Birth of an enzyme

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Mankind triumphed in a recent 'competition' against nature when scientists succeeded in creating a new type of enzyme for a reaction for which no naturally occurring enzyme has evolved. This achievement opens the door to the development of a variety of potential applications in medicine and industry.

Enzymes are, without a doubt, a valuable model for understanding the intricate works of nature. These molecular machines – which without them, life would not exist – are responsible for initiating chemical reactions within the body. Millions of years of natural selection have fine-tuned the activity of such enzymes, allowing chemical reactions to take place millions of times faster.

In order to create artificial enzymes, a comprehensive understanding of the structure of natural enzymes, their mode of action, as well as advanced protein engineering techniques is needed. A team of scientists from the University of Washington, Seattle, and the Weizmann Institute of Science, Israel, made a crucial breakthrough toward this endeavor. Their findings have recently been published in the scientific journal *Nature*.

Enzymes are biological catalysts that are made from a string of amino acids, which fold into specific three-dimensional protein structures. The scientists' aim was to create an enzyme for a specific chemical reaction whereby a proton (a positively charged hydrogen atom) is removed from carbon – a highly demanding reaction and rate-determining step in numerous processes for which no enzymes currently exist, but which

would be beneficial in helping to speed up the reaction. During the first heat of the 'competition,' the research team designed the 'heart' of the enzymatic machine – the active site – where the chemical reactions take place.

The second heat of the competition was to design the backbone of the enzyme, i.e., to determine the sequence of the 200 amino acids that make up the structure of the protein. This was no easy feat seeing as there is an infinite number of ways to arrange 20 different types of amino acids into strings of 200. But in practice, only a limited number of possibilities are available as the sequence of amino acids determines the structure of the enzyme, which in turn, determines its specific activity.

Prof. David Baker of the University of Washington, Seattle, used novel computational methodologies to scan tens of thousands of sequence possibilities, identifying about 60 computationally designed enzymes that had the potential to carry out the intended activity. Of these 60 sequences tested, eight advanced to the next 'round' having showed biological activity. Of these remaining eight, three sequences got through to the 'final stage,' which proved to be the most active. Drs. Orly Dym and Shira Albeck of the Weizmann Institute's Structural Biology Department solved the structure of one of the final contestants, and confirmed that the enzymes created were almost identical to the predicted computational design.

But the efficiency of the new enzymes could not compare to that of naturally-occurring enzymes that have evolved over millions of years. This is where 'mankind' was on the verge of losing the competition to nature, until Prof. Dan Tawfik and research student Olga Khersonsky of the Weizmann Institute's Biological Chemistry Department stepped in, whereby they developed a method allowing the synthetic enzymes to undergo 'evolution in a test tube' that mimics natural evolution. Their method is based on repeated rounds of random mutations followed by

scanning the mutant enzymes to find the ones who showed the most improvement in efficiency.

These enzymes then underwent further rounds of mutation and screening. Results show that it takes only seven rounds of evolution in a test tube to improve the enzymes' efficiency 200-fold compared with the efficiency of the computer-designed template, resulting in a million-fold increase in reaction rates compared with those that take place in the absence of an enzyme.

The scientists found that the mutations occurring in the area surrounding the enzyme's active site caused minor structural changes, which in turn, resulted in an increased chemical reaction rate. These mutations therefore seem to correct shortcomings in the computational design, by shedding light on what might be lacking in the original designs. Other mutations increased the flexibility of the enzymes, which helped to increase the speed of substrate release from the active site.

'Reproducing the breathtaking performances of natural enzymes is a daunting task, but the combination of computational design and molecular in vitro evolution opens up new horizons in the creation of synthetic enzymes,' says Tawfik. 'Thanks to this research, we have gained a better understanding of the structure of enzymes as well as their mode of action. This, in turn, will allow us to design and create enzymes that nature itself had not 'thought' of, which could be used in various processes, such as neutralizing poisons, developing medicines, as well as for many further potential applications.'

Source: Weizmann Institute of Science

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