A Novel Mechanism for the Inhibition of Nitric Oxide Synthase by Amino Acid and Amino Alcohol C_{60} Derivatives

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In recent years we have identified water-soluble fullerene derivatives as an exotic class of NOS inhibitors[1-2]. Studying a diverse group of fullerene derivatives we have accumulated significant data that suggests that there is a very interesting and sensitive structure-function relationship that affects their potency, isoform selectivity and even the pharmacologic mechanism of action. This suggests a molecular specific interaction of fullerenes with the enzyme.

We discovered recently that an important site of interaction of the amino acid fullerene derivatives with NOS is on the reductase domain of the enzyme, where these compounds block the electron transfer, perturbing the cytochrome c and $K_3[Fe(CN)_6]$ reductase activities of NOS. A particular derivative however, a sligthly modified adduct as an amino alcohol, L-threoninol bismalonate [60]fullerene monoadduct (Fig.1) paradoxically increased the NADPH oxidase activity of the neuronal isoform. This activity normally requires the functional and structural integrity of the entire electron transfer chain and has the oxygenase heme-bound O_2 as the acceptor. In the fully functional dimeric nNOS, electrons flow from NADPH to FAD to FMN within the reductase domain of one subunit of NOS and then are transferred to the heme-iron buried inside the oxygenase domain of the other subunit. In the absence of the L-Arginine substrate, nNOS is able to carry out the electron transfer in an "uncoupled" reaction that generates superoxide from heme-bound O_2. This reaction consumes the primary electron source, NADPH, in a process that could be monitored spectro-photometrically in real-time as NADPH oxidase activity.

After careful investigation it was determined that in the presence of L-Threoninol bismalonate monoadduct, the actual primary electron acceptor is the fullerene derivative itself. It appears that the fullerene works in tandem with the reductase domain of nNOS, since this phenomenon occurs at appreciable levels only in the presence of calmodulin, an activator protein that turns the enzyme on. The fullerene compound had no effect when incubated only with NADPH. We concluded that the electron transfer machinery of NOS is diverted to send electrons to the L-threoninol C_{60} bismalonate fullerene, instead of the natural heme-bound O_2 at the oxygenase domain. To date, this behavior is unique for this compound, in contrast with the effect of L-amino acid derivatives of C_{60} that block the electron transfer through the reductase domain of NOS *and* the NADPH oxidase activity.

Significance. This finding is our first indication of a particular water-soluble fullerene derivative being actively reduced by NOS in the process of inhibiting the enzyme. This emphasizes the electronic accepting ability of fullerenes as a potential key factor in the inhibition of NOS, an enzyme that works by a tightly regulated electron transfer mechanism. We envision that if this is true in the case of NOS there is an exciting possibility that *electronic competition* may constitute the underlying mechanism implicated in the inhibition of other redox enzymes (oxidoreductases, monooxygenases like P450 cytochromes, malate dehydrogenase etc.) by fullerenes.

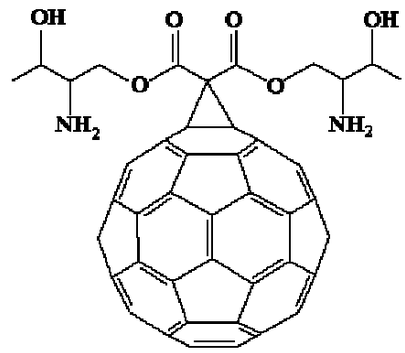

Fig. 1. L(-)-Threoninol Bismalonate C_{60} Monoadduct (Note. The configuration of the $-CH(CH_3)OH$ second chiral center is classically D- as in D-threose); in CIP notation the addend is 2S, 3R-Threoninol bismalonate).

References:

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