solution was used as crude bacterial transketolase after exhaustive dialysis against the same buffer.

Assay of O_2 Uptake. To determine the light-dependent uptake of O_2 by chromatophores, the following standard reaction mixture was used: Tricine–NaOH (pH 8.0), 25 mM; $MgCl_2$, 5 mM; chromatophores (0.19 mg of Bchl); DCPIP, 20 μM; and sodium ascorbate, 2 mM, in a total volume of 1 mL. Where applicable, the following reagents or enzymes were added to the reaction mixture: methyl viologen, 50 μM; superoxide dismutase, 360 units; catalase, 4000 units; NaN_3, 8.4 mM; and KCN, 8.7 mM. In an experiment shown in Figure 2, the amounts of superoxide dismutase were varied as indicated. The reaction mixture was placed in the chamber of a Rank-Bros O_2 electrode (Bottisham) and a light projector fitted with a red filter (Nikon R-60, DP-3) served as the light source. Light intensity was adjusted to 2.5 klx. The reactions were carried out at 25 °C, and the content of dissolved O_2 in the assay medium under this condition was determined to be 250 μM.

Transketolase-Catalyzed Glycolate Formation Utilizing Various Oxidants. The standard transketolase system contained the following components in a total volume of 0.25 mL: Hepes–NaOH (pH 7.8), 50 mM; $MgCl_2$, 8 mM; TPP, 3 mM; [U-^{14}C]fructose 6-P, 23.5 μM (1.25 μCi); and yeast transketolase (Sigma), 0.5 unit. In the reaction mixture containing the crude bacterial enzyme, transketolase was replaced with 330 μg of the crude enzyme prepared from the bacterial cells and TPP was omitted. Both reaction mixtures were coupled with the following oxidant or O_2^- generating systems: (i) potassium ferricyanide, 1 mM; (ii) xanthine (0.2 mM) and xanthine oxidase (39 μg); and (iii) H_2O_2, 2 mM (cf. Table I). Where applicable superoxide dismutase (160 units), catalase (2000 units), or Tiron (5 mM) was added to the assay mixture to determine their effect in each reaction system. After preincubation for 5 min at 25 °C, the reaction was started by adding oxidant or the O_2^- generating system and incubation continued for an additional 12 min at 250 μM O_2. However, in the coupled system containing chromatophores, the reaction mixture contained the following additional components: DCPIP, 50 μM; ascorbate, 2 mM; and chromatophores (88 μg of Bchl) (cf. Figure 3). The reaction was initiated with red light and terminated at selected reaction intervals by adding 0.2 mL of absolute ethanol and boiling the whole mixture at 80 °C for 1 min. An aliquot was subjected to high voltage paper electrophoresis at pH 5.0 (1.5 kV, 70 min) and the radioactivity in the glycolate region was determined in a liquid scintillation spectrometer using a nonaqueous scintillator system, the details of which have been reported previously (Asami et al., 1977). It was shown by additional paper chromatographic examination that the glycolate isolated by this electrophoretic technique was a homogeneous component.

Photosynthetic CO_2 Fixation. Basic experimental procedures for measuring photosynthetic CO_2 fixation using intact bacterial cells were previously reported (Asami and Akazawa, 1974). Unless noted otherwise, the reaction mixture contained: Hepes–NaOH (pH 7.8), 25 mM; Na_2S, 2 mM; $NaH^{14}CO_2$, 1 mM (11 μCi); potassium phosphate, 2 mM; $MgCl_2$, 1 mM; NH_4Cl, 2 mM; and bacterial cells (24 μg of Bchl) in a total volume of 1 mL. Where applicable varying amounts of methyl viologen (0.01–1 mM) or Tiron (1–10 mM) were added. The reaction mixture was bubbled with pure O_2 for 10 min, and the reaction started by adding $NaH^{14}CO_3$ and illuminating the mixture with white tungsten light (90 klx). The incubation was continued for 30 min at 35 °C. To determine the incorporation of $^{14}CO_2$ into glycolate and glycine, analytical methods using a Dowex column reported previously by Asami et al. (1977) were employed.

Enzymes. Baker's yeast transketolase (type X, lot no. 8580) was purchased from Sigma (St. Louis, Mo.) and dissolved in 25 mM Hepes–NaOH (pH 7.8) immediately before use. Spinach Cu,Zn-superoxide dismutase was a gift from Dr. K. Asada (Kyoto University) and used only after exhaustive dialysis against 10 mM potassium phosphate (pH 7.8) at 4 °C. Milk xanthine oxidase was purchased from Boehringer Mannheim and used after dialysis against 25 mM Tris-HCl (pH 7.5) containing 0.1 mM EDTA at 4 °C. Catalase (beef liver) was purchased from Sigma.

Results

Production of Superoxide Anion Radical (O_2^-) by Chromatium Chromatophores. We first examined the light-de-

TABLE I: Glycolate Formation by Transketolase System in the Presence of Various Oxidants.[a]

Enzyme system	Oxidants	Glycolate formed (nmol/12 min) None	+ superoxide dismutase[b]	+ catalase[d]	+ Tiron[d]
Transketolase (yeast)	Ferricyanide (1 mM)	1.21			
	Xanthine + xanthine oxidase	0.13	0.02	0.10	0.07
	H_2O_2 (2 mM)	0.04			
Chromatium crude enzyme	Ferricyanide (1 mM)	1.28			
	Xanthine + xanthine oxidase	0.07	0.04	0.05	0.05
	H_2O_2 (2 mM)	0.03			

[a] Experimental details of the assaying conditions and the analysis of glycolate formation are described in the text. [b] 160 units. [c] 2000 units. [d] 5 mM.

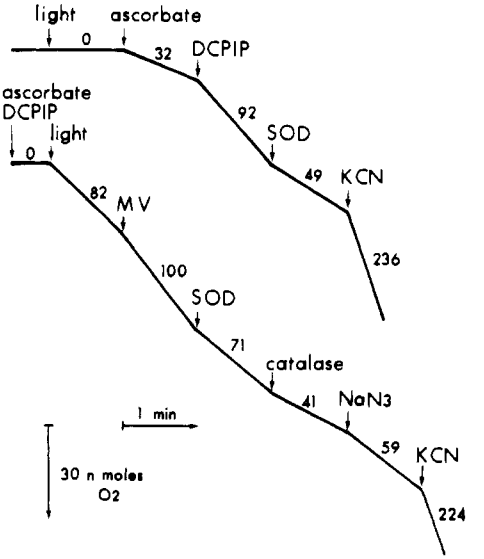

FIGURE 1: Light-dependent O_2 uptake by *Chromatium* chromatophores. Experimental details for assaying O_2 uptake with an O_2 electrode and the compositions of the reaction mixture are described in the text. At each respective arrow, the following quantities of reagents or enzymes were added to the assay mixture: ascorbate, 2 mM; DCPIP, 50 μM; methyl viologen, 50 μM; superoxide dismutase (SOD), 360 units; catalase, 4000 units; KCN, 8.7 mM; and NaN_3, 8.4 mM. The numbers along each trace are the relative rates of O_2 uptake, as a percentage of the rate in the presence of 50 μM methyl viologen (100%), which was 9.73 μmol (mg of Bchl)$^{-1}$ h^{-1}.

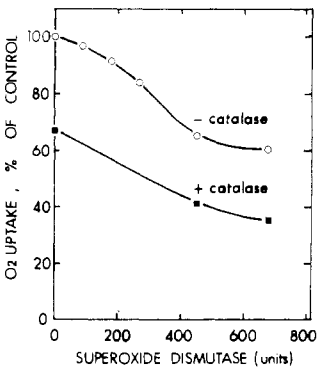

FIGURE 2: Inhibitory effect of superoxide dismutase and catalase on light-dependent O_2 uptake by *Chromatium* chromatophores. Experimental details of assaying O_2 uptake and the composition of the assay mixture were basically the same as for Figure 1, except methyl viologen was omitted and varying amounts of superoxide dismutase, ±catalase (4000 units), were added to the reaction mixture.

pendent uptake of O_2 by *Chromatium* chromatophores at 25 °C. Typical experimental results are shown in Figure 1. Unlike the case of higher plant chloroplasts or thyalkoid membranes, O_2 uptake was detectable only when appropriate electron donors were added to the reaction mixture. It was found that reduced DCPIP was effective in promoting O_2 uptake in the light, and this uptake was stimulated by methyl viologen. If the observed O_2 uptake was due to the formation of O_2^-, one can predict theoretically that the addition of excess superoxide dismutase should cause a 50% inhibition and an additional inhibition (75%) may result from catalase addition (cf. Allen and Hall, 1974). As can be seen from the tracing, the addition of spinach leaf Cu,Zn-superoxide dismutase caused a 29–47% inhibition and catalase resulted in further inhibition. The addition of NaN_3, an inhibitor of catalase, caused a partial restoration of O_2 uptake. The addition of KCN, an inhibitor of both Cu,Zn-superoxide dismutase and catalase, resulted in a marked stimulation of O_2 uptake. However, the stimulatory effect of KCN was observed even in the absence of exogenous superoxide dismutase and catalase (data not shown). Since *Chromatium* superoxide dismutase was shown to be an Fe-type enzyme and insensitive to KCN (Asada et al., 1976), it is difficult to attribute our KCN results to the contamination of the *Chromatium* chromatophores with endogenous superoxide dismutase. The overall results show that *Chromatium* chromatophores take up approximately 10 μmol of O_2 (mg of Bchl)$^{-1}$ h^{-1} under the air-saturated assay conditions at 25 °C employed. We found that the uptake of O_2 was proportional to the amount of chromatophore preparations added up to 0.4 mg of Bchl (data not shown). The inhibitory effect of varying amounts of superoxide dismutase was examined in the presence and absence of catalase and the results are presented in Figure 2.

Formation of Glycolate by a Transketolase System. The enzymic formation of glycolate via a transketolase (yeast) model system using [U-^{14}C]fructose 6-P as the substrate and three different oxidants, i.e., ferricyanide, O_2^-, and H_2O_2, was examined, and the results are summarized in Table I. We have previously reported on a transketolase-catalyzed formation of glycolate by cell-free extracts from *Chromatium* (Asami and Akazawa, 1975b), and in the present experiments ferricyanide (1 mM) proved to be far superior to two other oxidants, i.e., enzymically generated O_2^- and H_2O_2. It was found that in a reaction system containing xanthine and xanthine oxidase, the rate of glycolate formation was 0.5 nmol/h at 25 °C, ~2% of the [U-^{14}C]fructose 6-P supplied being converted to glycolate. The efficacy of H_2O_2 was still poorer. It should be noted that the molar quantity of O_2^- produced is far less than that of the

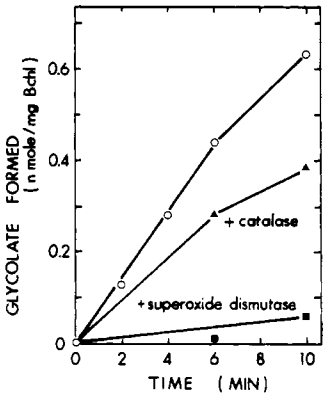

FIGURE 3: Glycolate formation by a yeast transketolase reaction utilizing O_2^- photogenerated by *Chromatium* chromatophores and the inhibitory effect of superoxide dismutase and catalase. Experimental details of the assay and the composition of the reaction mixture containing [U-^{14}C]-fructose 6-P (23.5 μM, 1.25 μCi) as substrate are described in the text. The quantities of catalase and superoxide dismutase added were 2000 units and 160 units, respectively.

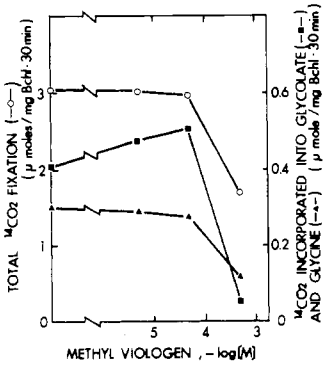

FIGURE 4: Effect of methyl viologen on photosynthetic $^{14}CO_2$ fixation and $^{14}CO_2$ incorporation into glycolate and glycine by photosynthesizing *Chromatium* cells in an O_2 atmosphere. The composition of the reaction mixture and the experimental details for assaying $^{14}CO_2$ fixation and $^{14}CO_2$ incorporation into glycolate and glycine are described in the text. The reaction mixture containing $NaH^{14}CO_3$ (1 mM, 11 μCi) was illuminated with white tungsten light (90 klx) to start the reaction and terminated after 30 min at 35 °C.

other two oxidants, as can be surmised from its large recombination constant (ca. 5×10^5 M^{-1} s^{-1}, pH 7.8) (Behar et al., 1970). In spite of such an apparently unfavorable reaction system, glycolate formation was substantially inhibited by superoxide dismutase (160 units) (85%) and the O_2^- scavenger Tiron (5 mM) (45%).

In the reaction system containing the crude bacterial enzyme as a source of transketolase, ferricyanide also proved to be the most efficient oxidant in promoting glycolate formation (Table I). A reaction system supplemented with xanthine and xanthine oxidase was partially effective, in promoting glycolate synthesis, but the rate was about 50% of that in the yeast transketolase model system. Again H_2O_2 was much inferior, but we cannot exclude a possibility that endogenous catalase and superoxide dismutase caused such a result.

We next examined a coupled system containing *Chromatium* chromatophores and baker's yeast transketolase, the former producing O_2^- in the light (cf. Figure 1). The results of the time-dependent labeling of glycolate from [U-^{14}C]-fructose 6-P are shown in Figure 3. The efficacy of O_2^- is not high, which is most probably due to the rapid reaction of the light-generated O_2^- with the ascorbate present in the assay mixture, lowering the reactivity of O_2^- in the transketolase system (cf. Elstner and Kramer, 1973; Ort and Izawa, 1974; Greenstock and Miller, 1975). The O_2^- dependent formation of glycolate was markedly suppressed by superoxide dismutase and not by catalase, indicating that H_2O_2, a secondary product of the photoreduction of O_2^-, does not serve as an efficient oxidant in the system.

Effect of Methyl Viologen and Tiron on Light-Dependent Glycolate Formation by Intact Chromatium Cells. In an attempt to examine the possible role of light-induced O_2^- production and its direct utilization for glycolate formation in photosynthesizing cells of *Chromatium,* we tested the effect of methyl viologen and Tiron. Photosynthesis of *Chromatium* was examined in the presence of various amounts of methyl viologen, and a typical experiment is presented in Figure 4. A final concentration of 10–100 μM methyl viologen did not affect photosynthetic $^{14}CO_2$ fixation, but 1 mM methyl viologen markedly inhibited photosynthesis. On the other hand, it was found that the addition of 10–100 μM exhibited a stimulatory effect (up to 25%) on $^{14}CO_2$ incorporation into glycolate, although the labeling pattern of glycine was essentially the same as that of total $^{14}CO_2$ fixation. It is well established that methyl viologen is reduced in the electron transport system of chloroplasts, and subsequently reacts with O_2 to form O_2^- (Allen and Hall, 1974; Asada et al., 1974; Epel and Neumann, 1973). It is thus possible that photogenerated O_2^- may serve as an oxidant in the light-dependent formation of glycolate in *Chromatium* cells. Our previous experiments have shown that in an O_2 atmosphere the bacteria produce not only glycolate but also glycine, and both are then excreted extracellularly (Asami and Akazawa, 1976). Results from radioisotopic experiments and total amino acid analyses showed that the glycolate–glycine transformation operates in *Chromatium.* As to the reason why glycine formation is not stimulated by the addition of methyl viologen, it is conceivable that the oxidation of glycolate and its subsequent transamination are rate limiting in the glycolate–glycine transformation processes (cf. Asami et al., 1977).

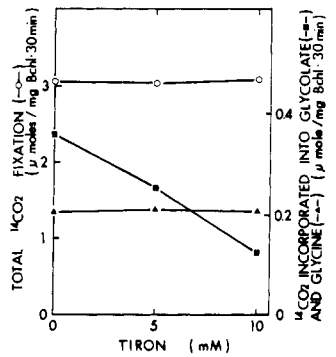

FIGURE 5: Effect of Tiron on photosynthetic $^{14}CO_2$ fixation and $^{14}CO_2$ incorporation into glycolate and glycine by photosynthesizing *Chromatium* cells in an O_2 atmosphere. The composition of the reaction mixture and the experimental details for measuring the photosynthetic activities were the same as for Figure 4.

We next tested the effect of Tiron, which is a potent scavenger of O_2^- (Greenstock and Miller, 1975; Hirata and Hayaishi, 1975), on glycolate formation in vivo and the results are presented in Figure 5. Both photosynthetic $^{14}CO_2$ fixation and $^{14}CO_2$ incorporation into glycine by *Chromatium* cells in an O_2 atmosphere were unaffected by up to 10 mM Tiron. However, a marked inhibitory effect of Tiron on the incorporation of $^{14}CO_2$ into glycolate was observed; this observation together with the one described in Figure 4 strongly suggest

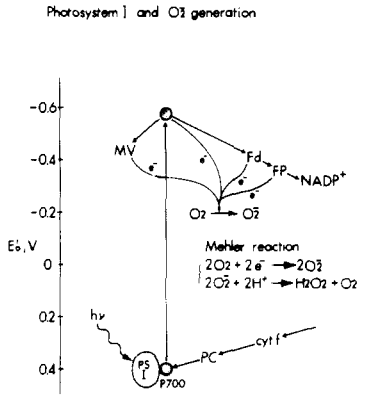

FIGURE 6: Light-dependent formation of O_2^- coupled to photosystem I.

that photosynthetically generated O_2^- can be utilized for glycolate formation in the bacterial cells.

Discussion

The present investigation has demonstrated that in the presence of an appropriate electron donor, i.e. reduced DCPIP, chromatophores from *Chromatium* exhibit a light-induced O_2 uptake which is attributed to a photogeneration of O_2^-. It was further found that light-produced O_2^- can be utilized for the oxidative formation of glycolate catalyzed by transketolase (Figure 3). This type of reaction mechanism was further supported by studies with a coupled system containing transketolase and xanthine + xanthine oxidase (Table I). These two reactions were appreciably inhibited by superoxide dismutase and Tiron. However, the rate of transketolase-catalyzed glycolate formation utilizing O_2^- was lower than that observed with ferricyanide as the oxidant, and does not account for the in vivo rate of glycolate formation determined in intact bacterial cells in an O_2 atmosphere (Asami and Akazawa, 1974, 1975a). Although we should bear in mind that these two reaction systems governing mechanisms may not be the same, the lower reactivity of O_2^- can be ascribed to its short-lived nature as reflected from the exceedingly large recombination constant recorded (Behar et al., 1970). Furthermore, the rapid reaction of the photogenerated O_2^- with ascorbate in the chromatophore reaction mixture potentially lowers its utilization in the transketolase system (cf. Elstner and Kramer, 1973; Ort and Izawa, 1974; Greenstock and Miller, 1975). In spite of such a low rate of glycolate formation in vitro utilizing O_2^-, it will be emphasized that methyl viologen enhances glycolate synthesis in photosynthesizing bacterial cells, and Tiron, an O_2^- scavenger, exhibits a potent inhibitory effect. Both observations suggest that photogenerated O_2^- is utilized in the biosynthesis of glycolate in vivo. To our knowledge this study provides the first evidence for the role of O_2^- in glycolate formation.

The mechanism of the transketolase-catalyzed formation of glycolate presently studied is basically analogous to the one proposed previously by Shain and Gibbs (1971) using broken chloroplast preparations. These authors have theorized that H_2O_2 generated during photosystem I electron flow serves as an oxidant in the cleavage of the C_2-TPP complex derived from fructose 6-P in the transketolase reaction. However, it is generally recognized that the primary molecular species produced from O_2 in the light reactions catalyzed by Fd-NADP reductase or Fd in chloroplast lamellae is O_2^-, not H_2O_2 (Massey et al., 1969; Misra and Fridovich, 1971) (Figure 6). It thus appears more likely that O_2^- is engaged in the transketolase-type formation of glycolate in photosynthetic organisms. Indeed our own experiments have shown that catalase was less effective than superoxide dismutase in inhibiting the glycolate-forming system containing yeast transketolase and illuminated chromatophores (cf. Figure 4).

It is believed that the ubiquitous distribution of superoxide dismutase in living organisms plays a role in protecting biological systems from the toxic effects of O_2^- produced by the monovalent reduction of O_2 by radiant energy and other mechanisms. However, the level of superoxide dismutase in organisms, including higher plants, is usually just sufficient to detoxify the O_2^- produced. Our previous experiments suggest that the production of glycolate by photosynthesizing *Chromatium* cells in an O_2 atmosphere represents the adaptive response of this organism, protecting from the harmful environmental atmosphere. In this connection it is interesting to recall that the viability of the blue-green alga, *Anacystis nidulans*, is variable depending on environmental conditions such as light intensity and O_2; moreover, it is highly correlated with the induction of superoxide dismutase activity in the organism (Abeliovich et al., 1974). It is thus noteworthy that, under conditions of high light intensity and high O_2 concentrations, the greatest amount of glycolate is produced by the alga and excreted extracellularly (Han and Eley, 1973).

It was recently found that with photosynthesizing *Chromatium* cells incubated in 100% $^{18}O_2$ or air (21% $^{18}O_2$), one atom of ^{18}O was incorporated into the carboxyl group of glycolate and the specific activity of the excreted glycolate was identical with that of the O_2 in the gas phase (Lorimer et al., 1976). The molecular mechanism of this O_2-dependent formation of glycolate can be readily explained by the RuP_2 oxygenase reaction utilizing molecular O_2 as substrate (Lorimer et al., 1973). It remains for future investigation to determine whether or not a transketolase-type formation of glycolate utilizing O_2^- as oxidant can account for the incorporation of ^{18}O into the carboxyl group of glycolate observed in vivo.

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Histone Messenger RNA from HeLa Cells: Evidence for Modified 5′ Termini†

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ABSTRACT: The distribution of [^{3}H]methyl radioactivity in cytoplasmic histone mRNA, isolated during the DNA synthetic (S) phase of the HeLa S_3 cell cycle, has been investigated. Evidence is presented that approximately 30% of the radioactivity is in m^7GpppX^mpYp oligonucleotides, where X^m represents 2′-*O*-methylated adenosine and guanosine with a molar ratio of 4:1, respectively. The remainder of the radioactivity is present as $m^7GpppX^mpY^mpZp$ oligonucleotides, where X^m is again 2′-*O*-methylated adenosine and guanosine (4:1) and where y^m represents 2′-*O*-methylated adenosine, guanosine, cytidine, and uridine with ratios of 2:1:1:1, respectively. While 48.6% of the [^{3}H]methyl radioactivity was present as N^6-methyladenosine in poly(adenylic acid)-terminated mRNA from S-phase cells, no evidence for N^6-methyladenosine was found in histone mRNA. It thus appears that histone mRNA which lacks 3′-terminal poly(adenylic acid) sequences and functions on cytoplasmic polyribosomes during a limited portion of the cell cycle is capped but lacks internal-modified nucleosides.

Inverted dinucleotides have recently been found at the 5′ termini of a variety of poly(A)[1]-containing mRNA species. These modified termini have the general structure $m^7G5'ppp5'X^m$(cap 1) and $m^7G5'ppp5'X^mpY^m$(cap 2). While cap 1 structures, where X^m is limited to 2′-*O*-methylated adenosine or guanosine, are the predominant termini synthesized by viral cores in vitro (Abraham et al., 1975; Furuichi and Miura, 1975; Furuichi et al., 1975a; Urushibara et al., 1975; Wei and Moss, 1975), both cap 1 and cap 2 structures have been found in poly(A)-terminated mRNA isolated from virus-infected and -uninfected mammalian cells (Adams and Cory, 1975; Cory and Adams, 1975; Furuichi et al., 1975b; Lavi and Shatkin, 1975; McGuire et al., 1976; Rose, 1975; Shatkin, personal communication; Wei et al., 1975a). One example of the heterogeneity of modified sequences found at the 5′ termini of cellular mRNA has appeared recently. Cory and Adams (1975) present evidence that poly(A)-containing mRNA from mouse myeloma cells contains a minimum of 27 5′-terminal sequences, with any of the four 2′-*O*-methylated nucleosides present at positions X^m and Y^m. The 5′ termini of low-molecular-weight nuclear RNAs also contain methylated nucleosides (Ro-Choi et al., 1974, 1975). In addition to modified nucleosides at the 5′ termini, poly(A)-containing cellular mRNA (Adams and Cory, 1975; Cory and Adams, 1975; Furuichi et al., 1975b; Desrosiers et al., 1974; Perry et al., 1975) and mRNA isolated from cells infected by SV40 virus (Lavi and Shatkin, 1975) and adenovirus (Shatkin, personal communication) contain internal N^6-methyladenosine residues, which are probably located near the 5′ end.

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[1] Abbreviations used are: poly(A), poly(adenylic acid); Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; DEAE, diethylaminoethyl.