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Photochemical Changes of Malonaldehyde by the Presence of Aflatoxins in Model Systems

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Aflatoxin 존재하에서의 수용액중 Malonadehyde의 광화학적 변화

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요 약

자외부흡광 스펙트럼의 변화와 티·비·에이(TBA) 반응으로 살필 때, 수용액 속에서의 말론알데하이드(malonaldehyde)는 파장 260mμ 부근의 자외선조명에 의하여 광화학적 변화를 받는다. 이 용액에 아프라톡신(aflatoxin)을 가하면 파장 360mμ 부근의 광선에 의해서 이와 같은 변화가 재빨리 일어나며, 또 보통 형광등을 이용한 실험실의 조명에 의해서도 비록 그 변화속도는 느리지만 마찬가지로 일어난다. 이와 같은 변화는 비가역적이며, 이 반응에서 아프라톡신은 다만 감광제로서의 역활을 한다. 또 이 반응의 기작은 말론알데하이드 분자의 평행적 이중결합체 형성에 의한 것 같다.

Abstract

On the basis of UV spectral changes and TBA reaction, malonaldehyde (MA) in aqueous solution receives considerable photochemical modification by UV light of short wavelengths around 260 mμ. When aflatoxin is added in the solution, UV light of long wavelengths around 360 mμ induces such changes quite rapidly and although the rate of change is rather slow, it is also true even with ordinary laboratory illumination(fluorescent). The modification is irreversible in nature and the role of aflatoxin in this reaction is identified as a photosensitizer. The mechanism involved in this modification is apparently due to the parallel dimerization of MA molecules, but not by head to tail combination of the molecules.

Introduction

Malonaldehyde (MA) occurs as an oxidation product of polyunsaturated fatty acids (Dahle *et al.*, 1962; Kwon and Olcott, 1966a). Since such fatty compounds of foods oxidize readily during processing and storage, determination of MA either by the 2-thiobarbituric acid (TBA) test (Tarladgis *et al.*, 1960) or ultraviolet (UV) spectrophotometry (Kwon and Watts, 1963) has been widely used as an index of lipid oxidation

of food products. However, some limitations of MA as a measure of lipid oxidation have been reported (Kwon and Olcott, 1966b). Aflatoxins are toxic metabolites of certain mold species of the genus *Aspergillus* (Wogan, 1966) and may accumulate in certain foods during fermentation and storage.

In the course of studies on the possible interaction of aflatoxins with food constituents, we observed significant changes in the UV absorption spectra of MA and toxin mixtures subjected to UV light irradiation. This paper describes the nature of the spectral changes and discusses the possible role of such changes in MA in foods.

Experimental

Aflatoxins. Afllatoxins B_1, B_2, G_1 and G_2 were eluted from preparative thin-layer chormatographic (TLC) separations of crude toxin extract, as described previously (Kwon and Ayres, 1967). B_1 obtained from the USDA, Northern Regional Research Laboratory, Peoria, Illinois, was also used. All toxins showed a single component on TLC plates.

MA. An aqueous hydrolyzate (Kwon and Watts, 1963) of MA bis-(diethyl acetal) was distilled twice at 100° in an all-glass apparatus. Ethanol in the hydrolyzate distills prior to pure aqueous MA; the yellowish polymeric MA remained in the distilling appratus. Purity of the MA preparation was checked by the pH dependent UV spectral properties (Kwon and Watts, 1963).

Reaction conditions. Equimolar concentration of the toxin and MA were mixed to give final concentrations of 2×10^{-5} *M* either in 0.05 *M* phosphate buffer, pH 7, or in 0.05 *M* KCl-0.0067 *M* HCl buffer, pH 2.2. A strong arc-discharge mercury vapor lamp (Matheson Scientific) was placed horizontally at a distance of 46cm from the reaction mixture in a sealed 1-cm quartz cell. This resulted in a UV ray (at 366 mμ) of 1680 microwatts per cm^2. At time intervals, the UV absorption spectrum of the reaction mixture was recorded (Beckman DK-2). The reaction mixtures were also subjected to TBA test and further examined on analytical TLC plates by using 3% methanol in chloroform (V/V) as a developing solvent.

In a separate experiment, a similar reaction was carried out in dichloromethane. MA was extracted with dichloromethane from the above MA preparation, concentrated by evaporation of the solvent, and dried with anhydrous sodium sulfate. B_1 toxin was eluted with dichloromethane directly from the preparative TLC plates. Then, both reactants were mixed and irradiated.

Results and Discussion

The reaction mixture of MA and B_1 in the neutral buffer solution had absorption bands at 267 and 363 mμ (Fig. 1). MA has a band at 267 mμ (Kwon and

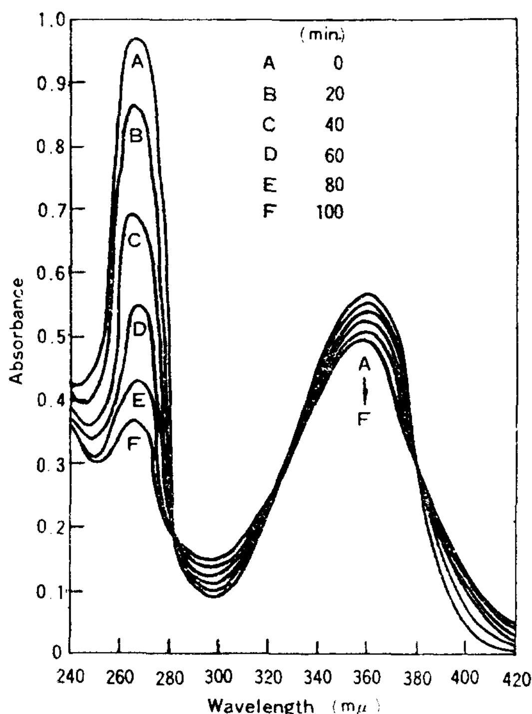

Fig. 1. Ultraviolet spectra of aflatoxin B_1 and malonaldehyde reaction mixture in a neutral buffer solution during irradiation by ultraviolet light at 366mμ. The final concentration of both reactants was 2×10^{-5} in 0.05 *M* phosphate buffer. pH 7. The spectra were obtained after different lengths of irradiation as indicated.

Watts. 1963) and B_1 has bands at 265 and 363 mμ in this buffer solution. Upon irradiation by UV light, the absor-

ption band at 267 mμ diminished quite rapidly, while that at 363mμ diminished rather slowly (Fig. 1). Moreover, the absorbance at 532 mμ of the reaction mixtures after TBA reaction also decreased linearly with that at 267mμ (Fig. 2). Eventually these mixtures became unreactive with TBA, indicating that MA is no

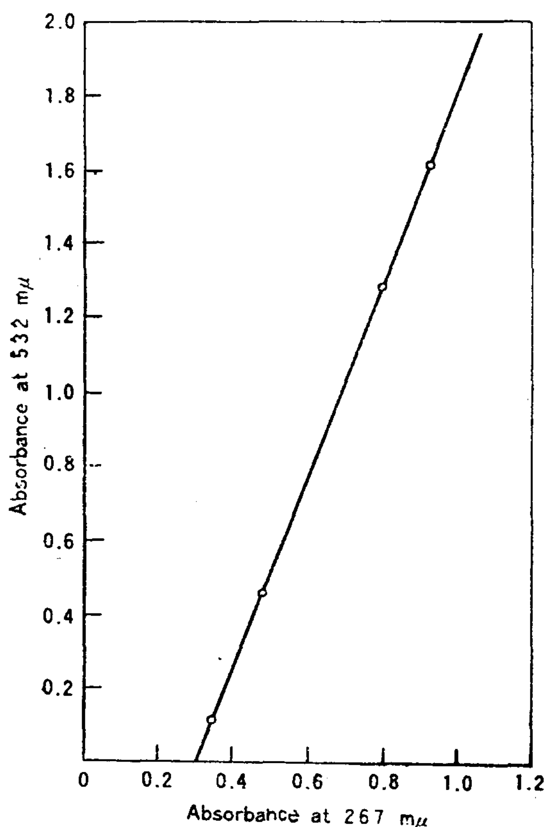

Fig. 2. Relationship between absorbance at 267 mμ and that of 532 mμ after TBA reaction of the reaction mixiures. The absorbance of 0.3 at the 267mμ intercept is due to the absorption of the toxin molecules.

longer present in the reaction mixture. This observation shows that MA undergoes a certain reaction in UV light and that the reaction is irreversible. The reaction mixture of MA with B_2 gave similar spectral changes, but with a faster rate than that with B_1(Fig. 3). Both reaction mixtures kept under ordinary laboratory illumination (fluorescent) also produced

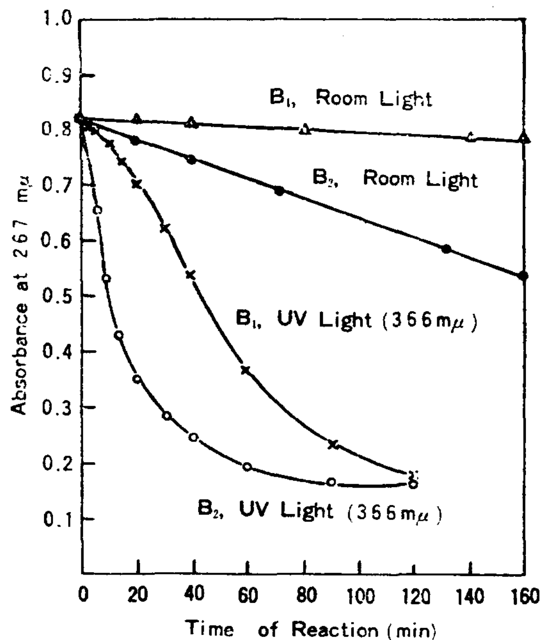

Fig. 3. Rate of spectral changes of the reaction mixtures at 267 mμ with different toxin and different type of light irradiated

such spectral changes although the rate of change was much slower than those induced by UV irradiation (Fig. 3). G_1 and G_2 acted similarly to B_1 and B_2. In all cases, however, no such changes were observed with reaction mixtures kept in the dark. Thus, the spectral changes are due to a photochemical interaction of the reactants. Irradiation by UV light at 366 mμ of the MA solution without the toxins did not produce any change in the spectrum of MA, suggesting a definite role of toxin molecules in this photochemical reaction.

Photochemical additions between olefins and carbonyl compounds have long been known to result in the formation of oxetane derivatives (Schneider and Meinwald, 1967; Turro *et al.*, 1967). The possibility that MA reacts with olefinic linkages in toxin molecules was tested. Apparently, toxin molecules are not directly involved in the reaction based on the following evidence: 1) the spectral changes observed at 363 mμ were not greater than those in the control toxin solution without MA; 2) no difference was observed in the TLC pattern of the reaction mixtures with and without MA after

UV irradiation; and 3) difference spectra of the reaction mixtures (against the control toxin solution without MA) after UV irradiation produced only typical spectra of MA. Consequently, the observed spectral changes are mainly attributed to a photochemical change in MA. Apparently, toxins respond toward UV irradiation in the same manner with and without MA in the solution, and thus toxin acts as a photosensitizer inducing photochemical changes in MA in the reaction mixtures.

A MA solution was exposed to a mineralight lamp (UVS-12, Ultra-Violet Products) emitting a weak UV light of short wavelengths. Although no change was observed in the spectrum of MA upon irradiaion of UV light at 366 mμ, there were gradual changes in the spectra of the MA solution irradiated with UV light of the shorter wavelengths as these were with the toxin-MA reaction mixtures. After 18 hr irradiation both absorbances at 267 mμ and at 532 mμ after TBA reaction were reduced to about 40% in neutral buffer solution, while those at 245 mμ and at 532 mμ after TBA reaction were reduced to about 36% in acidic buffer solution(Fig. 4).

The reaction mixture of MA and B_1 in the acidic buffer solution had absorption bands at 240 and 360mμ with a shoulder around 260mμ (Fig. 5). MA has an absorption band at 245 mμ at such pH values (Kwon and Watts, 1963). There was no change in the spectrum of B_1 in acidic solution from that in neutral ones. UV light gradaully reduced the absorption band at 240mμ

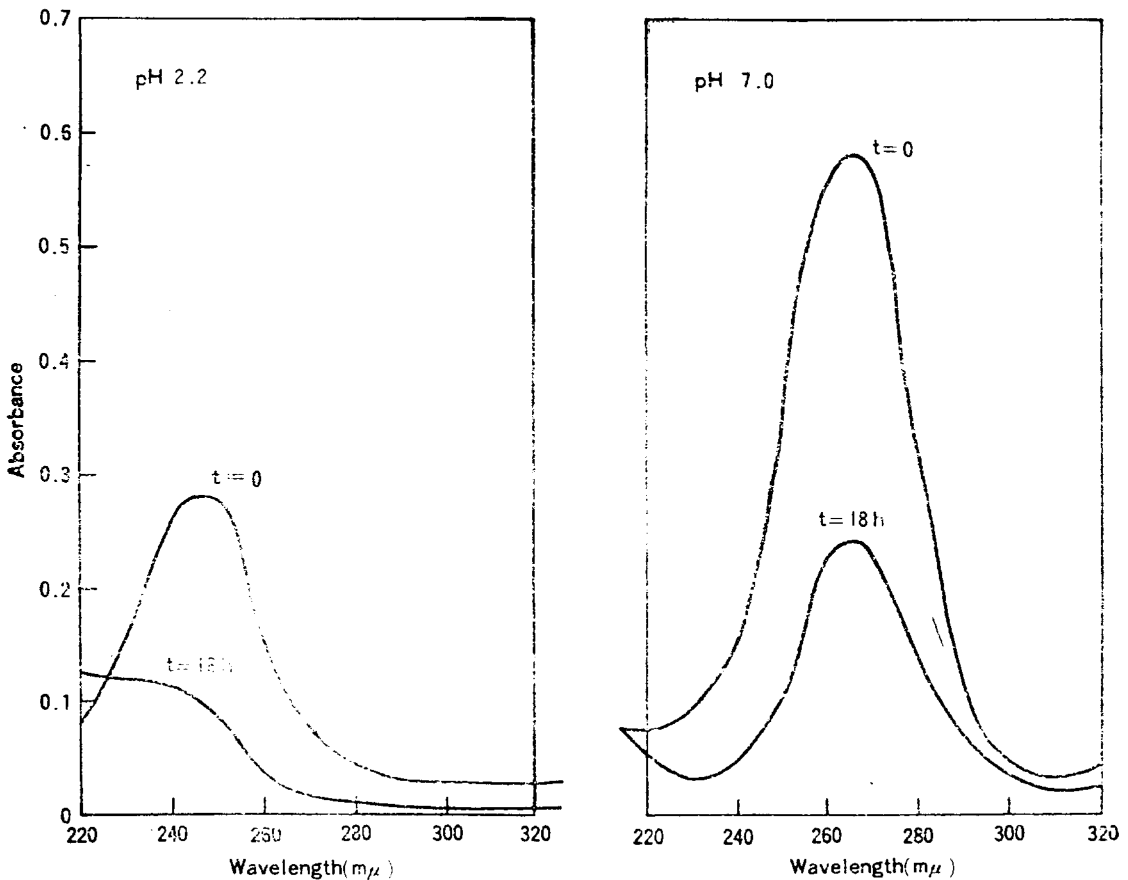

Fig. 4. Ultraviolet absorption spectra of MA either in acidic or neutral pH, before and after ultraviolet light irradiation. The MA solutions were irradiated at 3cm distance from a UVS-12 mineralight for 18 hr.

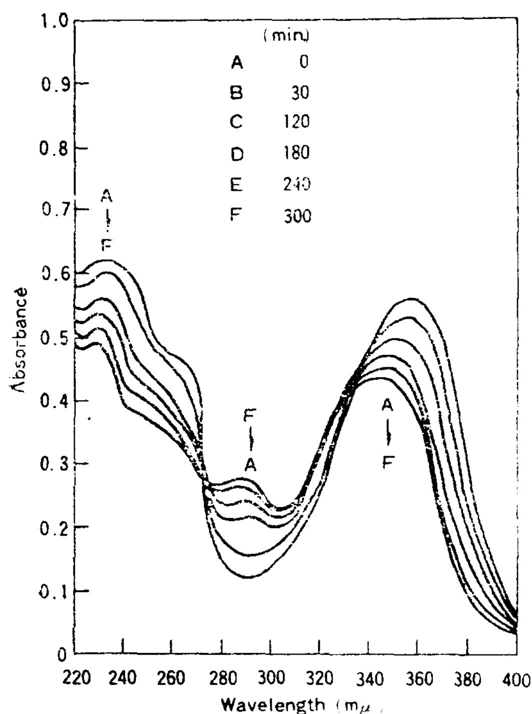

Fig. 5. Ultraviolet absorption spectra of aflatoxin B_1 and malonaldehyde reaction mixture in an acidic buffer solution during irradiation of ultraviolet light at 366mμ.

The final cocentration of both reactants was 2×10^{-5} *M* in 0.05 *M* KCl-0.0067 *M* HCl buffer, pH 2.2. The spectra were recorded after different lengths of irradiation as indicated.

and the shoulder, while the latter band shifted from 360mμ to 350mμ with reduced absorbance. An additional new band was formed at 290 mμ (Fig. 5). However, on the basis of the same criteria mentioned earlier, it was also concluded that only MA undergoes photochemical modification and the toxin acts as only a photosensitizer. Spectral changes were also observed when the reaction mixture in dichloromethane was subjected to UV irradiation, but with a slower rate than in the buffer solutions. In dichloromethane, MA occures predominantly as the diketo from with a small portion of *s-trans* enol form (Kwon and Van der Veen, 1967).

There are at least three possibilities for explanation of the photochemical reaction responsible for the spectral changes; namely, decomposition, dimerization and polymerization of MA. If MA decomposes, the rate of reaction must be first order. If MA polymerizes, the reaction will be much more complicated than second order. A kinetic treatment of the spectral changes (i. e., plotting reciprocal of absorbance at 267 mμ against reaction time) showed a straight line and thus indicated that the reaction for dimerization of MA is second order. If dimerization occurs by head to tail combination of MA molecules, the degree of conjugation of unsaturated double bonds must be increased, and thus, there should be an absorption band at longer wavelength than that of monomeric MA. Since there is no such new absorption band, the dimerization is possibly a parallel arrangement of the molecules to give saturated dimers which do not have any absorption in the UV range studied. A similar reaction may occur both in the acidic buffer solution and in dichloromethane. However, the reaction product was not isolated and the precise reaction mechanism is not yet understood.

Thus, MA undergoes considerable photochemical modification by UV light of wavelengths around 260 mμ and the modification is irreversible in nature. Even UV light of long wavelengths around 360mμ could be effective in photochemical changes of MA if foods contain certain photosensitive compounds such as aflatoxins. It is clear that, in the presence of aflatoxins as photosensitizers, MA undergoes irreversible changes with ordinary laboratory illumination. Thus, when MA contents of foods determined either by TBA or UV spectroscopic method are used as a measure of lipid oxidation, a consideration of whether samples have been exposed to certain light sources or not and, if any, the length of exposure, should be included in the final evaluation of the analytical data.

Summary

On the basis of UV spectral changes and TBA reaction, malonaldehyde (MA) in aqueous solution receives considerable photochemical modification by UV light of short wavelengths around 260 mμ. When aflatoxin is added in the solution, UV light of long wavelengths around 360 mμ induces such changes quite rapidly and although the rate of change is rather slow, it is also true even with ordinary laboratory illumination (fluorescent). The modificat-

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