Biomimetic Enzyme Nanocomplexes: Use as Antidotes and Preventive Measures for Alcohol Intoxication

In most living eukaryotic cells enzymes mediating sequential steps of a reaction are usually co-localized within organelles allowing more efficient completion of the reaction without much need for transport of intermediaries between different organelles. Similarly, multiple enzymes with complimentary functions involving multiple consecutive reactions are also spatially co-localized and enhance not only the overall efficiency but also minimize the toxicity of intermediate products generated during the reaction by prompt elimination of intermediate products toxic by the co-localized enzymes. The typical example of such organellar localization is the peroxisome where catalase enzyme detoxifies the hydrogen peroxide produced during enzymatic reactions occurring in the peroxisome.

Recently Liu et al demonstrated a design of an artificial method of creating enzyme complexes called nanocomplexes encapsulated within a crosslinked polymer nanocapsule.¹ The process involves conjugating enzymes having complementary or synergistic functions followed by encapsulation of the resultant nanocomplex within a crosslinked polymer nanocapsule. This protects the enzyme complex from breakdown by proteases without compromising on their enzymatic ability. First, inhibitors of each enzyme are conjugated to a specifically designed sequence of single-stranded DNA. These DNA molecules complementarily assemble to form a DNA-inhibitor scaffold (first step). Following this, binding of the inhibitors with the respective enzymes forms a multi-enzyme nanocomplex. Subsequently, in situ polymerization is performed to form a polymer network around each nanocomplex. This leads to the formation of nanocapsules containing a multi-enzyme core with a permeable shell (second step). In the last step the DNA-inhibitor scaffolds are removed to form the final multi-enzyme nanocomplex which is denoted by n (Enzymes), where 'Enzymes' refers to the enzymes within the core of the nanocapsules (third step) (Figure 1). These nanocomplexes can acquire both desired surface properties and targeting capability.

They have demonstrated the successful construction of these complexes by a model using horseradish peroxidase and glucose oxidase (Figure 1). This complex had more activity in metabolizing glucose compared to simple mixture of the constituent enzymes. The resultant hydrogen superoxide was rapidly metabolized by the peroxidase enzyme reducing its toxicity and promoting the glucose oxidase activity. The nanocapsules also demonstrated significantly greater stability when incubated at 65 °C for 60 min.

Subsequently they demonstrated the practical use of this technology in preventing and treating acute alcohol intoxication in mice using nanocomplexes containing alcohol oxidase (AOx) and catalase (Cat). For the prophylactic studies, mice were given an alcoholic diet containing native AOx, n (AOx), n (Cat), a mixture of n (AOx) and n (Cat), or n (AOx-Cat). n (AOx-Cat) rapidly metabolizes alcohol by allowing the toxic product of alcohol oxidase reaction (hydrogen peroxide) to be effectively detoxified by catalase and prevents hydrogen peroxide mediated inactivation of AOx. Elimination of hydrogen peroxide also regenerates oxygen needed for the AOx reaction. Co-ingestion of the nanocomplexes containing n (AOx-Cat) with alcohol resulted in shorter periods of intoxication in mice and lower blood alcohol content than with mixtures of individual nanocomplexes of AOx and Cat or with native AOx. For the antidote studies, phosphate buffer saline, native AOx, n (AOx), a mixture of n (Cat) and n (AOx), and n (AOx-Cat) were injected in tail-vein of intoxicated mice. Similarly, on injection of these nanocomplexes into the tail vein of mice already intoxicated with ethanol, the least alanine transaminase rise and most reduction of blood alcohol content was seen in mice receiving nanocomplexes containing AOx and Cat combination compared to mixtures of individual nanocomplexes or native enzymes alone.

This discovery of enzyme nanocomplexes opens the door for exciting research into possibly infinite tailor-made combinations of enzymes [n (Enzymes)] designed specifically for the metabolism of various compounds. For example, theoretically the addition of aldehyde dehydrogenase or aldehyde oxidase (ADOx) to AOx and Cat [n (AOx-ADOx-Cat)] triple-enzyme nanocomplex could virtually abolish the toxicity of alcohol by rapid elimination of acetaldehyde. Similarly, drug induced liver injury could be circumvented in large part by promoting disposal of toxic metabolites and promoting metabolism of drugs by relatively non-toxic pathways. Many animal studies have been performed principally in the treatment of organophosphate and cyanide poisoning, using various encapsulation or delivery systems like carrier erythrocytes, liposomal formulations and now synthetic polymer nanocapsules.^{2,3} However, further research is needed for the bench-to-bedside transition of this exciting new therapeutic mechanism. Delivery and release of these nanocomplexes into target tissues seems to be only the first of these challenges. Finding the right size of the nanoparticles with best pharmacokinetic properties for various routes of delivery in humans, ensuring bio-degradability without causing toxicity or inciting immune response

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Figure 1 Synthesis of enzyme nanocomplexes. Schematic illustration of the synthesis of a model triple-enzyme nanocomplex [invertase (Inv, A), glucose oxidase (GOx, B) and horseradish peroxidase (HRP, C); their respective competitive inhibitors—lactobionic acid (a), glucosamine (b) and 4-dimethylaminoantipyrine (c)] by DNA directed assembly and nano-encapsulation. Reproduced with permission (Liu Y et al Nat Nanotechnol 2013; 8:187–92).

while retaining its protective effect on the enclosed enzymes seem to be the major technological challenges that lie ahead in this field of research.

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