# Methylene Blue Sensitized Degradation of Sodium Hyaluronate through Photoinduced Electron Transfer

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Sodium hyaluonate (NaHA) and Methylene Blue (MB) formed a complex through charge transfer (CT) interaction or ion association in aqueous solution. Irradiation of the complex using a 313-nm light under argon caused degradation of NaHA. The mechanism proposed on the basis of the effect of excitation wavelength and oxygen, and by comparison with the degradation of NaHA using Rose Bengal (RB) and $TiO_2$, was that photoinduced electron transfer from NaHA to upper excited state MB initiated the degradation.

The liquefaction of vitreous body on aging has been explained as occurring due to the depolymerization of hyaluronic acid,[1] the mechanism of which has been thought until now to be initiated by reactive oxygen species generated in the body, such as hydroxy radical[2a, 3] ($OH^\bullet$) and superoxide anion radical[2] ($O_2^{\bullet -}$). We are particularly interested in the MB-sensitized degradation of NaHA originally reported by Chakrabati et al.,[4] who proposed that singlet oxygen ($^1O_2$) acted as the reactive oxygen. However, on the basis of the data presented here, we report that neither $^1O_2$ nor $OH^\bullet$ participates in the MB-sensitized degradation, which probably proceeds through photoinduced electron transfer (PET) from NaHA to upper excited state MB.

The photooxygenation of organic substrates by $^1O_2$ has been studied in great detail.[5] Our doubts as to whether $^1O_2$ is the reactive oxygen in the degradation of NaHA arose because there are no reactive sites in NaHA that would be attacked by $^1O_2$, which usually undergoes ene-reaction and cycloaddition at carbon-carbon double bonds, and also reacts with heteroatoms like sulfur.[4] Therefore, we reinvestigated the mechanism by analyzing the molecular weight of NaHA decomposed using a high pressure liquid chromatograph with a Shodex SB-806HQ packed column and a refractive index detector attached.

Firstly, it has been confirmed that the NaHA (Sigma, H-5388) used in our study does not undergo absorption in wavelength regions of longer than 290 nm; accordingly, it does not degrade on irradiation using a high-pressure mercury lamp through a Pyrex filter.

Secondly, the efficiency of the MB-sensitized degradation of NaHA (0.02 wt%) was compared with that for RB and Erythrosine (ET), which are more efficient $^1O_2$ sensitizers than MB: the quantum yield for production of $^1O_2 = 0.83$ for RB, 0.68 for ET, and 0.52 for MB.[6] As can be seen in Figure 1, when RB and ET were used as sensitizers, the depolymerization rate became significantly slower than that for MB on irradiation using a high-pressure mercury lamp through a Pyrex filter. In addition, we noticed the very important fact that the MB-sensitized degradation proceeded efficiently even without oxygen. Thus, it was concluded that the primary degradation of NaHA in the presence of MB was not caused by $^1O_2$.

Thirdly, we examined whether the carboxyl and *N*-acetyl groups in NaHA were essential for photodegradation. Several polysaccharides, the structures of which resemble NaHA were employed; namely, alginic acid (AA) and polygalacturonic acid (PGA) which have carboxyl but no *N*-acetyl groups, and also pullulan (PL) and methyl cellulose (MC) which have neither carboxyl nor *N*-acetyl groups. When the polysaccharides were irradiated under conditions similar to those used for NaHA, the polysaccharides with carboxyl groups (i.e. AA and PGA) decomposed, whereas those without carboxyl groups (i.e. PL and MC) did not. Thus, there can be no doubt that carboxyl groups play an important role in photodegradation. However, a monosaccharide with a carboxyl group like D-glucuronic acid did not decompose under similar conditions.

Fourthly, we investigated the photodegradation of the polysaccharides using $TiO_2$ as a photocatalyst to generate $OH\bullet$ in aqueous solution. The irradiation of the polysaccharides in the presence of powdered $TiO_2$ in aerated aqueous solution caused degradation of all the polymers. Therefore, we concluded that $OH^\bullet$ was not involved in the MB-sensitized degradation of NaHA.

Fifthly, it was found that the MB-sensitized degradation of NaHA depended on excitation wavelength. As shown in Figure 2, which plots main chain scission numbers ($M_0/M - 1$, MCSN) vs. irradiation time: $M_0$ = initial molecular weight of NaHA and M = molecular weight of NaHA after irradiation, when MB was excited by wavelengths of light longer than 430 nm, the use of which can generate $^1O_2$, degradation of NaHA did not occur at all; but, in contrast, it accelerated on excitation by wavelengths of light shorter than 430 nm. Thus, the observed wavelength dependence suggests the participation of the upper excited state of MB in photodegradation.

It has been demonstrated that, as the energy gaps between the upper excited state ($S_2$) and the lowest singlet excited state ($S_1$) for azulenes decrease, the fluorescence quantum yields from $S_2$ decrease due to the increased rates of internal conversion from $S_2$ to $S_1$; therefore, an energy gap of more than ca. 40 kcal/mol (1 kcal/mol = 4.184 kJ/mol) is necessary if the fluorescence process from $S_2$ is to compete with the internal conversion.[7] When MB was excited using 320- and 580-nm lights, no change in the fluorescence spectra was observed; however, on using a 320-nm light the intensity of fluorescence decreased markedly and fluorescence quenching occurred in proportion to the concentration of NaHA between ca. 0.0002 and 0.001 wt%. On the other hand, it was clear from the absorption spectrum that for MB there is a large energy gap (58 kcal/mol) between a visible absorption band at around 660 nm wavelength and an

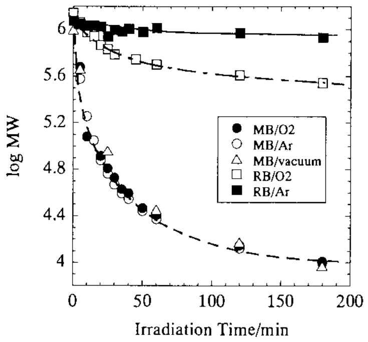


**Figure 1**. MB and RB-sensitized degradation of NaHA: log MW (molecular weight) of NaHA vs. irradiation time.

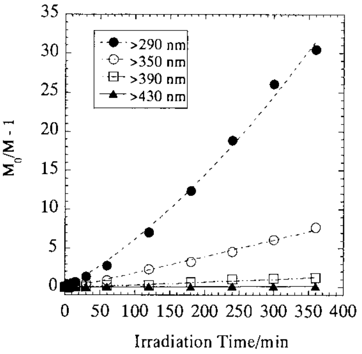


**Figure 2**. Effect of excitation wavelength on the degradation of NaHA.

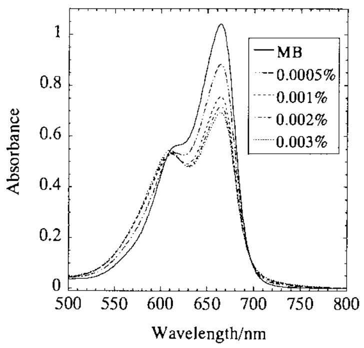
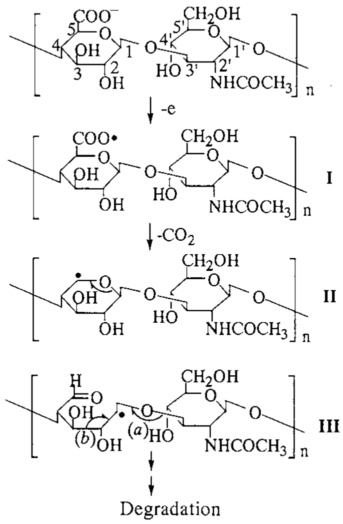


**Figure 3**. Formation of the charge-transfer complex between NaHA (0.0005-0.003 wt%) and MB (1 x $10^{-5}$ M) in aqueous solution.

ultraviolet absorption band at around 290 nm wavelength; therefore, it is possible that the energy gap accelerates the reaction of MB in the upper excited state.

Finally, it has been reported that methyl viologen ($MV^{2+}$) forms CT complexes with polymeric carboxylic acids such as poly(acrylic acid) and poly(methacrylic acid) as well as with acetic acid, acrylic acid, and methacrylic acid,[8] and also that only in the case of polymeric carboxylic acids does irradiation of the complexes cause decomposition of the acids and production of radical cation $MV^{\bullet+}$ through PET. We observed that NaHA formed the CT complex with $MV^{2+}$, irradiation of which using a 313-nm light caused degradation of NaHA. Furthermore, as shown in Figure 3, the absorption spectra for MB in aqueous solution changed in the presence of 0.0005 to 0.003 wt% NaHA, to indicate the formation of complex through CT interaction or simple ion association. It is noteworthy that Thionine (TN) also formed the complex and was as efficient a sensitizer for the degradation of NaHA as MB. It is known that TN forms an inclusion complex with cyclodextrin (CD) in aqueous solution.[9] Similarly, we observed that de-aggregation and aggregation of MB were induced in the presence of β-CD and γ-CD, respectively, as has been found in aqueous $SiO_2$ colloids.[9] When NaHA was irradiated in the presence of MB (3 × $10^{-5}$ M) and CD (1 × $10^{-2}$ M), the degradation of NaHA was significantly suppressed (MCSN = 27.2 for MB, 4.8 for MB/β-CD, and 6.0 for MB/γ-CD). This is probably because complexation of MB to CD suppressed that to NaHA. Therefore, we conclude that cationic dyes like MB and TN interact with the carboxyl groups in polysaccharides in the ground states, as in the case of $MV^{2+}$, and due to this interaction the photoreaction of MB in the upper excited state might compete with internal conversion from the upper state to $S_1$.

**Scheme 1.**



On the basis of the results described above, we propose a possible mechanism including a PET process between upper excited state MB and ground state NaHA. The electron transfer from the carboxylic anion to MB causes carbonyloxy radical **I**, which readily releases carbon dioxide to generate carbon center radical **II**, as shown in Scheme 1. The C1-O bond dissociation in C1-O-C5 from **II** gives another carbon center radical **III**, followed by further dissociation between the C3'-O bond (path *a*) or the C2-C3 bond (path *b*) to cause degradation of NaHA. The free energy change associated with the PET reaction was estimated to be exothermic if the oxidation potential ($E_{ox}$) of NaHA is less than ca. 1.7 V vs. SCE in $H_2O$, using a Rehm-Weller equation,[10] 98.6 kcal/mol as the energy of the upper excited state, and the reduction potential ($E_{red}$ = -0.19 V vs. SCE in $H_2O$) for MB. However, $E_{ox}$ was not determined using cyclic voltammetry because it was more than 1.5 V vs. SCE.

As shown in Figure 1, the degradation of NaHA was enhanced by oxygen for RB, but not for MB. Therefore, we are conducting further studies using other dyes to determine what factors control the degradation of NaHA.

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