Rate constants for reaction of hydroxyl radicals with Tris, Tricine and Hepes buffers

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Rate constants for the reactions of three commonly used organic buffers and hydroxyl radicals were measured using steady-state competition kinetics with thymine. For Hepes (4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid), Tricine (*N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine and Tris (2-amino-2-hydroxymethylpropane-1,3-diol) the rate constants were 5.1×10^9, 1.6×10^9 and 1.1×10^9 $l \cdot mol^{-1} \cdot s^{-1}$, respectively.

Hydroxyl radical *Organic buffer* *Hydroxyl radical scavenger* *Rate constant*

1. INTRODUCTION

Since their introduction by Good et al. [1], a range of organic buffers have been widely employed because of their efficient buffering capacity near physiological pH. In addition, detailed studies with soluble or membrane-bound enzymes and cultured cell lines have shown these buffers to be often superior to inorganic buffers such as phosphate, borate or bicarbonate in protecting many systems from denaturation [1–3]. The organic buffers have also been used in examinations of the formation and effects of free radicals in biological systems [4–6].

In the course of a study of the effects of free radicals on fatty acid membranes, it became clear that the presence of Tris complicated the interpretation of our results, because of its potential to scavenge hydroxyl radicals. Since this buffer is essential in stabilizing the membranes [7], a separate study of its reactivity towards the hydroxyl radical ($HO^{\cdot}$) was undertaken. Earlier reports [8–11] have indicated that Tris can scavenge $HO^{\cdot}$ radicals, but the rate constant for the reaction was not measured. We now report the rate constants for the reactions of $HO^{\cdot}$ with Tris, Tricine and Hepes. The values were obtained by steady-state competition kinetics with thymine.

2. MATERIALS AND METHODS

Tris, Tricine and Hepes were AR grade (Ultrol) and obtained from Calbiochem. Thymine was purchased from Sigma and recrystallized twice from hot triply distilled water and EDTA before use. All other chemicals were of AR grade from Ajax Chemicals. Water used for solution preparation was distilled then passed through a Milli-Q water purification system equipped with an Organex-Q cartridge for removal of trace organic residues.

Irradiations were performed in a 2500 Ci ^{60}Co gamma source. Dose rates were measured by Fricke dosimetry [12] with the *G* value for the formation of Fe^{3+} taken as 15.5 ions/100 eV [13]. The dose rates varied from 40 to 60 Gy/min. Loss of thymine was measured as the decrease in ab-

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Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; Tricine, *N*-[2-hydroxy-1,1-bis-(hydroxymethyl)ethyl]glycine; Tris, 2-amino-2-hydroxy-methylpropane-1,3-diol

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sorbance at 264 nm with the molar extinction coefficient of thymine taken as 7900 $l \cdot mol^{-1} \cdot cm^{-1}$ [14].

3. RESULTS AND DISCUSSION

Systems consisting of two solutes capable of reacting at comparable speeds with a third species are commonly used to measure reaction rate constants in solution. For such competing processes, the extent of each reaction is proportional to its individual rate constant. Here, the competing solutes were thymine (T) and a buffer (B), while the reactive species was the HO· radical generated by radiolysis of water. The reactions were thus:

$$HO^{\cdot} + T \xrightarrow{k_T} \text{T-products}$$

$$HO^{\cdot} + B \xrightarrow{k_B} \text{B-products}$$

It is convenient to measure radiation chemical yields in terms of 'G values'. These give the number of molecules of any species formed (+) or destroyed (−) for every 100 eV of energy absorbed. The loss of thymine chromophore on exposure to radiolytically produced HO· is given by:

$$G(-T) = G(HO^{\cdot}) \times \frac{k_T[T]}{k_T[T] + k_B[B]}$$

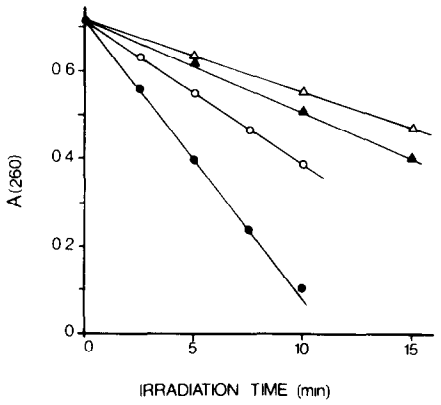

Fig.1. Loss of thymine chromophore in different thymine/Hepes mixtures as a function of absorbed radiation dose. Dose rate 42.25 Gy/min. Initial [thymine] = 0.09 mM. Initial [Hepes]: (●) 0, (○) 0.09, (▲) 0.18, (△) 0.27 mM.

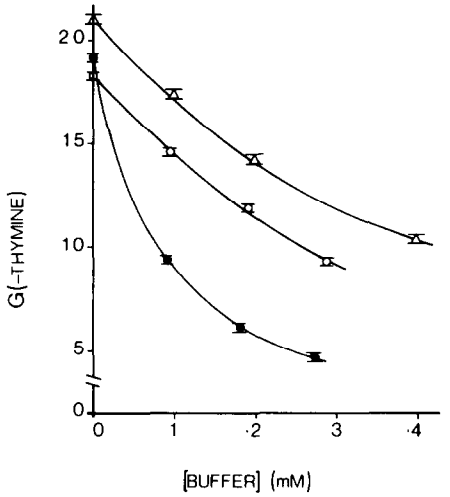

Fig.2. Decrease in radiation-induced loss of thymine chromophore as a function of buffer concentration. Each point is the mean of 5 determinations; the error bars are ± SE. (●) Hepes, (○) Tricine, (△) Tris. Dose rate was 42.25 Gy/min for Hepes and Tricine and 60 Gy/min for Tris.

In the experiments, [T] and [B] are arranged as required, the value of k_T is taken from the literature, $G(-T)$ is measured by following the loss of absorbance of irradiated thymine and the effective $G(HO^{\cdot})$ determined from the $G(-T)$ at zero buffer concentration. Thus, only the change in the concentration of one radical scavenger needs to be followed. When $G(-T)$ is determined for a

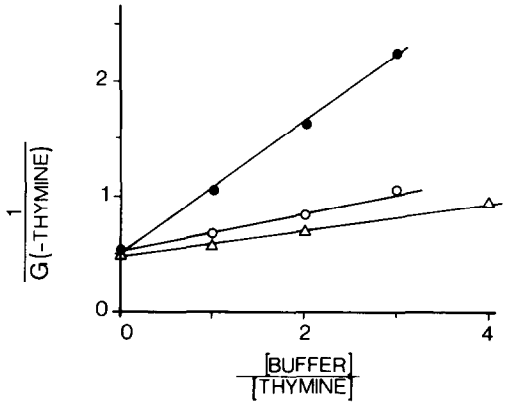

Fig.3. Reciprocal of $G(-$ thymine) as a function of the initial buffer/thymine ratios. (●) Hepes, (○) Tricine, (△) Tris. Correlation coefficients (r^2 values) for the equations of the lines are 0.998, 0.991 and 0.998 for Hepes, Tricine and Tris, respectively.

number of buffer/thymine ratios, k_B can be calculated from the slope of the plot of $(G(-T))^{-1}$ vs [B]/[T].

Aerated solutions of thymine (0.09 mM) in 6.6 mM Na_2HPO_4/KH_2PO_4 buffer at pH 8.0 were irradiated. The $G(-T)$ values were independent of initial thymine concentrations at around 0.1 mM and above, indicating complete scavenging of the HO· radicals. Fig.1 shows the effect of increasing concentration of Hepes on the loss of the thymine chromophore. Clearly, the buffer protected thymine by competing for the HO· radicals. Similar results (not shown) were obtained with Tris and Tricine. The effect of buffer concentration on the disappearance of thymine chromophore is shown in fig.2. In the absence of buffer, $G(-T)$ varied between 1.8 and 2.1, with an average of 1.9. Myers et al. [15] found similar variability. The G value of 1.9 shows that not all the HO· radicals generated reacted to saturate the 5–6 position of the thymine ring, because $G(HO^{\cdot})$ is 2.8 [16]. This agrees with the finding of several products derived from thymine/HO· reactions [15].

The data from fig.2 were transformed to give the results plotted in fig.3. Values of the rate constants were calculated from the relationship: slope = $k_B/G(HO^{\cdot})k_T$ using the absolute value of $k_T = 4.7 \times 10^9\ M^{-1}\cdot s^{-1}$ [17]. The results were: for Hepes, 5.1×10^9; for Tricine, 1.6×10^9; for Tris 1.1×10^9 (all in $M^{-1}\cdot s^{-1}$).

This study demonstrates that Hepes, Tricine and Tris are efficient scavengers of HO· radicals. It is likely that other organic buffers also act as scavengers with rate constants around $10^9\ M^{-1}\cdot s^{-1}$. The finding that under some conditions not all the HO· radical damage is inhibited by these buffers [4–6,18] suggests either that the secondary radicals derived from the buffers can initiate damage or that the HO· radicals are produced at sites inaccessible to the buffer molecules. There is a growing body of evidence for 'site-specific' formation of HO· which is not affected by scavengers present in the aqueous phase [19,20]. This is especially likely to occur in heterogeneous systems which are typical of biological cells and tissues.

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