BIOCHEMICAL JOURNAL LETTERS

Cellular effects of proteinase activity in commercial xanthine oxidase

The technique described by Gilbert & Bergel (1964) for preparing xanthine oxidase from milk, which is the method used routinely in commercial preparations, involves the addition of pancreatin (a crude enzyme mixture containing lipases and proteinases). A mixture of xanthine plus xanthine oxidase, which generates oxygen radicals, has been used as a cell-free model of the superoxide-generating system in the granulocyte (Rosen & Klebanoff, 1979) and is employed to study oxygen-mediated cytotoxicity (see, for example, Ohmari *et al.*, 1979; Kraut & Sagone, 1982). Waud *et al.* (1975) recognized that residual proteolytic activity existed in xanthine oxidase prepared by the method of Gilbert & Bergel (1964) – indeed, the subunit structure is affected as a consequence – and some workers therefore purify commercial xanthine oxidase to remove other contaminating enzymes. This further purification step is not, however, invariably undertaken, to judge from the cytotoxicity studies cited above, although it is common practice to remove $(\text{NH}_4)_2\text{SO}_4$ with a Sephadex G-25 column.

Substrate-independent effects of xanthine oxidase on platelets have been reported (Patscheke *et al.*, 1978; Levine *et al.*, 1981) and the factor responsible was subsequently separated from xanthine oxidase itself and found to have an M_r of about 17 000 (Ishii *et al.*, 1982). We found (Ager *et al.*, 1984) that, at the concentrations used to generate oxygen radicals, xanthine oxidase obtained from either Sigma or Boehringer Mannheim had substrate-independent effects on endothelial cells that included stimulation of potassium efflux and changes in morphology. These effects were blocked by soya bean trypsin inhibitor and were mimicked by pancreatin and by trypsin itself. In order to study the oxygen-dependent effects of xanthine plus xanthine oxidase on endothelial cells, we found it necessary to use xanthine oxidase that was uncontaminated by pancreatin (Ager & Gordon, 1984).

Thus, the residual pancreatin in commercial preparations of xanthine oxidase has biologically

important effects on mammalian cells at the concentrations normally used, apparently because of its trypsin-like activity. Forti (1977) briefly commented on a trypsin-like action of xanthine oxidase on chloroplast membranes. The purpose of this communication is to bring these effects to the notice of those who contemplate studying the actions of xanthine oxidase on mammalian cells and might be tempted to use a commercial preparation without removing proteinase contaminants. If even one person is saved from a time-consuming diversion of research effort, our own frustrating experience will not have been entirely useless.

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