The Reaction of Malonaldehyde or Oxidized Linolenic Acid with Sulfhydryl Compounds

Sir: In a recent paper by Shin et al. (Lipids 7:229 [1972]), the effect of malonaldehyde and oxidized linolenic acid on the sulfhydryl group of N-acetylcysteine was studied in a dilute aqueous system. The way the results of these experiments are presented in Figure 3 of the above paper leads one to conclude that malonaldehyde has no reactivity toward the sulfhydryl groups. However, considering the nature of the equilibrium reaction and the type of products formed between aldehydes and

$$\begin{gathered}\mathrm{RCHO + R'SH \rightleftharpoons RCHOHSR' \overset{H^+}{\rightleftharpoons}} \\ \mathrm{RCH\overset{+}{O}H_2SR' \overset{R'SH}{\underset{H_2O}{\rightleftharpoons}} RCH(SR')_2 + H^+}\end{gathered} \qquad [1]$$

mercaptans (Cecil and McPhee, Adv. Prot. Chem. 14:255 [1959]; Boyer, The Enzymes 1:511 [1959]), it would appear rather that the assay with the Ellman reagent is not suitable to detect the loss of sulfhydryls under the experimental conditions employed, since this reagent can influence the equilibrium position of the reaction. Although hemiacetals and thiohemiacetals of simple aldehydes are generally not sufficiently stable to permit isolation, the addition compounds of thioglycolic acid anilide and formaldehyde or butyric aldehyde $RCHOHSCH_2CONHC_6H_5$, as well as thiazolidine-4-carboxylic acid from cysteine, have been prepared (Schubert, J. Biol. Chem. 114:341 [1936]) and were shown to undergo in aqueous solution a rapid equilibrium with their reactants, aldehyde and thiol. Using the Ellman reagent 5,5'-dithiobis-(2-nitrobenzoic acid) to measure the extent of reaction [1] would deplete the thiol concentration by the mechanism of reaction [2] and shift the equilibrium of [1] to the left until all of the thiohemiacetal is dissociated.

$$\mathrm{RSSR + R'S^- \rightarrow RSSR' + RS^-} \qquad [2]$$

In our studies we found that the extent of the reaction between the functional groups NH_2 and SH of cysteine or other amino acids and malonaldehyde in aqueous solution ($\sim 3.8 \times 10^{-1}$ M) could be readily demonstrated by titration techniques involving proton equilibria of the remaining reactant $HSCH_2CHNH_2CO_2H$ as shown in Figure 1 of this letter.

The initial aldehyde-amine addition reactions [3] of aliphatic aldehydes and amines proceed in a manner similar to the hemiacetal formation and are in equilibrium with the Schiff base type products which can undergo further reactions of imine-amine type addition to the azomethine bond or by aldol type condensation with excess aldehyde (Layer, Chem. Revs. 63:489 [1963]).

$$\mathrm{RCHO + RNH_2 \rightleftharpoons RCHOHNHR \overset{H^+}{\underset{H_2O}{\rightleftharpoons}} RCH{=}NR} \qquad [3]$$

Trying to follow the glycine-malonaldehyde reaction (reactant concentrations 10^{-2}M) by the ninhydrin method (Fraenkel-Conrat, Methods Enzymol. 4:247 [1957]), we also failed to show a decrease in amino groups, even after heating the reaction mixture to 100 C. The reason for the failure of this technique to show any reaction is in principle the same as before. As the α-amino group of glycine reacts with the ninhydrin reagent, the equilibrium reaction [3] is shifted toward the left until all addition products are dissociated. Similarly, in attempting to prepare a semicarbazone derivative of the glycine-malonaldehyde monoaddition product, the malonaldehyde-bis-semicarbazone formed instead (Buttkus and Bose, J. Org. Chem. 36:3895 [1971]). When glycine was reacted with acetic or succinic anhydride, a decrease in the amino groups could be shown readily by the ninhydrin method.

$$\mathrm{RNH_2 + (RCO)_2O \rightarrow RNHCOR + RCOO^-} \qquad [4]$$

Also, when myosin was reacted with malonaldehyde in aqueous solution a decrease in its ϵ-amino groups could be followed by the ninhydrin method, the results possibly indicating further imine-amine addition reactions to produce more stable products.

The formation of more stable products by oxidized linolenic acid with thio compound in the biphasic or emulsion type system of Figure 3 (Lipids 7:229 [1972]) must have been due to reactions different from the malonaldehyde additions. Since photooxidized polyunsaturated lipids contain hydroperoxides, free radicals and epoxides, besides carbonyl compounds (Frankel, "Lipids and Their Oxidation," Avi Publishing Co., 1962, p. 51),

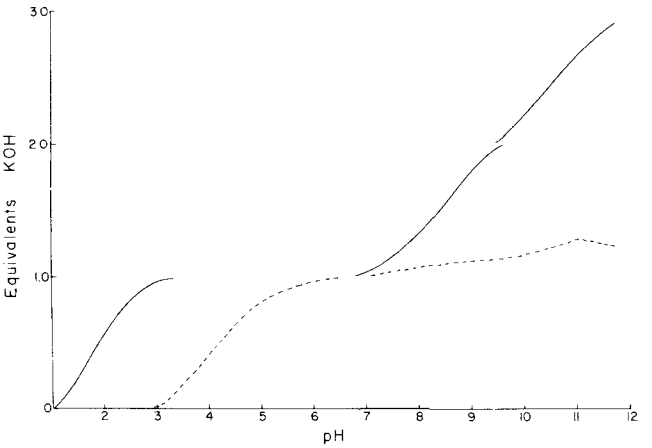

FIG. 1. Titration curves of cysteine (solid line) and cysteine in the presence of malonaldehyde (dashed line), demonstrating the small amounts of titrable NH_2 and SH groups (pk 8.36, 10.53) remaining, when the cysteine titration is carried out in the presence of aldehyde. Cysteine $HCl \cdot H_2O$, 0.0878 g were dissolved in 5.0 ml of water and adjusted to pH 7.0 while being gently flushed with nitrogen. Neutralized malonaldehyde solution, 4.5 ml, was then added. At a volume of 10.0 ml the concentration of the solution being titrated was ca. 0.05 M in cysteine and 0.33 M in malonaldehyde. The titration was carried out with 0.05 ml quantities of N KOH. A blank titration of malonaldehyde in the absence of cysteine was obtained and subtracted from the titration curve of the reaction mixture. When a cysteine solution without added reagents was titrated, a water blank was subtracted to obtain the final values recorded in the graph.

more stable products can be expected from their interaction with biological systems.

$$RSH + -HC\overset{O}{\diagdown\!\diagup}CH- \rightarrow RSCH\underset{|}{C}HOH \qquad [5]$$

$$2RS\cdot \rightarrow RSSR$$

Typical oxidation products -SS-, $-SO_3^=$, -SOSO- have been detected in such systems (Lewis and Wills, Biochem. Pharmacol. 11:901 [1962]).

The importance of the reaction of free radicals with each other and other cell constituents has generally been recognized; however these reactive constituents cannot be easily measured. Malonaldehyde, although possibly a less reactive product of lipid oxidation, by virtue of its thiobarbituric acid derivative or the fluorescent malonaldehyde-amine addition products, has provided working systems to measure lipid oxidation or lipid-protein interactions (Chio and Tappel, Biochemistry 8:2827, 2821 [1969]).

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[Received July 26, 1972]

Erratum

In the short communication, "Selective Hydrogenation Catalyzed by Polymeric Palladium and Platinum Complexes," (Bruner and Bailar, JAOCS 49:533[1972]), there is an error in Reference 7. The reference should read: Haag, W.O., and D.D. Whitehurst, Belgian Patent No. 721,686, 1969.