Binding to proteins and their fragmentation by resveratrol in the presence of copper ions[i]

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Resveratrol is a natural polyphenol with promising anticancer properties. We have earlier reported its DNA-damaging ability in the presence of Cu(II) with the underlying prooxidant mechanism involving the generation of free radicals. Here, we report protein fragmentation by resveratrol in the presence of Cu(II), which further characterizes its prooxidant activity in the presence of copper ions, leading to macromolecular damage. Studies involving bathocuproine and scavengers of oxygen free radicals suggest that the fragmentation of BSA by resveratrol-Cu(II) involves a pathway similar to that responsible for the DNA cleavage activity, and is predominantly mediated by hydrogen peroxide and superoxide free radicals. We also studied the interaction of resveratrol with proteins. Fluorescence absorption studies show binding of resveratrol to BSA and to other proteins. A comparison of the pattern of binding of resveratrol to proteins with different L-tryptophan content indicates that resveratrol, in addition to tryptophan, may bind to other amino acids and also, non-specifically to the proteins. Given the recent interest in protein conformational changes and protein damage induced by anticancer agents with resulting altered sensitivity to therapies, our work proposes a closer examination of such activity of putative anticancer drugs.

Keywords: *Resveratrol, polyphenols, copper, pro-oxidant, oxidative protein damage*

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AUTHOR CONTRIBUTIONS

AA and MF performed experiments; AA, TA, HZ, SA and AF analyzed results; SMH supervised the study; AA and SMH prepared the first draft; All authors edited and proofread the manuscript.

ETHICAL CONSIDERATIONS

Not applicable.

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CONFLICT OF INTEREST

None.

REFERENCES

1. Ahmad A., Farhan Asad S., Singh S., Hadi S.M. (2000) DNA breakage by resveratrol and Cu(II): reaction mechanism and bacteriophage inactivation. *Cancer Lett.*, 154: 29-37.
2. Ahmad A., Syed F.A., Singh S., Hadi S.M. (2005) Prooxidant activity of resveratrol in the presence of copper ions: mutagenicity in plasmid DNA. *Toxicol Lett*, 159: 1-12.
3. Ahmed M.S., Ainley K., Parish J.H., Hadi S.M. (1994) Free radical-induced fragmentation of proteins by quercetin. *Carcinogenesis*, 15: 1627-30.
4. Davies K.J., Lin S.W., Pacifici R.E. (1987) Protein damage and degradation by oxygen radicals. IV. Degradation of denatured protein. *J. Biol. Chem.*, 262: 9914-20.
5. Dean R.T., Wolff S.P., McElligott M.A. (1989) Histidine and proline are important sites of free radical damage to proteins. *Free Radic. Res. Commun.*, 7: 97-103.
6. Farhan M., Rizvi A. (2022) Understanding the prooxidant action of plant polyphenols in the cellular microenvironment of malignant cells: Role of copper and therapeutic implications. *Front. Pharmacol.*, 13: 929853.
7. Farhan M., Ullah M.F., Faisal M., Farooqi A.A., Sabitaliyevich U.Y., Biersack B., Ahmad A. (2019) Differential methylation and acetylation as the epigenetic basis of resveratrol's anticancer activity. *Medicines (Basel)*, 6(1): 24.
8. Gieseg S.P., Simpson J.A., Charlton T.S., Duncan M.W., Dean R.T. (1993) Protein-bound 3,4-dihydroxyphenylalanine is a major reductant formed during hydroxyl radical damage to proteins. *Biochemistry*, 32: 4780-4786.
9. Hadi S.M., Asad S.F., Singh S., Ahmad A. (2000) Putative mechanism for anticancer and apoptosis-inducing properties of plant-derived polyphenolic compounds. *IUBMB Life*, 50: 167-171.
10. Hou X., Rooklin D., Fang H., Zhang Y. (2016) Resveratrol serves as a protein-substrate interaction stabilizer in human SIRT1 activation. *Sci. Rep.*, 6: 38186.
11. Iliadis S., Papanikolaou N.A. (2024) Reactive oxygen species mechanisms that regulate protein-protein interactions in cancer. *Int. J. Mol. Sci.*, 25(17): 9255.
12. Jeandet P., Sbaghi M., Bessis R. (1992) The production of resveratrol (3,5,4'-trihydroxy-stilbene) by grapevine in vitro cultures, and its application to screening for grey mould resistance. *Journal of Wine Research*, 3: 47-57.
13. Jojima K., Tanaka A., Node K. (2023) Resveratrol supplementation: a therapeutic potential for cardiac remodeling in hypertensive heart disease. *Hypertens. Res.*, 46: 1596-98.
14. Kim S., Kim E., Park M., Kim S.H., Kim B.G., Na S., Sadongo V.W., Wijesinghe W.C.B., Eom Y.G., Yoon G., Jeong H., Hwang E., Lee C., Myung K., Kim C.U., Choi J.M., Min S.K., Kwon T.H., Min D. (2024) Hidden route of protein damage through oxygen-confined photooxidation. *Nat. Commun.*, 15: 10873.
15. Leinisch F., Mariotti M., Hagglund P., Davies M.J. (2018) Structural and functional changes in RNAse A originating from tyrosine and histidine cross-linking and oxidation induced by singlet oxygen and peroxyl radicals. *Free Radic. Biol. Med.*, 126: 73-86.
16. Levine R.L. (1977) Fluorescence-quenching studies of the binding of bilirubin to albumin. *Clin. Chem.*, 23: 2292-2301.
17. Liu F., Lai S., Tong H.,. Lakey P.S.J, Shiraiwa M., Weller M.G., Poschl U., Kampf C.J. (2017) Release of free amino acids upon oxidation of peptides and proteins by hydroxyl radicals. *Anal. Bioanal. Chem.*, 409: 2411-2420.
18. Liu J.C., Yang J., Lei S.X., Wang M.F., Ma Y.N., Yang R. (2023) Natural phytoalexins inspired the discovery of new biphenyls as potent antifungal agents for treatment of invasive fungal infections. *Eur.*

J. Med. Chem., 261: 115842.
19. Mehringer J., Navarro J.A., Touraud D., Schneuwly S., Kunz W. (2022) Phosphorylated resveratrol as a protein aggregation suppressor *in vitro* and *in vivo*. *RSC Chem. Biol.*, 3: 250-260.
20. Meyer M.C., Guttman D.E. (1968) The binding of drugs by plasma proteins. *J. Pharm. Sci.*, 57: 895-918.
21. Nakamura H., Takada K. (2021) Reactive oxygen species in cancer: Current findings and future directions. *Cancer Sci.*, 112: 3945-52.
22. Orru R., Pazmino D.E., Fraaije M.W., Mattevi A. (2010) Joint functions of protein residues and NADP(H) in oxygen activation by flavin-containing monooxygenase. *J. Biol. Chem.*, 285: 35021-35028.
23. Radeva L., Yoncheva K. (2025) Resveratrol - a promising therapeutic agent with problematic properties. *Pharmaceutics*, 17(1):134.
24. Ren B., Kwah M.X., Liu C., Ma Z., Shanmugam M.K., Ding L., Xiang X., Ho P.C., Wang L., Ong P.S., Goh B.C. (2021) Resveratrol for cancer therapy: Challenges and future perspectives. *Cancer Lett.*, 515: 63-72.
25. Ruzza, P., Honisch C., Hussain R., Siligardi G. (2021) Free radicals and ROS induce protein denaturation by UV photostability assay. *Int. J. Mol. Sci.*, 22(12): 6512.
26. Shao F., Li Z., Xiao H., Chen Y., Zhang Y., Li L., Peng Y., Li X., Hou Y., Li B., Chu X., Ran M., Tang D., Han X., Yao J., Zhang C., Wang L., Li H., Shao N., Miao K., Xu X., Shi, Y. Zhang C., Yan J., Lin Y., Deng C.X. (2025) Suppressing protein damage response to overcome multidrug resistance in cancer therapy. *Cell Discov.*, 11: 80.
27. Subramaniam S., Vohra I., Iyer A., Nair N.K., Mittra I. (2015) A paradoxical relationship between Resveratrol and copper (II) with respect to degradation of DNA and RNA. *F1000Res*, 4: 1145.
28. Tang X., Yan Z., Miao Y., Ha W., Li Z., Yang L., Mi D. (2023) Copper in cancer: from limiting nutrient to therapeutic target. *Front. Oncol.*, 13: 1209156.
29. Xu Y., Fang M., Li X., Wang D., Yu L., Ma F., Jiang J., Zhang L., Li P. (2024) Contributions of common foods to resveratrol intake in the Chinese diet. *Foods*, 13 (8): 1267.
30. Zhang L.X., Li C.X., Kakar M.U., Khan M.S., Wu P.F., Amir R.M., Dai D.F., Naveed M., Li Q.Y., Saeed M., Shen J.Q., Rajput S.A., Li. J.H. (2021) Resveratrol (RV): A pharmacological review and call for further research. *Biomed/ Pharmacother*, 143: 112164.

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