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ON THE REACTION MECHANISM OF THE RADIOLYSIS OF THE AQUEOUS NICOTINAMIDE ADENINE DINUCLEOTIDE

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The radiolysis studies of aqueous NAD^+ solutions have been performed. It is suggested that NADH and $(NAD)_2$ are produced in the irradiated solution. A reaction scheme including a dimerization of NAD free radicals and the formation of NADH is presented.

Many studies have been reported about the mechanism of oxidation and reduction induced on nicotinamide adenine dinucleotide (NAD), one of the coenzymes concerned with oxidation and reduction in vivo.[1,2] Most of those studies have been carried out under biological conditions.

A series of pulse radiolysis studies of NAD have been reported by Land and Swallow.[3,4] They reported that when NAD^+ was subjected to radiolysis it was reduced to form a single free radical (Reaction 1), which reacted with another radical to give a dimer (Reaction 2).

$$NAD^+ \rightsquigarrow NAD\cdot \qquad (1)$$

$$2NAD\cdot \longrightarrow (NAD)_2 \qquad (2)$$

In this work we have studied radiolysis of aqueous NAD^+ solutions to elucidate the mechanism. An absorption spectral analysis has shown the formation of NADH (Reaction 3) in addition to dimerization of the radicals.

$$2NAD\cdot \xrightarrow{H^+} NADH + NAD^+ \qquad (3)$$

NAD^+ and NADH were obtained from Boehringer Manheim GmbH and used without further purification. Sodium formate was reagent grade. Solutions usually contained $1.5\text{-}2.0\times10^{-4}$M NAD^+ or NADH with 10^{-1}M sodium formate. Solutions were degassed by the method of freeze-thaw cycle in a spherical glass vessel, and then transferred into the reaction tube for irradiation. Solutions were irradiated with ^{60}Co γ-rays at room temperature. A Shimazu Double-40R Wide spectrophotometer was used to measure the absorption spectra of irradiated solutions. An unirradiated solution was placed in the reference side of the spectrophotometer and a differential spectrum between the irradiated and the unirradiated solution was measured.

The differential spectra are shown in Fig. 1. The solid and dotted lines show spectra observed immediately after irradiation and two days after irradiation, respectively. The former has two maxima at about 283 nm and 340 nm (we shall call these spectra as absorption band 1 and absorption band 2, respectively). When the solutions were left in air after irradiation, the absorption band 2 decreased to half in about 100 min and vanished in two days, whereas the absorption band 1 decreased slightly in two days.

The behavior of the two absorptions differed from each other in the decay and dose dependence. The ε·G values of those two absorptions are plotted against the absorbed dose in Fig. 2. The absorption band 1 increases with an increase of the dose to about 2×10^4 rad and then decreases slowly. The absorption band 2 decreases rapidly above about 1×10^4 rad. The different behavior of the absorption bands 1 and 2 suggests that at least two different kinds of species are

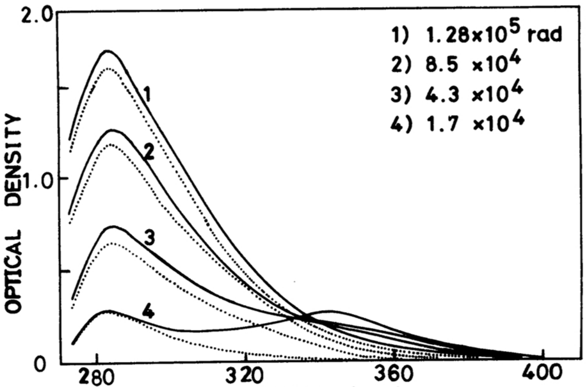


Fig. 1. The differential spectra of irradiated NAD$^+$ solutions (2×10^{-4}M) immediately after irradiation (solid line) and two days after irradiation (dotted line). Dose rate was 2.8×10^4 rad·hr^{-1}.

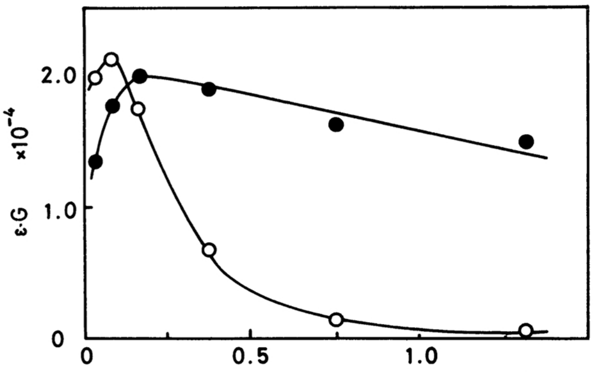


Fig. 2. The ε·G values of irradiated NAD$^+$ solution (2×10^{-4}M). Dose rate was 2.8×10^4 rad·hr^{-1}. Observed at 283 nm (closed circles) and 340 nm (open circles).

produced in the irradiated NAD^+ solution.

The spectral shape and location of the absorption band 2 are identical with those of NADH in the region from about 320 nm to about 400 nm. In order to test the possibility that the absorption band 2 is due to NADH, irradiation of NADH was also investigated. The absorption due to NADH at 340 nm decreased with irradiation. The behavior of the absorption band is approximately the same with that of the absorption band 2 observed above about 2.5×10^4 rad. When the both solutions were left in air after irradiation, the absorption at 340 nm decreased or vanished. Thus, it may be concluded that the absorption band 2 is due to NADH.

Dose rate dependences of the absorption bands 1 and 2 were also investigated. Figure 3 shows that the $\varepsilon\cdot G$ values of the absorption band 1 increase with the increase of dose rate. This finding suggests that the absorption band 1 arises from a dimer, $(NAD)_2$. The dimer is considered to be stable during irradiation. With irradiation of NAD^+ at a large dose, the absorption band 1 was observed predominantly (see Figs. 1 and 2). The experimental result is well explained if we take account of the stability of the dimer.

Pulse radiolysis study of NAD^+ solution was also carried out in order to examine the above assignment. The transient absorption at 400 nm, which has

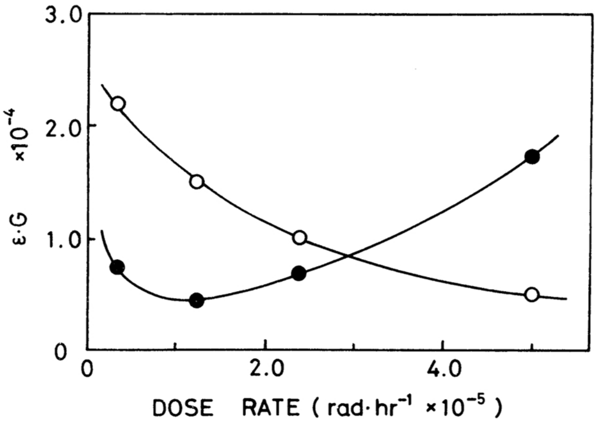


Fig. 3. The $\varepsilon\cdot G$ values at 283 nm (closed circles) and 340 nm (open circles) against dose rate. (Total dose: 3×10^4 rad, 1.5×10^{-4}M NAD^+)

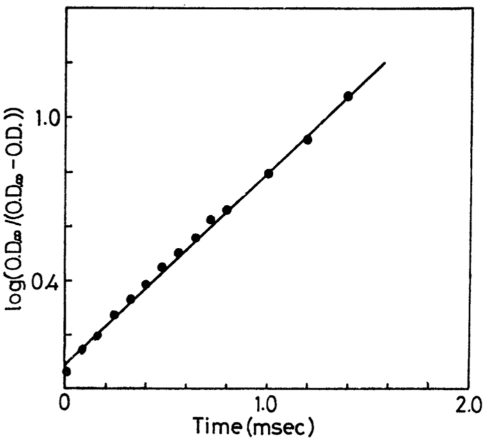
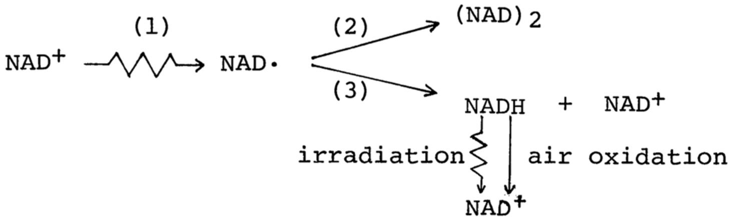


Fig. 4. First-order plot of appearance of the absorption at 340 nm observed by pulse radiolysis. (8×10^{-4}M NAD^+)

already been assigned to NAD radical,[3] appeared after electron pulse (4 MeV electron accelerator; 1.6 μs pulse width, 2 krad per pulse) and decayed predominantly according to a second order kinetics. The absorption at 340 nm, however, built up with a first order kinetics as is shown in Fig. 4. The transient behavior also supports the results obtained by the study of γ-radiolysis.

In summary, it has been shown that when aqueous NAD^+ solution is irradiated, NADH and $(NAD)_2$ are produced. A possible reaction scheme is given below.



The scheme contains a dimerization (Reaction 2) and the formation of NADH (Reaction 3) from NAD free radicals. It is assumed that $(NAD)_2$, which gives strong absorption at 283 nm, is rather stable and remains after irradiation, whereas NADH formed by the reaction 3 gives NAD^+ upon irradiation and air oxidation. It is suggested that light absorbing species at 340 nm is NADH, which Land and Swallow[3,4] failed to identify.

References

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