Radiation Physics and Chemistry 79 (2010) 960–965

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Efficiency of superoxide anions in the inactivation of selected dehydrogenases

Aleksandra Rodacka [a,*], Eligiusz Serafin [b,1], Mieczyslaw Puchala [a,1]

[a] Department of Molecular Biophysics, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland
[b] Laboratory of Computer and Analytical Techniques, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

ARTICLE INFO

Article history:
Received 5 February 2010
Accepted 1 April 2010

Keywords:
Radiation
Superoxide anion radical
Reactive oxygen species
Dehydrogenase
Xanthine oxidase
Enzyme inactivation

ABSTRACT

The most ubiquitous of the primary reactive oxygen species, formed in all aerobes, is the superoxide free radical. It is believed that the superoxide anion radical shows low reactivity and in oxidative stress it is regarded mainly as an initiator of more reactive species such as $^{\bullet}OH$ and $ONOO^{-}$.

In this paper, the effectiveness of inactivation of selected enzymes by radiation-generated superoxide radicals in comparison with the effectiveness of the other products of water radiolysis is examined. We investigate three enzymes: glyceraldehyde-3-phosphate dehydrogenase (GAPDH), alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH).

We show that the direct contribution of the superoxide anion radical to GAPDH and ADH inactivation is significant. The effectiveness of the superoxide anion in the inactivation of GAPDH and ADG was only 2.4 and 2.8 times smaller, respectively, in comparison with hydroxyl radical. LDH was practically not inactivated by the superoxide anion.

Despite the fact that the studied dehydrogenases belong to the same class of enzymes (oxidoreductases), all have a similar molecular weight and are tetramers, their susceptibility to free-radical damage varies. The differences in the radiosensitivity of the enzymes are not determined by the basic structural parameters analyzed. A significant role in inactivation susceptibility is played by the type of amino acid residues and their localization within enzyme molecules.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

The superoxide anion radical demonstrated considerably smaller reactivity with proteins in comparison to the $^{\bullet}OH$ radical, and therefore in some papers its reaction with proteins is neglected (Bielski and Cabelli, 1995; Bielski et al., 1985; Schüssler and Puchala, 2004; Gebicki, 2006). The lower reactivity of $O_2^{\bullet -}$ is connected with its longer half-life time in comparison to the hydroxyl radical. In effect, it can diffuse at great distances and react with amino acid residues, which are the most important for protein functioning. If these residues are situated inside or near the protein's active site, the superoxide radical can also cause significant inactivation of the enzyme.

Moore et al. (2000) in the work on the radiolysis of protein A estimated that superoxide is as efficient as the hydroxyl radical in this protein inactivation. Similar observations regarding alcohol dehydrogenase were made by Badiello et al. (1974).

The aim of this paper was to estimate the effectiveness of the superoxide radical in the inactivation of selected enzymes in comparison to the effectiveness of other products of water radiolysis. It was also attempted to find a connection between the structure of the examined enzymes and their sensitivity to free-radical inactivation.

2. Experimental

2.1. Materials

Alcohol dehydrogenase (ADH) (alcohol:NAD^+ oxidoreductase, EC 1.1.1.1), molecular weight of 147 kDa from baker's yeast, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (D-glyceraldehyde-3-phosphate: NAD^+ oxidoreductase, EC 1.2.1.12), molecular weight of 143 kDa from a rabbit muscle, lactate dehydrogenase (LDH) (L-lactate:NAD^+ oxidoreductase, EC 1.1.1.27), molecular weight of 140 kDa from a rabbit muscle, xanthine oxidase (XO) (xanthine:oxygen oxidoreductase, EC 1.17.3.2) from bovine milk, xanthine (X), superoxide dismutase (SOD) (superoxide:superoxide oxidoreductase, EC 1.15.1.1) from bovine erythrocytes, catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) from bovine liver, cytochrom c from bovine heart were purchased from the Sigma Chemical (St. Louis, MO, USA). All other chemicals were of analytical grade and were purchased from Sigma (St. Louis, MO, USA) or POCH (Gliwice, Poland). All solutions were made with water purified with the Milli-Q system.

* Corresponding author. Tel.: +48 426354480; fax: +48 426354473.
E-mail addresses: olakow@biol.uni.lodz.pl (A. Rodacka), serafin@biol.uni.lodz.pl (E. Serafin), puchala@biol.uni.lodz.pl (M. Puchala).
[1] Fax: +48 426354473.

0969-806X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.radphyschem.2010.04.001

2.2. Conditions of irradiation

The dehydrogenases were dissolved in 0.05 M potassium phosphate buffer, pH 7.4 and irradiated in the presence of or without mannitol (0.05 M), SOD (40 U/ml) and catalase (50 U/ml). Enzyme concentration was 0.05 mg/ml, which corresponds to molar concentrations: 3.5×10^{-7} M for GAPDH, 3.4×10^{-7} M for ADH and 3.6×10^{-7} M for LDH. Solutions were irradiated in air using Stabilipan an X-ray machine (Siemens, Germany) 180 kV, 18 mA, with an aluminium filter (2 mm). The dose rate estimated with a modified Fricke dosimeter was 4.1 Gy/min.

2.3. The determinants of protein concentration

Concentration of the dehydrogenases was determined on the basis of the absorbance measurements at 280 nm using the absorption coefficient of $E^{1\%}_{280}=14.6$ for ADH (Buhner and Sund, 1969), $E^{1\%}_{280}=10.2$ for GAPDH (Murdock and Koeppe, 1964) and $E^{1\%}_{280}=14.9$ for LDH (Bartholmes et al., 1973).

2.4. Calculations

The radiation yield of G_{inact} for enzymes irradiated was calculated on the basis of the value of the dose D_{37} according to the formula

$$G_{inact}=\frac{[E]}{\rho D_{37}} \quad (1)$$

where $[E]$ is the enzyme concentration in mol dm^{-3}, D_{37} the irradiation dose (in Gy) at which the enzyme activity decreased to 37% of the initial activity and ρ the density of solution ≈ 1 kg dm^{-3}.

If it is assumed that each of the radical species acts independently of the other, and reactions between the products of water radiolysis are neglected, then radiation yield of inactivation can be described as

$$G_{inact}=\sum_i f_i G_i \quad (2)$$

where f_i is the efficiency of inactivation by an individual radical and G_i the yield of this radical under conditions of the irradiation.

2.5. Generation and measurement of the superoxide anion radical in chemical reaction

The superoxide anion radical was generated in the xanthine/xanthine oxidase system (X/XO). The reaction was initiated by adding XO (0.02 U/ml) to a solution of xanthine (0.5 mM) containing DTPA (0.01 mM) in potassium phosphate buffer (0.05 M, pH 7.4).

The superoxide anion radical generated in the XO/X reaction was measured spectrophotometrically on the basis of reduction of cytochrome c (0.1 mM) at 550 nm using an absorption coefficient of 21 mM^{-1} cm^{-1} (reaction (1)) (Hodges et al., 2000).

$$\text{Cyt c}(Fe^{3+})+O_2^{\bullet-}\rightarrow\text{Cyt c}(Fe^{2+})+O_2 \quad (1)$$

All the assays were performed in the presence of a catalase (330 U/ml).

2.6. Conditions of incubation of the enzymes with superoxide radical anion chemically generated

The studied dehydrogenases (the same concentration as in the irradiation, Section 2.2) were incubated at room temperature in the presence of xanthine oxidase (0.02 U/ml), xanthine (0.5 mM) and catalase (330 U/ml). After a given time, the dehydrogenase activities were determined according to the procedures described below. The enzyme activities were expressed as a percent of the activity of enzymes incubated with xanthine and catalase without xanthine oxidase.

2.7. Determination of enzyme activities

ADH activity was measured by the method of Bonnichsen and Brink (1955), GAPDH activity was measured by the method of Amelunxen and Carr (1967) and LDH activity was measured according to Wroblewski and La Due (1955). The activities of enzymes were determined on the basis of the rate of reduction of NAD^+ to NADH (for ADH and GAPDH) or the rate of oxidation of NADH to NAD^+ (for LDH). The formation of NADH and NAD^+ was estimated by the measurement of absorbance at $\lambda=340$ nm.

All spectrophotometric measurements were carried out at room temperature in a CARY-1 apparatus (Varian, Melbourne, Australia).

2.8. Comparative analysis of selected structural parameters of examined dehydrogenase

The comparative analysis of the structure parameters of examined protein tetramers was performed on the basis of their 3-D structures. In the case of GAPDH from rabbit muscle the 1JOX structure from the Protein Data Bank was used. This structure was obtained by the method of X-ray diffraction (Cowan-Jacobs et al., 2003). In the case of two other dehydrogenases, the experimentally determined tetramer structures are not accessible yet and that is why the models of these tetramers were used. In the case of baker's yeasts' ADH, the tetramer models from the *Protein Quaternary Structure at EBI* were used (*2hcy*_1 and *2hcy*_2). The LDH tetramer model was obtained by the method of homological modeling by means of an *Automated Comparative Protein Modeling Server* (Arnold et al., 2006, Kopp and Schwede, 2004, Schwede et al., 2003, Guex and Peitsch, 1997, Peitsch, 1995). The *Sus strofa* LDH (PDB code 9 LDT) (Penel et al., 2009) was chosen as the pattern model because it was the most phylogenetic ally similar tetramer with a known model of a quaternary structure.

The accessibility of the amino acid residues in the studied enzymes was determined with a Swiss-PDB Viewer (DeepView) program (Guex and Peitsch, 1997).

3. Results

3.1. Efficiency of radiation-generated radicals in inactivation of dehydrogenases

In this paper, the radiation inactivation of three enzymes: GAPDH, ADH and LDH was determined. The solutions of enzymes were irradiated under air with doses of up to 120 Gy in four systems:

1 in the absence of other compounds;
2 in the presence of mannitol;
3 in the presence of mannitol and SOD and
4 in the presence of mannitol, SOD and catalase.

The dependence of log (% activity) on the radiation dose for the studied systems is presented in Figs. 1–3. In all the cases, these dependences are linear, which indicates pseudo-first-order kinetics. From the obtained dependences in Figs. 1–3, D_{37} values were determined, which served as a basis for the calculation of

Download English Version:

https://daneshyari.com/en/article/1886573

Download Persian Version:

https://daneshyari.com/article/1886573

Daneshyari.com