Purification of superoxide dismutase from porcine erythrocyte and determination of activity

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ABSTRACT

This study includes two parts. The first part of the study is comprisedof three methods that examine SOD activity. Various bovine SOD activitylevels are used to measure and determine the effectiveness of each method.Standard curves are then made for later reference in purification of SODfrom porcine erythrocyte. In the second part, the purification andcharacterization techniques of SOD from porcine erythrocyte are studied. Thexanthine oxidase method shows that the bovine SOD is most sensitive to a0.05 U content of xanthine oxidase. When SOD activity ranges from 0.25 U to5.0 U, the changes in absorbance reveal the possible presence of bovine SOD.The xanthine oxidase solution becomes obsolete for determining SOD activityafter 24 hours of exposure at room temperature. During the photoreduction ofNBT, bovine SOD proves to be most sensitive at a pH of 7.8, as proven from aseries of experimental runs under various pH levels. If SOD is not present,the average absorbance increase rate is 0.054 /min for the first 6 minutes.After 1.0 U of SOD is added, about 50% of photoreduction reaction isinhibited. This NBT method more effectively determines the SOD activity thanthe xanthine - xanthine oxidase method. Experimental results reveal that theNBT method is suitable to d etermine the presence of bovine SOD in the range0.0625 U and 5.0 U. The NBT solution becomes obsolete in determining SODactivity after 4 days of exposure at room temperature. Experimental resultsreveal that pyrogallol autoxidation method is very effective to determinebovine SOD activity if its range is between 0.0625 U and 5.0 U. This happensto be same range as the NBT photoreduction method. However, the pyrogallolsolution will not becomes obsolete until two weeks of exposure at roomtemperature. Comparison of these methods reveals that the autoxidationmethod is most convenient for determining SOD activity because a long periodof purification of SOD is needed. About 60 mg of rough SOD, which has aspecific activity of 3700 U/mg, can be obtained from 4 liter porcine blood.Two kinds of SOD are identified from the isolation on native-PAGE. One has asimilar charge or molecular weight to that of bovine SOD, and the other mayhave a larger charge or smaller molecular weight than bovine SOD. Furtherpurification using gel filtration chromatography and SDS-PAGE, we can obtainporcine SOD, which has a molecular weight of 34,000 Da, approximately. ThisSOD can be div ided into two units through heating, each with a molecularweight of 17,000 Da. The porcine SOD has been identified to be Cu,Zn-SOD bytreatment of inhibitors. The pH has no effect on the activity of porcineSOD, if the pH is in the range of 4.0 and 7.0. The activity of the bovineSOD decreases at higher pH values (8.0 - 11.0). When SOD has been heated at70 °C for 30 minutes, at least 60% of the original activity remains. Whenheated at 100 °C for 60 minutes, only 1% of the original activity survives.

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