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DECOMPOSITION OF VITAMIN A BY NO_2

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Reactions of gaseous NO_2 with Vitamin A's (retinol and retinal) dissolved in CCl_4 were carried out in test tubes. The concentrations of Vitamin A's were monitored by HPLC using UV-VIS absorption spectrometry for each compound. These Vitamin A's were found to be easily decomposed by bubbling NO_2 through the solution at concentrations of the order of ppm in air at the flow rate of 30 ml/min.

In our previous paper,[1] we have reported the gas-phase rate constants for the reactions of NO_2 with conjugated olefins at room temperature, and the results showed that the rate constants for conjugated olefins were larger than those for simple monoolefins by factors of 10^3-10^4. The finding that the conjugated diolefins have a large reactivity towards NO_2 stimulated us to study the liquid phase reaction of biomolecules having conjugated carbon-carbon double bonds with gas phase NO_2. We chose two Vitamin A's, i.e. retinol and retinal, which have five conjugated carbon-carbon double bonds. As far as we know, no report on reactions of NO_2 with biomolecular conjugated polyolefins has been made from the viewpoint of possible biological effect by NO_2, so far.

After the ampoules of retinol and retinal (Sigma Chem. Co.) were opened, the contents were dissolved in CCl_4 (Kanto Kagaku) and the solutions were kept in the refrigerator (-20 °C). Just before the experiments, the original solutions were further diluted with the same solvent and the final concentrations were 14.3 ppm for retinal and 13.8 ppm for retinol, respectively. About 15 ml of the solution was put into a glass tube of 1.6 cm diameter. Nitrogen gas containing NO_2 (96.0

ppm, Nippon Sanso) was bubbled into the solution at the rate of 30 ml/min through a glass ball filter (Kinoshita No.4). After a given period of bubbling, a portion of the solutions was placed in a UV-VIS spectrometer cell. Figure 1 shows the variation of UV-VIS spectra of retinal before and after some intervals of bubbling. Figure 2 shows the results for retinol. These figures indicate that the absorption due to Vitamin A's decreased and absorption at lower wavelength due to products increased, as the bubbling time elapsed.

In order to obtain a rough idea of the reactivity of Vitamin A's, we followed the decay profile of retinal in CCl_4 with bubbling NO_2. Throughout the experiments, shielding against room light was necessary to prohibit the isomerization or decomposition of retinal. Retinal was analyzed by HPLC, using a 15 cm Zorbax-ODS column, by observing the absorption at 360 nm. Acetonitrile was used as the eluent. Reactions and analysis were performed at room temperature. The results are shown in Fig. 3. Bubbling pure N_2 or O_2 at the rate of 30 ml/min did not decrease the

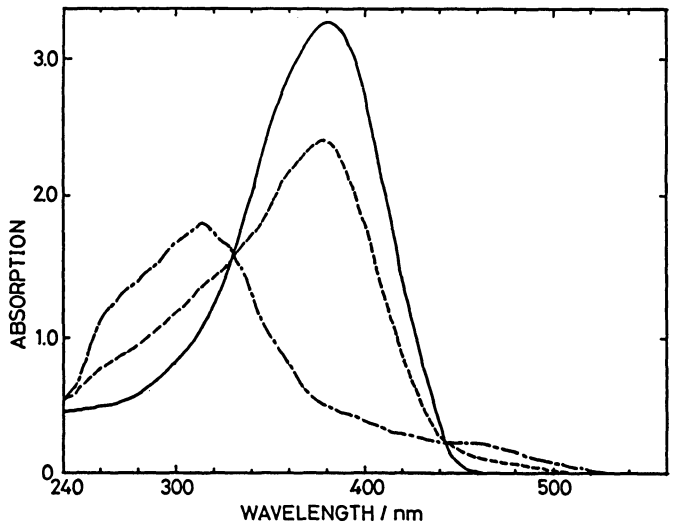

Fig. 1. Change of UV-VIS spectra of retinal by bubbling NO_2.
$[retinal]_0$ = 14.3 ppm in CCl_4,
$[NO_2]$ = 96 ppm in N_2 (30 ml/min)
———— , before;
--------- , 10 min bubbling;
—·—·—·—, 40 min bubbling.

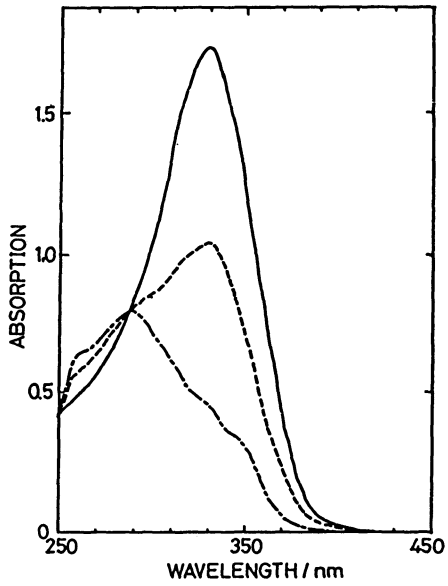

Fig. 2. Change of UV-VIS spectra of retinol by bubbling NO_2.
$[retinol]_0$ = 13.8 ppm in CCl_4,
$[NO_2]$ = 96 ppm in N_2 (30ml/min)
———— , before;
---------- , 16 min bubbling;
—·—·—·— , 24 min bubbling.

absorption of retinal under the present experimental conditions. However, bubbling of the standard NO_2 gas in pure N_2 (96 ppm) and the gas diluted with O_2 to a concentrattion of 16 ppm of NO_2 caused a decrease in the absorption. Similar results were obtained for retinol, but the reaction appeared to proceed faster. The appearence of a few peaks on the LC-chromatograms before the peak of Vitamin A was noticed and this suggested the formation of degraded products.

It is interesting to know how fast the reaction proceeds, but it is difficult to determine the absolute rate constants between substances in liquid phase and in bubbling gas. A competitive reaction between retinol and α-terpinene towards NO_2 was carried out in CCl_4 to obtain a relative rate constant of retinol against α-terpinene. The initial concentration of retinol was 6.9 ppm, and that of α-terpinene was 8.4 ppm. The gas for bubbling contained 38.4 ppm of NO_2, 40% of N_2 and 60% of O_2, and total flow rate was 25 ml/min. The plots of $\ln([\alpha\text{-terpinene}]_0/[\alpha\text{-terpinene}]_t)$ versus $\ln([retinol]_0/[retinol]_t)$ for the experiment is shown in Fig. 4. The gradient (6.8) gave the relative rate constant of retinol. The gas

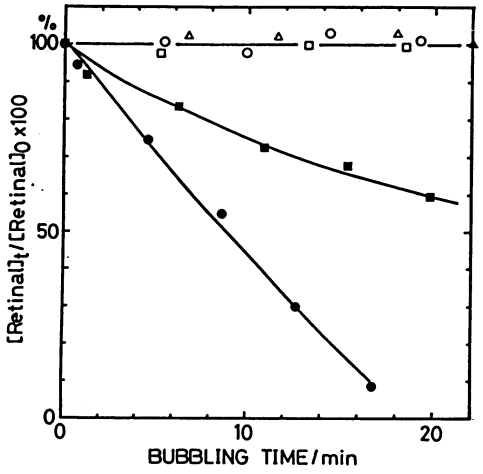

Fig. 3. Decomposition of retinal by NO_2.
△, no bubbling;
○, bubbling of N_2;
□, bubbling of O_2;
●, 96 ppm NO_2 in N_2;
■, 16 ppm NO_2 in O_2 and N_2;
total flow rate was 30 ml/min.

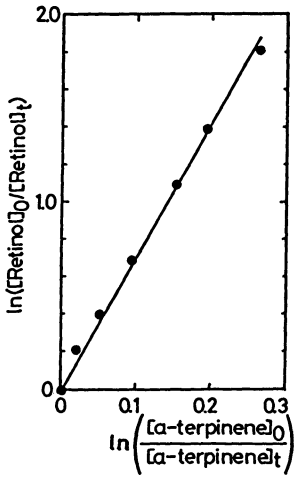

Fig. 4. Decay plots of retinol against α-terpinene by the reaction with NO_2 in CCl_4.
$[retinol]_0$ = 6.9 ppm;
$[\alpha\text{-terpinene}]_0$ = 8.4 ppm;
$[NO_2]_0$ = 38.4 ppm;
$[N_2]$ = 40 %, $[O_2]$ = 60%.

phase rate constant for the reaction of α-terpinene with NO_2 was recently reported to be $(6.5 \pm 1.4) \times 10^{-18}$ $cm^3 molecule^{-1} s^{-1}$, by Atkinson et al.[2] In the gas phase, α-terpinene reacts with NO_2 so fast that we can observe the loss of reactants over a period of a few minutes. In view of the finding that retinol was decomposed in CCl_4 by NO_2 6.8 times faster than α-terpinene, Vitamin A might be decomposed in human body at an observable rate, if it is exposed to NO_2 at ppm levels.

Shalamberidze reported[3] that exposure of NO_2 of 0.14 mg/m^3 (75 ppb) suppressed a dark adaptaion. (When the light-stimulus is discontinued, there is a certain period of "dark adaptation" necessary before the eye regains its maximum sensitivity. "Dark adaptation" is usually measured as a decrease in the threshold with time in the dark.[4]) If in vivo exposure to NO_2 caused the decrease of Vitamin A, as was observed in vitro, loss of Vitamin A might explain the suppression of the dark adaptation. On the other hand, it is worthwhile to examine whether the reaction of NO_2 with Vitamin A occurres in human lungs and causes any damage, because Vitamin A storing cells are located in the alveolar septum between the blood capillary and epithelium, i.e. in the fibroblast of connective tissue,[5] and Vitamin A possesses anticarcinogenic activity.[6] Further studies are required to clarify the biological effects of the loss of Vitamin A by the reaction proposed in this paper.

References

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