Chapter 17
Mechanism of Intermolecular Electron Transfer in Bionanostructures

A. Gruodis, N. Galikova, K. Šarka, R. Saulė, D. Batiuškaitė, and G. Saulis

Abstract Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide. Most patients are inoperable and hepatoma cells are resistant to conventional chemotherapies. Thus, the development of novel therapies for HCC treatment is of paramount importance. Amongst different alimentary factors, vitamin C and vitamin K_3 In the present work, it has been shown that the treatment of mouse hepatoma MH-22A cells by vitamin C and vitamin K_3 at the ratio of 100:1 greatly enhanced their cytotoxicity. When cells were subjected to vitamin C at 200 μM or to vitamin K_3 at 2 μM separately, their viability reduced by only about 10%. However, when vitamins C and K_3 were combined at the same concentrations, they killed more than 90% of cells. To elucidate the mechanism of the synergistic cytotoxicity of the C&K_3 mixture, theoretical quantum-chemical analysis of the dynamics of intermolecular electron transfer (IET) processes within the complexes containing C (five forms) and K_3 (one form) has been carried out. Optimization of the ground state complex geometry has been provided by means of *GAUSSIAN03* package. Simulation of the IET has been carried out using *NUVOLA* package, in the framework of molecular orbitals (MO). The rate of IET has been calculated using Fermi Golden rule. The results of simulations allow us to create the preliminary model of the reaction pathway.

Keywords Ascorbate • Menadione • Cytotoxicity • Chemotherapy • Quantum-chemical analysis • Fermi Golden rule

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17.1 Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide [1]. So far, the only curative therapy options have been liver resection or orthotopic liver transplantation [2]. Unfortunately, most patients (80%) are inoperable and hepatoma cells are resistant to conventional chemotherapies [3]. Hence, the development of novel therapies for treatment of HCC is of paramount importance.

The influence of dietary components on tumor growth and development has recently become a subject of major interest [4]. Amongst different alimentary factors, vitamin C (*L*-ascorbate) and vitamin K_3 (menadione) have also been considered as possible antitumor agents [5]. The tumor growth-inhibiting and chemotherapy-potentiating effects of vitamin C and K_3 combinations have been evaluated using a variety of human tumor cell lines [6] and a new type of cell death – *autoschizis* – has been described [7].

It is assumed, that the synergistic anticancer effect of the C/K_3 combination is likely explained by the redox-cycling that occurs between these compounds [8]. Menadione has been used experimentally as a chemotherapy agent for cancer since 1947. Menadione in combination with vitamin C is being studied as a potential treatment for prostate cancer. However, the detailed mechanism has not been known.

The aim of this work is to study the cytotoxicity of vitamins C and K_3 and their mixture on mouse hepatoma MH-22A cells *in vitro* and to elucidate the plausible mechanism of the synergism of their anticancer action by theoretical quantum-chemical analysis of the dynamic electron transfer processes within the complexes containing various forms of vitamins C and K_3.

17.2 Materials and Methods

17.2.1 Experiments with Mouse Hepatoma MH-22A Cells In Vitro

The experiments were performed with mouse hepatoma MH-22A cells. The cells were grown in monolayer cultures in 25-cm^2 (60-ml) flasks at 37°C in a humidified 5% CO_2–95% O_2 atmosphere in a water-jacketed incubator IR AutoFlow NU-2500E (NuAire, Plymouth, MN, USA). All manipulations that required sterile conditions were done in a vertical laminar flow cabinet (Aura Vertical SD4, BIOAIR Instruments, Siziano, Italy).

The cells were seeded in a duplicate (200–300 cells per 40 mm diameter) Petri dish (Techno Plastic Products) in the culture medium, which was additionally supplemented with 90 U/ml penicillin and 90 μg/ml streptomycin (growth media)

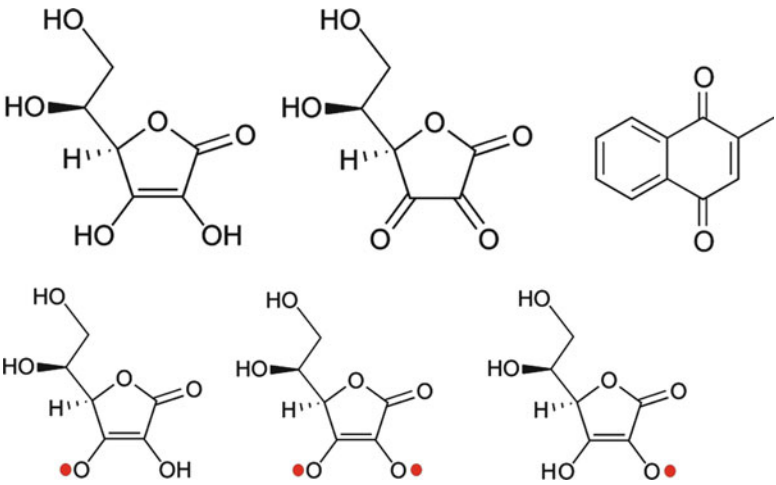

Fig. 17.1 Molecular structures. *Top* – two forms of vitamin C (C1, ascorbic acid and C2, de-hydro-ascorbic acid) and vitamin K_3. *Bottom* – three forms of ionized ascorbic acid (C3, C4, C5)

and the appropriate concentration of vitamin C or K_3 separately or in their combination. The cells were incubated with vitamins for 6 days at 37°C and 5% CO_2. The cytotoxicity of vitamins C and K_3 alone and their mixture were estimated from the reduction of the cell viability.

The cell viability was determined by means of a colony-forming assay [9]. After incubation of cells seeded in Petri dishes at 37°C and 5% CO_2 for 9 days, the formed colonies were fixed with 96% ethanol, and stained with a gram's crystal violet solution (Fluka Chemie, Buchs, Germany). Then, the colonies were counted under a binocular light microscope and the survival of the cells treated with vitamins was calculated as the percentage of the colonies obtained from the untreated control cells.

17.2.2 Theoretical Quantum-Chemical Analysis

Vitamin C could be presented in two molecular forms (C1, ascorbic acid, 2-oxo-*L*-threo-hexono-1,4- lactone-2,3-enediol and C2, de-hydro-ascorbic acid, (*R*)-3,4-dihydroxy-5-((*S*)-1,2-dihydroxy-ethyl)-furan-2(5*H*)-one) and three ionized forms (C3, C4, C5) – see Fig. 17.1. Vitamin K_3 (2-methyl-naphthalene-1,4-dione; menaphthone) belongs to the class of quinines.

We have examined several [C..$_3$] complexes in order to establish the most probable geometry. Optimization of the ground state complex geometry was provided by means of *Gaussian03* package using Hartree–Fock (HF) method and several basic sets of gaussian type: 6-311G and 6-311G(2df,2pd). Diffusion functions were not included [10].

Dynamical IET process between two molecular systems, the so called donor and acceptor, respectively, is defined by rate of IET k. In our case, donor (C) and acceptor (K_3) were presented as two resonant molecular systems. Fermi golden rule (Eq. 17.1) and Marcus theory [11] were used for the process modeling by means of *Nuvola* [12] package. α and β represent electronic states belonging to the donor and acceptor, respectively.

Electronic states α and β of separated molecular systems must be of one-particle state type according to Fermi golden rule definition. We have generated molecular orbitals (MO) in the framework of atomic orbitals (AO).

H represents electronic hamiltonian of interactions between these molecular systems as intermolecular electronic coupling (presented in ket-bra notation). *Delta* function $\delta(x)$ expresses the strongest resonant condition (band halfwidth converges to zero):

$$k = \frac{2\pi}{\hbar} \langle \alpha | \hat{H} | \beta \rangle^2 \delta(E_\alpha - E_\beta). \tag{17.1}$$

17.3 Results and Discussion

First, the cytotoxicities of vitamins C and vitamin K_3 were estimated. It can be seen from Fig. 17.2 that at the concentrations of 200 and 400 μM, vitamin C did not influence the survival of the mouse hepatoma MH-22A cells. The concentration of vitamin C required to reduce the survival of mouse hepatoma MH-22A cell by 50% (50% growth inhibitory dose ID_{50}) was 0.95 mM.

When MH-22A cells were cultured in the media with 2 μM of vitamin K_3, no inhibition of cell proliferation was observed, and only 10% viability reduction was obtained at 4 μM concentration (Fig. 17.1). Vitamin K_3 exhibited toxicity of 50% over the control at 6.2 μM.

Then, *in vitro,* cytotoxicity of the mixture of vitamins C and K_3 at the ratio of 100:1 on MH-22A cells was examined. It turned out that the treatment of cells by both vitamins at the ratio of 100:1 (VC:VK3) had greatly enhanced their cytotoxicities related to these cells. The mixture of 200 μM of vitamin C and 2 μM of vitamin K_3 had killed about 25% of cells, while only 6% of cells had survived in the mixture of two times higher concentrations.

Fifty percent growth inhibitory dose ID_{50} for vitamin K_3 became 2.4 μM, that is, it was reduced by 2.6 times. Therefore, vitamin C synergistically increased the cytotoxicity of vitamin K_3. It is assumed that such enhanced cytotoxicity is due to the redox cycling between ascorbate and menadione generating hydrogen peroxide (H_2O_2) [8]. However, the detailed mechanism is not known.

In the attempt to elucidate the plausible mechanism of the synergistic action of vitamins C and K_3, theoretical quantum-chemical analysis of the dynamics of

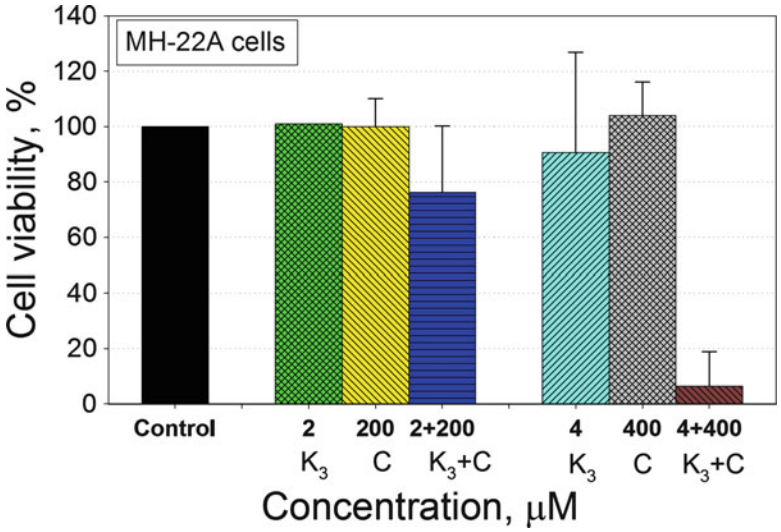

Fig. 17.2 Dependences of the viability of mouse hepatoma MH-22A cells on the concentration of vitamins C and K_3 alone and their mixture at the ratio of 1:100 (K_3 + C)

intermolecular electron transfer processes within the complexes containing C (five forms) and K_3 (one form) has been carried out.

Two concurrent pathways must be analyzed. First, the increase of acidity in the near surrounding of complex must be treated as a significant factor influencing redox cycling. Second, the creation of the most stable [C2K] complexes allows stabilizing pH (acidity increases and saturates at quite a high level).

Figure 17.3 represents two most typical pseudo-planar complexes of vitamin K_3 with C1, ascorbic acid [C1K] and C2, de-hydro-ascorbic acid [C2K], which could be titled as the most stable.

It could be concluded that such two complexes are of different electronic nature due to the different position of the van der Waals junction. Because the molecule of vitamin K_3 has no symmetry, the position of the methyl group plays an essential role in the associate formation process. For [C1K], the complex is formed at the side of the methyl group, meanwhile, for [C2K], the complex is formed at the opposite side.

The spectra of IET represent the different nature of complexes. There are several possibilities for electron transfer for the less stable [C1K] complex (several resonant states with a quite high transfer rate k). On the other hand, for the more stable [C2K] complex, the number of resonant states significantly decreases (see Fig. 17.3, bottom). This phenomenon confirms the preliminary assumption about the surrounding effect: the creation of complex [C1K] escalates the increase in acidity (number of protons increases).

Finally, two factors – the surrounding effects and the creation of complexes – must be estimated by explaining the phenomenon of cytotoxicity.

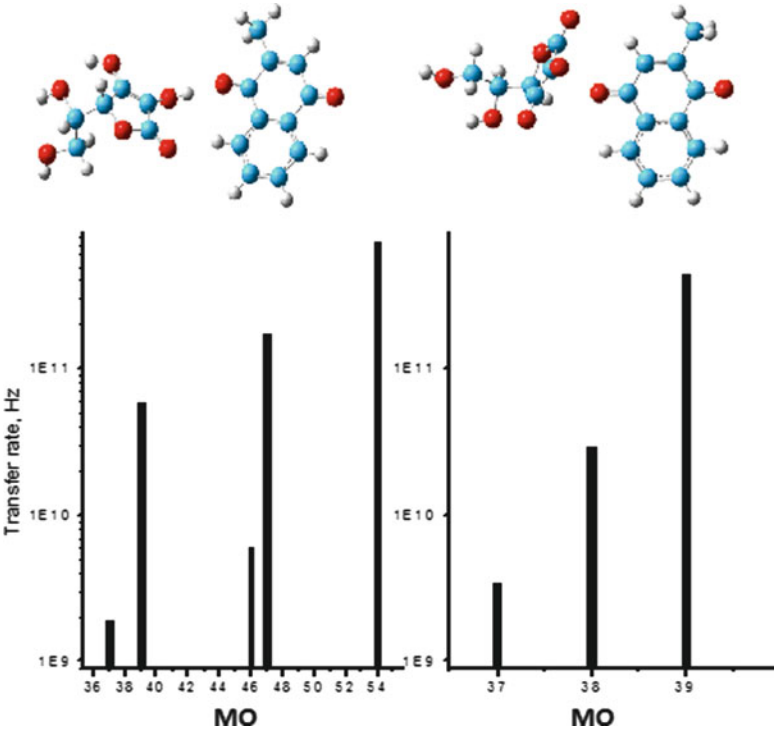

Fig. 17.3 At the *top* – two most typical pseudo-planar complexes of vitamin K_3 with C1, ascorbic acid [C1K] and C2, de-hydro-ascorbic acid [C2K]. At the *bottom* – IET spectra of [C1K] and [C2K] complexes (band position presented as a *stick*)

References

1. Boudreau CR, Yang I, Liau LM (2005) Gliomas: advances in molecular analysis and characterization. Surg Neurol 64:286–294
2. Barbieri F, Sparatore F, Bonavia R, Bruzzo C, Schettini G, Alama A (2002) Chemosensitivity of glioblastoma cells during treatment with the organo-tin compound triethyltin(IV) lupinylsulfide hydrochloride. J Neurooncol 60:109–116
3. Prados MD, Levin V (2000) Biology and treatment of malignant glioma. Semin Oncol 27:1–10
4. Milner JA (2008) Nutrition and cancer: essential elements for a roadmap. Cancer Lett 269:189–194
5. Taper HS, Jamison JM, Gilloteaux J, Summers JL, Calderon PB (2004) Inhibition of the development of metastases by dietary vitamin C:K_3 combination. Life Sci 75:955–967
6. De Loecker W, Janssens J, Bonte J, Taper HS (1993) Effects of sodium ascorbate (vitamin C) and 2-methyl-1,4-naphthoquinone (vitamin K3) treatment on human tumor cell growth *in vitro*. II. Synergism with combined chemotherapy action. Anticancer Res 13:103–106
7. Gilloteaux J, Jamison JM, Neal DR, Summers JL (2005) Cell death by autoschizis in TRAMP prostate carcinoma cells as a result of treatment by ascorbate: menadione combination. Ultrastruct Pathol 29:221–235

8. Verrax J, Cadrobbi J, Marques C, Taper H, Habraken Y, Piette J, Calderon PB (2004) Ascorbate potentiates the cytotoxicity of menadione leading to an oxidative stress that kills cancer cells by a non-apoptotic caspase-3 independent form of cell death. Apoptosis 9:223–233
9. Freshney IR (2000) Culture of animal cells: a manual of basic techniques. Wiley, New York
10. Frisch MJ et al (2004) Gaussian 03, revision D.01. Gaussian, Inc., Wallingford
11. Marcus RA, Sutin N (1992) Electron transfers in chemistry and biology. Biochim Biophys Acta 811:265–322
12. Galikova N, Gruodis A (2008) Innov Infotech Sci Bus Educ 2(3):12.1