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Diastrophic dysplasia is recessively inherited chondrodysplasias due to mutations in the diastrophic dysplasia sulfate transporter (DTDST), a sulfate/chloride antiporter present on the cell membrane of chondrocytes and several other cell types, whose function is crucial for the uptake of inorganic sulfate, important for proteoglycan sulfation. Because sulfation is a post-translational modification, many other macromolecules should be undersulfated. 2-D Electrophoresis protein separation followed by western blotting, using antibodies against sulphated proteins, should be a valid approach in order to study protein sulfation.

Proteomic analysis of cartilage presents several problems because of the high amount of proteoglycans which interfere with focusing, resolution and reproducibility of 2-DE. It is reported that voltage rehydration loading facilitate the entry of large proteins into IPG strips; thus in order to reduce or prevent the entry of large proteins (*i.e.*, proteoglycans) in the IPG strip, we employed passive rehydration loading. Using this approach I set up a consistent and reproducible method by which proteoglycans were not adsorbed in the IPG strip gel and thus did not interfere with IEF¹. This method was applied to analysis of sulfated proteins in cartilage of a mouse model of diastrophic dysplasia (dtd mouse). Proteins to be studied were selected using public database (<http://www.ncbi.nlm.nih.gov/> and <http://www.expasy.ch>) and by using the on-line software “Sulfinator” (<http://www.expasy.ch/tools/sulfinator/>).

So far I have performed a 2-D Electrophoresis followed by western blotting using a monoclonal antibody against CKR-5 protein. Obtained results have shown no differences in protein concentration and isoforms position for *C-C chemokine receptor type 5*.

References

1. *A quantitative and qualitative method for direct 2-DE analysis of murine cartilage.* - Pecora F., Forlino A., Gualeni B., Lupi A., Sofia G., Marchese L., Stoppini M., Tenni R., Cetta G. and Rossi A. - *Proteomics* 7, 4003–4007 (2007)