BIOCHEMICAL LETTERS

Tris buffer—a case for caution in its use in copper-containing systems

The use of Tris, which has a pK_a value of 8.1 (Bai & Martell, 1969), as a buffering agent in the region pH 7–9 is well established. Nevertheless, care should be taken when working with copper-containing systems because Tris itself exhibits copper-complexing ability and hence may remove copper from certain key sites in the system being investigated. We have now demonstrated this in the case of the copper-containing enzyme, bovine superoxide dismutase (Scrimgeour, 1977), using e.p.r. spectroscopy.

E.p.r. spectra were obtained at 77 K from a solution of the enzyme (1%, w/v) and solutions of the enzyme (1%, w/v) with Tris (5%, w/v) at pH 10.4, 8.6 and 8.0. The solution at pH 10.4 was prepared from Tris alone, while the solutions at lower pH were prepared by adjustment with HCl. For comparison, spectra were also obtained from solutions of (a) CuSO_4 (10 mM) and (b) CuSO_4 (10 mM) with Tris (50 mM).

Fig. 1(a) shows the spectrum obtained from the CuSO_4 solution; the signal can be attributed to the species $\text{Cu}(\text{H}_2\text{O})_6^{2+}$. The spectrum obtained from the CuSO_4 /Tris solution is shown in Fig. 1(b). It is apparent that the cupric ion has been complexed by the Tris, with none of the free hydrated species remaining in solution. Indeed if the CuSO_4 /Tris solution is examined at room temperature (Fig. 2), hyperfine structure resulting from the interaction of the unpaired electron with the nuclei of bound N atoms can be seen at high field. The 1:2:3:2:1 intensity ratio of this quintet is indicative of co-ordination via two magnetically equivalent nitrogen atoms, and must, therefore, arise from a complex with two Tris ions bound to the copper.

The spectrum obtained from the aqueous superoxide dismutase, in which all of the copper present is bound to the enzyme, is shown in Fig. 1(c). In contrast, the spectrum obtained from the enzyme/Tris solution at pH 10.4 (Fig. 1d) indicates that a substantial proportion of the copper is in the form of a Tris complex.

Fig. 1(e) shows the spectrum obtained from the Tris/enzyme solution of pH 8.6 and, although the Tris/copper signal is difficult to detect in the parallel region of the spectrum, the off-axis turning point (marked by arrows in Figs. 1b, 1d and 1e), is

clearly observed, indicating the presence of the complex at this lower pH. Indeed the spectrum obtained at pH 8.0 (not shown) still displays this feature although it has further diminished by approx. 40%. The presence of the copper-Tris complex could also be observed in the fluid solution spectra of all the enzyme solutions that contained Tris. In these cases no peaks corresponding to copper in the superoxide dismutase are

Fig. 1. E.p.r. spectra at 77 K of copper-containing samples (a) $\text{Cu}(\text{H}_2\text{O})_6^{2+}$ aq.; (b) solution of CuSO_4 (10 mM) and Tris (50 mM); (c) superoxide dismutase (1%, w/v); (d) superoxide dismutase (1%, w/v) and Tris (5%, w/v) at pH 10.4; (e) superoxide dismutase (1%, w/v) and Tris (5%, w/v) at pH 8.6.

Fig. 2. Room temperature, second-derivative e.p.r. spectrum of a solution of CuSO_4 (10mM) and Tris (50mM)

seen because of the low intensity of the anisotropic spectrum that arises as a result of the slow tumbling motion of the large enzyme molecule.

In order to determine whether all of the Tris-bound copper was removed from the enzyme or some held as a copper-Tris complex on the enzyme, a solution similar to that which produced the spectrum of Fig. 1(d) was dialysed against water for 20h. The dialysed enzyme produced a spectrum that was indistinguishable from that of the untreated enzyme (Fig. 1c) and the concentrated diffusible material gave a spectrum identical to that of the copper-Tris complex.

Despite the use of a high concentration of Tris relative to the number of enzyme copper sites, it must be stressed that even at pH 10.4, where the Tris is fully ionized, only approx. one-half of the copper-enzyme signal was removed by the buffer and that appreciably smaller amounts of copper were removed at lower pH values. These results do, however, indicate that caution should be exercised when using Tris buffer in combination with copper-containing systems, and may be especially important in chromatographic work if the buffer is contained in a column eluent, where the amounts

of Tris in contact with the enzyme will be relatively large compared with a static situation. It is also interesting to note that several other complexing and chelating agents have not been able to remove copper from the enzyme site (Heikkila & Cohen, 1977). These aspects of the work require investigation.

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(Received 10 April 1984)

Bai, K. S. & Martell, A. E. (1969) *J. Inorg. Nucl. Chem.* **31**, 1697-1707

Scrimgeour, K. G. (1977) *Chemistry and Control of Enzyme Reactions*, pp. 362-365, Academic Press, London, New York and San Francisco

Heikkila, R. E. & Cohen, G. (1977) in *Superoxide and Superoxide Dismutases* (Michelson, A. M., McCord, J. M. & Fridovich, I., eds.), pp. 367-373, Academic Press, London, New York and San Francisco