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ENZYMATIC SYNTHESIS OF MALONALDEHYDE

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SUMMARY

Malonaldehyde was prepared from 1,3-propanediol by alcohol dehydrogenase. The K_m for 1,3-propanediol was about 1.7 mM. The reaction proceeded best at low ionic strength and at pH 9. The reaction was unaffected by pyrophosphate, phosphate, bicarbonate, or N-ethylmorpholine buffers, or by Mg^{+2}, Ca^{+2}, EDTA, or citrate. However, the reaction was inhibited 50% by 1.5 mM borate, 1 mM cyanide, and 5 mM azide. Thiols, such as dithioerythritol, inhibited the reaction 50% at 50-100 μM, while others, such as mercaptoacetate, inhibited 50% at concentrations over 1 mM. Malonaldehyde was removed from the reaction mixture by evaporation at pH 3 and condensation at -78°C. No other products associated with lipid peroxidation were produced. The method was useful for preparation of radiolabeled malonaldehyde.

INTRODUCTION

Malonaldehyde has been implicated in aging (1), carcinogenesis (2), and radiation damage (3). It has been shown to crosslink amino groups on phospholipids (4), proteins (5), and nucleic acids (6). Therefore, the study of reactions that involve malonaldehyde is of interest in many biochemical areas. Until now, it was necessary to prepare malonaldehyde by the high-temperature, acidic hydrolysis of a 1,1,3,3-tetraalkoxypropane (7) or by shaking with protonated resins (8). In either case, rapid polymerization occurs if the concentration of malonaldehyde exceeds a few millimolar. Also, since no radiolabeled tetraalkoxypropanes are available, tracer studies cannot be done. For these reasons, a new synthesis was developed.

MATERIALS AND METHODS

Equine liver alcohol dehydrogenase (EC 1.1.1.1), rabbit muscle lactic dehydrogenase (EC 1.1.1.27), and NAD were obtained from Sigma Chemical Co.; sodium phosphate, pyrophosphate, bicarbonate, and citrate from Mallinckrodt Chemical Works; sodium pyruvate and N-ethylmorpholine from Nutritional Biochemicals Corp.; and 1,3-propanediol and 1,1,3,3-tetramethoxypropane from Eastman Organic Chemicals. The 1,3-propanediol was distilled twice, and only the fraction boiling above 200°C was used.

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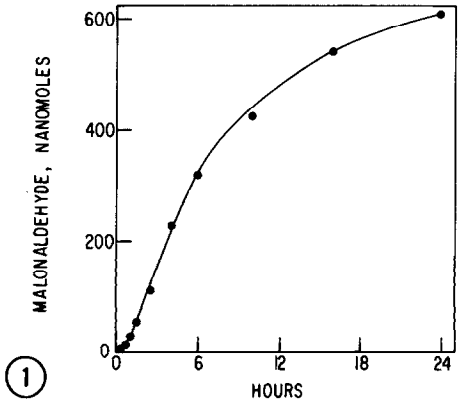


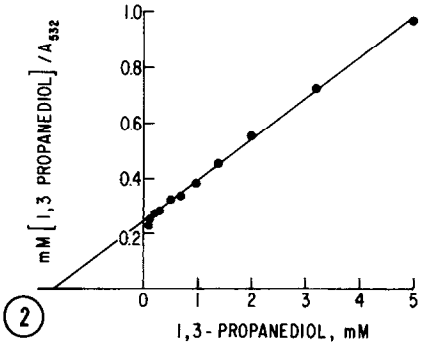


FIGURE 1. Time course for production of malonaldehyde.

FIGURE 2. Hanes-Woolf plot of enzyme activity as a function of substrate concentration.

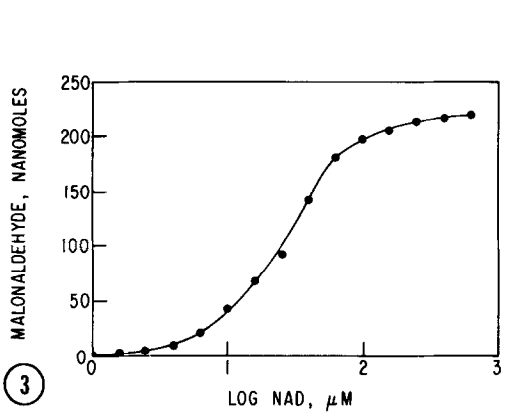
Unless otherwise stated, all reactions were carried out at 25°C in 50 mM pyrophosphate buffer, pH 9, with 0.0015 unit (1 unit is equivalent to 1 µmole product/min) of alcohol dehydrogenase, 0.005 unit of lactic dehydrogenase, 50 µM NAD, 1 mM 1,3-propanediol, and 20 mM pyruvate in a final volume of 1 ml. [1-^{14}C]-1,3-Propanediol for the preparation of ^{14}C-malonaldehyde was obtained from ICN Chemical and Radioisotope Division. Malonaldehyde was determined by a modification of the thiobarbituric acid reaction (9). The reaction mixture, or an appropriate aliquot thereof diluted to 1 ml, was mixed with 1 ml of glacial acetic acid and 3 ml of 10 mM thiobarbituric acid and the mixture was heated in a covered test tube in a boiling water bath for 1 hr. Tetramethoxypropane was used as a standard, and the absorbance was read at 532 nm.

RESULTS

The time course of the reaction is shown in Figure 1. After an initial lag of about 20 min, malonaldehyde was formed at about 50 nanomoles/hr. The rate of the reaction was increased by higher temperatures, but the yield did not increase beyond about 65%.

Figure 2 illustrates the effect of 1,3-propanediol concentration on the rate of the reaction. The reaction was allowed to proceed for 4 hr, thus minimizing the effect of the initial lag. The plot reveals a K_m of about 1.7 mM 1,3-propanediol. Though this value is large, the K_m of alcohol dehydrogenase for many non-biological alcohols is much greater than for ethanol (10).

Figure 3 displays the NAD requirement of the reaction. The determining factor was the K_d of alcohol dehydrogenase for NAD, about 30 µM (11).



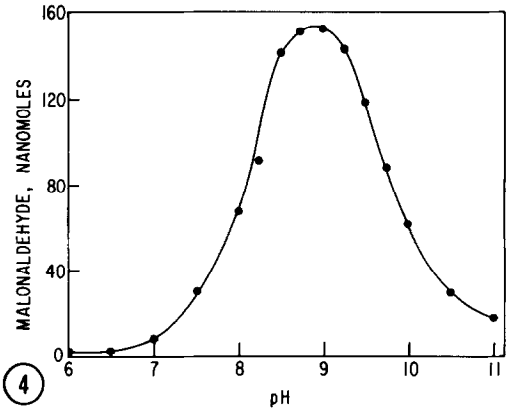


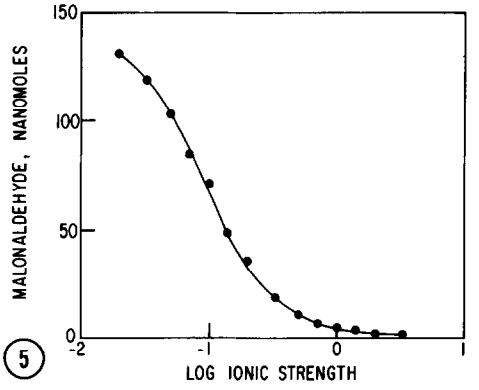
FIGURE 3. Dependency of reaction rate on NAD concentration.

FIGURE 4. Enzyme activity as a function of pH.

Figure 4 depicts the effect of pH on the reaction. Though optimum activity was observed at pH 9, there was still significant activity at pH 8 or 10. The left side of the curve may be steeper because lactic dehydrogenase has optimum activity at about pH 7.5.

Figure 5 shows the effect of ionic strength on the reaction. Here, the pyruvate concentration was 10 mM, and 10 mM N-ethylmorpholine was used so that ionic strengths as low as 0.02 could be used. The results showed no maximum activity at any ionic strength above 0.02, but substantial activity at ionic strengths below 0.1. Thus, for good activity, the ionic strength should not be increased unnecessarily.

The rate of the reaction was not significantly changed by the use of N-ethylmorpholine, phosphate, or bicarbonate buffers; however, 1.5 mM borate caused 50% inhibition by affecting the lactic dehydrogenase. There was also a 50% inhibition by 1 mM cyanide and 5 mM azide, but Ca^{+2}, Mg^{+2}, citrate, and EDTA had little effect. Thiols containing hydroxyl groups, such as dithioerythritol and mercaptoethanol, caused 50% inhibition at 50-100 μM, while others, such as cysteine, required 2-5 mM concentration to cause 50% inhibition.



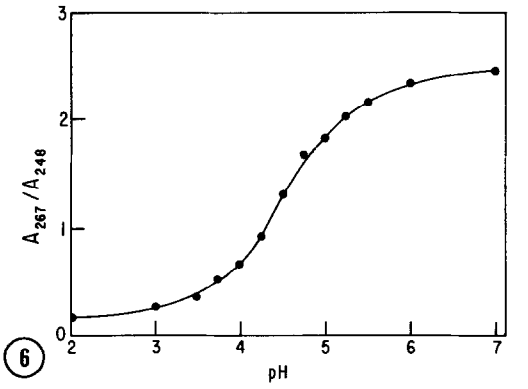


FIGURE 5. Effect of ionic strength on enzyme activity.

FIGURE 6. Effect of pH on the ultraviolet absorption spectrum of malonaldehyde.

Kwon and Watts (7) have shown that the ultraviolet absorption spectrum of malonaldehyde changes with pH. Since the volatility of malonaldehyde is affected by pH, these spectral changes were used to determine its pK. Figure 6 reveals a pK of about 4.5. With this information, a scheme for removal of malonaldehyde from the reaction mixture was devised. When the reaction had proceeded for 24 h, the reaction mixture was adjusted to pH 3 and heated to 50°C. The malonaldehyde was volatilized by an air stream and then condensed at -78°C. The condensate was adjusted to pH 7 and concentrated by evaporation at 35°C. By this method, concentrations of up to 40 mM malonaldehyde were obtained. The solution was stable if stored at pH 7 and 4°C.

DISCUSSION

Alcohol dehydrogenase converts a wide variety of alcohols to their corresponding aldehydes (10). In this study, the substrate was a diol, and since only malonaldehyde was detected, an initial lag would be expected for a steady-state concentration of hydroxypropionaldehyde to be reached. If the reaction reached equilibrium, over 90% conversion would be expected on a thermodynamic basis. However, it is quite possible that at this concentration, the malonaldehyde was reacting with itself as rapidly as it was formed. Since

higher substrate concentrations resulted in higher product concentrations, it is not likely that malonaldehyde was inactivating the enzyme.

The study of reactions involving malonaldehyde would be facilitated by a system for its generation in a reaction mixture that contains the molecule with which it is to react. This type of system more closely resembles in vivo conditions, in which a constant, low concentration is present, than do most in vitro systems. Different target molecules may require different buffers, salt concentrations, divalent cations, chelators, or thiols; bactericides such as azide may be necessary for long reaction times. Therefore, the system described may prove useful for a variety of investigations. Malonaldehyde can also be generated in situ by treating polyunsaturated fatty acid micelles with hydrogen peroxide (6) or lipoxygenase. However, oxygen or peroxide must be used, the yield of malonaldehyde is very low, and many other reactive products can be formed.

The system described can be used anaerobically if necessary, and it generates no peroxides or other reactive products. Thus, malonaldehyde can be prepared as a thiobarbituric acid reaction or ultraviolet absorption standard and also can be used to prepare secondary products related to food rancidity, toxicity, or biochemical damage. Finally, [1-^{14}C]-malonaldehyde was prepared from commercially available [1-^{14}C]-1,3-propanediol by the methods described; it is thus possible to prepare radiolabelled malonaldehyde for tracer studies.

ACKNOWLEDGMENT

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REFERENCES

1. Munkres, K. D. (1976) Mech. Aging Devel. 5, 171-191.
2. Kubinski, H., Strangstalin, M. A., Baird, W. M., and Bountwell, R. K. (1973) Can. Res. 33, 3103-3107.
3. Packer, L., Deamer, D. W., and Heath, R. L. (1967) Advan. Gerontol. Res. 2, 23-64.

4. Bidlack, W. R., and Tappel, A. L. (1973) Lipids 8, 203-207.

5. Chio, K. S., and Tappel, A. L. (1969) Biochemistry 8, 2827-2832.

6. Reiss, U., Chio, K. S., and Tappel, A. L. (1972) Biochem. Biophys. Res. Commun. 48, 921-925.

7. Kwon, T., and Watts, B. M. (1963) J. Food Sci. 28, 627-630.

8. Brooks, R. B., and Klamerth, O. L. (1968) Eur. J. Biochem. 5, 178-182.

9. Wills, E. D. (1966) Biochem. J. 99, 667-676.

10. Dickinson, F. M., and Dalziel, K. (1967) Biochem. J. 104, 165-172.

11. Theorell, H., and Bonnichsen, R. K. (1951) Acta Chem. Scand. 5, 1105-1114.