ACTA CHEMICA SCANDINAVICA 4 (1950) 245–250

On the Autoxidation of Linoleic Acid in Aqueous Colloidal Solution

SUNE BERGSTRÖM, R. BLOMSTRAND and S. LAURELL

Department of Physiological Chemistry, University of Lund, Lund, Sweden

The autoxidation of the unsaturated fatty acids has in recent years been extensively studied in a number of laboratories. The results have been reviewed by Farmer [1], Bergström and Holman [2], Swern, Scanlan and Knight [3] and Lundberg [4].

This work has mainly been concerned with the early phases of the autoxidation at lower temperatures and, in order to minimize the influence of the carboxyl group, esters have generally been used as such or diluted with hydrocarbon solvents. In several cases it has been found that the main primary autoxidation product contains a hydroperoxidic group attached to a carbon atom in α-position to a double bond.

In the case of linoleic acid and similar compounds with methylene interrupted double bonds, it has further been found that an absorption band at 232 mμ appears that is caused by the conjugation of the double bonds [5–9]. In the earlier stages of the autoxidation the absorption band increases parallel with the oxygen uptake and hydroperoxide formation. When the reaction is continued the autoxidation of this conjugated system leads to concurrent extensive polymerisation which generally occurs when substances containing conjugated double bonds autoxidize. The reaction product also becomes progressively more complex as the autoxidation proceeds due to secondary reactions of the hydroperoxidic group, resulting in the formation of hydroxylic, epoxidic, carbonylic, carboxylic groups etc.

The free radical mechanism that has been proposed by Farmer for the hydroperoxide formation and the double bond conjugation seems to have been well established through kinetic studies in some cases [10]. However, very little is known about the later stages of the autoxidation when the hydroperoxides decompose in various ways and the second molecule of oxygen is absorbed.

The spectral changes and the products formed in the enzymatic oxidation of linoleic acid and other similar compounds in aqueous media with soy bean lipoxidase has been investigated by Bergström [11,13], Holman [12,14] and others.

In this very rapid reaction the same spectral changes as in the ordinary autoxidation were found to occur and the same hydroxystearic acids were isolated after hydrogenation of the reaction products. With the pure crystalline enzyme at low temperatures (0—5°) the increase in absorption at 232 mμ corresponded to the formation of one pair of conjugated double bonds for every oxygen molecule absorbed [13,14]. In the ordinary autoxidation at 20—50° temperatures the absorption only reaches at most two thirds of this value. In the enzymatic oxidation, however, extinction values in this range were also observed when the reaction was run at higher temperatures (37°).

The action of the enzyme lipoxidase is apparently limited to the uptake of one mole oxygen per mole linoleate whereas in the autoxidation of methyl linoleate there is no sharp break in the oxygen uptake that continues even after the uptake of more than two moles oxygen per mole fatty acid ester (Franke [15]).

We were interested to know how the aqueous suspension of sodium linoleate behaved in this respect and to compare the autoxidation in aqueous media with the enzymatic oxidation under similar conditions. The autoxidation in an aqueous system also seems of interest from a biochemical point of view.

The rate of oxygen uptake in the earlier phases of the autoxidation of sodium linoleate in phosphate buffers has been investigated by Smith and Stotz [16]. They found the rate depending on the amount of copper ions in the solution and also investigated the influence of various antioxidants forming complexes with copper ions. These authors or earlier workers in this field do not seem to have followed the ultraviolet spectrum during the autoxidation of linoleate in aqueous media nor the autoxidation to the final stages.

RESULTS

We have followed the autoxidation of sodium linoleate in a system similar to that used for the lipoxidase work referred to above. The sodium salt of 2 mg linoleic acid in 1.5 ml redistilled water containing borate buffer pH 9 was shaken in Warburg vessels at 37° in an atmosphere of air. The oxygen uptake curves shown in Fig. 1 show the influence of copper ions at different concentrations. Without any addition of copper salts an induction time of more than 24 hours was observed and this time was progressively shortened as the concentration of copper ions increased. At the same time the maximum speed of oxygen

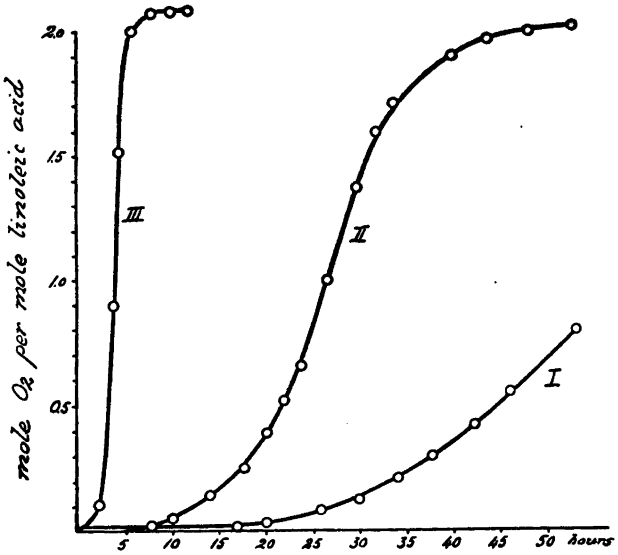


Fig. 1. Influence of cupric ions on the rate of oxygen uptake of sodium linoleate in aqueous colloidal solution. I. No addition of $CuCl_2$. II. 10^{-6} M $CuCl_2$. III. 10^{-5} M $CuCl_2$.

uptake increased. The more or less S-shaped curves always levelled off when 2 moles of oxygen per mole fatty acid had been taken up.

In similar runs in 10^{-5} molar $CuCl_2$ solution the reaction was stopped at different levels. A sample was taken out and diluted with ethanol as described in the experimental part and the ultraviolet absorption spectrum determined. In Fig. 2 the molar extinction of the linoleate at 232 mμ from a number of different runs are plotted against the oxygen uptake.

If the extinction is calculated per mole oxygen absorbed the values of the earlier parts of the curve are in the range $\varepsilon = 15$—$20\,000$. These parts thus resemble the corresponding curve for the autoxidation of methyl linoleate when shaken as such in an oxygen-containing atmosphere at this temperature and also the earlier phases of the enzymatic oxidation at this temperature although the speed is very much slower ($>$ 100 times) in the nonenzymatic reaction.

As the oxydation proceeded (Fig. 2) the ultraviolet absorption increases at a progressively slower rate. It then leveled off when approximately one mole of oxygen had been absorbed, and then gradually decreased so that when the oxygen uptake had ceased at two moles the absorption band at 232 mμ had almost disappeared.

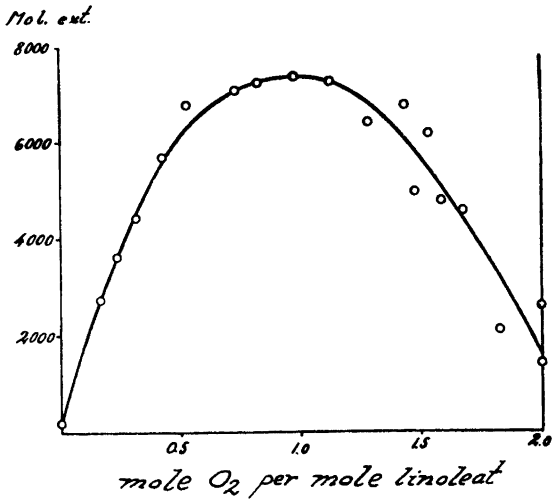


Fig. 2. Molar extinction at 232 mμ of linoleate at different stages of the autoxidation in aqueous medium.

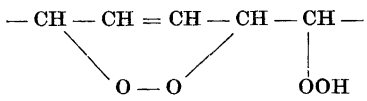
That a similar decrease of the ultraviolet absorption at 232 mμ occurs when ethyl linoleate is oxidized as such appears probable from the figures in a paper by Holman [21], where the spectrum has been determined on autoxidizing linoleate containing carotene. Recently Allen, Jackson and Kummerow [22] have published similar observations.

The peroxide content was also determined on aliquots with the ferric thiocyanate method of Balls *et al.* [17]. The curve showing the peroxide content at various oxidation levels roughly had the same shape as the curve of the ultraviolet absorption. The peroxide content reached a maximum when about one mole oxygen per mole fatty acids had been absorbed and then decreased as the oxygen uptake proceeded. The content, however, was not reduced to such a low level as the ultraviolet absorption but about 0.5—1 mol of peroxidic groups remained. However, this method is known to give too high values [18].

In a preparative run 2 g linoleic acid were oxidized under similar conditions until two moles oxygen per mole fatty acid had been absorbed. The reaction products were extracted from the aqueous phase after careful acidification with hydrochloric acid. The ether residue was a light mobile oil with a strong odor indicating that some chain splitting had taken place.

The dispersion in micellar form in the aqueous medium thus seems to have inhibited the extensive polymerization that occurs when the undiluted acid or ester autoxidizes (*cf.* Treibs [19]). The sharp break in the oxygen uptake curve after the uptake of two moles of oxygen also indicates that the product of the autoxidation might be of a fairly reproducible composition.

It appears possible that monomeric peroxides of the general structure I are formed by autoxidation of the primary hydroperoxide with conjugated



double bonds. This method might be of value to obtain material to determine the structure of the compound of linoleate with two moles of oxygen, a compound normally difficult to obtain due to the marked polymerization in the ordinary autoxidation [19].

Further discussions as to the structure of the primary and secondary reaction products and the reaction mechanism must await further chemical work. These preliminary results are being published because the work must be discontinued for the present.

EXPERIMENTAL

Autoxidation of sodium linoleate. To 100 mg linoleic acid was added 0.37 ml 1.0 *N* NaOH. The resulting gel was stirred until homogeneous and distilled water was then added drop by drop with vigorous stirring. The solution was then made up to 50 ml in a volumetric flask with 0.1 *M* sodium borate buffer pH 9. One ml of this solution and 0.5 ml of the cupric chloride solution was added in the main compartment of the Warburg vessel. These autoxidations were made in air at 37° with 100 oscillations per minute.

The ultraviolet absorption was determined with a Beckman ultraviolet spectrophotometer on suitable dilutions of the reaction mixture with alcohol. Peroxide determinations were made according to Balls, Axelrod and Kies [17]. The determinations were made at 535 mμ in a Coleman spectrophotometer. Linoleic acid was prepared by the method of McCutcheon [20].

SUMMARY

The autoxidation of sodium linoleate in aqueous solution under conditions similar to those of the lipoxidase standardization has been investigated.

The rate of oxygen uptake is dependent on the concentration of cupric ions. The maximum oxygen uptake is two moles per mole linoleate. The absorption at 232 mμ first increases and then decreases to low values.

The reaction product with two moles of oxygen does not show any signs of polymerization.

This work is part of an investigation supported by *Statens naturvetenskapliga forskningsråd.*

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Received December 22, 1949.