Description for general public

Pyridoxal 5'-phosphate (PLP) is the major biologically active form of vitamin B6 in the human body. It acts as an essential co-factor in amino acid metabolism, e.g., for cystathionine β-synthase and cystathionine γ-lyase that participate in the transsulfuration of homocysteine (Hcy) to cysteine (Cys). On the other hand, Cys and Hcy are major sulfur aminoacids in human biofluids. Hcy is a precursor of Cys in the transsulfuration pathway as well as for methionine (Met) in the remethylation pathway. Hcy and Cys levels are closely related to human diseases [1–3]. For example, disorders of Cys metabolism include cystinosis and cystinuria, while elevated plasma Hcy levels are observed in patients with heart and brain diseases [4–7]. *In vitro*, Cys and Hcy are known to undergo facile condensation with aldehydes, including PLP, affording 1,3-thiazolidine-4-carboxylic acids and tetrahydro-1,4-thiazine-4-carboxylic acids, respectively [8–13]. Since commonly present in biological fluids Cys can react with PLP *in vitro* forming 2-pyridoxyl-1,3-thiazolidine-4-carboxylic acid (PTCA), this reaction if occurring *in vivo*, would deplete total pool of PLP. Because PLP-depletion is detrimental to living organisms, it is important to investigate whether this kind of PLP-depletion occurs in humans. Importantly, the problem concerning presence and role of PLP-derived thiazolidine in humans has received no attention as yet. Completion of the proposed study will generate some brand new information concerning the thiazolidine presence and will facilitate in the future investigations of a potential role of PTCA as a marker in the human pathophysiology.

Our project will be successfully accomplished by realization of few main aims centered around:

- ✓ synthesis of PTCA with the use of PLP and Cys as substrates,
- ✓ confirmation of its structure with the use of spectroscopic methods (NMR, IR) as well as mass spectrometry,
- ✓ testing of the stability of PTCA in water solutions including human biofluids,
- ✓ optimization of biological sample preparation (plasma, urine, saliva) for PTCA chromatographic determination,
- ✓ elaboration of a new chromatographic methods (HPLC-UV, GC-MS) enabling PTCA determination in water solutions as well as in human plasma, urine and saliva,
- ✓ revelation of PTCA presence in biological fluids and application of elaborated procedures to samples donated by apparently healthy volunteers in order to prove utility of the methods.

The condensation reactions between aldehydes and Cys to 1,3-tiazolidines are reversible, particularly at alkaline pH [8,10]. So, it is interesting to check whether PTCA can acts as specific reservoir of PLP *in vivo*. On the other hand PTCA formation may cause PLP depletion, e.g. in humans. Present project is undertaken to verify these thesis.

1. Himmelfarb J, McMenamin E, McMonagle E, *Kidney Int*. 61 (2002) 705–16.
2. Stampfer MJ, Malinow MR,Willett WC, Newcomer LM, Upson B, Ullmann D, et al., *J. Am. Med. Assoc.* 268 (1992) 877–81.
3. Kleinman WA, Richie JP, *Biochem. Pharmacol*. 60 (2000) 19–29.
4. Andersson A, Ankerst J, Lindgren A, Larsson K, Hultberg B, *Clin. Chem. Lab. Med.* 39 (2001) 229–33.
5. Rojkovich B, Nagy E, Prohle T, Poor G, Gergely P, *Clin. Diagn. Lab. Immunol.* 6 (1999) 683–5.
6. Ueland PM, Mansoor MA, Guttermsen AB, Muller F, Aukrust P, Refsum H, et al., *J. Nutr.* 126 (1996) 1281–4.
7. Jakubowski H, Borowczyk K, Głowacki R, Nygård O, *Circulation*,132 (2015) A19250.
8. Ratner S, Clarke HT, *J. Am. Chem. Soc*. 59 (1937) 200–6.
9. Schubert MP, *J. Biol. Chem*. 114 (1936) 341–50.
10. Wriston Jr JC, Mackenzie CG, *J. Biol. Chem.* 225 (1957) 607–13.
11. Buell MV, Hansen RE, *J. Am. Chem. Soc.* 82 (1960) 6042–9.
12. Griffiths R, Williams DC, O'Neill C, Dewhurst IC, Ekuwem CE, Sinclair CD, *Eur. J. Biochem*. 137 (1983) 467–78.
13. Jakubowski H, *Cell. Mol. Life Sci.* 61 (2004) 470–87.