Free Rad. Res. Comms., Vol. 19, No. 5, pp. 315-321
Reprints available directly from the publisher
Photocopying permitted by license only

INTERACTIONS OF HYDROXYL RADICALS WITH TRIS (HYDROXYMETHYL) AMINOMETHANE AND GOOD'S BUFFERS CONTAINING HYDROXYMETHYL OR HYDROXYETHYL RESIDUES PRODUCE FORMALDEHYDE

HIROYUKI SHIRAISHI, MASANORI KATAOKA, YUTAKA MORITA and JUNJI UMEMOTO

Research Laboratory, Maruho Co. Ltd., 1-8-23 Oyodo-naka, Kita-ku Osaka 531 Japan

(Received November 25th 1992; in revised form June 29th 1993)

The production of formaldehyde from tris(hydroxymethyl) aminomethane(Tris) by interaction with hydroxyl radicals(·OH) was studied, since the reaction mixture from the Fenton reaction performed in Tris/HCl buffer was found to be color-developed by colorimetric determination of formaldehyde. The absorption spectrum of chromogens was identical to that of authentic formaldehyde. Color development, which required the presence of Tris, hydrogen peroxide and cupric ions in the Fenton reaction mixture, was inhibited by the addition of hydroxyl radical scavengers such as glucose or hyaluronic acid. These results indicated that formaldehyde was produced when Tris interacted with ·OH. With structures similar to Tris, Good's buffers were also found to produce formaldehyde by interaction with ·OH. Analysis of formaldehyde derived from these buffers may provide a simple and convenient assay for detecting ·OH generation. In evaluating effects of ·OH on the biological system in Tris/HCl buffer or certain Good's Buffers, ·OH loss may be due to interactions of ·OH with these buffers. The formaldehyde produced as a result of such interactions may affect biological systems.

KEY WORDS: Fenton reaction; hydroxyl radical; Tris(hydroxymethyl)aminomethane; Tris; Good's buffers; Formaldehyde; HEPES.

INTRODUCTION

Dimethyl sulfoxide (DMSO) is a potent hydroxyl radical scavenging agent.[1] Formaldehyde production from DMSO by interaction with ·OH has been used to indicate an ·OH generating system.[2] While determining formaldehyde produced from DMSO by the copper-catalyzed Fenton reaction[3] with the colorimetric method of the Hantzsch reaction,[4] we observed color development in the reaction mixture, even in the absence of DMSO. However, this phenomenon was observed only after the copper-mediated Fenton reaction[3] was performed in Tris(hydroxymethyl)aminomethane/hydrogen chloride (Tris/HCl) buffer.

If formaldehyde is produced from Tris by interaction with ·OH, this phenomenon may provide a basis to develop a simple and convenient assay for detecting the presence of ·OH in certain biological systems. In evaluating effects of ·OH on

Correspondence address: J. Umemoto, Research Laboratory, Maruho Co. Ltd., 1-8-23 Oyodo-naka, Kita-ku, Osaka, 531 Japan.

certain biological systems in Tris/HCl buffer, the buffer should not be considered as an inert medium. In other words, ·OH loss may occur because of the interaction of ·OH with Tris. The formaldehyde produced as a result of such an interaction may affect the biological systems.

The present study attempted to detect the formaldehyde derived from Tris by interaction with ·OH, which formed during the copper-mediated Fenton reaction.[3] Formaldehyde formation from certain Good's buffers[5-7] by interaction with ·OH in the copper-mediated Fenton reaction[3] was also studied since the structures of certain Good's buffers[5-7] resemble that of Tris.

MATERIALS AND METHODS

Tris, Good's buffers[5-7] and catalase (EC 1.11.1.6, 40,000 units/mg protein) were obtained from Wako Pure Chemicals Co. (Osaka, Japan). Hyaluronic Acid(Mr $= 1.81 \times 10^6$) was a gift from Shiseido Co. (Tokyo, Japan). All other chemicals used were of analytical grade. The copper-mediated Fenton reaction[3] was performed in two kinds of reaction mixtures. Reaction mixtured (I) was composed of 50 mM potassium phosphate buffer (pH 7.5), 0.1 mM or 0.5 mM Tris, or certain Good's buffers,[5-7] 0.1 mM cupric sulfate and 5 mM hydrogen peroxide, adjusted to a final volume of 1.5 ml. Reaction mixture (II) was composed of 0.5 mM or 20 mM Tris/HCl buffer, or Good's buffer,[5-7] and the above concentrations of Cu^{2+} and hydrogen peroxide were used. The pH of the reaction mixture (II) was adjusted to the midpoint of the buffering range of each buffer.[5-7] Incubation was carried out in triplicate at 37°C for 30 min. Reactions were initiated by adding hydrogen peroxide and the incubation at 37°C was terminated by incubation with 10 μl catalase (250 units) for 5 min. Blank control solutions were incubated without hydrogen peroxide. An 1.5 ml aliquot was then assayed colorimetrically for the presence of formaldehyde by the Hantzsch reaction.[4] An another aliquot (1.0 ml) was assayed following a colorimetrical reaction with chromotropic acid.[8] Amount and absorption spectrum of chromogens depicted by the test solution were determined and compared using the blank as a reference. Hydrogen peroxide was determined colorimetrically by the iodine-starch method.[9]

RESULTS AND DISCUSSION

Detection of Formaldehyde Derived from Tris by Interaction with ·OH

In view of the report[3] describing copper ions as a more effective catalyst than Fe^{2+} under physiological conditions, the copper-catalyzed Fenton reaction was used for the ·OH generation system. Reaction mixture(I) was used for examining products derived from Tris during the copper-catalyzed Fenton reaction.[3] Figure 1(A) illustrates the absorption spectra of chromogens derived from a solution of authentic formaldehyde (0.01 mM) by the Hantzsch reaction[4], and from products derived during the copper-mediated Fenton reaction[3] in the presence of Tris. Similar spectra with an absorption peak at 412 nm were observed. Figure 1(B) shows the absorption spectra of chromogens formed by another color reaction for formaldehyde.[8] Similar spectral patterns with maximum and submaximum absorption were detected at approximately 570 nm and 480 nm, respectively. These results indicated that the

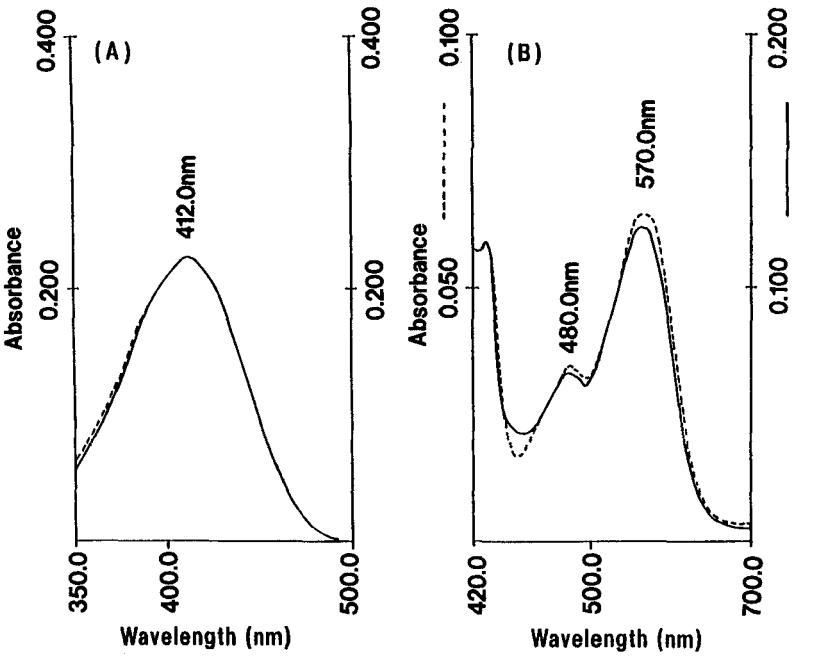

FIGURE 1 Absorption spectra of chromogens formed by Hantzsch reaction (A) and by the reaction with chromotropic acid(B) from formaldehyde(– – –) and from products (—) of the copper-mediated Fenton reaction. Reaction mixture of the copper-mediated Fenton reaction was composed of 50 mM potassium phosphate buffer(pH 7.5), 0.5 mM tris(hydroxymethyl)aminomethane, 0.1 mM cupric sulfate and 5 mM hydrogenperoxide. Incubation was carried out at 37°C for 30 min. Reaction was initiated by adding hydrogen peroxide and was terminated by incubation with catalase (250 units) at 37°C for 5 min.

product formed by the copper-catalyzed Fenton reaction[3] was formaldehyde. Figure 2 shows the time course of formaldehyde production during the copper-catalyzed Fenton reaction.[3] Increase in formaldehyde production in the reaction mixture was observed over time. However, formaldehyde production was not seen when Cu^{2+} or Tris was omitted from the reaction mixture or when 250 units of catalase were added at time zero. The results showed that formaldehyde was formed from Tris with participation of hydrogen peroxide, although formaldehyde formation was not a result of direct interaction between Tris and hydrogen peroxide. Glucose[10,11] and hyaluronic acid[12,13] are ·OH scavengers. D-glucose and hyaluronic acid were used to examine their effects on formaldehyde production derived from Tris during the copper-mediated Fenton reaction.[3] As demonstrated in Figure 3(A) and (B), D-glucose and hyaluronic acid inhibited the production of formaldehyde in a dose-dependent manner. More potent inhibitory effects of D-glucose were observed in reaction mixtures with lower Tris concentrations. This result indicated that formaldehyde was produced by the interaction of ·OH with Tris, whereas D-glucose and Tris might have competed to react with ·OH.

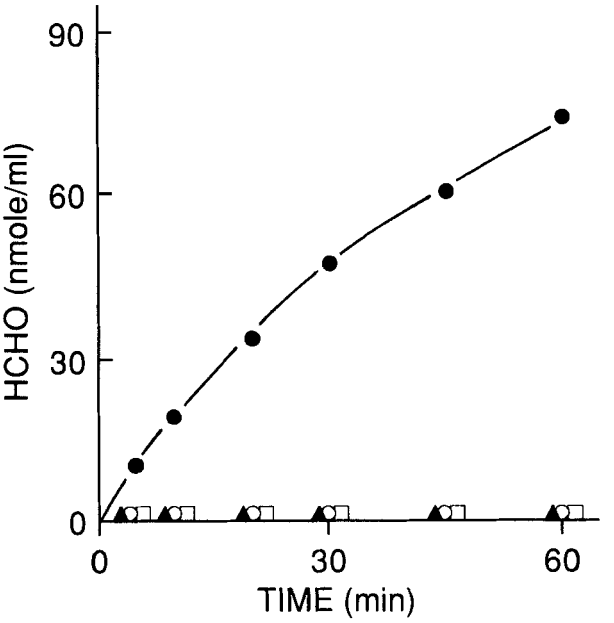

FIGURE 2 Time course of formaldehyde production derived from tris(hydroxymethyl)aminomethane(Tris) during the copper-mediated Fenton reaction. Reaction mixture was composed of 50 mM potassium phosphate buffer (pH 7.5), 0.5 mM Tris, 0.1 mM cupric sulfate and 5 mM hydrogen peroxide (●), without cupric sulfate (○), without Tris (□) or with the addition of 250 units catalase at time zero (▲). Reactions at 37°C were initiated by adding hydrogen peroxide and terminated by incubation with catalase(250 units) at 37°C for 5 min.

Formation of Formaldehyde from Tris and Good's Buffers by Interaction with ·OH

The reaction mechanism involved in formaldehyde formation from Tris is unclear as yet. However, formaldehyde is evidently derived from trishydroxymethyl residue of Tris because formaldehyde is formed from deoxyribose by interaction with ·OH.[14] It is possible that formaldehyde is produced from the Good's buffers[5-7] by ·OH formed during the copper-mediated Fenton reaction[3] because, similar to Tris, Good's buffers[5-7] such as N-Tris-(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), N-Tris-(hydroxymethyl)methyl-2-hydroxy-3-aminopropanesulfonic acid (TAPSO), N-Tris(hydroxymethyl) methyl-2-aminoethanesulfonic acid (TES), and Tris(hydroxymethyl)methylglycine (Tricine) have a trishydroxymethyl residue in their structures. Formaldehyde formation from these buffers by interaction with ·OH was examined. In addition to these, buffers with a N,N-Bis-2-hydroxyethyl residue such as N,N-Bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), N,N-Bis(2-hydroxyethyl)glycine (Bicine) and 3-[N,N-Bis(2-hydroxyethyl) amino]-2-hydroxypropanesulfonic acid (DIPSO), buffers with a N-2-hydroxyethyl residue such as N-2-Hydroxyethylpiperazine-N'-3-propanesulfonic acid (EPPS), N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) and N-2-Hydroxyethylpiperazine-N'-2-hydroxypropane-3-sulfonic acid (HEPPSO), or buffer with both trishydroxymethyl and N,N-Bis-2-hydroxyethyl residues such as Bis(2-

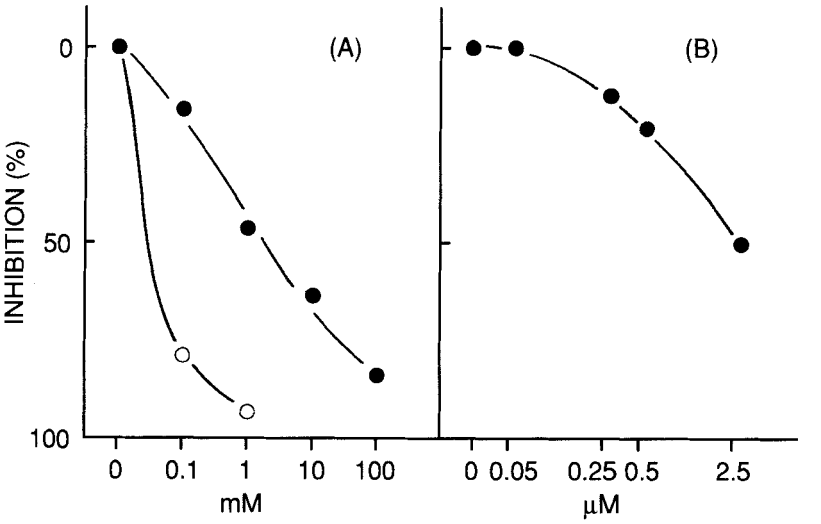

FIGURE 3 Inhibitory effects of D-glucose and hyaluronic acid on formaldehyde production derived from tris(hydroxymethyl)aminomethane(Tris) during the copper-mediated Fenton reaction. Reaction mixture was composed of 50 mM potassium phosphate buffer (ph 7.5), 0.1 mM cupric sulfate and 5 mM hydrogen peroxide and Tris. Incubation was carried out at 37°C for 30 min. Reactions were initiated by adding hydrogen peroxide and terminated by incubation with catalase (250 units) at 37°C for 5 min. D-glucose was added to the reaction mixture containing 0.1 mM (○) or 0.5 mM (●) Tris (A), and hyaluronic acid was added to the mixture containing 0.5 mM (●) Tris (B).

hydroxyethyl) iminotris(hydroxymethyl)methane (Bis-Tris) were also examined. Table 1 shows the results of formaldehyde formation from Tris or Good's buffers[5-7] when the copper-mediated Fenton reaction[3] was carried out in reaction mixture(I). Formaldehyde production was seen in the reaction mixture containing Tris, TAPSO, DIPSO, HEPES, EPPS or HEPPSO, showing that these buffers were effective in trapping ·OH in such reaction systems. Similar studies with reaction mixture(II) revealed formaldehyde was produced from Tris or Good's buffers[5-7] other than Tricine, Bis-Tris and Bicine at both buffer concentrations. Tricine, Bis-Tris and Bicine were poor reactants of ·OH in both reaction mixtures, but Good's buffers[5-7] such as TES, TAPS and BES have different reactivity with ·OH in the two reaction mixtures (Tables 1 and 2). The relationships between buffer structures and their reactivity with respect to interaction with ·OH are not yet clear. The radical, ·OH, has been detected chemically with methional,[15] benzoic acid,[16] DMSO[2] or salicylic acid[17] and quantified chemically with p-nitrosodimethylaniline[18] and DMSO.[19] Although it is not yet clear whether Tris or the Good's buffer[5-7] is the more sensitive index of ·OH generation, the production of formaldehyde from Tris or some of Good's buffers[5-7] by interaction with ·OH may provide a simple and convenient assay for detecting ·OH generation. Further studies on the application of these buffers as an index for ·OH quantification are warranted.

Reactions involving ·OH have been studied in Tris/HCl buffer.[20,21] Good's buffer,[5-7] especially HEPES, is often added to the cell or tissue culture medium as a buffer. HEPES reportedly stimulates ·OH generation by copper ions and hydrogen peroxide.[22] In evaluating the biological effect of ·OH in these biological reaction

TABLE 1
Formaldehyde formation from 0.5 mM Tris and Good's buffer by ·OH formed during copper-catalyzed Fenton reaction in 50 mM potassium phosphate buffer (ph7.5)

Buffer	Formaldehyde production(nmol/ml)	Reactive residues
Tris	50.7	trishydroxymethyl
TES	4.0	
TAPSO	30.7	
TAPS	2.0	
Tricine	1.3	
Bicine	0.7	N,N-bis-2-hydroxyethyl
BES	2.0	
DIPSO	24.7	
HEPES	45.3	N-2-hydroxyethyl
EPPS	58.7	
HEPPSO	52.0	
Bis-Tris	2.0	trishydroxymethyl N,N-bis-2-hydroxyethyl

Buffers used were Tris(hydroxymethyl)aminomethane (Tris); N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES); N-Tris(hydroxymethyl)methyl-2-hydroxy-3-aminopropanesulfonic acid (TAPSO); N-Tris-(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS); Tris(hydroxymethyl) methylglycine (Tricine); N,N-Bis(2-hydroxyethyl)glycine(Bicine); N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES); 3-[N,N-Bis(2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (DIPSO); N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid(HEPES); N-2-Hydroxyethylpiperazine-N'-3-propanesulfonic acid (EPPS); N-2-Hydroxyethylpiperazine-N'-2-hydroxypropane-3-sulfonic acid (HEPPSO); Bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane (Bis-Tris).

TABLE 2
Formaldehyde formation from Tris/HCL buffer and Good's buffers by ·OH formed during copper-catalyzed Fenton reaction

		Formaldehyde production(nmol/ml)	
		Concentration	
Buffer	pH	0.5 mM	20 mM
Tris	8.2	44.0	66.0
TES	7.6	45.3	189.3
TAPSO	7.6	29.3	152.0
TAPS	8.4	20.7	500.7
Tricine	8.3	7.3	8.0
Bicine	8.4	1.3	0.7
BES	7.3	23.3	114.7
DIPSO	7.5	22.7	282.7
HEPES	7.5	28.7	203.3
EPPS	8.0	37.3	210.0
HEPPSO	8.0	45.3	352.7
Bis-Tris	6.5	0.7	0.7

systems, it should be considered that these buffers are not inert; loss of ·OH might have resulted from the interaction of ·OH with the buffer, and that the formaldehyde resulting from such a reaction might affect the biological system.

References

1. M. Anbar and P. Neta (1967) A compilation of specific biomolecular rate constants for the reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals with inorganic and organic compounds in aqueous solution. *International Journal of Applied Radiation and Isotopes*, **18**, 493–528.
2. S.M. Klein, G. Cohen, and A.I. Cederbaum (1981) Production of formaldehyde during metabolism of dimethyl sufoxide by hydroxyl radical generating systems. *Biochemistry*, **20**, 6006–6012.
3. K. Uchida and S. Kawakishi (1988) Interaction of (1 → 4) and (1 → 6)-linked disaccharides with the fenton reagent under physiological conditions. *Carbohydrate Research*, **173**, 89–99.
4. T. Nash (1953) The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochemical Journal* 416–421.
5. N.E. Good, G.D. Winget, W. Winter, T.N. Connolly, S. Izawa, and R.M.M. Singh (1966) Hydrogen ion buffers for biological research. *Biochemistry*, **5**, 467–477.
6. N. E. Good and S. Izawa (1972) Hydrogen ion buffers. In *Methods in Enzymology*, vol. **XXIV** (ed. Pietro, A.S.), Academic Press, New York and London pp. 53–68.
7. W.J. Feruguson, K.I. Braunschweiger, W.R. Braunschweiger, J.R. Smith, J.J. McDormick, C.C. Wasmann, N.P. Jarvis, D.H. Bell, and N.E. Good (1980) Hydrogen ion buffers for biological research. *Analytical Biochemistry*, **104**, 300–310.
8. M. Rambert and A.C. Neish (1950) Rapid method for estimation of glycerol in fermentation solutions. *Canadian Journal of Research*, **28B**, 83–89.
9. E. Graf and J.T. Penniston (1980) Method for determination of hydrogen peroxide, with its application illustrated by glucose assay. *Clinical Chemistry*, **26**, 658–660.
10. A.L. Sagone, J. Greenwald, E.H. Kraut, J. Bianchine and D. Singh (1983) Glucose: a role as a free radical scavenger in biological systems. *Journal of Laboratory and Clinical Medicine*, **101**, 97–104.
11. B. Halliwell and J.M.C. Gutteridge (1986) Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. *Archives of Biochemistry and Biophysica*, **246**, 501–514.
12. H. Sato, T. Takahashi, H. Ide, T. Hukushima, M.,Tabata, F. Sekine, K. Kobayashi, M. Negishi and Y. Niwa (1988) Antioxidant activity of synovial fluid, hyaluronic acid, and two subcomponents of hyaluronic acid: Synovial fluid scavenging effect is enhanced in rheumatoid arthritis patients. *Arthritis and Rheumatism*, **31**, 63–71.
13. P. Myint, D.J. Deeble, P.C. Beaumont, S.M. Blake and G.O. Phillips (1987) The reactivity of various free radicals with hyaluronic acid: steady-state and pulse radiolysis studies. Biochima Biophysica Acta, **925**, 194–202.
14. S. Khan, R. Krishnamurthy and K.P. Pandya (1990) Generation of hydroxyl radicals during benzene toxity. *Biochemical Pharmacology*, **39**, 1393–1395.
15. A.I. Tauber and B.M. Babior (1977) Evidence for hydroxyl radical production by human neutrophils. *Journal of Clinical Investigation*, **69**, 374–379.
16. A.L. Sagone, M.A. Decker, Jr, R.M. Wells and C. Democko (1980) A new method for the detection of hydroxyl radical production by phagocytic cells. *Biochimica Biophysica Acta*, **628**, 90–97.
17. Y. Yoshimura, K. Otsuka, K. Uchiyama, H. Tanaka, K. Tamura, K. Ohsawa and K. Imaeda (1989) Detection of hydroxyl radicals with salicylic acid. *Analytical Science*, **5**, 161–164.
18. W. Bors, C. Michel and M. Saran (1979) On the nature of biochemically generated hydroxyl radicals: studies using the bleaching of p-Nitrosodimetylaniline as a direct assay method. *European Journal of Biochemistry*, **95**, 621–627.
19. M.G. Steiner and C.F. Babbs (1990) Quantitation of hydroxyl radical by reaction with dimethyl sulfoxide. *Archives of Biochemistry and Biophysics*, **278**, 478–481.
20. E. Graf, J.R. Mahoney, R.G. Bryant and J.W. Eaton (1984) Iron-catalyzed hydroxyl radical formation. Stringent requirement for free iron coordination site. *Journal of Biological Chemistry*, **259**, 3620–3624.
21. E. Graf, K.L. Empson and J.W. Eaton (1987) Phytic acid. A natural antioxidant. *Journal of Biological Chemistry*, **262**, 11647–11650.
22. J.A. Simpson, K.H. Cheeseman, S.E. Smith and R.T. Dean (1988) Free radical generation by copper ions and hydrogen peroxide: Stimulation by Hepes buffer. *Biochemical Journal*, **254**, 519–523.

Accepted by Prof. T. Yoshikawa