## Communications to the Editor

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# An Application of Molecular Orbital Theory for Screening Research of Antifungal Drugs

The relationship between antifungal activity and the energy of the lowest unoccupied molecular orbital (LUMO) of several phenolic compounds was examined using MINDO/3 method. Among the phenols examined, all the compounds which are highly active in inhibiting the growth of fungi possess a low-lying LUMO as compared with the compounds poor in antifungal activity. From these results, it is conceivable that the LUMO energy of a molecule should be one of the useful indices for screening antifungal drugs.

**Keywords**——antifungal activity; phenolic compound; MINDO/3 method; lowest unoccupied molecular orbital (LUMO); electronic structure; DFP optimization; Self-Consistent Field molecular orbital

Recently, one of the authors, Kurita,[1] found that the antifungal activity of four kinds of unsaturated aldehyde obtained from the essential oils of higher plants is closely correlated with the energy level of the lowest unoccupied molecular orbital (LUMO) calculated by the Hückel MO method with $\pi$-electron approximation. He also suggested, by studies of difference spectra, that the phenomenon is due to the electron-accepting ability of the aldehydes in addition to their reactivity with sulfhydryl group.

Regardless of the validity of the suggestion, however, the correlation may provide a hopeful technique for screening a new antifungal drugs, if there exists a simple relationship between antifungal activity and LUMO energy over a wide variety of compounds. We examined the applicability of the screening technique by the calculation of the LUMO energy using phenolic compounds, and obtained promising results.

This brief communication outlines the research.

### Methods

Molecular orbital calculations were carried out by using a semiempirical Self-Consistent Field (SCF) MO procedure considering all valence electrons[2] with MINDO/3 parameters.[3] Prior to the calculation of the electronic structure, the chemical structure of compounds was optimized using DFP (Davidon-Fletcher-Powell) method[4] based on the MINDO/3 calculations.

Assays of antifungal activity were carried out at 27° on 2% glucose Sabouraud agar slants containing a compound to be tested. The activity of each compound employed was examined by using 7 kinds of fungi.

### Results and Discussion

Results are summarized in Table I, where relationship between antifungal activity and LUMO energy is clearly demonstrated for 7 kinds of phenolic compounds. Results on cinnamaldehyde, the most active compound in the previous research, are also shown, where its LUMO energy was recalculated by the MINDO/3 method after DFP optimization.

#### Antifungal Activity

Phenol and *p*-cresol, at a concentration of 1 mM, were almost ineffective in inhibiting the growth of any of the fungi employed. *p*-Acetoxyphenol inhibited the growth of *Blastomyces*

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2) J.A. Pople, D.L. Beveridge, and P.A. Dobosh, *J. Chem. Phys.*, **47**, 2026 (1967).
3) R.C. Bingham, M.J.S. Dewar, and D.L.Lo, *J. Am. Chem. Soc.*, **97**, 1285 (1975).
4) R. Fletcher and M.J.D. Powell, *Computer J.*, **6**, 163 (1963); W.C. Davidon, *ibid.*, **10**, 406 (1968).

Table I. Antifungal Activity and LUMO Energy of Phenolic Compounds

| Compound | | Duration of growth inhibition (day) ($D$) | | | | | | | LUMO energy (eV) ($E_{LUMO}$) |
|---|---|---|---|---|---|---|---|---|---|
| | | *Blastomyces dermatitidis* | *Histoplasma capsulatum* | *Trichophyton rubrum* (H) | *Fonsecaea pedrosoi* (T) | *Aspergillus nidulans* | *Penicillium frequentans* | *Penicillium cyclopium* | |
| Phenol | 1 mM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.146 |
| *p*-Cresol | 1 mM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.143 |
| *p*-Acetoxyphenol | 1 mM | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0.431 |
| *p*-Cyanophenol | 0.5 mM | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0.848 |
| | 1 mM | >20 | >20 | 2 | 0 | 0 | 0 | 0 | |
| *o*-Nitrophenol | 0.5 mM | 0 | 4 | 1 | 4 | 2 | 4 | 0 | 0.119 |
| | 1 mM | 4 | >20 | 9 | >20 | 6 | >20 | 4 | |
| *m*-Nitrophenol | 0.5 mM | 1 | 10 | 2 | 1 | 0 | 0 | 0 | −0.552 |
| | 1 mM | >20 | >20 | >20 | >20 | 2 | 1 | 1 | |
| *p*-Nitrophenol | 0.5 mM | >20 | >20 | >20 | >20 | 0 | 2 | | −0.319 |
| | 1 mM | >20 | >20 | >20 | >20 | >20 | >20 | >20 | |
| Cinnamaldehyde | 0.33 mM | >20 | >20 | 3 | 0 | 1 | 0 | 0 | −0.157 |
| | 0.66 mM | >20 | >20 | >20 | 2 | >20 | 1 | 1 | |

*dematitidis* and *Histoplasma capsulatum* for 4 days, but failed to exhibit a definite inhibitory effect on the growth of the other fingi at a concentration of 1 mM. *p*-Cyanophenol, at a concentration of 1 mM, inhibited the growth of *B. dermatitidis* and *H. capsulatum* for more than 20 days, and the growth of *Trichophyton rubrum* (Hagiwara) for 2 days, but failed to exert a definite inhibitory effect on the growth of the other fungi.

*p*-Nitrophenol, at a concentration of 1 mM, inhibited the growth of all the fungi employed, and at 0.5 mM the growth of *B. dermatitidis*, *H. capsulatum*, *T. rubrum* (Hagiwara) and *Fonsecaea pedrosoi* (Tsuchiya) for more than 20 days. *m*-Nitrophenol, *o*-nitrophenol and cinnamaldehyde also possess a potent antifungal activity.

**LUMO Energy**

The LUMO energies of *p*-nitrophenol, *m*-nitrophenol and cinnamaldehyde, all highly active in the antifungal action, are negative-valued under the MINDO/3 parameters, while those of the other phenols poor in this activity are all positive-valued and much higher than those of the nitrophenols and cinnamaldehyde.

**Correlation Analysis**

The data collected in the Table I were examined by the method of correlation analysis. It was found that the structure–activity relationships between the antifungal activities ($D$) and LUMO energies $E_{LUMO}$ are expressed by the equations (1) and (2) for *T. rubrum* (H) and *F. pedrosoi* (T), respectively. Both the equations fit the data at a level of significance $P=0.01$ by the F-test.

| | $n$ | $r$ | $s$ | |
|---|---|---|---|---|
| $D = [-13.4(\pm 5.7)]E_{LUMO} + 13.3(\pm 4.0)$ | 8 | 0.921 | 4.08 | (1) |
| | $n$ | $r$ | $s$ | |
| $D = [-14.5(\pm 7.0)]E_{LUMO} + 14.8(\pm 4.9)$ | 8 | 0.901 | 5.01 | (2) |

where the figures in parentheses are the 95% confidence intervals by the $t$-test, $n$ is the number of points, $r$ is the correlation coefficient and $s$ is the standard deviation from regression.

These results suggest that the LUMO energy of a molecule should be one of useful indices for screening antifungal substances, though other factors such as steric properties and cell-membrane permeability are also important.

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# Measurement of Carbohydrate Contents in Multiple Forms of Hog Pancreatic Kallikrein and Their Behavior on Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis[1]

Carbohydrate content of kallikrein B was about twice as much as that of kallikrein A in neutral hexsose and glucosamine. Total sugar contents of kallikrein B-III and B-IV (main micro-heterogeneous forms derived from kallikrein B) were also about twice as much as that of kallikrein A-II (one of the main micro-heterogeneous forms derived from kallikrein A). Contents of sialic acid in heterogeneous form, A, (more acidic than B) contain rather less amount than that of B, while contents of sialic acid in the molecules of the microheterogeneous forms derived from both kallikreins A and B were different from each other and this difference was seemed to reflect their isoelectric points.

After the reduction of hog pancreatic kallikreins A and B with 0.04% 2-mercapto-ethanol in 0.01 M Tris-HCl buffer pH 7.4 containing 1% SDS, two protein bands were detected in each sample on polyacrylamide gel electrophoresis. However, although staining of carbohydrate moiety of the reduced preparation of kallikrein B by PAS reagent also showed two bands identical with the protein bands, only one of the protein bands of the reduced preparation of kallikrein A was stained for carbohydrate. From these results, it was revealed that both kallikreins A and B are consisted of two peptide chains, and one of the peptide chains of kallikrein A has no carbohydrate moiety, whereas the carbohydrate moieties are bound to both peptide chains in case of kallikrein B.

**Keywords**——hog pancreatic kallikrein; multiple forms of kallikrein; micro-heterogeneous forms; carbohydrate contents of kallikreins; sialic acid; isoelectric focusing; sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Recently, it has been revealed that the glandular kallikreins, such as human urinary, hog pancreatic, rat submandibular kallikreins and so on, have multiple forms which are usually separable on Ampholine isoelectric focusing.[2] Multiple forms of hog pancreatic kallikrein were early observed by Moriya and Shimazawa (called $a_1$ and $a_2$ on paper electrophoresis Fig. 3. in ref.[3]) which were separable by electrophoresis with starch gel.[3] Afterwards, these two forms of kallikrein were certainly clarified and separated by other groups[4,5] named as kallikreins A and B. Moreover, kallikreins A and B were further separated into several

1) Enzymes: kallikrein (EC 3.4.21.8); neuraminidase (EC 3.2.1.18). Abbreviations: sodium dodecyl sulfate, SDS; Kallikrein Unit, KU; periodic acid-Schiff reagent, PAS.
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