[Chem. Pharm. Bull. 16(3)556—558 (1968)]

UDC 547.831.1.09 : 547.96.09 : 541.69

Interaction of the Carcinogen 4-Nitroquinoline 1-Oxide with Protein and Aromatic Amino Acids[1]

4-Nitroquinoline 1-oxide (NQO) was first synthesized by Ochiai, *et al.*,[2] and its potent carcinogenic activity has been discovered by Nakahara, *et al.*[3] As to its *in vitro* interactions with cellular materials, Nagata, *et al.*[4] and Malkin, *et al.*[5] showed that it is bound to DNA, Okano, *et al.*[6,7] proved that the binding is worked mainly through charge-transfer interactions between NQO and the base moieties of DNA, and Endo[8] examined its chemical reactivity toward sulfhydryl group of cysteine and glutathione in connection with the biological activity of this compound. No paper, however, has come out dealing with molecular interactions of NQO with protein or amino acids. In this communication we wish to report our observations on the interaction of NQO with protein as well as with aromatic amino acids, which is best explainable in terms of charge-transfer interactions. Reference will be made also to the behavior of 4-nitropyridine 1-oxide (NPO), which is non-carcinogenic (see ref. 8), in comparison with the quinoline compound.

Albumin was chosen as an example of protein, and the crystallized, lyophilized preparation of bovine serum albumin (BSA) obtained from Sigma Chemical Co. was used. Recrystallized, spectrophotometrically pure samples of L-tryptophan (Try), L-histidine (His), L-tyrosine (Tyr), and L-phenylalanine (Phe) were used. NQO,[9] mp 153—154°, and NPO,[10] mp 159°, were synthesized in this laboratory. All solutions, prepared with Clark-Lubs' phosphate buffer mixture (pH 7.0), were shielded from the light throughout the experimental procedure. Making use of a matched pair of 1 cm cells, difference spectra were recorded on a Shimadzu MPS-50 spectrophotometer and positions and intensities of absorption maxima were determined more accurately with a Shimadzu QV-50 spectrophotometer. Because of limited solubilities of NQO and certain amino acids, the measurements were carried out at a relatively elevated temperature, 35.0°.

In Fig. 1 the difference spectra of mixtures of BSA plus NQO (or NPO) *versus* free NQO (or NPO) are represented. Intense, broad absorption bands are exhibited in the vicinity of 430 mμ (BSA—NQO system) and in the vicinity of 382 mμ (BSA—NPO system), the absorbance of the former system (A_{430}=0.545, under the concentration conditions indicated) being much larger than that of the latter (A_{382}=0.210, under the same conditions).

The difference spectra of mixtures of Try plus NQO (or NPO) *versus* free NQO (or NPO) are shown in Fig. 2, which have been cited as typical examples of spectral changes brought about by interactions between aromatic amino acids and N-oxide compounds. In the figure intense, broad absorption bands with wavelength maxima at about 433 mμ (Try—NQO system) and at about 383 mμ (Try—NPO system) are exhibited, the absorbance of the former system (A_{433}=0.510) being much larger than that of the latter (A_{383}=0.240). The locations of absorption maxima of these new absorption bands shifted toward the red with increasing

1) This paper constitutes Part XIX of a series entitled "Electronic Properties of N-Heteroaromatics." Part XVIII: *Yakugaku Zasshi*, **88**, 211 (1968).
2) E. Ochiai, M. Ishikawa, and Z. Sai, *Yakugaku Zasshi*, **63**, 280 (1943).
3) W. Nakahara, F. Fukuoka, and T. Sugimura, *Gann*, **48**, 129 (1957).
4) C. Nagata, M. Kodama, Y. Tagashira, and A. Imamura, *Biopolymers*, **4**, 409 (1966).
5) M.F. Malkin and A.C. Zahalsky, *Science*, **154**, 1665 (1966).
6) T. Okano and K. Uekama, *Chem. Pharm. Bull.* (Tokyo), **15**, 1251 (1967).
7) T. Okano and K. Uekama, *Chem. Pharm. Bull.* (Tokyo), **15**, 1812 (1967).
8) H. Endo, *Gann*, **49**, 151 (1958).
9) M. Hamana and T. Nagayoshi, *Chem. Pharm. Bull.* (Tokyo), **14**, 319 (1966).
10) E. Ochiai, *J. Org. Chem.*, **18**, 534 (1953).

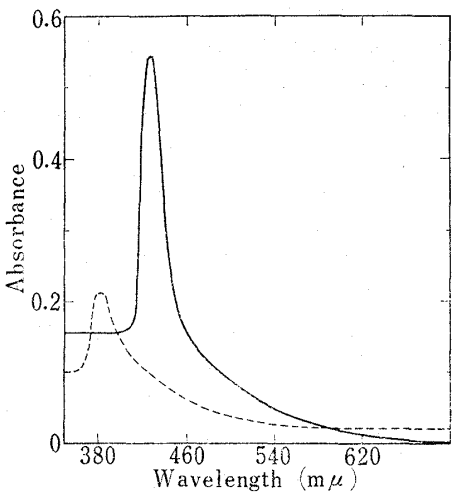

Fig. 1. Difference Spectra of Mixtures of BSA plus NQO or NPO *versus* the Respective N–Oxide Compounds

Solid line : BSA (0.5 mM)+NQO (5 mM) *vs.* NQO (5 mM)
Broken line : BSA (0.5 mM)+NPO (5 mM) *vs.* NPO (5 mM)

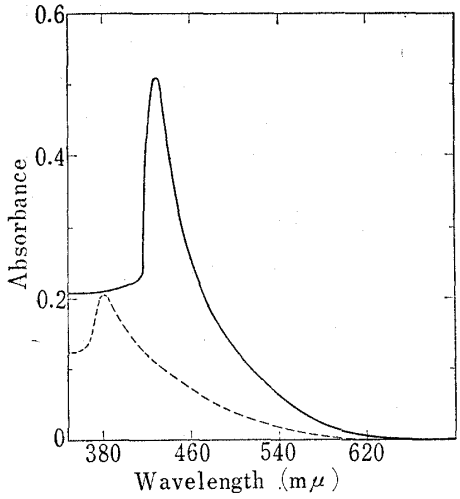

Fig. 2. Difference Spectra of Mixtures of Try plus NQO or NPO *versus* the Respective N–Oxide Compounds

Solid line : Try (25 mM)+NQO (5 mM) *vs.* NQO (5 mM)
Broken line : Try (25 mM)+NPO (5 mM) *vs.* NPO (5 mM)

concentration of N–oxide compounds, but at a fixed NQO (or NPO) concentration absorbance of each system increased linearly with increasing concentration of Try. Difference spectra of similar character have been observed for mixed systems of each of the other aromatic amino acids and either NQO or NPO (see Table I for approx. λ_{max} values). In the case of the mixed systems of NQO with Try, His, and Phe where absorbances were sufficiently large, through Benesi–Hildebrand plots[11] of the experimental data it was possible to confirm the formation of complexes of 1:1 molar ratio and also to evaluate molar extinction coefficient, ε, and formation constant, K, of each complex, which are listed in Table I.

Table I. Analysis of the Difference Bands of Mixtures of Aromatic Amino Acids and 4–Nitroquinoline 1–Oxide or 4–Nitropyridine 1–Oxide *versus* the Respective N–Oxide Compounds

Amino acid	NQO–systems			NPO–systems	λ h.o.[b]
	λ_{max} (mμ)[a]	ε	K	λ_{max} (mμ)[a]	(β unit)
Try	433	1299	5.54	383	0.53
His	426	120	2.63	382	0.66
Tyr	426	—	—	381	0.79
Phe	426	257	2.30	379	0.91

a) Values obtained with mixed systems where concentration of the respective N–oxide compounds in both sample and reference beam cells was fixed at 5 mM.
b) These values have been represented by energies of the π–eletcron systems of indole, imidazole, phenol, and toluol (for Try, His, Tyr, and Phe, respectively) (ref. 12).

In view of the electron–donor character of aromatic amino acids[12] and the electron–acceptor character of NQO[13,14] and NPO,[13,15] we are led quite naturally to the presumption that the observed new absorption bands might have arisen from charge–transfer interactions.

11) H.A. Benesi and J.H. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703 (1949).
12) B. Pullman and A. Pullman, "Quantum Biochemistry," 320 (1963). Interscience Publishers, New York & London.
13) C. Nagata, A. Imamura, K. Fukui, and H. Saito, *Gann*, **54**, 401 (1963).
14) G. Karreman, *Annals N.Y. Acad. Sci.*, **96**, 1029 (1962).
15) T. Okano, K. Uekama, Y. Isawa, and K. Tsukuda, *Yakugaku Zasshi*, **87**, 1309 (1967).

In Table I values for the energy level of the highest molecular orbital, λh.o., of each aromatic amino acid[12] are also listed. And an inversely correspondent relationship is seen to exist between the K values of the NQO–systems and the λh.o. values. What is more, the relative magnitudes of these K values as contrasted with the corresponding λh.o. values are comparable to those found in the charge–transfer interaction systems of nucleoside—NQO.[7] Furthermore, wavelength maximum of any one of the NQO–systems was situated on the longer wavelength side of that of the corresponding NPO–system, which is in accord with the fact that NQO is more efficient as a π–acceptor than NPO[13,16] (this implies that the tendency of NPO to form complexes with aromatic amino acids might be smaller than that of NQO). In another comparison, the tendencies of either NQO or NPO to bind with aromatic amino acids expressed by the magnitude of λ_{max} values is also seen to run parallel with the order of the magnitude of the λh.o. values, though the parallelism is more distinct in the case of the NPO–systems than in the case of the NQO–systems. All of these are in support of the above presumption.

It is not practicable at the present stage of investigation to correlate the results obtained at the level of amino acid quantitatively to those obtained with the macromolecule. It is noteworthy, however, that the features (locations and shapes) of the difference bands of the amino acid—N–oxide compound systems are well reflected on the difference spectra of the BSA—N–oxide compound systems. It has been known that one molecule of BSA (mol. wt. 66000) contains 64 aromatic amino acid residues (Try_2, His_{17}, Try_{19}, and Phe_{26}).[17] And it may be quite within the bounds of possibility that the BSA—N–oxide compound interactions involve charge–transfer interactions between N–oxide compounds and the π–electron systems of aromatic amino acid residues of BSA.

The present investigation show a remarkable fact that the carcinogen NQO does bind with a protein, perhaps more firmly than non–carcinogenic NPO does. And since the observed BSA—NQO interaction also points to the possibility of a more general pattern of protein—NQO interactions, it seems likely that this potentiality of NQO to bind with cellular proteins (or enzymes), besides its ability to bind with cellular DNA,[18] might have some relations to the biological activity of this carcinogen. Work on these lines is being continued, and further details will be published later.

Pharmaceutical Institute,
Tohoku University School of Medicine
85 Kita–yobancho,
Sendai, Miyagi

Teisuke Okano
Shoji Takenaka
Yukio Sato

Received November 6, 1967

16) M. Tachibana, S. Sawaki, and Y. Kawazoe, *Chem. Pharm. Bull.* (Tokyo), **15**, 1112 (1967).
17) H.E. Schultze, N. Heimburger, and G. Frank, *Biochem. Z.*, **336**, 388 (1962).
18) Y. Matsushima, I. Kofuna, and T. Sugimura, *Seikagaku*, **39**, 311 (1967) (an abstract).