Can someone please explain to me what PEG-PAL is and how it works?

This question came to us via the PKU community and prompted the following response:

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WHAT IS PAL?

Phenylalanine ammonia lyase (PAL), is a protein, an enzyme, found in plants, fungi and bacteria, which converts L-phenylalanine to *trans*-cinnamic acid and ammonia (1, 2).

WHY WAS PAL THE CHOICE OF ENZYME AND HOW DOES IT WORK?

PKU and related hyperphenylalaninemias result from compromised phenylalanine hydroxylase (PAH) activity, causing excess phenylalanine (Phe) accumulation (3). The harmfully augmented levels of Phe can be diminished by phenylalanine metabolizing enzymes via *enzyme replacement* with PAH or *enzyme substitution* with PAL.

Enzyme replacement therapy with the native PAH (the primary choice), requiring a multi-enzyme complex to function, presents a number of challenges. PAH is inherently unstable; its complex activity and cofactor (BH_4), oxygen and iron requirements, in addition to the inherent degradation sensitivity and potential immunogenicity in a person lacking the functional enzyme, further complicates its therapeutic viability (3).

PAL is a more stable enzyme and does not require a cofactor to function. Therefore enzyme substitution therapy with PAL alone could metabolize Phe. PAL enzyme can act as a surrogate to the deficient PAH enzyme and convert the excess phenylalanine to *trans*-cinnamic acid and ammonia. *Trans*-cinnamic acid is a harmless metabolite, as it degrades and is rapidly excreted in the urine as hippuric acid, and the ammonia does not accumulate in sufficient quantity to pose a threat of hyperammonemia (1, 2, 4-6).

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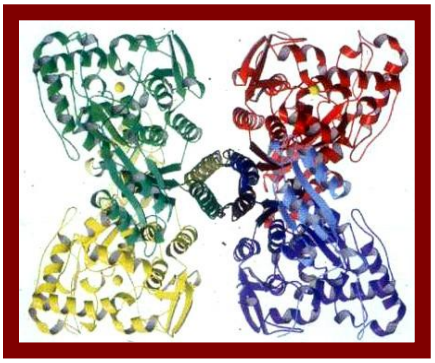
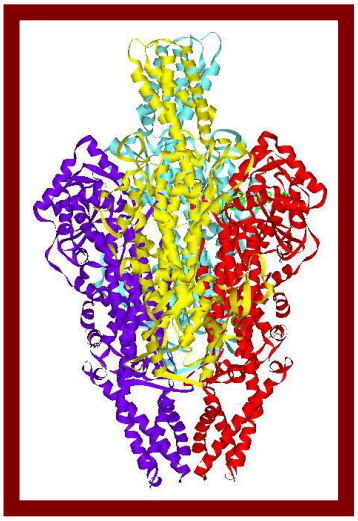
3-D crystal structures:



Phenylalanine Hydroxylase (PAH)



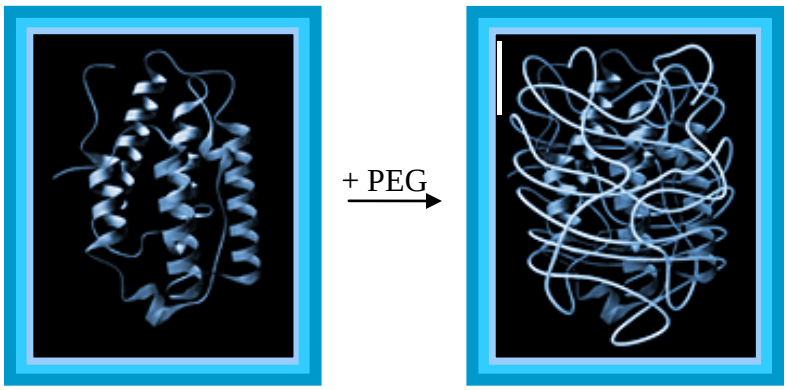
Phenylalanine Ammonia Lyase (PAL)

Courtesy of Dr. R.C. Stevens' Laboratory, The Scripps Research Institute, CA, USA.

WHAT IS PEG?

PEG stands for polyethylene glycol. It is a water soluble and non-toxic compound that is safely cleared from the body. Its primary use to date in currently approved drugs has been to favourably alter the properties of biologics (7). The attachment of PEG to a therapeutic protein (a process called PEGylation) can improve its biopharmaceutical properties by increasing its stability and resistance towards inactivation and directed degradation. It also diminishes immunogenicity (ability of a foreign protein (drug) to elicit an immune response), by masking the presence of the drug from the host's immune system, and enhances and extends its performance by reducing clearance and prolonging circulatory time (8).

Schematic Drawing of PEGylation Technology:



UnPEGylated protein PEGylated protein

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WHY IS PAL PEGYLATED FOR THERAPEUTIC USE?

An injectable form of phenylalanine ammonia lyase (PAL) could have a therapeutic profile for PKU. However PAL is a non-human protein that the immune system recognizes as foreign. Long-term reduction of phenylalanine levels by PAL is hampered by clearance of the enzyme via an immune response; PAL alone (without PEGylation), although shown to be harmless upon repeated dosing to the PKU mouse model, permanently loses its ability to metabolize systemic phenylalanine one week following initial administration (2).

PEGylation of PAL controls its immunogenicity. Modification of primary amino groups (largely confined to the protein surface of PAL) by attachment of activated forms of PEG (producing PEG-PAL), reduces and nearly eliminates the recognition of the now tailored enzyme by the immune system, and therefore prolongs its life while retaining its function (6).

RESEARCH:

We have shown that the blood phenylalanine concentration in the PKU mouse model can safely be reduced down to normal levels by the administration of PEG-PAL. All PEG-PAL formulations that we tested were effective catalysts; they had prolonged activity and certain forms had profoundly suppressed immunogenicity, a desirable feature for clinical use (2, 4).

Applied to humans with PKU, this type of substitute enzyme therapy could provide substantial relief from the rigors of the diet and improve compliance, metabolic control, and clinical outcome in this relatively frequent disorder (2).

Phase I clinical study of PEG-PAL for the treatment of PKU was initiated on May 20th, 2008 (9).

ACKNOWLEDGEMENTS:

These developments would not have occurred without the contributions of Drs. Charles R. Scriver, Raymond C. Stevens, Lin Wang and all our colleagues at BioMarin Pharmaceutical Inc.

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