

Electron Transfer from Biological System to Dioxygen Mediated by Fullerene Derivative

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Investigation of the biological effects of fullerene and its derivatives with regard to the development of new and efficient pharmaceuticals is important.

Now, much attention has been focused on the antioxidant activity of carboxy fullerene¹. We have reported that the carboxy fullerenes decrease active oxygen toxicity².

Contrast to the carboxy fullerenes, we found bacteriostatic effect of cationic fullerene derivatives³. We have also shown that C₆₀-bis(*N,N*-dimethylpyrrolidinium iodide) (**1**) (mixture of regio isomers) inhibited the dioxygen uptake caused by *E. coli* and glucose. This result indicates that the mechanism of bacteriostatic effect is an inhibition of energy metabolism.

In this paper, we isolated regio isomers of **1** and studied their effect on respiratory chain. We found an electron transfer activity of the fullerene derivatives from respiratory chain to dioxygen which produced active oxygen species.

According to Lu *et al.*⁴, mixture of C₆₀-bis(*N*-methylpyrrolidine) regio isomer was prepared, then, *t*-2 and *t*-4 isomers were purified by silica gel column chromatography. Both isomers were treated with methyl iodide to give *t*-2 and *t*-4 C₆₀-bis(*N,N*-dimethylpyrrolidinium iodide).

The activity of respiratory chain was determined by dioxygen uptake rate caused by inner-membrane fraction of *E. coli* and NADH.

The effect of fullerene derivatives on the dioxygen uptake was biphasic. At low concentration, both isomers inhibited the dioxygen uptake, whereas, at high concentration, both enhanced it. At 2.5 μM, *t*-2 isomer was effective in the inhibition than *t*-4, but, from 5.0 μM to 12.5 μM, *t*-4 was a more effective inhibitor. Higher concentration than 25 μM, dioxygen uptake rate was accelerated.

To elucidate a mechanism of the dioxygen uptake enhancement, an effect of catalase was investigated. An addition of catalase to the reaction mixture (*t*-2 12.5 μM) reduced dioxygen uptake rate to half, whereas the effect of catalase was small in *t*-4 at 12.5 μM. In the case of *t*-2, dioxygen was reduced to produce H₂O₂ not

to H₂O because catalase returns half amount of H₂O₂ back to O₂.

Then, cytochrome *c* reduction was investigated. Cytochrome *c* was reduced by complete system, which contains inner membrane fraction, NADH and the fullerene derivative but a reduction rate was very slow in the absence of the fullerene derivative. SOD, superoxide dismutase, suppressed the reduction. SOD inhibition showed that cytochrome *c* reduction was mediated by superoxide.

On the basis of these data, the fullerene derivatives must be reduced by a respiratory chain system then they transfer an electron to dioxygen which produced superoxide and H₂O₂.

Then, we examined a reaction of reduced fullerene derivatives with dioxygen by electrochemical method and found that the reduced fullerene derivatives reacted with dioxygen.

This is the first report that fullerene derivative is reduced by a biological system then transfer electron to dioxygen.

Reference

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