SOME OBSERVATIONS ON THE REACTION OF GLYOXAL WITH 2-THIOBARBITURIC ACID

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The application of the thiobarbituric acid (TBA) reaction to the products of periodic acid oxidation of certain carbohydrates has recently appeared in the literature. Two compounds have been reported as being the precursors of the soluble red pigment that is formed in acid solution; malonaldehyde and formyl pyruvic acid. Srinivasan and Sprinson (1959); Warren (1959); Weissbach and Hurwitz (1959).

In this laboratory, studies have been carried out on the nature of the reaction of TBA with radiation sterilized meats. In addition to the presence of malonaldehyde, it has been shown that glyoxal is also present and reacts with TBA. In view of this fact, a more extensive study of the glyoxal-TBA reaction has been carried out.

Experimental

The TBA reagent was prepared using 0.72% 2-thiobarbituric acid (EKCO #660) weight per volume in aqueous 10% trichloroacetic acid. Fresh reagent was made up for each run.

The glyoxal used in these experiments was obtained from Carbide and Carbon Chemical Company as a 30% aqueous solution. This solution was standardized using the method of Salomaa (1956). A 0.29% (w/v) solution was prepared and kept in the refrigerator. This concentration was selected as it is 1:1 with TBA on the molar basis.

Some of the experiments were run using crystalline glyoxal sodium

bisulfite (Delta Chemical).

Spectral data were obtained using Beckman model DU or a Cary model 11 recording spectrophotometer.

Results

Glyoxal and TBA reagent in a molar ratio of one mole glyoxal to three moles of TBA were heated for 10 minutes in a boiling water bath. The resultant solution was an intense red. The absorption spectrum of this solution showed distinct absorption maxima at 525 mμ and 552 mμ. Chromatographic analysis of the reaction mixture demonstrated the presence of two pigments. Using paper buffered at pH 8·7 with borate buffer and using the upper phase of the system, phenol; isopropanol: formic acid: water (80:10:10:100, w/w), the compound with the 525 mμ absorption maximum gave an R_f of 0.43 and that with 552 mμ absorption maximum an R_f of 0.24.

The effect of variation in heating time on the glyoxal-TBA reaction in acid solution was studied spectrophotometrically in systems in which the glyoxal/TBA ratio varied from 1:1 to 1:9. Irrespective of the ratio, it was found that a maximum absorption was obtained after heating for between 10 and 15 minutes with both the 525 and 552 mμ absorption peaks. Further heating had little effect on the 525 peak but resulted in a substantial decrease in absorption at the 552 maximum.

Allowing a mixture of glyoxal solution and TBA reagent to stand at room temperature resulted in the formation of a yellow solution with a single absorption maximum at 454 mμ. This solution upon heating in a boiling water bath produced the typical red solution and maxima and a loss of the 454 mμ peak. Even after standing overnight at room temperature, the yellow solution would turn red with heating. Malonaldehyde does not give an absorption maximum in this region on mixing with the TBA reagent and standing at room temperature.

Effects of Variation in the Reaction Medium. The reaction occurs only in acid solution. At pH 12 no pigment was produced and at pH 7 only a small tint was observed in the reaction mixture. The test was run routinely in 10%

trichloroacetic acid at pH 0.9. If the reaction is carried out in either orthophosphoric acid or sulfuric acid at a similar pH, the absorption maximum at 525 mμ is again obtained but only a small inflection of the absorption curve is observed at 552 mμ in contrast to the well defined absorption maximum obtained in 10% trichloroacetic acid.

Reaction With 5 Substituted 2-Thiobarbituric Acid. In order to more fully understand the reaction, the probable reaction site of the TBA molecule with the glyoxal was sought. It was known that reaction with barbituric acid produced a yellow colored solution. Using sodium pentothal (sodium 5 ethyl-5 (1 methyl butyl)-2 thiobarbituric acid) in 15% trichloroacetic acid and heating times up to 40 minutes, did not produce a colored solution. The lack of hydrogen at C-5 appeared to block the formation of the chromogen.

TBA-Glyoxal Ratios. A particular phenomenon has been noted with glyoxal in its reaction with varying molar quantities of TBA reagent. A constant amount of glyoxal (50 μ moles) in 15 ml of 15% trichloroacetic acid was heated in a boiling water bath for ten minutes with varying amounts of thiobarbituric acid. The TBA/glyoxal ratio was varied from 0.1 to 16 and the spectral curves of the resulting solution were measured between 500 to 560 mμ. A plot of absorbancy vs TBA/glyoxal molar ratio for each absorption maximum is given in Figure 1.

These results indicate that the compound absorbing at 525 mμ is not modified to a large extent by excess thiobarbituric acid. The 552 mμ absorbing compound shows a maximum concentration at a TBA/glyoxal ratio of 2; increasing the TBA/glyoxal ratio results in either the decomposition or further modification of the compound to other compound(s) which do not absorb in this portion of the spectrum. This observation has been confirmed by separating the two pigments chromatographically, eluting and then heating the individual pigments with some TBA reagent. The compound absorbing at 552 mμ is rapidly decolorized whereas the compound absorbing at 525 mμ was not affected by this procedure.

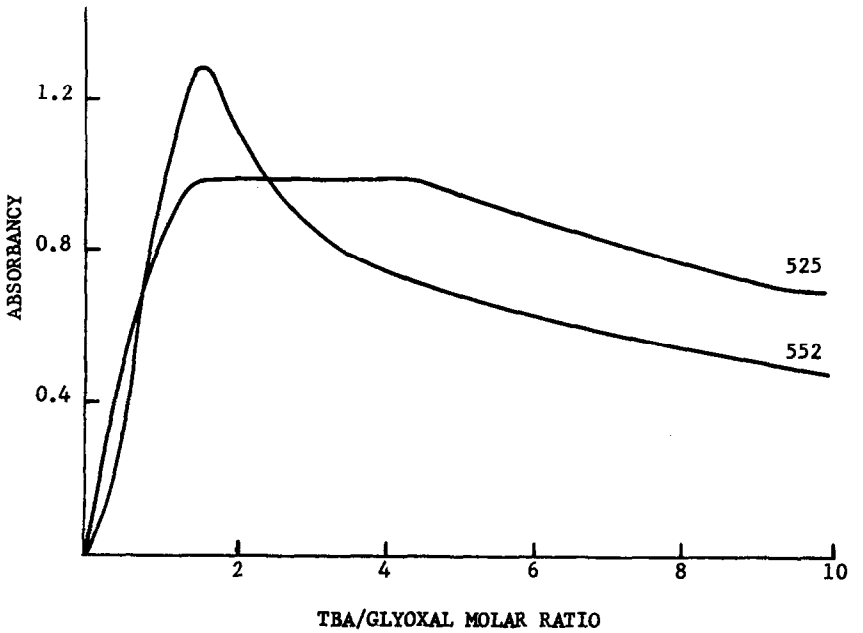

Figure 1. Effect of TBA/Glyoxal Molar Ratio on the 525 and 552 mμ Absorption Maxima

Malonaldehyde-Glyoxal Interaction. When malonaldehyde is heated in trichloroacetic acid with TBA, a red solution is produced. This solution has an absorption maximum at 534 mμ.

In the irradiated meat samples it was known that glyoxal as well as malonaldehyde contributed to the red pigmentation. The presence of two pigments has been verified by separation on paper chromatograms followed by elution, concentration and spectrophotometric measurement. In addition, glyoxal had been measured quantitiatively in these meat samples by the method of Arijamma (1928).

However, the spectrophotometric measurement of the TBA reaction on irradiated meats indicated only one broad peak at 534 mμ. Using equal concentrations of malonaldehyde and glyoxal in mixed solutions, it was seen that only one peak at 534 mμ was formed. The addition of larger amounts of glyoxal to a constant level of malonaldehyde did not produce an appreciable change in the curve. The molar absorbancy indexes for the 525 mμ and 552 mμ absorption maxima of the glyoxal-TBA system are 236 and 130 liters/mole cm respectively. A value of 1.56×10^5 liters/mole cm has been reported for the

molar absorbancy index of the malonaldehyde-TBA pigment, Sinnhuber and Yu (1958). The differences in the molar absorbancy indexes of the TBA pigments of these compounds are so great that small amounts of malonaldehyde can mask the presence of glyoxal. Thus, more than spectral evidence is needed before the identity of the pigments can be assured.

References

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