Free Radical Conservation and Quantitative Research on Radiation Effects

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Abstract

The purpose of this article is to quantitatively study the influence of direct and indirect action on the radiation effect and its production mechanism. As the indirect action of radiation effects is triggered by the radiolysis of free water molecules, the free radical conservation equation for the same radiation effect is deduced by the definition and theoretical analysis of the radiation effects of microbial cells and biologically active macromolecules with different water contents. Through the free radicals conservation equation, these values were quantitatively calculated with different water contents, which were the D37 value of microbial cells or biological macromolecules, the proportion of direct action and indirect action, the influence on the target volume and number of targets, and the influence on radiation effects. These quantitative calculation results from the free radical conservation equation show that as the water content increases from 0, the radiation sensitivity of RNase and DNA molecules continues to increase, the direct actions gradually decrease, and the indirect actions continue to increase. The quantitative calculation value of D37 decreases with the increase of water content, and the quantitative calculation value of RNase target volume increases with the increase of water content. These quantitative calculation results are completely consistent with the theories and research results of radiation chemistry and radiation biology, fully revealing that the free radical conservation equation is in accordance with objective reality and is scientifically effective. It has laid a scientific theoretical foundation for the quantitative research and application of radiation effects in the future.

Introduction

The research of radiation chemistry and radiation biology believes that biological cell is a water-containing system, and the effects of radiation on biological cells include direct action and indirect action\(^1\). The D\(_{10}\) and D\(_{37}\) value refer to the radiation dose respectively required to kill 90%, 63% of microorganisms and other biological cells or inactivate 90%, 63% of biological active substances by ionizing radiation in the field of radiation biology. They are the characteristic index of traditional radiation biology to measure the radiation sensitivity of different organisms, and mathematical expression of the relationship between the amount of deposition of radiation energy the organism exposed and the damage or even inactivation or death of organisms\(^2,3\).

Over the past decades, a large number of experimental results have shown that the same kind of microorganisms have different D\(_{10}\) values in different experiments\(^4,5\). The same microorganisms have different D\(_{10}\) values in different media, sometimes differ by several times or even more than ten times or dozens of times\(^6,7\). It shows that the same biological cell or biologically active macromolecule has different radiation effect in different experiments\(^8\). The values of D\(_{10}\) and D\(_{37}\) can directly reflect the degree of participation of direct action and indirect action in radiation effects. This phenomenon has always attracted the attention of radiation chemistry and radiation biologists\(^9,10\). At the molecular level, it has been studied how free radicals derived from water decomposed by radiation ionizing indirectly cause damage to biological macromolecules in cells and what kind of damage. And it was concluded that the radiation sensitivity of microorganisms and biological cells is proportional to their water content\(^11-14\). However, the quantitative relationship between direct action, indirect action and its influence on the production mechanism of radiation effects are rarely reported.

Results

According to the theory and definition of radiation biology, the following formula can be deduced.

The mass-energy absorption coefficient of biomaterials is calculated as follows\(^15\).
\[ \mu_{\text{en}}/\rho = 2.6691 + 2.6419y \quad (1) \]

In formula 1,

\[ \mu_{\text{en}}/\rho, \text{- mass-energy absorption coefficient of a biomaterial, unit: } 10^{-3}\text{m}^2/\text{kg.} \]

\[ y, \text{- hydrogen content of a biological material.} \]

**Conversion of absorbed dose**\(^{16}\),

\[ D_m = (\mu_{\text{en}}/\rho)_m / (\mu_{\text{en}}/\rho)_d \times D_d \quad (2) \]

In formula 2,

\[ D_m, \text{- absorbed dose of a substance to be being measured, unit: Gy.} \]

\[ (\mu_{\text{en}}/\rho)_m, \text{- mass-energy absorption coefficient of a substance tested, unit: m}^2/\text{kg.} \]

\[ D_d, \text{- absorbed dose of a substance to be been tested, unit: Gy.} \]

\[ (\mu_{\text{en}}/\rho)_d, \text {-mass-energy absorption coefficient of a substance to be been tested, unit: m}^2/\text{kg.} \]

**Calculation formula of free radicals yield**\( (M_m) \) **of biomacromolecule in cells**, 

According to the definition of free radicals,

\[ M_m = W \times D_m \times G_m \quad (3) \]

In formula 3,

\[ W, \text{- Mass of dry matter of microbial cells, unit: kg, } 0 < W \leq 1.0. \]

\[ D_m, \text{- Absorption rate of rays by Biomacromolecule in microbial cells.} \]

\[ G_m, \text{- Free radical yield of Biomacromolecule in microbial cells, unit: mol/J.} \]

**Calculation formula of free radical yield**\( (M_{\text{H}_2\text{O}}) \) **of water in microbial cells**, 

\[ M_{\text{H}_2\text{O}} = (1-W) \times D_{\text{H}_2\text{O}} \times G_{\text{H}_2\text{O}} \quad (4) \]

In formula 4,

\[ 1-W, \text{- Mass of water in microbial cells, unit: kg, } 0 < 1-W \leq 1.0. \]

\[ D_{\text{H}_2\text{O}}, \text{- Absorption rate of ray by H}_2\text{O in microbial cells.} \]

\[ G_{\text{H}_2\text{O}}, \text{- Free radical yield of H}_2\text{O in microbial cells, unit: mol/J.} \]

**Calculation of total free radicals**\( (M_t) \),
\[ M_t = D_{\text{KAgCr2O7}} \times W \times D_m \times G_m + (1-W) \times D_{\text{H2O}} \times G_{\text{H2O}} \] (5)

In formula 5,

\( D_{\text{KAgCr2O7}} \) - Absorbed dose corresponding to a certain biological effect, unit: Gy.

**Free radicals conservation equation**, according to the definition of total free radicals and the principle of free radicals conservation.

\[ D_i \times W_i \times D_m \times G_m + (1-W_i) \times D_{\text{H2O}} \times G_{\text{H2O}} \]

\[ = D_n \times W_n \times D_m \times G_m + (1-W_n) \times D_{\text{H2O}} \times G_{\text{H2O}} \] (6)

In formula 6,

\( D_i, D_n \) - Absorbed dose of microorganisms or bioactive macromolecules with different water content when the same biological effect caused, unit: Gy.

**Theory application and verification**. The free radical conservation equation was applied to quantitative calculation, and the calculation results were analyzed and verified.

**Calculation of dose absorption rate of RNase**. The H content of RNase calculated from the molecular formula of RNase (C_{9}H_{14}N_{4}O_{3}) is 0.06237. The \( D_{37} \) value of RNase was obtained from literature\(^7\), which are \( D_{37}(\text{RNase}) = 420 \) kGy, \( D_{37}(0.5\%\text{RNase}) = 4.0 \) kGy.

The H value and \( D_{37} \) value are substituted into formula 1 and formula 2 and calculated, then obtained \( D_{\text{RNase}} = 0.9574 \times D_{\text{KAgCr2O7}} \).

**Calculation of free radical yield of RNase**. \( D_{\text{H2O}} = 1.0017 \times D_{\text{KAgCr2O7}}, D_{\text{RNase}} = 0.9574 \times D_{\text{KAgCr2O7}}, G_{\text{H2O}} = 600.088 \times 10^{-9} \) mol/J, \( D_{37}(\text{RNase}) = 420 \) kGy, \( D_{37}(0.5\%\text{RNase}) = 4 \) kGy.

The algebraic value above are substituted into formula 6 to calculate, and obtained \( G_{\text{RNase}} = 5.958 \times 10^{-9} \) mol/J.

**Calculation of free radical of RNase and value of \( D_{37} \) at low water content**. Let the water content of RNase be: 0.00, 10%, 20%, 30%, 40%, 50% and 60%. The values of including water content and related parameters of \( D_{\text{H2O}}, D_{\text{RNase}}, G_{\text{H2O}}, G_{\text{RNase}} \) were substituted into formula 3, formula 4, and formula 6. The percentage of direct and indirect action (That is: free radicals percentage) and the corresponding \( D_{37} \) value at different water content was calculated. The results are shown in Table 1.

Table 1 ratios of indirect and Direct action and corresponding \( D_{37} \) values at different water contents
In Table 1, the quantitative calculation results by the free radical conservation equation show that when RNase is in a dry state, the biological effects of radiation are completely produced by the direct action of rays. The $D_{37}$ value is as high as 420 kGy without indirect action at this time. When the water content rises to 10%, more than 90% of the radiation effect is produced by the indirect action derived from water radiolysis, and the direct action ratio is less than 10%, and the $D_{37}$ value drops from 420 kGy to 36.73 kGy. When the water content of RNase reaches 50%, the ratio of direct action is less than 1%. These calculation results are consistent with radiation biology theory and research results.

**Calculation of free radical of RNase and $D_{37}$ value at high water content.** Let the water content of RNase be: 0.99500, 0.99750, 0.99900, 0.99950, 0.99975, 0.99990. The values of water content and related parameters are substituted into formula 3, formula 4, and formula 6 and calculated. The results are shown in Table 2.

| Water content (%) | 0.99500 | 0.99750 | 0.99900 | 0.99950 | 0.99975 | 0.99990 |
|-------------------|---------|---------|---------|---------|---------|---------|
| $G_{RNase} (x10^{-9} \text{ mol/J})$ | 0.02852 | 0.01426 | 0.00570 | 0.00285 | 0.00143 | 0.00057 |
| $G_{RNase}/(G_{H20}G_{RNase}) \times 10^{-7}$ | 477     | 238     | 95.0     | 47.5     | 23.7     | 9.49    |
| $D_{37} (\text{ Gy})$ | 4007.0  | 3997.0  | 3991.0  | 3989.0  | 3988.0  | 3987.0  |

In Table 2, the quantitative calculation results of the free radical conservation equation show that when the water content of RNase reaches 99.50%, the biological effects of radiation are mainly caused by indirect action, and the ratio of direct action is less than 5/100,000. When it reaches 99.90%, the direct action ratio is less than one hundred thousandths. Its $D_{37}$ value also drops below 4.00 kGy, and gradually changes linearly. These quantitative calculation results are consistent with actual results. See Fig. 1 for details.

This result is completely consistent with the research result of Yijing Chen$^{17}$. It also shows that the free radical conservation equation is in line with objective reality.

**Influence of water content on target volume.** According to target theory, by analysis of the RNase activity survival curve, RNase is a biological macromolecule that can be inactivated with a single target click. The target volume can be calculated according to the formula $v = 1/D_{37}^3$. See Table 3.

| Water content (%) | 0.000 | 0.100 | 0.200 | 0.300 | 0.400 | 0.500 | 0.600 |
|-------------------|-------|-------|-------|-------|-------|-------|-------|
| $D_{37} (\text{ kGy})$ | 420.0 | 36.73 | 18.96 | 13.00 | 9.83  | 7.900 | 6.600 |
| Target volume ($\times 10^{-20} \text{ cm}^3$) | 3.897 | 44.56 | 86.32 | 125.90 | 166.50 | 207.07 | 247.98 |
In Table 3, the calculation results of the free radical conservation equation show that the target volume of RNase is positively correlated with the water content of RNase. When the water content of RNase increases by 10%, the target volume increases by more than 11 times. When the water content increases to 50%, the target volume increased by 53 times. Although the target theory calculates all doses on the target volume, which reduces the calculated target volume by hundreds of times, the increase in water content does not affect the multiple of the target volume expansion. As the target volume increases, the energy of radiation absorbed increases, and the free radicals generated increase, thereby the dose of radiation has been greatly reduced. This result explains the reason why the value of $D_{37}$ drops by 90% when the water content increases from zero to 10% by the view of target theory.

Relationship between water content and number of target. When drawing the dose survival curve of a biological cell at any water content in a radiation experiment, the free radical conservation equation can be used to calculate the $D_{37}$ value of the biological cell at any water content, and multiple cell survival curves with different slopes can be drawn. It can be seen that for the same biological cell, when its water content is different, the N value (number of targets or number of hits) derived from the survival curve is also different. According to the Target Theory, as the water content increases, the number of times of hit also increases. It can also be said that as the water content increases, the number of targets hit also increases. This proves the reliability of the free radical conservation equation based on the target theory.

Quantitative calculation of radiation effects of DNA molecules. The radiation death of biological cells is mainly caused by the double-strand break or aberration of DNA molecules. A large number of cell dose survival curves show that the survival rate of biological cells is linearly related to the absorbed dose.

In an aerobic environment, calf thymus DNA powder was irradiated with $\gamma$-rays, and the double-strand breaks rate was 0.16 molecules/100 eV, and the single-strand breaks rate was 3.4 molecules/100eV. The free radical yield of DNA molecules calculated, $G_{DNA}=3.72$ molecules/100 eV, which is converted into $G_{DNA}=3.854 \times 10^{-7}$ mol/J. According to the molecular formula of calf thymus DNA, the hydrogen content $Y_{H(calf thymus DNA)} = 0.0252$. The Y value Substituted into formula 1 and calculated,

$$\frac{\mu_{en}}{\rho}_{DNA} = 2.7357 \times 10^{-3} \text{m}^2/\text{kg}$$

the parameters of $(\mu_{en}/\rho)_{DNA}$ and $(\mu_{en}/\rho)_{KAgCr2O_7}$ Substituted into formula 2 and calculated,

$$D_{DNA}/D_{KAgCr2O_7} = 0.9242$$

$$D_{H2O}/D_{KAgCr2O_7} = 1.0017, G_{H2O}=6.0088 \times 10^{-7} \text{mol/J}$$.

If the ratio of double-strand breaks caused by the radiation dose of 3 Gy absorbed by dry DNA powder is used as the basis for the calculation of radiation effects, then $D_{DNA(calf thymus)} = 3$ Gy. Set the water content of DNA to 0, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and substituted parameters such as $G_{DNA}$, $D_{DNA}/D_{KAgCr2O_7}$, $G_{H2O}$, $D_{H2O}/D_{KAgCr2O_7}$ into formula 3, formula 4, formula 5, formula 6, and calculated. The results are shown in Table 4.
In Table 4, the calculation results of the free radical conservation equation show that in the radiation process of DNA molecular, with the increase of water content, the direct action decreases and the indirect action increases. The magnitude of this change decreases with the increase of water content. The absorbed dose required to achieve the same radiation effect also changes according to this law.

Combined with the influence of water content on the relevant parameters of RNase, it can be seen that the stronger the anti-radiation ability of biological cells or biologically active macromolecules, the more significant the indirect action derived from water and the greater the range of absorbed dose changes. These quantitative calculation results are completely consistent with the radiation chemistry theory and research results.

Characterization parameters of radiation sensitivity and quantification. Due to the difference of water content, the absorbed dose when reaching a certain radiation effect is changing. A kind of biological cell can have multiple water content and different $D_{37}$ values, so $D_{37}$ cannot correctly express radiation sensitivity of biological cells or active macromolecules. However, higher biological cells are difficult to survive when the water content is less than 65%. The absorbed dose with a dried state is only a virtual value, and it is not suitable to represent the radiation sensitivity of biological cells.

In the free radical conservation equation, there is an important parameter that can reflect biological radiation sensitivity. That is, the DG value (the product of dose absorption rate and free radical yield).

According to the definition,

$$DG = W_1D_1G_1 + W_2D_2G_2 + W_3D_3G_3 + \ldots + W_iD_iG_i + \ldots + W_nD_nG_n,$$

in which of this formula, $D_i$ is the relative dose absorption coefficient of component i in biological cells, and $G_i$ is the free radical yield of component i in biological cells.

When the external radiation environment is constant, DG is a constant value for a specific biological cell or biological macromolecule and does not change with changes of water content. A biological cell or a biological macromolecule has only one DG value. The stronger the radiation resistance, the smaller the DG value. The larger the DG value, the stronger the radiation sensitivity. Although the DG value of biological cells is extremely difficult to measure and calculate, it is very convenient to use the free radical conservation equation to calculate. Therefore, the DG value is very suitable for characterizing the radiation sensitivity of biological cells or biologically active macromolecules.

| Water content (%) | 0.00 | 0.10 | 0.20 | 0.30 | 0.40 | 0.50 | 0.60 | 0.70 | 0.80 | 0.90 |
|-------------------|------|------|------|------|------|------|------|------|------|------|
| Direct action (%) | 1.000 | 0.8419 | 0.7030 | 0.5800 | 0.4703 | 0.3718 | 0.2829 | 0.2023 | 0.1289 | 0.0617 |
| Indirect action (%) | 0.0000 | 0.1581 | 0.2970 | 0.4200 | 0.5297 | 0.6282 | 0.7171 | 0.7977 | 0.8711 | 0.9383 |
| Dose absorbed (Gy) | 3.00 | 2.81 | 2.64 | 2.49 | 2.35 | 2.23 | 2.12 | 2.02 | 1.93 | 1.85 |


**Discussion**

Through the free radical conservation equation, the D value, free radical yield, direct action and indirect action, even the number of chemical bond breaks and other important parameters in radiation effects can be accurately and quantitatively calculated. It has laid a scientific theoretical foundation for the quantitative research of radiation effects in the future. It has important scientific guiding significance for the practical application of radiobiology. In actual production applications, the free radical conservation equation can accurately guide the inactivation process of living bacteria or active biological macromolecules of fermentation products under different conditions, such as yeast extract, yeast powder, lysine, and threonine. In the field of radiotherapy, the tissues or cells in different parts that you want to be removed can be accurately killed to achieve the purpose of precision medicine.

**Methods**

**Energy transfer and main radiation chemical reactions of biological macromolecules.** Under the direct action of rays, biological macromolecules are excited or ionized to form excited molecules, excited ions or slow electrons. And then the radiation energy is transferred to neighboring biological macromolecules through collisions to form new excited molecules or excited State ions\(^{22,23}\). At the same time, the excitation energy of part of the molecules moves inside the molecule, eventually makes the chemical bond with the lowest bond energy breaking and decomposing into free radicals. Similarly, biological macromolecules that are indirectly excited or ionized will also Decompose to generate free radicals\(^{24}\).

\[
\begin{align*}
AB^* & \rightarrow A^* + B^- \\
RH^* & \rightarrow R^* + H^- \\
AB + e^- & \rightarrow A^* + B^- 
\end{align*}
\]

When water-containing RNase is irradiated, with the exception of the direct actions, the main indirect actions are caused by water radiolysis. Water molecules are excited or ionized to form a variety of reactive intermediates. Small molecule free radicals will further promote the transfer of radiant energy between biological macromolecules, and accelerate the transfer and diffusion of radiant energy in aqueous biological systems\(^{25}\). This effect increases with the increase of water content. The main radiation chemical reactions include decomposition reactions, coupling reactions, and disproportionation reactions. The most important ones are the radiation oxidation reaction of sulfhydryl amino acids and the hydrolysis reaction of peptide chains\(^{20,21,26}\).

\[
\begin{align*}
2\text{HOOCCH(NH}_2\text{)CH}_2\text{SH} + 2\text{HO}^* & \rightarrow \text{HOOCCH(NH}_2\text{)CH}_2\text{SSCH}_2\text{CH(NH}_2\text{)COOH} + 2\text{H}_2\text{O} & \text{(Cysteine)} \\
\text{RCONHCHR}_2 + \text{HO}^* & \rightarrow \text{RCONHCR}_2 + \text{H}_2\text{O} & \rightarrow \text{RCONH}_2 + \text{RCOR}
\end{align*}
\]

This type of reaction plays a decisive role in the inactivation of RNase radiation.

**Absorbed dose and free radical yield.** A certain amount of radiant energy produces a certain amount of free radicals. And a certain amount of free radicals induces a certain amount of chemical and biological effects. The processes of radiation polymerization, radiation grafting, radiation degradation and radiation crosslinking in polymer radiation chemistry have fully proved this principle, and radiation therapy also reflects the important role of this principle\(^3,21\).

When the external conditions of the radiation process are constant, and reaching the same definite biological effect for the same biological cell or biologically active macromolecule, of which the D\(_{37}\) value will decrease with the
increase of the water content, but the total amount of free radicals is constant\textsuperscript{2,27,28}.

**Related concepts and basic parameters. Absorption coefficient**, refers to the share of energy absorbed by certain substance when γ-ray or χ-ray passes through the substance with unit thickness. Unit: m\(^2\)/kg.

**Calculation of mass-energy absorption coefficient of H\(_2\)O**, calculated from the molecular formula of H\(_2\)O, \(y_{H2O}=0.111898\).

\(y_{H2O}\) Substituted into formula 1, \((\mu_{en}/\rho)_{H2O}=2.965\times10^{-3}\) m\(^2\)/kg.

**Mass-energy absorption coefficient of potassium dichromate silver(KAgCr\(_2\)O\(_7\)) dose agent**, according to literature\textsuperscript{16},

\((\mu_{en}/\rho)_{KAgCr2O7}=2.960\times10^{-3}\) m\(^2\)/kg.

**Dose absorption rate of H\(_2\)O**, the following two algebraic modules Substituted into formula 2, \((\mu_{en}/\rho)_{KAgCr2O7}=2.960\times10^{-3}\) m\(^2\)/kg, \((\mu_{en}/\rho)_{H2O}=2.965\times10^{-3}\) m\(^2\)/kg, then calculated, \(D_{H2O}= 1.0017D_{KAgCr2O7}\).

**Free radical yield of H\(_2\)O**, the amount of free radicals produced from water irradiated by a certain dose of ray, unit: mol/J.

According to the literature\textsuperscript{21}, \(G_{H2O}=5.8\) molecules/100ev, which is converted to, \(G_{H2O} = 6.0088 \times 10^{-7}\) mol/J.

**Data availability**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Declarations**

**Competing interests**

The authors declare no competing interests.

**Author contributions**

H.Z.X. constructed the concept and methodology, and wrote the original manuscript. S.H.W. inquired and collected datas. J.H.Q. tested and verified results. L.T. provided fund support and managed project progress. Y.X.C. reviewed the manuscript. M.L.C. wrote, reviewed and edited manuscript.
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References

1. Rodemann, H. P. & Blaese, M. A. Responses of Normal Cells to Ionizing Radiation. Seminars in Radiation Oncology. 17, 81-88 (2007).

2. Hall, E. J. & Giaccia, A. J. Radiobiology for the Radiologist. 8th ed. Philadelphia: Lippincott Williams &Wilkins. 67-110 (2019).

3. Gong, S. L. Radiation cytobiology. 1st ed. Beijing: China Atomic Energy Press. 12-40 (2014).

4. Geng, Y. S., Yuan, Q. K., Li T., et al. A study on the resistance and stability of *bacillus pumilus* E601 against radiation. Chin J Epidemiol. 20, 334-337 (1999).

5. Kong, Q. L., Yue, L., Qi, W. Y., et al. The effect of E-beam irradiation on the sterilization for calcium lactate. Journal of radiation research and radiation process. 29, 33-37 (2011).

6. Borsaa, J., Lacroixb, M., Ouattarab, B., et al. Radiosensitization: enhancing the radiation inactivation of foodborne bacteria. Radiation Physics and Chemistry. 71,135–139 (2004).

7. Huang, G. L., Feng, Y. D. and Wu, M. L. Fundamentals of radiation chemistry of polymers. Chengdu: Sichuan University Press. 328-356 (1993).

8. Ballarini, F. From DNA radiation damage to cell death: theoretical approaches. Journal of Nucleic Acids. 1-8 (2010).

9. Dizdaroglu, M. & Jaruga, P. Mechanisms of free radical-induced damage to DNA. Free Radical Research. 46, 382–419 (2012).

10. Krisch, R. E., Flick, M. B., and Trumbore, C. N. Radiation chemical mechanisms of single- and double-strand break formation in irradiated SV40 DNA. Radiation Research. 126, 251-259 (1991).

11. Shao, C. L., Saito, M., and Yu, Z. L. Mechanisms of Formation of DNA Strand Breaks Induced by γ-Rays. Acta Biochimica et Biophysica Sinica. 31, 159-162 (1999).

12. Osman, R., Pardo, L., Banfelder, J., et al. Molecular Mechanisms of Radiation Induced DNA Damage: H-Addition to Bases, Direct Ionization and Double Strand Break. Free Radical Research Communications. 13, 465-467 (1991).

13. Kelsey, C. A., Heintz, P. H., Sandoval, D. J., et al. Radiation Biology of Medical Imaging, 1st ed. New Jersey: John Wiley & Sons Inc, 125-140 (2014).

14. Luo, X. L., Zheng, Y., Sanche, L. DNA strand breaks and crosslinks induced by transient anions in the range 2-20 eV. The Journal of Chemical Physics. 140, 155101-155111 (2014).

15. Zheng, Z., Chen, J. S., Zhu, J.T., et al. Mass-energy absorption coefficient of biomaterials and its calculation formula. Nuclear Agriculture Bulletin. 18, 63-71 (1997).
16. GB/T 15447-2008. Conversion method of absorbed doses in different materials irradiated by x, γ rays and electron beams. National standard of the people's Republic of China. (2008).

17. Chen, Y. J. & Ha, P. F. Study on radiation-induced deactivation of catalase in dilute aqueous solution and protective effect. Journal of radiation research and radiation process. 11, 18-22 (1993).

18. Huang, W. D. & Yu, Z. L. A dose-survival model for low energy ion irradiation. Int J Radiat Biol. 83, 133-139 (2007).

19. Osborne, J. C., Miller, J. H., and Kempner, E. S. Molecular mass and volume in radiation target theory. Biophys. J. 78, 1698-1702 (2000).

20. Hitschke, K., Bühler, R., Apell, H. J., et al. Inactivation of the Na, K-ATPase by radiation-induced free radicals evidence for a radical-chain mechanism. FEBS Letters. 353, 297-300 (1994).

21. Qiu, G. Y. & Feng, S. Y. Radiation biophysics. Wuhan, Wuhan University Press. 54-113 (1990).

22. Ruchardt, C. Basic Principles of Reactivity in Free Radical Chemistry. Free Radical Research Communications. 2, 197-216 (1987).

23. Liu, S. Z., Ju, J. Z., Li, X. Y., et al. Medical radiobiology. 3rd ed. Beijing: China Atomic Energy Press. 2010; 6-31 (2010).

24. Hoe, S. T., Bisby, R. H., Cundall, B., et al. Free Radical Reactions with Proteins and Enzymes: the Inactivation of Bovine Carbonic Anhydrase B. Biochimica et Biophysica Acta. 662, 55-64 (1981).

25. Rao, V. S., Goldstein, S., Czapski, G. The Relative Efficiency of Radicals in Radiation Damage to Deoxyribose. Free Radical Research Communications. 12, 67-73 (1991).

26. Yamazaki, I. Free radical Mechanisms in Enzyme Reactions. Free Radical Biology & Medicine. 3, 397-404 (1987).

27. Alpen, E. Survival curve and its significance. Radiation Biophysics, Newyork: Academic Press. 169-193 (1998).

28. Seymour, C. B. & Mothersill, C. Relative contribution of bystander and targeted cell killing to the low-dose region of the radiation dose–response curve. Radiation Research. 153, 508-511 (2000).

**Figures**
Figure 1

Curve of D37 in dilute solution of RNase