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# FREE RADICAL PRODUCT FORMED BY THE REACTION OF DEHYDROASCORBIC ACID WITH AMINO ACIDS

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A short time heating of the reaction mixture of dehydroascorbic acid and amino acid was found to give a fairly stable free radical product(s) which could be isolated on tlc as a blue fraction and showed the hyperfine esr spectra.

The redox reaction between ascorbic acid (AsA) and dehydroascorbic acid (DHA) involving free radical intermediate has been studied from various viewpoints because of its biological importance. On the other hand, the reaction of these compounds with amino acids have been noticed in food chemistry in relation to the quality changes as the formation of a red pigment by the reaction of DHA with amino acids.[1)]

In the course of our studies on the radical development in the amino carbonyl reactions of various materials of biological importance, we have found a formation of fairly stable free radical products by the reaction of DHA with various amino acids or amines, and this communication is concerned with the isolation and some properties of these radical products.

DHA was prepared from AsA by the method of von Euler[2)] using p-benzoquinone as the oxidizing reagent. AsA, amino acid and other reagents used were Guaranteed grade and distilled water prepared with a Pyrex apparatus or purified ethanol was used as the reaction medium. The reactions were done in a Pyrex test tube and the esr spectra were measured in a quartz tube with a JES-ME-1X esr spectrometer. UV and visible spectra were measured with a Hitachi EPS-3T spectrometer. Tlc was made on microcrystalline cellulose (Avicel) with ethylacetate-pyridine-water.

A mixture of equimolar DHA and α-alanine (each, 0.6 M) prepared with 95% ethanol was heated in a boiling water bath. The reaction mixture colored pink immediately

after the heating was started, it turned to a characteristic wine red in few minutes and then gradually to red brown with further heating. On this reaction, it was found that an apparent esr signal was developed as soon as the reaction was started and it increased rapidly at an early stage of the reaction and remained fairly long time with some changes in the shape and amplitude of the spectrum during further heating. Fig. 1-a shows the spectrum at the heating for 3 minutes which is considered from the following investigations to be composed at least two kinds of the spectra, a triplet one (Spectrum A) and a quintet-doublet one (Spectrum B). The prolonged heating over 10 minutes brought about an increase in (B) with a decrease in (A) accompanied by the color change to brown. It was also observed that the addition of water on the ethanol reaction mixture arose a significant enhancement of the triplet signal (A).

In the case of the reaction employed in an aqueous system (each, 1.2 M), the color changes as well as the development and disappearance of the esr signal occured in a similar way but proceeded far more quickly than the above case. Fig. 1-b shows the spectrum at the initial stage of the reaction (after 1 minute of the heating), which is also assumed to be composed of at least two spectra, the spectrum A and another multiplet one. There was no significant difference in the shape and amplitude of the spectrum between the esr spectra observed in the aqueous reaction mixtures heated in an open tube and in the closed one previously evacuated at $10^{-4}$mmHg in a frozen state. Therefore it seems to say that oxygen does not play any significant role in the formation of the free radical product(s), though it seems to effect considerably on the life time of the radical product(s).

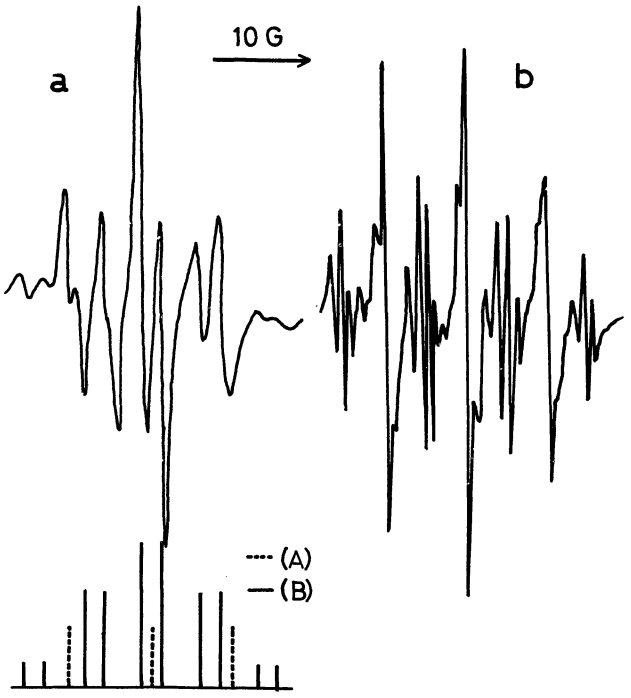


Fig. 1 Fsr spectra of free radical produced in the presence of DHA with α-alanine. a) in alcohol, b) in water

A typical thin layer chromatogram of the reaction mixture with ethanol was illustrated in Fig. 2. There were several visible spots and the two spots of the materials colored with ninhydrin or 2,4-DNP. Among several colored

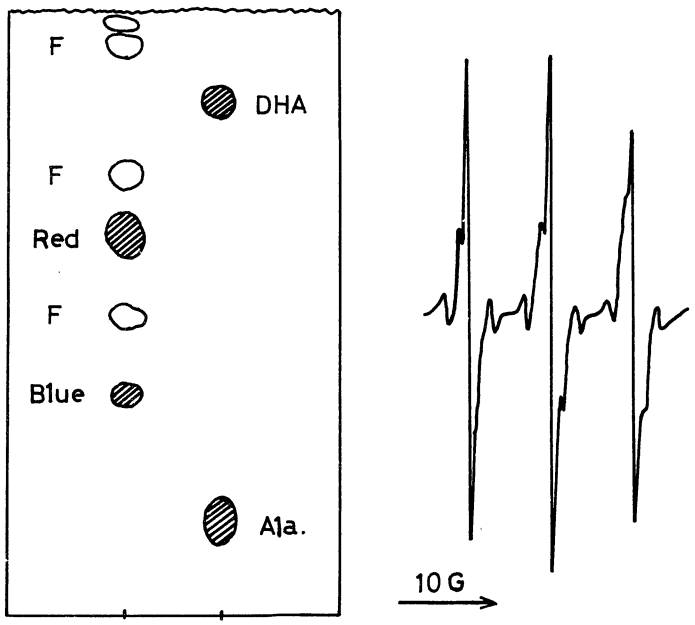


Fig. 2 Tlc of the reaction products of DHA and α-alanine in alcohol, and esr spectrum of water extract of the blue spot on the tlc. F: fluorescence

spots, a most significant one is known to correspond to the so-called red pigment,[1] and below this spot the presence of a blue one of unknown product could be observed. Then, each of the different colored bands in the preparative tlc was scraped off and employed to the esr measurement. Consequently it was known that the esr signal could be detected only in the Avicel powders from the blue band. The water extract of the blue band showed the esr spectrum involving a triplet signal of the type A as shown in Fig. 2, and at the same time, it colored blue and gave the absorption spectrum having the maxima at 248, 386, 520 and 646 nm. The free radical product in the water extract appeared to be oxidized easily by oxygen at room temperature because the triplet signal was decreased rapidly and disappeared greatly after about 2 hours in the presence of air, while it remained for longer time in nitrogen at 0°C. Together with this abolishment of the esr spectrum, the blue color as well as the peak at 646 nm was disappeared and the solution turned to red along with an apparent increase in the peaks at 386 and 520 nm. Moreover, it was demonstrated that the addition of a slight amount of benzoquinone on the reaction mixture of DHA with α-alanine resulted in a complete abolishment of the esr signal and at the same time the disappearance of the blue product observed on the tlc. Thus, it seems very probable that the free radical product formed by the reaction of DHA with amino acid is identical with the blue product found on the tlc of the reaction mixture, which was easily oxidized by the actions of oxygen, and other weak oxidizing reagents as benzoquinone to give the red pigment of no free radical.

The structure of the radical product isolated here is not yet clear because it is not so stable as it could be obtained in a pure crystalline form, but the

esr spectrum of type A, as shown in Fig. 2, a sharp triplet with a ratio of 1:1:1 with a splitting constant of 8.4 G, suggests that the presence of an unpaired electron located on the nitrogen nucleus and has no neighboring proton. However, the futher hyperfine splitting of each signal of the triplet was not yet clearly assigned.

It was also observed that the similar reactions done with other amino acids than α-alanine gave essentially the same pattern in the esr spectrum.

Relating to this free radical formation in the reaction of DHA with amino acid, the development of free radical product has been observed in the reaction of AsA with hydrazine when the mixture was kept in alkali under aerobic condition,[3,4] and it was proposed that some reaction product from AsA and hydrazine would be a precursor to give a radical product by oxidation in alkali.[3] We have observed that the reaction of DHA with hydrazine gave the esr spectrum identical with that has been presented by Burlamacchi et al.[4] in the case of AsA and hydrazine, and this esr spectrum could be observed even in the absence of air and at neutral pH though no such signal in the case of AsA instead of DHA with α-alanine. Thus, it seems reasonable to consider that even in the case of the reaction of AsA with hydrazine, the oxidation of AsA to DHA by oxygen is preceded to the radical formation by the reaction with amino acid.

The details of the experiments and the further investigations on the structure of the radical product(s) will be presented elsewhere.

## References

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