Title

A SENSITIVE CHEMICAL PROCEDURE FOR MONITORING THE RADIOLYTIC OXIDATION OF PROTEINS AT y-RAY DOSAGES DOWN TO 1 KRAD

Authors

Jayko, M.E.
Garrison, W.M.

Publication Date

1986-02-01

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

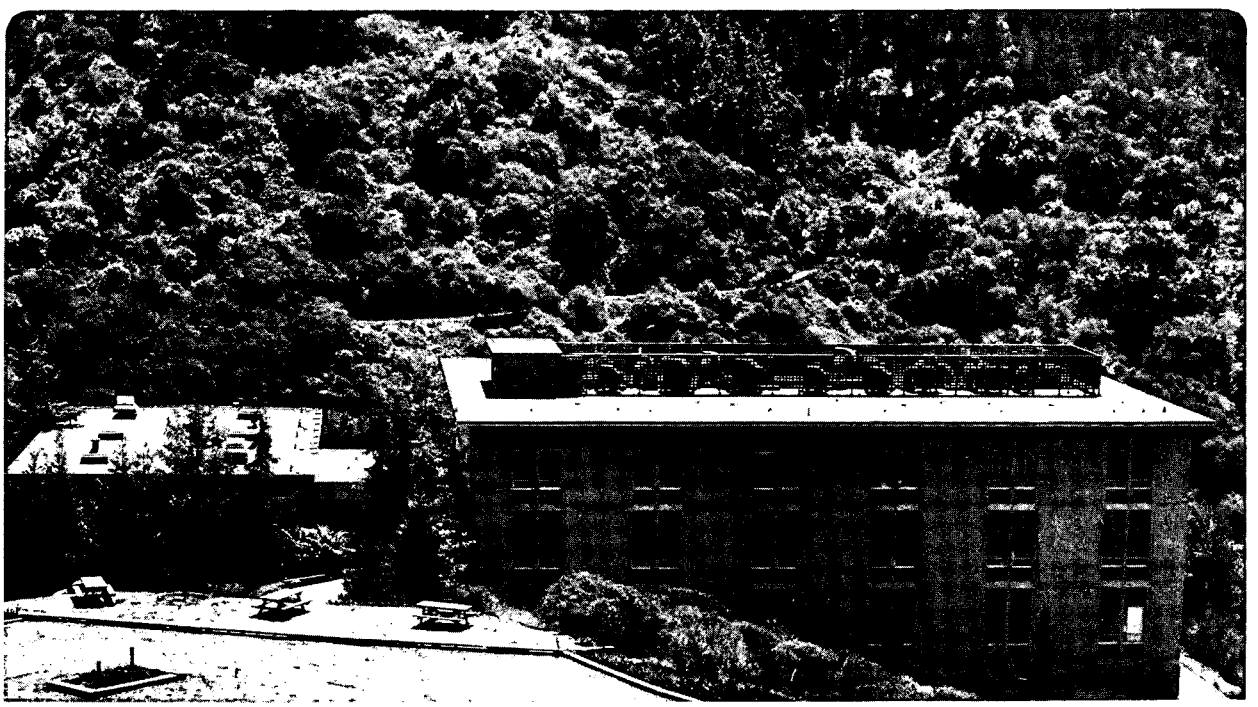
Materials & Molecular Research Division

Submitted to Radiation Effects

A SENSITIVE CHEMICAL PROCEDURE FOR MONITORING THE RADIOLYTIC OXIDATION OF PROTEINS AT γ-RAY DOSAGES DOWN TO 1 KRAD

M.E. Jayko and W.M. Garrison

February 1986



Prepared for the U.S. Department of Energy under Contract DE-AC03-76SF00098

DISCLAIMER

LBL-20335

A SENSITIVE CHEMICAL PROCEDURE FOR MONITORING THE RADIOLYTIC OXIDATION OF PROTEINS AT γ-RAY DOSAGES DOWN TO 1 Krad

Michael E. Jayko and Warren M. Garrison

Lawrence Berkeley Laboratory
University of California
Berkeley, CA 94720

This work was supported by the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

Summary: The radiolytic degradation of proteins in oxygenated solution is initiated by the attack of OH radicals at a multiplicity of main-chain and side-chain loci to give a wide variety of oxidized protein sites. A major fraction of these oxidation reactions lead to the introduction of reactive carbonyl groups into the protein co-valent structure. With γ-rays the total carbonyl yield correspond to $G(>CO)_t \simeq 1$ for a number of different proteins. Outlined here is a sensitive chemical-spectrophotometric procedure for quantitatively monitoring $G(>CO)_t$ over a dose range of interest in radiation biology (1 to 50 Krad). Data obtained with pepsin, α-chymotrypsin and β-lactoglobulin are discussed.

Radiation-chemical damage to protein in oxygenated solution arises from chemical change initiated by the attack of OH radicals formed in the radiation-induced decomposition of water.[1] For many protein systems, a major over-all chemistry can be generalized in terms of the schematics

$$H_2O + O_2 \rightsquigarrow HO_2 + OH \quad (1)$$

$$PH + OH \rightarrow \dot{P} + H_2O \quad (2)$$

$$\dot{P} + O_2 \rightarrow \dot{P}O_2 \quad (3)$$

$$\dot{P}O_2 + HO_2 \rightarrow \text{oxidation products.} \quad (4)$$

Because of the complex chemical composition of proteins, the attack of OH radicals via reaction 2 occurs at a multiplicity of main-chain and side-chain loci to give a wide variety of protein free-radical sites as evidenced by product analysis,[2] pulse-radiolysis,[3,4] and spin-trapping[5] techniques. A major fraction of the subsequent oxidation reactions 3, 4 leads to the introduction of carbonyl groups (>C=O) into the protein co-valent structure.[2] Carbonyl groups are produced in the radiolytic oxidation of (1) the peptide main-chain to yield α-keto acid functions $RCOCONHCHR_2$ (2) the aliphatic side-chain groups of serine, threonine, lysine, and arginine to yield RCHO and R_2CO carbonyls and (3) the unsaturated side-chains particularly tryptophan which yields a carbonyl of the type Ar COR. The detailed mechanisms of the various radiation induced reactions at main-chain and side-chain loci of peptides, polypeptides and proteins are the subject of a recent monograph.[6]

In previous studies we have shown that the combined yield of carbonyl groups formed at these various sites can be determined by a

chemical-spectrophotometric procedure that involves the formation of the 2,4-dinitrophenyl hydrazone derivatives. The phenylhydrazone derivatives of most monocarbonyls show essentially the same absorption spectrum in basic solution and have very similar extinction coefficients at the wave length of maximum absorption with λ_{max} = 440 mμ, $E_{440} = 2.4 \times 10^4$. Carbonyl analysis of oxygen-saturated solutions of pepsin, α-chymotrypsin and β-lactoglobulin after irradiation with ^{60}Co γ-rays were found to give total carbonyl yields, $G(>CO)_t$ of 1.20, 0.89, and 1.25 respectively over the dose range 50-500 Krad.[2] These yield values account for a major fraction of the OH radicals formed in step 1 with G(OH) ≈ 2.8.

We now find that this spectrophotometric procedure, under certain specific conditions, can be adapted to quantitatively monitor the radiolytic oxidation of aqueous proteins at γ-ray dosages as low as 1 krad (and lower under optimum experimental conditions). In a typical "low-dose" procedure, an aqueous pepsin solution (0.5%, pH 3) is equilibrated with air or oxygen and irradiated in a sealed pyrex vial (10 ml) with ^{60}Co γ-rays from a 200 curie source at a dose rate of ~ 1 krad/min. Immediately after irradiation, the solution is dialyzed in cellophane against water until hydrogen peroxide cannot be detected with titanium sulfate reagent. The dialyzed protein solution is then acidified to 0.1N with hydrochloric acid, treated with 2,4-dinitrophenylhydrazine (2,4 DNPH) reagent to give a five to ten-fold excess of reagent over the estimated carbonyl concentration and allowed to stand at room temperature (25°C) for 4 hours to effect the quantitative formation of the hydrazone derivatives. Higher 2,4-DNPH concentra-

tions, longer reaction times, and reaction temperatures above ~ 25°C favor a "dark-reaction" between protein carboxyl groups and 2,4-DNPH which yields interferring chromophoric protein-hydrazides.[2] The reaction mixture is then dialyzed in cellophane against water at 25°C for a period of 15 to 18 hours to quantitatively remove excess reagent. Aliquots of the dialyzed solution are made 1.5N in potassium hydroxide to develop the anion chromophores of the protein hydrazones and the absorption spectra are immediately recorded over the 350 to 600 mμ range using a Cory spectrophotometer Model 14, with a 1 cm or longer path. Control samples of the unirradiated protein solutions are carried through the entire procedure in parallel with the irradiated samples; the calculations of carbonyl yields are based on the differential spectra.

Figure 1 compares relative spectra of the hydrazone derivatives of irradiated pepsin with hydrazones derived from a number of monocarbonyls of the type $RCOCONHCHR_2$, R_2CO, RCHO and ArCOR.[2,7-9] Very similar spectra are obtained with the hydrazone derived from irradiated α-chymotrypsin, β-lactoglobulin and soluble collagen (gelatin).

Figure 2 shows the observed optical densities of the hydrazone anions derived from irradiated pepsin as a function of dose under the experimental conditions described here. The control corrections for unirradiated pepsin, α-chymotrypsin and β-lactoglobulin are uniformly low. At dosages of 5 Krad and above the corrected optical densities of the irradiated samples are reproducible to ± 2 percent. At 1 Krad the optical density of the control amounts to ~ 20 percent of the irradiated value and the reproducibility at this dose level is within ± 6 percent.

The experimental procedure described here provides us, for the first time, with a technique for quantitatively determining major products formed in the radiolysis of proteins in oxygenated solution at biologically relevant dosages down to at least 1 krad. We are presently using this low-dose technique in studies of the radiolytic oxidation of aqueous histone and nucleohistone with the objective of adapting the procedure to permit a quantitative study of the radiolytic oxidation of chromosomal proteins in-vivo.

References

1. Allen, A. O., "The Radiation Chemistry of Water and Aqueous Solutions". D. Van Nostrand Co. Inc. New York, 1961.

2. Garrison, W. M., Jayko, M. E. and Bennett, W., Radiat. Res., 16 483 (1962).

3. Clement, J. R., Armstrong, D. W., Klassen, N. V. and Gillis, H. A., Can. J. Chem., 50, 2833, (1972).

4. Lichtin, N. H., Ogden, J., Stein, G., Biochim Biophys. Acta 276 124 (1972).

5. Joshi, A., Rustgi, S., Moss, H., and Riesz, P., Int. J. Radiat. Biol. 33, 205 (1978).

6. Garrison, W. M., "Reaction Mechanisms in the Radiolysis of Peptides, Polypeptides and Proteins". LBL-18613, 1985 Lawrence Berkeley Laboratory, Berkeley, CA.

7. Cennamo, C., Carafoli, B., and Bonetti, E., J. Am. Chem. Soc., 78, 3523 (1956).

8. Wells, C. F., Tetrahedron, 22 2685 (1966).

9. Jones, L. A., Holmes, J. C., and Seligman, R. B., Anal. Chem. 28 191 (1956).

FIGURE CAPTIONS

Fig. 1. Relative absorption spectra of 2,4-dinitrophenylhydrazone derivatives of (1) irradiated pepsin —— (2) RCHO(acetaldehyde) —·— (3) $RCONHCHR_2$(pyruvoylalanine) — — — (4) R_2CO(acetone) ———— (5) ArCOR(acetylphenone) ——··—— [in 1.5N KOH]

Fig. 2. Optical density of irradiated pepsin hydrazones —●—, and unirradiated control, —o— [in 1.5N KOH].

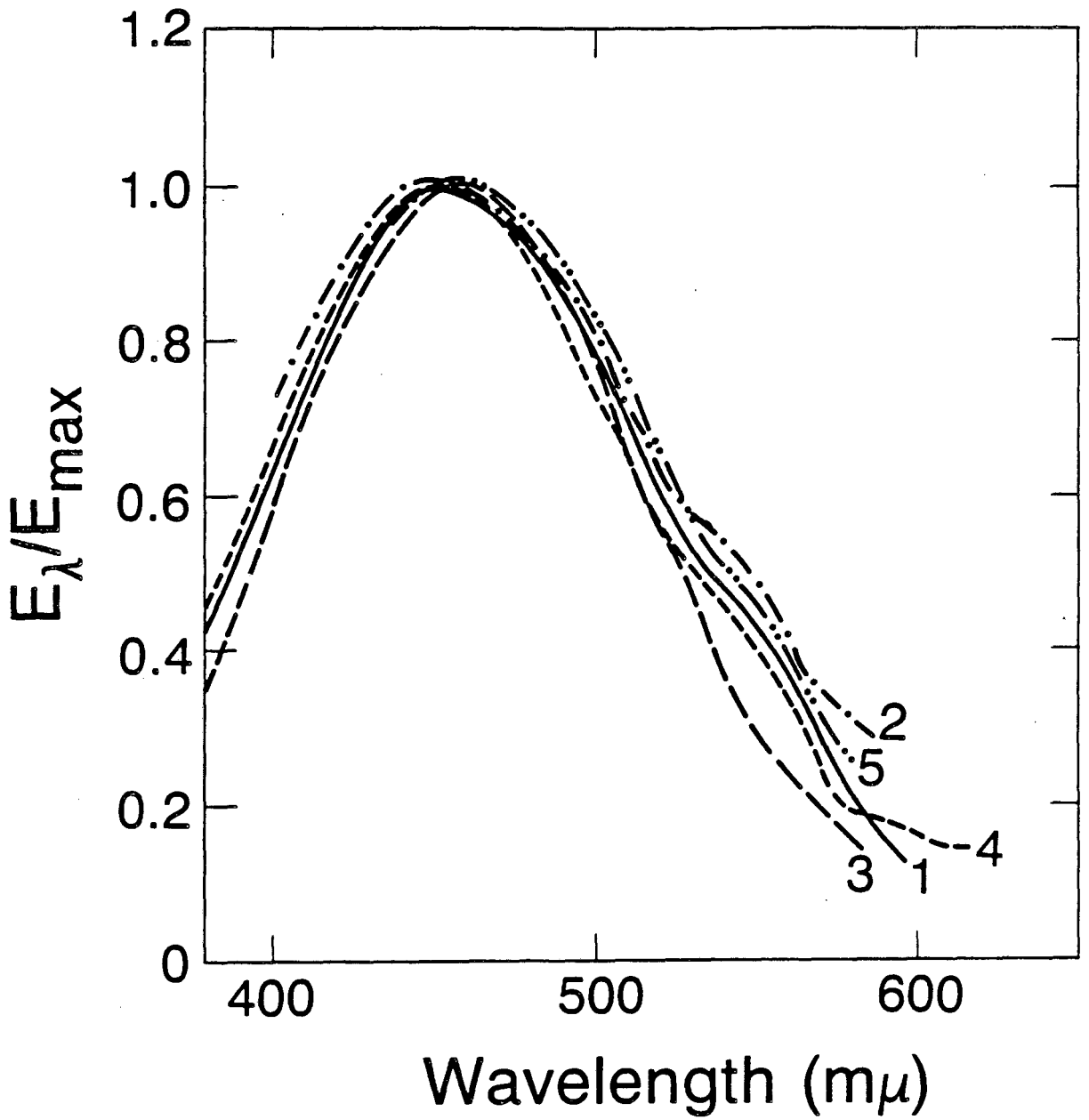
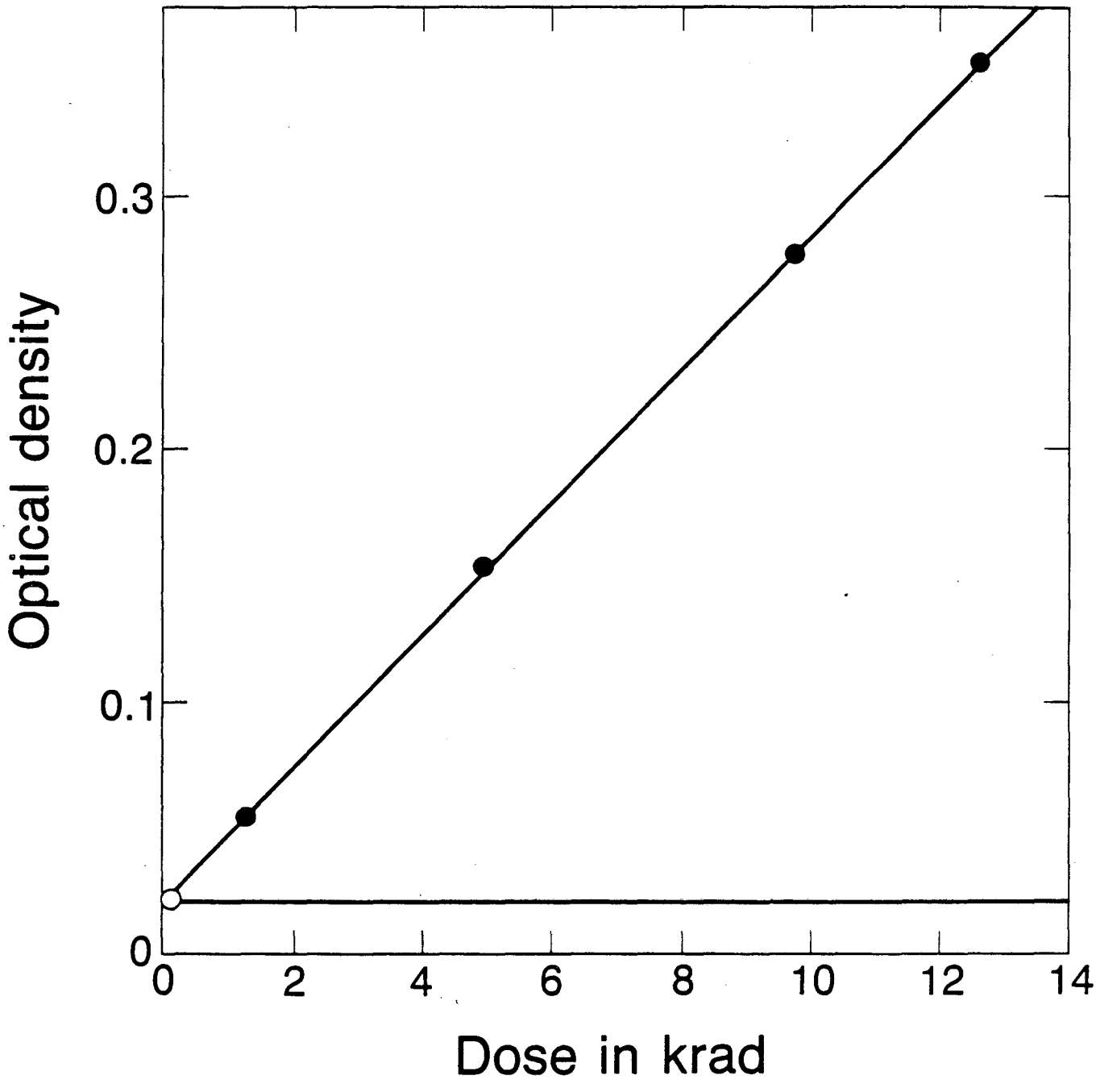

1.2
1.0
0.8
0.6
0.4
0.2
0
E_λ/E_{max}
400
500
600
Wavelength (mμ)
1
2
3
4
5
XBL 8511-11544

Fig. 1



XBL 8511-11536

Fig. 2

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.

LAWRENCE BERKELEY LABORATORY
TECHNICAL INFORMATION DEPARTMENT
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720