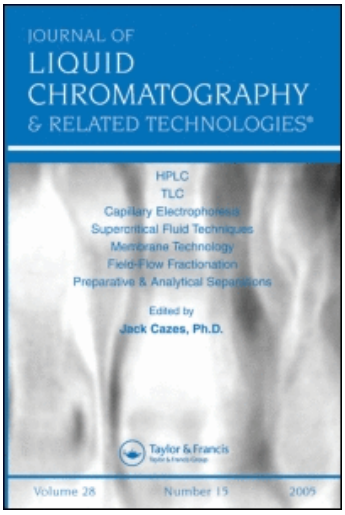

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QUANTITATIVE COMPUTATIONAL CHEMICAL ANALYSIS OF THE SENSITIVITY OF CHEMILUMINESCENCE DETECTION

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ABSTRACT

The relative sensitivity of chemiluminescence detection in liquid chromatography was analyzed by properties calculated using computational chemistry. The important reaction process was considered as the keto–enol form rearrangement. According to radical reaction, the keto–enol rearrangement produces superoxide, and then the superoxide reacts with luminol or lusigenin to produce chemiluminescence. The partial charge of carbon atoms of the carbonyl group changed significantly and correlated well with the relative sensitivity.

The computational chemical analytical method can predict the relative sensitivity detected by the chemiluminescence reaction using luminol and lusigenin. Computational chemical analysis can help to estimate sensate detection in liquid chromatography. The reaction mechanisms of other compounds, under similar conditions, should be the same as that described here. Further

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computational study will elucidate the reaction mechanisms of chemiluminescence and the sensitivity differences.

INTRODUCTION

Chemiluminescence methods have been developed as analytical systems with high sensitivity, selectivity, and wide dynamic range. Chemiluminescence analysis has been applied for a variety of atoms and compounds in different techniques, such as gas-chromatography, liquid chromatography, supercritical fluid chromatography, capillary electrophoresis, chip analysis, flow injection analysis, and immunoassay. The high reactivity is suitable as a post-column reaction detection for chromatography and flow injection analysis. One targeted glycosylated albumin was analyzed[1] using chemiluminescence detection without separation from glycosylated albumin mixtures in the Meillard reaction products,[2] where fluorescence detection required chromatographic separation of such complex mixtures.

The efficiency of a chemiluminescence reaction can be expressed as the number of light-emitting molecules, relative to the number of all exited molecules (luminescence efficiency). Peroxyoxalate luminescence has been used to assay hydrogen peroxide or the number of fluorophores. However, most of the compounds assayed by peroxyoxalate chemiluminescence do not possess luminescence and a suitable fluorescence tagging operation must proceed the actual assay.[3] On the other hand, reducing agents have been analyzed without pre-derivatization. Organic reducing compounds, reducing sugars, ascorbic acid, uric acid, etc., were detected by the chemiluminescence method using lucigenin and luminol.[4–6]

The reaction process is considered the same for similar compounds, but the sensitivity of chemiluminescence has been suggested to be structure-dependent.[3,7] Thus, the reactivities of compounds contribute to the sensitivity of similar compounds. Phenacylalcohol derivatives were detected with different sensitivities in chemiluminescence analysis.[8,9] According to radical reaction, the reaction process can be explained as follows: in buffered solutions, a compound such as phenacylalcohol is easily attacked by oxidation if a trace of copper or iron salt is present,[10] and the superoxide reacts with luminol or lucigenine to produce chemiluminescence.[11]

Previously, the relative sensitivities of phenacylesters and steroids measured by chemiluminescence detection in liquid chromatography using luminol were analyzed quantitatively using computational chemistry. The chemiluminescence reaction of lucigenin with reducing sugars was proposed as the formation of the 1,2 enediol tautomer. This intermediate enediol is oxidized by lucigenin in subsequent reaction steps.[12] Therefore, the most important reaction process was

considered to be that of keto–enol form rearrangement. The partial charge of the carbon atom of the carbonyl group changed significantly and was correlated well with the relative sensitivity. The correlation coefficient (r^2) was 0.970 ($n = 5$) and 0.965 ($n = 8$) for phenacylesters and steroids, respectively.[13]

The chemiluminescence detection mechanisms of phenacylesters and steroids have been suggested to be the same, due to their chemical structures, and the differences in sensitivity were suggested to be due to differences in the stability of compounds in alkaline solution. Further computational chemical analyses of the relative sensitivity of other compounds, measured by liquid chromatography and flow injection analysis,[8,14] were performed quantitatively as the reactivity of analytes.

EXPERIMENTAL

A variety of molecules were constructed using the molecular editor of the CAChe™ program, and their properties were calculated using MOPAC after optimizing their structure using the molecular mechanics feature of the CAChe™ program from Fujitsu (Tokyo, Japan). The properties were analyzed using the CA Cricket-Graph™ program from Computer Associates (San Diego, CA, USA) on a modified Macintosh 8100/250 computer. The changes in the partial charge of key atoms, before and after their keto–enol form rearrangement, were used to analyze their reactivity.

RESULTS AND DISCUSSION

As the chemiluminescence reaction detects superoxide, the sensitivities depended on the amount of superoxide under the same reaction conditions. The reaction process from the keto form to the enol form was considered to be an important process, similar to that observed for phenacyl alcohols and steroids.[13] The reaction process was the same, but the reactivity was different, and the amount of superoxide may have been related to those of enol form compound. The process of rearrangement from the keto to the enol form was considered to be the key for analyzing the sensitivity and the changes in the properties of the key atoms during the reaction process to predict sensitivity. The relative sensitivity of derivatized phenacylalcohols from references and the calculated balance of partial charges of carbonyl carbon are summarized in Table 1.

Relative sensitivities of acetylated phenacyl alcohols, measured by different systems, were related to balance of partial charges of carbonyl carbon between original and Amadori rearranged forms. The correlation coefficient (r^2) was 0.807 using lucigenin[8] and 0.826 using luminol.[9] These results indicated that

Table 1. Relative Sensitivity and Partial Charges of Key Carbon of Phencylalcohols

Chemicals			Relative Sensitivity		
R_1[a]	R_2[a]	Δpc	RS[b]	RS[b]	RS[c]
Hydrogen	Benzoyl	0.1918	0.65	—	—
Bromo	Benzoyl	0.2029	0.13	—	—
Nitro	Benzoyl	0.2170	1.25	—	—
Phenyl	Benzoyl	0.1908	0.39	—	—
Hydrogen	Hydroxyl	0.1990	1.00	1.00	1.00
Hydrogen	Acetyl	0.1986	—	0.83	1.09
Bromo	Acetyl	0.2045	—	1.54	2.07
Nitro	Acetyl	0.2124	—	1.51	3.61
Phenyl	Acetyl	0.1967	—	0.39	1.11
	r^2		0.349	0.807	0.826

[a]$R_1-C_6H_4-CO-CH_2-R_2$.
[b]From Ref. 8.
[c]From Ref. 9.

the relative sensitivity can be predicted from the partial charge calculated by computational chemical methods, as explained previously.[13] However, this relationship for benzoylesters was poor. The r^2 was 0.349 ($n = 5$). This previous result was based on experimented data in which the data point for 4′-bromophenacyl benzoylester was 0.13. This value may actually be 1.3 and, thus, r^2 should be 0.804 ($n = 5$), based on the results obtained for acetylated compounds. Unfortunately, this old result could not be reconfirmed.

Atomic partial charges were subjected to analysis of the detection limits of a variety of compounds measured by the same system. The detection limits and atomic partial charges are shown in Table 2. The partial charges of saccharides were calculated as the aldehyde-form, but these sugars exist as hexoses and the concentration of aldehyde-form was not known under these experimental conditions. Therefore, these values were eliminated from the correlation coefficient calculations. The sensitivity of creatinine was very low. Therefore, creatinine was also eliminated from the calculations. The correlation coefficients (r^2) were 0.701 ($n = 7$) for group 1, and 0.922 ($n = 8$) for group 2. In the above calculation, uric acid and ascorbic acid have two carbonyl groups and, therefore, two partial charges were combined.

The HOMO density, frontier density, superdelocalizability, LUMO density, and partial charge (au) of all elements were calculated using the MOPAC program after optimizing their structures with molecular mechanics. Electron densities of

Table 2. Chemiluminescence Intensity and Partial Charge

Chemicals	pc	Detection Limit (pmol) Group 1[a]	Group 2[b]
Uric acid	0.4421	1.7	—
Phenacylalcohol	0.1990	3	267
Cortisone	0.3725	4	42
Ascorbic acid	0.4428	6	—
Corticosterone	0.3165	16	—
Glutathione	0.1368	55	—
Cysteine	0.0477	62	—
Fructose	0.3605	600	—
Glucose	0.1463	1,500	1
Creatinine	0.1962	15,000	—
Galactose	0.1463	—	1
Mannose	0.1463	—	1
Glucosamine	0.1789	—	1.9
Gluceraldehyde	0.2837	—	138
Glycoaldehyde	0.2792	—	181
Cortisol	0.3280	—	41
Tetrahydrocortisol	0.3450	—	47
Dihydroxyacetone	0.2458	—	212
Benzoin	0.2460	—	173
	r^2:	0.701	0.922

[a]From Ref. 14.
[b]From Ref. 8.

keto- and enol-forms of phenacylalcohol were constructed using the CAChe™ system and are shown in Fig. 1. The electron density of carbonyl carbon was significantly reduced from keto- to enol-form.

Generally, the electron density of LUMO is a key property for studying the reactivity, but the values did not explain the sensitivity difference and the electron density of HOMO was also not useful to explain the sensitivity; thus, the electron density values of LUMO and HOMO are not given. The frontier density and the superdelocalizability also could not be used for this purpose, and their values are also not given.

These changes in partial charges of key atoms are useful for studying the feasibility of the keto–enol form rearrangement, as the balances of partial charges of these atoms were correlated with their sensitivity. High correlation coefficients were obtained between the relative sensitivity and the balance of the partial charge of series of compounds such as phenacyl alcohol derivatives.

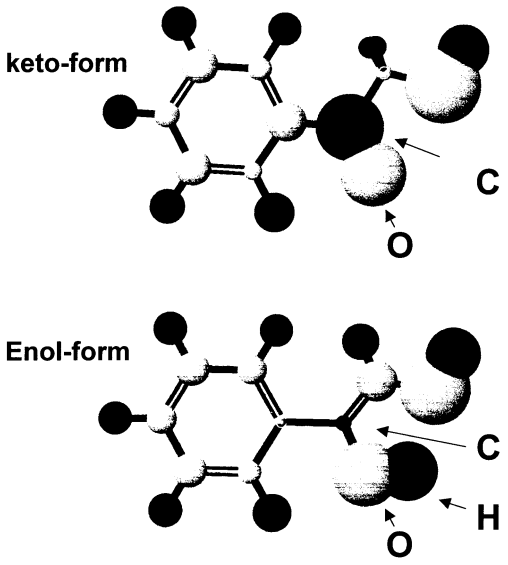

Figure 1. Electron density of phenacylalcohol.

The analytical method described here can predict the relative sensitivity detected by the chemiluminescence reaction using luminol and lucigenine, and computational chemical analysis can help to estimate sensitivity of detection in liquid chromatography and ion-chromatography.[15] In the latter, reaction products are not easily obtained. Moreover, the reaction mechanisms of other compounds under similar conditions should be the same as those described for the above compounds. Further computational chemical studies will elucidate the reaction mechanisms of chemiluminescence and the sensitivity differences, and facilitate further improvement of sensitivity.

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