Development of a novel enzyme replacement therapy against phenylketonuria based on a stabilized version of AvPAL using the IC-Tagging system

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Phenylketonuria is the most common hereditary defect in amino acid metabolism and is characterized by the presence of excessive levels of phenylalanine in blood and tissues. Currently, the only available treatment of this pathology is based on a strict diet without phenylalanine. In this context, our work proposes the use of the IC-Tagging system patented in our laboratory to develop an enzyme replacement therapy. Based on the production of a stabilized version of the enzyme phenylalanine ammonium lyase from *Anabaena variabilis* (AvPAL), which can reduce the phenylalanine levels of patients. The IC-Tagging methodology is a protein labeling platform based on the use of the avian reovirus-derived muNS-Mi protein (Brandariz-Nuñez A et al. (2010) PLoS One 5.11 e13961). This system is able to produce protein micro- or nanospheres (MS or NS) that can be loaded with proteins of interest in any expression system through their tagging with a tag called IC. Our results show that it is posible to use our system to produce NS in bacteria loaded with the stabilized AvPAL enzyme maintaining its catalytic activity, correct folding and without altering its kinetic parameters in an inexpensive and simple way. The encapsulation of the AvPAL enzyme in these NS significantly improves its thermoresistance, has a stabilizing effect over a wide range of pH values and provides great resistance to incubations with low pH values. This nanoencapsulation also provides formidable protection against denaturing agents and proteolytic digestion and increases its long-term storage stability. Moreover, it is possible to coat the NS with different polymers without losing specific activity to avoid degradation and increase the oral bioavailability as we demonstrated in *in vivo* biodistribution assays. In conclusion, our work presents a new and disruptive approach to develop an orally administered enzyme replacement therapy against phenylketonuria.