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Physicochemical Interaction of the Carcinogen 4-Nitroquinoline 1-Oxide and Its Analogues with Purine and Pyrimidine Bases as the Component of Nucleic Acid[1]

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The potent carcinogenicity[3] and other biological activities[4–6] of 4-nitroquinoline 1-oxide (4NQO)[7] are well known. The mode of physicochemical interactions of 4NQO and its analogues with DNA has therefore become an important subject in connection with possible mechanisms involved in the biological activity at the molecular level. The pioneer work by Nagata and his colleagues[8] using the flow dichroism method showed that 4NQO and its quinoline derivatives, but not 4-nitropyridine 1-oxide (4NPO)[9] which lacks such biological activities[4,6,10] 4NQO exhibits, intercalate between the plane of bases of DNA. The work further referred to the base specificity of interactions of 4NQO with DNA suggesting purine bases as the active site.

Since some years ago a series of biogenetical investigations on the multiple drug resistance (*R*) factor in *Escherichia coli* has been in progress in our laboratory. At the earlier stage of the research, we found that 4NPO, which was shown not to be as effective for the elimination of *R* factors in *E. coli* giving drug sensitive cells as 4NQO,[11] could be much more effective for the selective isolation of proline-requiring mutants of *E. coli* in high efficiency than 4NQO.[12] We were then interested in the difference between unique biological activities 4NQO and 4NPO exhibit, and began to investigate on the problem from both biogenetical and physicochemical sides. In the present paper we will deal with some results on the physicochemical interaction of 4NQO and its reductive derivative *in vivo*,[13–15] 4-hydroxyl aminoquinoline 1-oxide (4-HAQO),[16] and 4NPO and its reductive derivative *in vivo*,[11] 4-hydroxylaminopyridine 1-oxide (4HAPO),[17] with adenine, guanine, cytosine, thymine, and uracil, using the difference spectrum method. In the mean time, some papers by Nagata, *et al.*[18] and Okano, *et al.*[19,20] on the problem

1) The work was reported at the 25th Annual Meeting of the Pharmaceutical Society of Japan in Tokyo, April 1968.
2) Location: *Takara-machi, Kanazawa.*
3) W. Nakahara, F. Fukuoka and T. Sugimura, *Gann*, **48**, 129 (1956).
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12) Y. Hirota, M. Inuzuka and M. Tomoeda, *J. Bacteriol.*, **91**, 2392 (1966).
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14) Y. Hashimoto, M. Terasawa and N. Toriyama, *Seikagaku*, **36**, 557 (1964).
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17) E. Ochiai and H. Mitarashi, *Ann. Rept. Itsuu Lab.*, **13**, 19 (1963).
18) M. Kodama, Y. Tagashira and Ch. Nagata, *J. Biochem.*, **64**, 167 (1968).
19) T. Okano, A. Niitsuya, A. Takadate and K. Uekama, *Gann*, **60**, 97 (1969).
20) T. Okano, K. Uekama and E. Taguchi, *Gann*, **60**, 295 (1969).

using also the difference spectrum method appeared very recently which dealt with physicochemical interactions of 4NQO and its analogues with DNA and deoxyribonucleosieds, showing that 4NQO interacts much stronger than 4NPO, and that complexes between 4NQO analogues and deoxyribonucleosides may be formed by simultaneous participation of charge transfer of π–π and n–π types. We found that the results and conclusions obtained by those authors are principally in accord with ours.

Experimental

Materials——4NQO,[7] 4HAQO,[16] 4NPO,[9] and 4HAPO[17] were synthesized in our laboratory according to the literature. Adenine and guanine of Wako Pure Chemical Industries, cytosine of Sigma Chemical Co., thymine of Tokyo Chemical Industry Co., and uracil of Katayama Chemical Co. were used without further purification. The distilled water utilized was deionized by column deionizer (Organo Co.).

Preparation of Solutions——Solvents used were five-fold diluted Sörensen's buffer solutions, namely, 0.01M phosphate buffer solutions of pH 7.10 and 8.01, 0.01M borate buffer solutions of pH 8.30, 8.66, 9.05, 9.40 and 10.31, and 0.02M glycocol buffer solution of pH 10.40. The final concentrations of 4NQO or other drugs and adenine or other bases in the solution for the measurement of spectra in the UV and visible regions were around 5.3×10^{-5}M and 5.0×10^{-3}M respectively. Experimental errors of final pH of diluted Sörensen's buffer solutions thus made was found to be within ±0.15.

For examination of an effect of neutral salt in the solvent toward the difference spectra, 0.2M NaCl was added to the solution for the measurement, its final concentration being 0.02M.

Difference Spectral Measurements——The measurements were carried out on a Hitachi EPS-2U Automatic Recording Spectrometer equipped with either tandem cells (quartz cubic double cells or four compartments) or quartz cubic cells (two compartments). The cells had 1 cm light path. Measurements with tandem cells were designed to subtract the contribution of both two components, *i.e.* drug and base, to the difference spectrum directly in a single operation. The measurements were made in the range of 230—500 mμ.

Results

All the measurements were carried out at room temperature (approx. 20°). Change of temperature in the range of several degrees was found not to give any meaningful effect toward the measurement of spectra.

Difference Spectra of the 4NQO-Base System

Using tandem cells (four compartments), difference spectra of the 4NQO-base system containing these two components of relative ratios of 4:1, 2:1, 1:1, 1:2, and 1:4 respectively, were measured. Using solutions of variable concentrations around 5.3×10^{-5}M of 4NQO and bases, difference spectra of negative sign could be observed at around 260 mμ but not in the visible region. Representative results at pH 9.4 or 10.3 are shown in Fig. 1. Intensities (OD) of difference spectra observed were up to -0.12 and varied according to the system used. Positions of difference spectra appeared to vary also according to the relative ratio of 4NQO and bases. A general tendency observed was that the smaller than 1:1 the ratio of 4NQO to base was, the stronger the intensity of observed difference spectra was. Furthermore, the ratio of 1:2 of 4NQO and bases appeared to give the strongest difference spectra in this region. The appearance of difference spectra of negative sign in the UV region thus observed was supported by the fact that using pairs of cells (two compartments), hypochromic effects of 4NQO on the absorption spectra of bases and vise versa were observed at the same rigion as reported in the literature.[19]

The 4NQO-base system of higher concentrations or around 5.0×10^{-3}M with relative ratios of 1:1.9—1:15 of 4NQO and bases gave difference spectra of positive sign in the visible region or at around 410 mμ (tandem cells). Representative results with adenine and uracil are shown in Fig. 2. As shown in Fig. 2, the intensities of spectra increased linearly as a function of the ratio of 4NQO and bases. The result of the visible region was in agreement with the literature.[18–20]

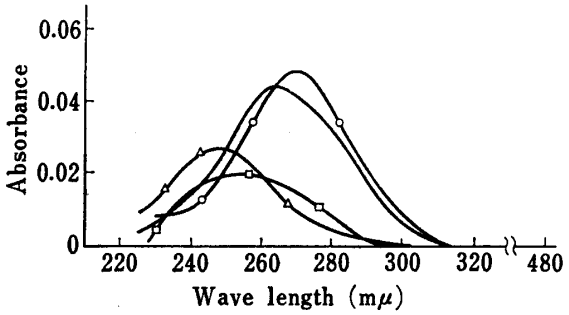

Fig. 1a. 4NQO–Adenine System

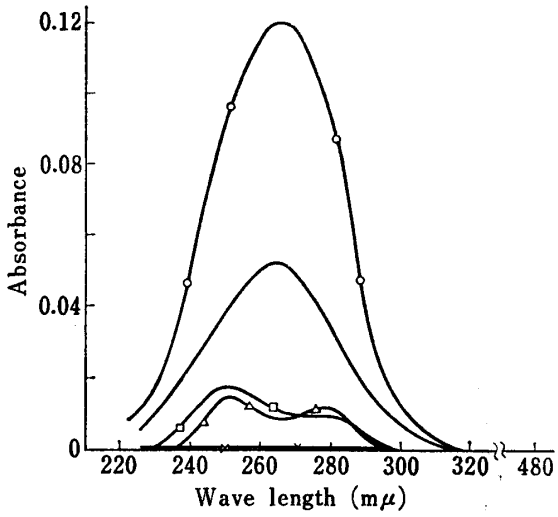

Fig. 1b. 4NQO–Guanine System

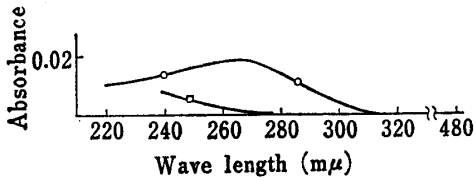

Fig. 1c. 4NQO–Cytosine System

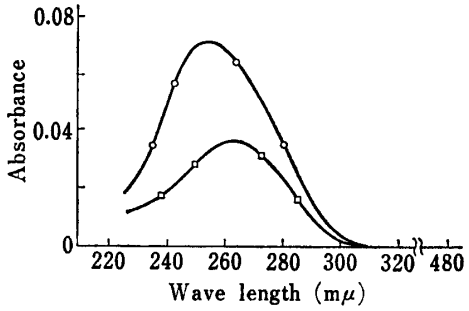

Fig. 1d. 4NQO–Thymine System

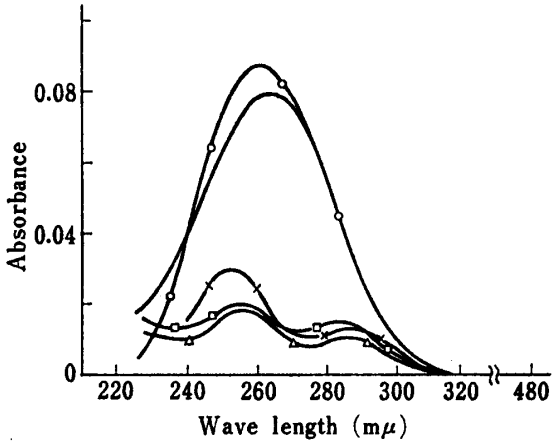

Fig. 1e. 4NQO–Uracil System

Fig. 1. Difference Spectra of the 4NQO–Base System in the UV Region measured with Pairs of Double Cubic Cells or Four Compartments

a) 4NQO–adenine system (pH 10.3) *b*) 4NQO–guanine system (pH 9.4)
c) 4NQO–cytosine system (pH 9.4) *d*) 4NQO–thymine system (pH 10.3)
e) 4NQO–uracil system (pH 10.3) concentrations of 4NQO and bases, and their relative ratios in parent heses: 4NQO, ———: 1.3×10^{-5}M(1:4)
—○—: 2.7×10^{-5}M (1:2) —□—: 5.3×10^{-5}M(1:1) —△—: 1.1×10^{-4}M(2:1)
—×—: 2.1×10^{-4}M (4:1); bases, 5.3×10^{-5}M

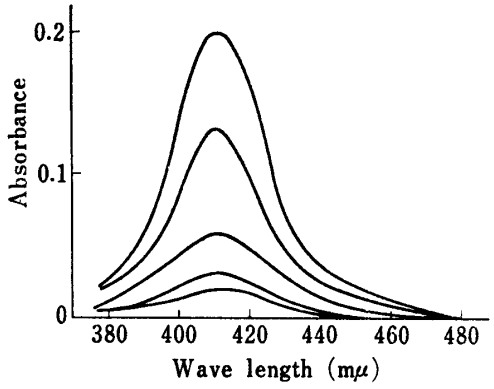

Fig. 2a. 4NQO–Adenine System

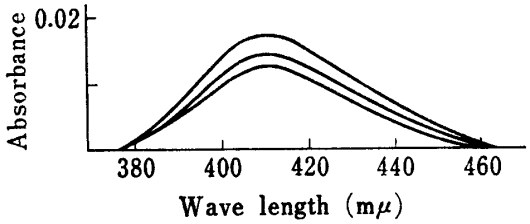

Fig. 2b. 4NQO–Uracil System

Fig. 2. Difference Spectra of the 4NQO-Base System in the Visible Region measured by Pairs of Double Cubic Cells or Four Compartments.

a) 4NQO–adenine system (pH 7.0); concentrations of 4NQO and adenine, and their relative ratios in parentheses: 4NQO, 1.0×10^{-3}M; adenine from top to bottom, 1.5×10^{-3}M(1:15), 1.0×10^{-3}M (1:10), 5.0×10^{-3}M (1:5), 2.5×10^{-3}M(1:2.5), 1.9×10^{-3}M (1:1.9)

b) 4NQO–uracil system (pH 7.0); concentrations of 4NQO and uracil, and their relative ratios in parentheses: 4NQO, 1.0×10^{-3}M; uracil from top to bottom, 1.5×10^{-3}M (1:15), 1.0×10^{-3}M (1:10), 5.0×10^{-3}M (1:5)

Difference Spectra of the 4HAQO-Base System

Using tandem cells, difference spectra in the visible region of the 4HAQO-base system of variable concentrations around 5.0×10^{-3}M with these two components in relative ratios of 1:10, 1:20 and 1:30 respectively, were measured. As bases, adenine and uracil were used. Difference spectra of positive sign could be observed at around 385 mμ. A representative result with adenine is shown in Fig. 3. The

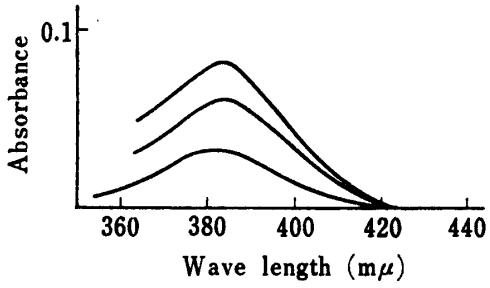

Fig. 3. Difference Spectrum of the 4HAQO–Base (Adenine) System in the Visible Region measured with Pairs of Double Cubic Cells or Four Compartments

concentrations of 4HAQO and adenine, and their relative ratios in parentheses: 4HAQO 5.0×10^{-4}M; adenine from top to bottom, 1.5×10^{-3}M (1:30), 1.0×10^{-3}M (1:20), 5.0×10^{-3}M (1:10). pH of solutions, 7.0

result was in agreement with that of 4NQO mentioned above, and also that of 4HAQO and deoxyribonucleosides in the literature.[18]

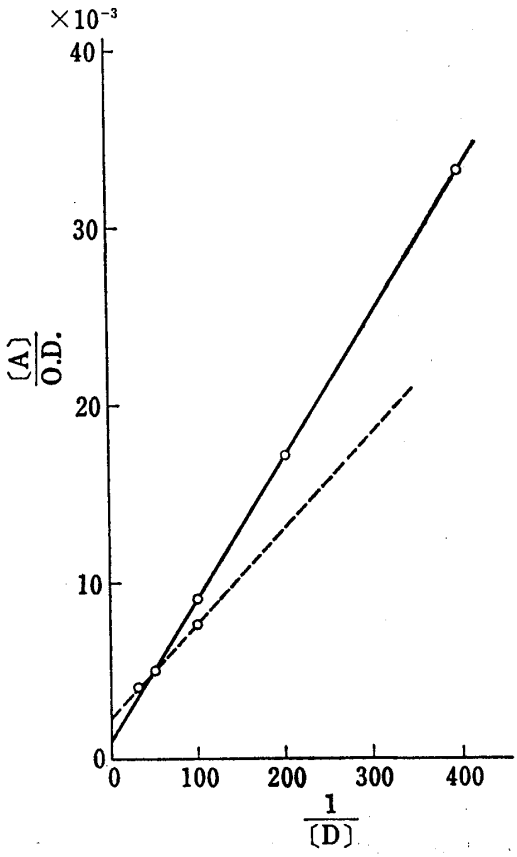

Fig. 4. Benesi-Hildebrand Plots of $\frac{[A]}{O.D.}$ versus $\frac{1}{[D]}$ for —o— the 4NQO-Adenine System, and ---o--- the 4HAQO–Adenine System

Analysis of Difference Spectra of the 4NQO- and 4HAQO-Base Systems in the Visible Region using the Benesi-Hildebrand Equation

The difference spectra observed with the 4NQO- and 4HAQO-base systems in the visible region were analyzed using the Benesi–Hildebrand equation.[21] If the difference spectra were derived from a one–to–one change transfer complex between 4NQO or 4HAQO as an electron acceptor and bases as an electron donor in the equilibrium, $A+D \rightleftarrows A \cdot D$, where A denotes the electron acceptor and D the electron donor, the association constants K for the equilibrium represented in the equation (1) could be estimated using the Benesi–Hildebrand equation in

$$K=\frac{[A \cdot D]}{\{[A]-[A \cdot D]\}\{[D]-[A \cdot D]\}} \tag{1}$$

$$\frac{[A]}{O.D.}=\frac{l}{K\varepsilon[D]}+\frac{l}{\varepsilon} \tag{2}$$

the form (2) (for 1 cm cells) where O.D. is the optical density due to the complex only, ε the extinction coefficient of the complex at the wave length of measurement, [A] the fixed concentration of the acceptor and [D] the variable concentration of the donor. The Benesi–Hildebrand plots of [A]/O.D. versus l/[D] for 4NQO and 4HAQO with $A=1.0\times10^{-3}$M and 1.0×10^{-4}M respectively, were then made showing straight line slopes as shown in Fig. 4. The result supported the origin of the spectra in the visible region as a one–to–one charge–transfer complex formation between drugs and bases. Analysis of the result then led to the calculation of ε to be 890 for 4NQO and 880 for 4HAQO respectively, and K as 14.0 for both 4NQO and 4HAQO. The literature[20] reported the value of ε and K for the 4NQO-deoxyadenosine system to be 800 and 11.5 respectively.

Experiments with 4NPO and 4HAPO

Measurements of difference spectra of the 4NPO- and 4HAPO-base systems were made under almost same conditions as with 4NQO and 4HAQO. However, any meaningful difference spectra could not be observed both in the UV and visible regions, so far as the condition used in the present investigation were concerned. The line of the result was in agreement with the literature.[19,20]

Effect of pH toward Difference Spectra

Effect of pH of Sörensen's buffer solutions toward difference spectra was examined using the 1:1 4NQO-base system. It was shown that difference spectra were generally observed in more alkaline solutions than pH 9.4, the line of the result being in accord with the literature.[19,20]

Effect of Salt

Effect of addition of extra neutral salt to Sörensen's buffer solutions on the difference spectra was examined making final NaCl concentration of solutions as 10^{-2}M. No effect was observed by addition of extra NaCl.

Discussion

The data presented in this paper showed that the carcinogens 4NQO and 4HAQO, when mixed in relative ratios of 4:1—1:30 with purine and pyrimidine bases of nucleic acid in Sörensen's buffer solutions of several pH, give rise to difference spectra not only in the visible region (spectra with positive sign) but also in the UV region (spectra with negative sign) according to the concentration of drugs and bases used. On the other hand, non–carcinogens 4NPO and 4HAPO, when mixed with those bases, were found not to give any meaningful difference

21) H.A. Benesi and J.H. Hildebrand, *J. Amer. Chem. Soc.*, 71, 2703 (1946).

spectra within the experimental conditions used in the present investigation. The results were found to be principally in accord with the earlier observation[19,20] that 4NQO derivatives interact with DNA and nucleosides more strongly than 4NPO derivatives.

The nature of physicochemical interaction (s) between 4NQO, 4NPO and their derivatives with purine or pyrimidine bases, whether it might be strong or weak, may principally be a charge transfer or shift of electron. In fact, an analysis of difference spectra observed with the 4NQO- and 4HAQO-base systems in the visible region using the Benesi–Hildebrand equation and plots supported the presence of a one–to–one charge transfer complex between those drugs and bases in buffer solution, the K values being different somewhat from those reported in the literature.[20] Importance of a charge transfer complex with 4NQO derivatives as electron acceptor[19–23] has very recently been confirmed by Kubota and his colleagues,[24] who showed that under certain conditions 4NQO as well as 4NPO can be electron acceptors to form a charge–transfer complex with tetramethyl-1,4-phenylenediamine, hydroquinone and their analogues as electron donors. It thus appears that an important contribution of a charge–transfer complex for the difference spectra between 4NQO, 4NPO and their related compounds and purine and pyrimidine bases of nucleic acids has well been proved.

Meanwhile, the difference spectra observed in the UV region in our study appeared to be strongest when the relative ratio of drug and base was 1:2. Furthermore the position and number of absorption maxima appeared to vary according to the ratio of drug and base used, the feature being quite different from that of original absorption spectra of base and drug themselves. The nature of those difference spectra in the UV region is still obscure, and there is no definite explanation for the abnormal phenomena mentioned above.

Our biogenetical work[25] on the inhibitory action of 4NQO and 4NPO derivatives on the transforming DNA in *Bacillus subtilis* has shown that among representative derivatives of 4NQO and 4NPO, only 4HAQO and 4HAPO are effective in inhibiting the transforming activity of DNA, that the inhibitory action of 4HAQO and 4HAPO is effective under aerobic condition but not under anaerobic condition, and that the proximate compound responsible for such activity may be transitory radical (s), for instance, $-\dot{N}-OH$ or $-\overset{H}{\overset{|}{\dot{N}}}-O\cdot$. The work has also revealed that 4HAPO could interacts with DNA as strong as 4HAQO. Now it appears that on an assumption that DNA is the primary target of 4NQO and its analogues for their unique biological activities, physicochemical interactions between 4NQO and its analogues and purine or pyrimidine bases, if present, could not at least be the main factor governing the biological activity of those drugs. Further experiments particularly from the biogenetical side would be necessary to clarify the real mechanism of biological activities 4NQO and 4NPO derivatives exhibit.

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22) G. Karreman, *Ann. N.Y. Acad. Sci.*, **96**, 1029 (1962).
23) Ch. Nagata, *Gann*, **54**, 401 (1963).
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25) H. Adachi, M. Inuzuka and M. Tomoeda, *Japan. J. Genetics.*, **44**, 31 (1969).