Structural characterization of human ADPGK and GPD2 – enzymes involved in modified glucose metabolism of rapidly proliferating cells

Dr. Przemysław Grudnik

Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow

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In the early twentieth century German physiologist Otto Warburg discovered that cancer cells and other rapidly proliferating cells produce energy mainly in the fermentation strangely even in the presence of oxygen because usually fermentation is mainly found in anaerobic conditions. This paradox was named after discoverer: *the Warburg effect*. Recently published studies have linked activated lymphocytes - cells of the immune system - and the presence of the Warburg effect, to the production of reactive oxygen species (ROS) by mitochondria. Reactive oxygen species by activating transcription factors influence the production of proteins necessary for the process of multiplication, differentiation and cell survival. The exact molecular pathway leading from lymphocyte activation to an increased ROS level is still unclear, however, two proteins, crucial for this process, have been identified: ADP-dependent glucokinase (ADPGK) and the mitochondrial glycerol-3-phosphate dehydrogenase (GPD2).

The main scientific objective of the proposed project is the structural characterization of human enzymes ADPGK and GPD2 complexed with inhibitors and the development of new highly specific inhibitors of these proteins.

METHODOLOGY: Human proteins ADPGK and GPD2 will be obtained by the use of genetic engineering and bacterial protein production systems. For the identification of potential inhibitors we will employ biochemical methods and screening of the chemical compounds that are analogs of the typical substrates of these proteins. The strongest inhibitors will be used for crystallization trials. We plan to obtain crystals of proteins with bound inhibitors, which will contribute to our understanding of the atomic structure of the enzymes by using methods of X-ray crystallography.

THE IMPORTANCE OF THE PROJECT: One of the most intriguing issues concerning the research on human ADPGK and GPD2 is their role in the excessive multiplication of cells. Since elevated expression levels of ADPGK were described for blood cancer cells the pharmacological blockade of ADPGK activity may be critical for such diseases as lymphoma and leukemia. Furthermore ADPGK activity and to some extent GPD2 activity, is essential for lymphocyte activation which makes these proteins potential targets of compounds that modulate immunity. Such *modulators* of activity could be used to treat diseases mediated by lymphocytes (e.g. AIDS, multiple sclerosis, rheumatoid arthritis, diabetes).

Increased activity of ADPGK and GPD2 leads to the production of ROS and consequently stimulates the production of proteins involved in the inflammation process. Elevated mitochondrial ROS production is often observed among the various types of malignancies, autoimmune diseases and diseases of the cardiovascular system. Therefore, strategies for the inhibition of ROS production may have particular clinical importance, and contribute to the development of new therapeutic approaches. Scheduled studies using analogs of substrates and inhibitors, in combination with cell biology studies will contribute to the identification and development of such specific immune modulators. The developed inhibitors of ADPGK and GPD2 will be primarily used to metabolic pathways, allowing to block specific individual stages of glycolysis. In summary, the proposed project aims to clarify the mechanisms underlying key processes involved in the immune response, carcinogenesis, and inflammation.