Re-evaluation of Cytotoxicity and Iron Chelation Activity of Three β-Diketones by Semiempirical Molecular Orbital Method

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Abstract. We investigated the chelating effect of FeCl₃ on three β-diketones, curcumin [1], (-)-3-(trifluoroacetyl)camphor [2] and 3-formylchromone [3], as judged by changes in their cytotoxicity and absorption spectra. Addition of an equimolar concentration of FeCl₃ almost completely abrogated the cytotoxicity and changed the pattern of absorption spectra (decrease in the peak height at 430 nm) of [1], without affecting those of [2] and [3]. A semiempirical molecular orbital method (CAChe), based on these experimental data, proposed the estimated structure of [1] chelated with FeCl₃ at 1:1 molar ratio. The present study suggests that [1], but not [2] and [3], is an iron chelator.

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Materials and Methods

Materials. The following chemicals and reagents were obtained from the indicated companies: [1] (MW=368) (Tokyo Kasei Kogyo Co., Tokyo, Japan); [2] (MW=248), [3] (MW=174) (structure shown in Figure 1) (Aldrich Chemical Co., Inc., Milwaukee, USA). Dulbecco’s modified Eagle medium (DMEM) (Gibco BRL, Grand Island, NY, USA); fetal bovine serum (FBS) (JRH Bioscience, Lenexa, KS, USA); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Chem. Co., St. Louis, MO, USA); FeCl₃, dimethyl sulfoxide (DMSO) (Wako Pure Chem. Ind., Ltd., Osaka, Japan).

Cell culture. HSC-2 cells were cultured in DMEM supplemented with 10% heat-inactivated FBS under a humidified 5% CO₂ atmosphere.

Assay for cytotoxic activity. The cytotoxic activity of samples was determined by the MTT method and expressed as absorbance at 540 nm of the MTT-stained cells. The 50% cytotoxic concentration (CC₅₀) was determined from the dose-response curve (17).

Calculation methods. The molecular geometries of [1] were optimized by a semiempirical molecular orbital method, the MOPAC PM3 method (CAChe). The computer programs of the INDO/1 were provided by the CAChe system.

Results and Discussion

Cytotoxicity. [1] dose-dependently reduced the viable cell number of HSC-2 cells (CC₅₀=8.5 μM). The cytotoxic activity of [1] was significantly reduced by addition of 250 μM FeCl₃ (CC₅₀=120 μM) (Figure 2A). The cytotoxic activities of [2] and [3] were slightly lower than [1] (CC₅₀=58 and 14 μM, respectively), and were not affected by addition of FeCl₃ (Figure 2B, C).
The cytotoxic activity of [1] (CC_{50}=8.6 \mu M) was reduced slightly by a less than equimolar concentration of FeCl_{3} ([1]: FeCl_{3}=1:1/3) (CC_{50}=19.5 \mu M), but potently by equimolar and higher concentrations of FeCl_{3} ([1]: FeCl_{3}=1:1, 1:2, 1:3) (CC_{50}>100 \mu M)(Figure 3A). On the other hand, the cytotoxic activities of [2] and [3] were not reduced, but rather slightly enhanced by addition of FeCl_{3} at 1/3, 1 or 2 molar ratio to [1] (Figure 3B, C).

Absorption spectra. [1] showed small peaks at 225 and 260 nm, a shoulder peak at 360 nm, and a large peak at 430 nm (Figure 4A). Addition of FeCl_{3} at 1/3 or 1/2 molar ratio to [1] slightly reduced the peak height at 430 nm (Figure 4A). Addition of FeCl_{3} at 1, 2 or 3 molar ratio to [1] further reduced the peak height at 430 nm and increased the peak height at 260 nm (Figure 4B), accompanied by coloration and improvement of water-solubility (data not shown). [2] produced two peaks at 225 and 304 nm, and addition of FeCl_{3} at 1 and 3 molar ratio did not significantly change the absorption profile (Figure 4C). [3] produced four peaks at 225, 260, 304 and 360 nm, and addition of FeCl_{3} at 1 and 3 molar ratio to [1] did not significantly change the profile of absorption spectra of [3] (Figure 4D).
The absorption spectra data further supported that only [1], but not [2] and [3], interacts with iron. Assuming that [1] and Fe$^{3+}$ makes a chelation complex at 1:1 molar ratio, the structure of this complex was predicted with ZINDO (Figure 5). The estimated absorption spectra of [1] showed three peaks at 260, 330 and 430 nm (Figure 6A). Addition of Fe$^{3+}$ at 1:1 ratio to [1] resulted in the disappearance of the large peak at 430 nm, and shifts of other peaks to different wavelengths (Figure 6B), which corresponded with the experimental data which shows that addition of Fe$^{3+}$ reduced the peak height at 430 nm (Figure 4A, B).

When [1] forms the chelation complex with Fe$^{3+}$, the six-membered ring becomes stabilized. A straight chain structure of [1] gives it a degree of freedom. When FeCl$_3$ and [1] are added to the culture medium, Fe(OH)$_2$, Fe$^{3+}$ and [1] are co-existent there. Fe$^{3+}$ begins to make a chelation complex with [1]. Since this complex is stable, the equilibration between Fe(OH)$_2$ and Fe$^{3+}$ favors the formation of Fe$^{3+}$, all of which is used to make a chelation complex. The more the complex is formed, the lesser percentage of free [1], which exerts its actions on the cells, remains.

On the other hand, [2] becomes less stable, upon the formation of a chelation complex with Fe$^{3+}$. The ring structure of [2] eliminates a degree of freedom. Therefore, the chelation with Fe$^{3+}$ creates a strain to [2]. Since the chelation complex with Fe$^{1+}$ is unstable, most of the [2] remains unchelated, and therefore can exert its actions on the cells.

There is little or no likelihood for [3] to make a chelation complex with Fe$^{3+}$, since [3] cannot be enolized (13, 14). Such a conformational limitation enforces [3] to continuously exert an action on the cells, even in the presence of Fe$^{3+}$.

The present study suggests that [1], but not [2] and [3], chelates with FeCl$_3$, although these three compounds belong to the β-diketones. The biological action of [1] may be significantly affected by the Fe$^{3+}$ concentration in both intracellular and extracellular milieu. It remains to be investigated by which mechanism other than metal chelation [2] and [3] induce cytotoxicity.

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Figure 5. Predicted conformation of [1] and Fe$^{3+}$ complex. White color, H; black color, C; green color, Cl; red color (center), Fe; red color (in other places), O.

Figure 6. Predicted absorption spectra of [1] by INDO/1 in the absence (A) or presence (B) of equimolar concentration of FeCl$_3$. 
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References


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