CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
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Project Number
S0422

Project Title
The Effect of the Superoxide Anion on Cytochrome C

Abstract

Objectives/Goals
My goal in this experiment is to determine whether O2- causes damage to proteins, which are essential to all forms of life. Hopefully, this can lead to further research on the specific results of the damage that O2- causes to proteins. I hypothesize that upon exposure to the superoxide anion, cytochrome C will indeed be damaged, and hopefully this will be evident in my data.

Methods/Materials
Xanthine; Xanthine Oxidase; Spectrophotometer; Pipettes; Test Tubes; Cytochrome C; Superoxide Dismutase; Potassium Phosphate Buffer (pH 7.8).

Results
Cytochrome C was damaged in the presence of the superoxide anion, which was produced in a reaction consisting of xanthine, oxygen gas, water, and xanthine oxidase. Using the data from the spectrophotometer, I calculated the amount of cytochrome c that was damaged by the superoxide radical. After five minutes, approximately 16.7% of the protein had been damaged. With the addition of SOD(superoxide dismutase), the damage to cytochrome c slowed down drastically. I calculated the amount of cytochrome c that was damaged in the solution included SOD (which removes the superoxide radical). After five minutes, only 6.7% of the protein had been damaged. As you can see, the presence of SOD slows the damage to cytochrome c significantly. Therefore, the superoxide radical must be responsible for the damage to cytochrome c (because SOD removes it).

Conclusions/Discussion
My hypothesis was indeed correct. After observing that the spectrum of the cytochrome c solution experienced a marked change when exposed to the superoxide anion, I have concluded that cytochrome c was indeed transformed, and in fact damaged, by this reactive oxygen species. Upon further research, I discovered the exact reaction for cytochrome c and the superoxide anion:

O2- + cytochrome c[Fe(III)]--> O2 + cytochrome c [Fe(II)}

Hence, in the presence of the superoxide anion, cytochrome C was reduced, and thus damaged. The addition of an extra electron to cytochrome c causes the protein to change its inherent shape, and therefore lose its function. In the absence of antioxidant enzymes, I have shown that the superoxide anion can damage proteins, and as a result, it can impair essential cell processes. It is no wonder that oxidative stress can play a major role in Parkinson's disease, diabetes, Alzheimer's disease, and many other significant

Summary Statement
I discovered that the superoxide anion damages proteins significantly, but this damage can be reduced if superoxide dismutase is injected into the solution because this enzyme removes the destructive anions.

Help Received
I used lab equipment at the Charles R. Drew Medical School under the supervision of my father, Dr. Ram K. Sindhu.