REACTION MECHANISM

Reaction of Malonaldehyde with Glycine

DAVID L. CRAWFORD,
T. C. YU, and
RUSSELL O. SINNHUBER
Department of Food Science and
Technology, Oregon State
University, Corvallis, Ore.

The reaction of malonaldehyde with the amino function of glycine has been investigated. It appears to involve a 1,4-addition of the nucleophilic nitrogen atom of glycine to the enolic carbon atom of the alpha,beta-unsaturated carbonyl system of the enol of malonaldehyde to form the enamine, N-prop-2-enal aminoacetic acid.

MALONALDEHYDE has been found to be one of the decomposition products of autoxidized, polyunsaturated lipid materials and its presence as determined by reaction with 2-thiobarbituric acid (15, 18) serves as a measure of oxidative rancidity (1, 3, 6, 10, 11, 14, 17). It also may be derived by further oxidation of secondary oxidation products of lipids (8).

The purpose of this research was to investigate the conditions and mechanism of the reaction between malonaldehyde and the amino function of glycine. Since malonaldehyde in an aqueous system exists primarily as an alpha, beta-unsaturated carbonyl (9, 13), this investigation would also contribute insight into the probably similar reaction of other carbonyl compounds of this type. The reaction of these carbonyl compounds, derived from oxidized lipids, with the amino function of proteins and amino acids in vivo and in vitro may be of nutritional and physiological significance.

Methods and Results

Malonaldehyde obtained by hydrolysis of malonaldehyde acetals or from the enolic sodium salt, sodium β-oxyacrolein, reacts with glycine under acid conditions to form the enamine, N-prop-2-enal aminoacetic acid.

Synthesis. 1, 1, 3 - Trimethoxy - 3-ethoxypropane (26.7 grams, 0.15 mole) was mixed with 14.2 ml. of $1N$ HCl with warming until the liquids became miscible. After being cooled to room temperature, this solution was added with stirring to 7.5 grams (0.1 mole) of glycine in 20 ml. of water. After stirring for 1 hour, the solid product was filtered off and washed three times with 20-ml. portions of cold water. Upon drying in vacuum at 50° C., 11.6 to 12.0 grams (45 to 47% yield) of yellow-orange crystals were obtained. The crude product (5.0 grams) was neutralized with $3.5N$ NaOH, diluted to 50 ml. with water, and precipitated by slow addition of $1N$ HCl with rapid stirring, to yield 3.7 grams (74%) of nearly white crystals. The once-precipitated product (3.7 grams) was

neutralized with $3.5N$ NaOH, diluted to 30 ml. with water, and added to 2 volumes of MeOH. To the hot, aqueous MeOH solution, 150 ml. of boiling acetone was added slowly. After being allowed to cool slightly, the solution was filtered rapidly with suction. Crystallization at room temperature yielded 3.3 grams (65%) of a nearly white, crystalline sodium salt. Recrystallization from the aqueous MeOH-acetone solvent system was repeated twice and then the free acid was precipitated from aqueous solution (5 grams per 50 ml.) by addition of $1N$ HCl to yield a nearly white, crystalline product. This product melted, with decomposition, at 157–58° C. Titration with NaOH revealed 1.007 equivalents per 129.114 grams (theoretical molecular weight). Reaction with 2-thiobarbituric acid (15, 18) cleaved the molecule and yielded 1.003 moles of malonaldehyde per theoretical molecular weight. The semi-carbazone of N-prop-2-enal aminoacetic acid melted at 225–27°. The ultraviolet absorption spectrum (Beckman, Model DU) showed a maximum in water at 271 to 272 mμ (ε 22,434). An infrared spectrum (Beckman, Model IR-5A) of this compound in a KBr pellet revealed a single N—H stretching absorption at 3255 cm.$^{-1}$

Analysis. Calculated for $C_5H_7O_3N$: C, 46.51; H, 5.46, N, 10.85. Found: C, 46.34; H, 5.35; N, 10.78.

Reaction Kinetics. As malonaldehyde reacts with glycine to form N-prop-2-enal aminoacetic acid under acid conditions, there results a bathochromic shift in the ultraviolet absorption spectrum of the reaction mixture from that of the chelated enol of malonaldehyde at 245 mμ (ε 12,960) (13) to that of N-prop-2-enal aminoacetic acid at 272 mμ (ε 22,434).

The formation of N-prop-2-enal aminoacetic acid and its hydrolysis were spectrophotometrically followed using a Beckman, Model DK1 recording spectrophotometer, equipped with a constant temperature cell holder. The reactions were carried out at 20° C. in a 1-cm. silica cell and distilled water was used as a reference. The concentration of N-prop-2-enal aminoacetic acid in the

reaction mixture at any time, t, during the reaction was based on $E_{1cm.}^{272m\mu}$ and the molecular absorbancy. The observed rate constants were determined graphically from the appropriate first- or second-order plots of the data. The pH of the reaction mixtures was determined using a Beckman, Zeromatic pH meter. Glycine in the experiment was recrystallized once from water. Malonaldehyde in the form of the enolic sodium salt, sodium β-oxyacrolein (5), was prepared according to a modified procedure of Protopopova and Skoldinov (12), from 1,1,3,3-tetraethoxypropane. The hydrolysis of the acetal and simultaneous neutralization of the enol were carried out slowly at −14° C. The product was directly separated from the reaction mixture by the slow addition of excess acetone in the cold and purified by fractional precipitation from methanol with ethyl ether.

Kinetic experiments showed that the over-all reaction of malonaldehyde with glycine to form N-prop-2-enal aminoacetic acid conforms to a S_N^2 mechanism and that the reaction rate is very dependent on the hydrogen ion concentration (Table I, Figure 1). A maximum reaction rate was observed near pH 4.20. The rate of the reaction decreased rapidly at hydrogen ion concentrations greater and less than pH 4.20. The rate constants listed in Table I should be considered as observed rate constants, since under the acidic conditions of the reaction the amino function of glycine exists largely as the protonated amine, which is not a nucleophile. N-Prop-2-enal aminoacetic acid is readily hydrolyzed under acidic conditions to malonaldehyde and glycine by a reaction exhibiting kinetics which apparently conform to a S_N^1 mechanism (Table II, Figure 2).

Discussion

Chemical and spectral evidence indicates that malonaldehyde and glycine react to form the enamine, N-prop-2-enal aminoacetic acid. The presence of one titratable carboxyl function and one mole of malonaldehyde per theoretical

Table I. Experimental Conditions for Reaction of Malonaldehyde with Glycine and Rate Constants Obtained under Those Conditions
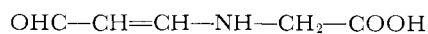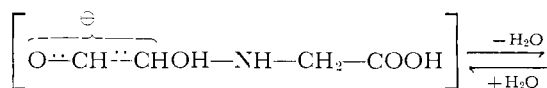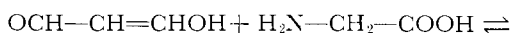
Reaction Number	Malonaldehyde, μmoles/liter	Glycine, μmoles/liter × 10⁻³	HCl, μmoles/liter	pH	k_{obd} × 10⁷, Liter μMoles⁻¹ Min.⁻¹, 20° C.
1	40.0	10.0	7500.0	2.60	0.567
2	40.0	10.0	2500.0	3.20	0.903
3	40.0	10.0	1000.0	3.60	1.153
4	40.0	10.0	500.0	3.90	1.453
5	40.0	10.0	300.0	4.10	1.693
6	40.0	10.0	250.0	4.24	2.073
7	40.0	10.0	125.0	4.64	1.674
8	40.0	10.0	62.5	5.00	0.510
9	5.0	50.0	780.5	4.20	1.669
10	10.0	50.0	790.0	4.20	2.022
11	20.0	50.0	800.0	4.20	2.333
12	30.0	50.0	810.0	4.20	2.791
13	40.0	50.0	820.0	4.20	3.011

Table II. Experimental Conditions for Hydrolysis of Malonaldehyde and Rate Constants Obtained under Those Conditions

Reaction Number	N-Prop-2-enal aminoacetic acid, μmoles/liter	HCl, μmoles/liter	pH	k_{obs} × 10⁴, Min.⁻¹, 20° C.
14	40.0	15.6	3.03	14.1
15	40.0	62.0	2.35	47.0
16	40.0	250.0	1.82	77.9
17	40.0	1000.0	1.25	186.7
18	60.0	1000.0	1.25	197.4
19	80.0	1000.0	1.25	184.2

Figure 1. Reaction of malonaldehyde and glycine as a function of malonaldehyde concentration

Numbers in parenthesis correspond to reactions described in Table I

Figure 2. Hydrolysis of N-prop-2-enal aminoacetic acid as a function of hydrogen ion concentration

Numbers in parenthesis correspond to reactions described in Table II

molecular weight indicates a condensation in a 1 to 1 molar ratio. This conclusion is further verified by the formation of a semicarbazone of the free carbonyl group. The absorption (log ε 4.35) in the ultraviolet region at 272 mμ reveals the strong $\pi \rightarrow \pi^*$ electronic transitions of a conjugated system in the molecule. This would preclude the presence of an imine linkage between malonaldehyde and glycine. This conclusion is verified by a sharp, single N—H stretching absorption band at 3255 cm.⁻¹ Elemental analysis confirmed the above conclusions made on the basis of chemical and spectral evidence.

The proposed mechanism of the reaction of malonaldehyde and glycine involves the nucleophilic attack of the amino nitrogen of glycine upon the enol carbon atom of the alpha,beta-unsaturated carbonyl system of the enol of malonaldehyde which is followed by loss of water to form the enamine:

centration would not change significantly during the reaction, the kinetics appear to follow only a S_N1 mechanism.

Cromwell (2) has postulated a similar mechanism for the reaction of 1,3-diketones which exist primarily in the enol form—that is, the 1,4-addition of the nucleophilic nitrogen of the amine to the enolic carbon atom, yielding a hydroxy intermediate which subsequently loses water to form either the imine or the enamine. Like the keto-enol tautomerism exhibited by carbonyls, imines exhibit an imine-enamine tautomerism (4, 7). The enamine structure is favored in most cases where there are possibilities for conjugation, resulting in resonance stabilization of the molecule. Imines derived from carbonyl compounds of types RCOCH₂COR, RCOCH₂CO₂R, and RCOCH₂CN exist as enamines, although under certain conditions they may exist as imines (7).

The marked dependence of the rate of

$$OCH—CH=CHOH + H_2N—CH_2—COOH \rightleftharpoons$$

$$\left[O^{-}CH^{-}CHOH—NH—CH_2—COOH \right] \xrightleftharpoons[+H_2O]{-H_2O}$$

$$OHC—CH=CH—NH—CH_2—COOH$$

The hydrolysis of N-prop-2-enal aminoacetic acid, although kinetically conforming to a S_N1 mechanism, probably proceeds by a S_N2 mechanism involving the nucleophilic attack by water on the protonated enamine. Since the attacking nucleophile is the solvent and its con-

the reaction on the hydrogen ion concentration indicates that a particular molecular species of malonaldehyde is most susceptible to nucleophilic attack by the amino nitrogen of glycine. Malonaldehyde exists in aqueous solution predominantly in the enol form (9, 13)
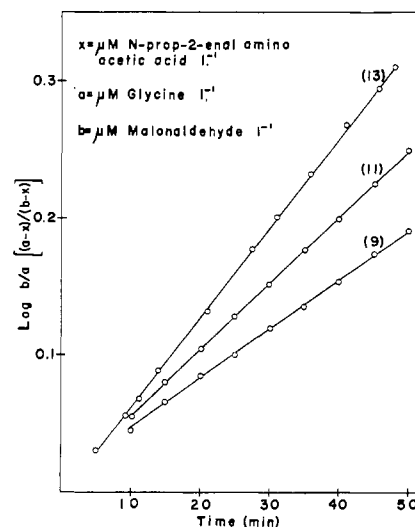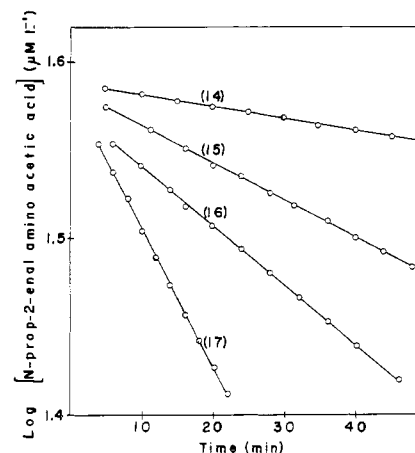
and below pH 4.5 largely as the cyclic chelated enol (13). The enolic hydroxyl function has a pKₐ of 4.6 (9) and forms stable enolic salts (5, 12). Malonaldehyde in the cyclic chelate or in the enolate ion form would tend to stabilize the enolic hydroxyl and make it difficult to displace by nucleophilic attack. The protonated enolic hydroxyl of malonaldehyde would be most susceptible toward nucleophilic displacement. The enolic form of malonaldehyde would exist in highest concentration at a pH slightly below its pKₐ.

It is possible that alpha,beta-unsaturated aldehydes and ketones as well as various dicarbonyl compounds derived from oxidized lipids could undergo similar reactions. Ideal conditions for

the reaction of malonaldehyde and other secondary oxidation products of lipids with various biologically important nucleophiles exist in food products, in the mammalian gastrointestinal tract, and possibly at a cellular level in various tissues.

Enamines have chemical properties which make them of great interest. Stork (16) has indicated the great ease with which enamines of aldehydes and ketones undergo alkylation and acylation by numerous electrophiles. This could point to a possible mechanism for the formation of C—C bonds between certain aldehydes and ketones derived from oxidized lipids and various electrophiles of a biological origin.

Acknowledgment

Elemental analysis was conducted by Galbraith Laboratories, Inc., P. O. Box 1187, Knoxville 21, Tenn.

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