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URIC ACID PROTECTS MEMBRANES AND LINOLENIC ACID FROM OZONE-INDUCED OXIDATION

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Aqueous preparations of linolenic acid, bovine serum albumin, and bovine erythrocyte membrane fragments were bubbled with ozone in the presence or absence of uric acid. Ozonation of the membrane fragments or the bovine serum albumin did not result in protein degradation. After 15 min of ozonation, the absorbance of the thiobarbituric acid-reactive material increased by 0.34 in the linolenic acid preparation and by 0.08 in the suspension of membrane fragments. In the presence of uric acid, these changes in absorbance were reduced to 0.14 for the fatty acid and to 0.01 for the membrane fragments. This result indicates that uric acid protects lipids from ozone-induced oxidation.

Plasma uric acid has been hypothesized to provide antioxidant protection for erythrocytes, and evidence from several studies supports the idea that uric acid functions as a free radical scavenger (1-7). While ozone is not a free radical, the damage it causes is thought to be mediated by free radicals. The toxic effects of ozone may be due to its reaction with cell membranes. The unsaturated fatty acids in membrane lipids are a possible target for ozone attack. Exposure of red blood cells to ozone in vitro results in lipid peroxidation (8) and a decrease in the relative amounts of polyunsaturated fatty acids (9). Other studies point to oxidation of membrane proteins as the significant event in ozone damage (10,11).

The possibility that uric acid could protect the fatty acids in membrane lipids from oxidation by ozone was suggested by studies which demonstrated that uric acid inhibits oxidation of linoleic acid, linolenic acid, and arachidonic acid (2,12) and that uric acid protects nucleobases and erythrocytes from ozone-induced damage (13,14). Uric acid inhibits

ABBREVIATIONS: BSA = bovine serum albumin; TBA = thiobarbituric acid.

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ozone-induced hemolysis, methemoglobin formation, and production of TBA-reactive materials in bovine and swine erythrocytes (14). This report demonstrates that uric acid may protect erythrocyte membranes from ozone damage by inhibiting fatty acid oxidation.

MATERIALS AND METHODS

Chemicals: All chemicals were purchased from Sigma Chemical Company, St. Louis, Missouri.
Linolenic acid: The linolenic acid (12.8 mg) was dissolved in 1 ml of 0.65 N NaOH and diluted to 100 ml with 0.2 M borate buffer, pH 9.0. For protection studies, the buffer contained uric acid at a concentration of 1 mM.
Bovine serum albumin: BSA was prepared in 10 mM phosphate buffer, pH 7.4, to give a final concentration of either 0.5 mg/ml or 2.0 mg/ml.
Membrane fragments: Saline-washed bovine erythrocytes were prepared as described by Ames et al. (1). The lysed cells were washed with 10 mM phosphate buffer until they were no longer pink. For cavitation, a Parr cell disruption bomb, Model 4639, from Parr Instrument Company, Moline, Illinois, was used. The membranes were cavitated under nitrogen at 1000 psi for 20 min and then lyophilyzed. Cavitation allowed maximum reaction within 15 min so that losses from foaming during ozonation were not a factor. An antifoaming agent was not added because of reports that it affected experimental results (15). For ozone treatment, 0.25 g (dry weight) of membrane fragments were suspended in 10 mM phosphate buffer with or without uric acid.
Ozonation: A silent electric discharge apparatus, Model 03V1-0, from Ozone Research and Equipment Corporation, Phoenix, Arizona, was used to generate ozone from a stream of oxygen gas. Ozone delivery was 1.7 umole/min with a gas flow rate of 70 ml/min. The KI method (10,16) was used to measure ozone concentration. The ozone/oxygen mixture was bubbled through a 100-ml sample solution containing linolenic acid, BSA, or membrane fragments. Periodically 1-2 ml of sample were removed for assay. Control samples were bubbled with oxygen under the same conditions used for ozonation or were exposed to room air without bubbling.
Assays: To measure oxidation of linolenic acid and lipids in the membrane fragments, a modified TBA method (12) was used. Protein content in both the BSA and the membrane fragments was determined according to Lowry et al. (17). Uric acid, at the concentration used in the protection studies, did not interfere with the Lowry assay. Ultraviolet absorbance spectra were measured from 240 to 340 nm with a Beckman Model 25 spectrophotometer. The breakdown of uric acid alone or in the presence of linolenic acid was followed spectrophotometrically at 292 nm. In the membrane preparation, uric acid degradation was determined by extraction of 1 ml of sample with 0.25 ml of cold 50% TCA. After centrifugation, 0.1 ml of the supernatant was added to 1.4 ml of 0.1 M phosphate buffer, pH 7.4, and the absorbance read at 292 nm.

RESULTS

Control treatment of samples was not associated with an increase in TBA-reactive material, protein loss, or changes in the ultraviolet absorbance spectrum of BSA. Ozonation of the linolenic acid solution resulted in an increase in the amount of TBA-reactive material (Fig. 1). In the absence of

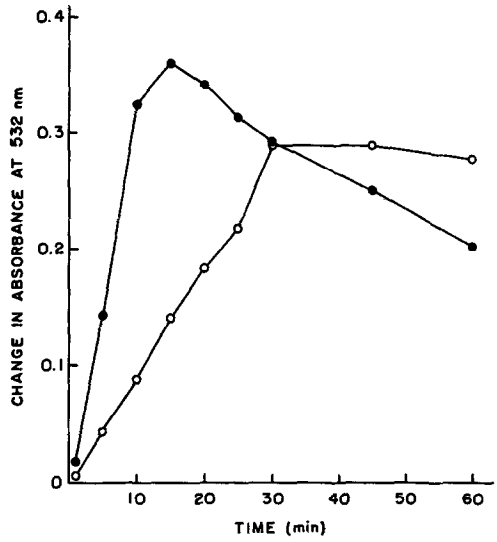

Fig. 1. Formation of TBA-reactive material as a result of ozonation of linolenic acid. Linolenic acid (12.8 mg) was diluted to 100 ml with borate buffer, pH 9.0. Ozone flow = 1.7 umole/min. Linolenic acid alone (●) or in buffer containing 1 mM uric acid (○). Data points represent means from 3 experiments.

uric acid, TBA-positive material reached a maximum at 15 min and declined slightly between 15 and 60 min. When uric acid was in the buffer, TBA-reactive material was formed at a slower rate with the presence of uric acid tripling the time required to reach an absorbance of 0.20. In the solution containing uric acid, the maximum absorbance of TBA-reactive material occurred at 30 min. At the respective maximum levels, more TBA-positive material (absorbance=0.36) was formed in the solution without uric acid than in the one containing it (absorbance=0.29). At 30 min of treatment, the levels of TBA-reactive material in the somples with and without uric acid were similar. After that time, the higher level of TBA-reactive material in the samples containing uric acid presumably is due to the fact that at this point the linolenic acid concentration is lower in the uric acid-free mixture than in the solution containing the antioxidant. Pryor has suggested that the TBA-reactive material is a non-volatile malonaldehyde precursor, possibly a labile endoperoxide (18). The lability of this precursor could account for the decrease in TBA-positive material as ozonation is prolonged.

Ozonation of the BSA or membrane fragments did not result in a loss of protein as measured by the Lowry method. At the 15-min sampling time, more than 96% of the protein originally present remained in the samples treated with ozone. Examination of the ultraviolet spectra of ozonized BSA showed changes characteristic of ozone reaction with tryptophan (19,20). These changes include decreased absorbance between 275 and 280 nm, increased absorbance at 320 nm, and a new peak at 260 nm.

Treatment of the membrane suspensions with ozone resulted in an increase in TBA-positive material, but when uric acid was present during ozonation, the increase was much smaller (Fig. 2). After 15 min of ozonation, the samples without uric acid had an absorbance of 0.080, whereas in the presence of uric acid, the absorbance at 15 min was 0.010.

When ozonized, uric acid alone or in the presence of linolenic acid or membrane fragments exhibited the same degradation rate. The time required to

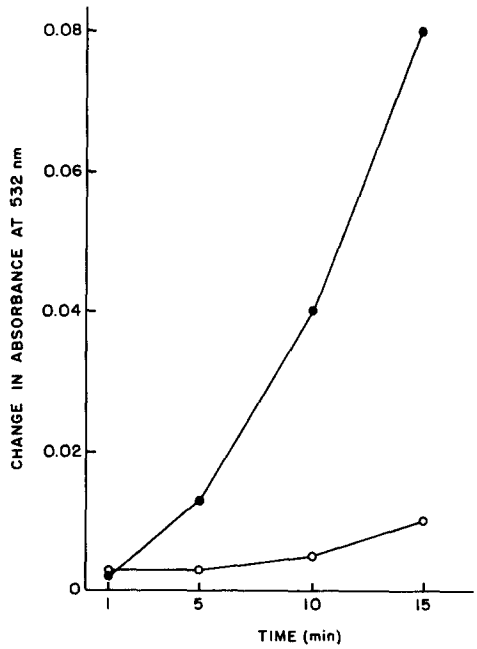

Fig. 2. Increase in TBA-positive material in cavitated erythrocyte membranes as a result of ozone treatment. Cavitated membranes (0.25 g dry weight) were suspended in 100 ml 10 mM phosphate buffer, pH 7.4. Ozone delivery was 1.7 umole/min. Cavitated membranes in buffer only (●) or in buffer plus 1 mM uric acid (○). Data points represent the means from 3 treatments.

decrease the initial concentration by one-half was approximately 18 min in each case.

DISCUSSION

Urate has been shown to be an excellent scavenger of singlet oxygen (1). Uric acid is very reactive with peroxyl radicals (7,21), and it will also scavenge hydroxyl radicals (21).

In aqueous solutions above pH 5 or 6, ozone produces hydroxyl radicals (22). It has not been determined whether ozone-induced free radicals react with fatty acids and lead to peroxidation with the production of more radicals or if the ozonation of unsaturated fatty acids results in formation of adducts that decompose to form free radicals which initiate peroxidation (9). In either case the ability of uric acid to scavenge both the hydroxyl and peroxyl radical could account for its antioxidant effect with fatty acids.

The lack of ozone-induced protein degradation in this system is consistent with studies which have shown that ozone primarily affects certain reactive amino acid residues (23-25) and that polypeptide chains are not degraded to any significant extent (26). This finding does not diminish the possibility that protein oxidation may play a key role in ozone toxicity. Many enzymes have one or more of these amino acids at their active site, and ozonation has been shown to result in a loss of activity by enzymes containing tryptophan or cysteine at their active sites (11,23).

The ability of uric acid to protect the bovine membrane fragments from lipid oxidation is consistent with research by Ames et al. (1) and Smith and Lawing (2) which demonstrated the effectiveness of uric acid in protecting bovine and human erythrocyte membranes from peroxidation by t-butyl hydroperoxide. A recent study (27) with methyl linoleate, phosphatidyl choline, and erythrocyte ghosts using a water soluble radical initiator has suggested that uric acid may act as an antioxidant by scavenging radicals in the aqueous phase. A similar mechanism may account for the protection seen in the ozone system.

If, in fact, the oxidation of membrane lipids is important in free radical toxicity, then the ability of uric acid to inhibit lipid oxidation is probably significant, particularly in humans where uric acid is close to saturation levels in plasma.

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