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Clinical analysis of plasma proteins by electrospray ionization mass spectrometry

A thesis submitted in (partial) fulfilment of the
requirements for the award of the degree of

MASTER OF SCIENCE - RESEARCH

from



University of Wollongong

by

Margey Tadesse
Master of Science-Coursework

Department of Chemistry
2005

CERTIFICATION

I, Margey Tadesse, declare that this thesis, submitted in partial fulfilment of the requirements for the award of Master of Science-Research, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualification at any other academic institution.

Margey Tadesse

16th August 2005

ABSTRACT

Electrospray ionization mass spectrometry (ESI-MS) was assessed as a tool for the analysis of different glycosylated and iron forms of transferrin (tf). Carbohydrate deficient transferrin (CDT) is clinically relevant in the diagnosis of congenital disorders of glycosylation (CDG) and in monitoring compliance in rehabilitation programs for alcoholism. The iron load of transferrin is an indicator of iron deficiency (or sufficiency). The concentration ranges (5 – 15 μ M) over which the ESI-MS response for tf was λ ive α p were determined in 0.1 M NH_4HCO_3 , pH 8.2, and in 1% formic acid. In order to develop a method for analysis by ESI-MS, optimal conditions were determined for obtaining CDT from commercial apo-tf by treatment with neuraminidase or PNGase. The resulting CDT forms were separated by HPLC and analysed by ESI-MS. The abundant human serum albumin was removed from plasma by loading 25-75 μ l of plasma onto a Micro Bio-Spin column containing Cibacron Blue-agarose. A high quality ESI mass spectrum of tf was obtained from the eluent. Using ESI-MS it was possible to distinguish between commercial holo-tf (Fe_2 -tf) and apo-tf (Fe_0 -tf). The rapid analysis of transferrin from plasma by ESI-MS lays the foundation for development of a clinical method for analysing CDT and possibly for determining the iron load of circulating tf.

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ABBREVIATIONS

A ₂₈₀	Absorption at 280 nm
A ₄₇₀	Absorption at 470 nm
Amu	Atomic mass unit
Apo-tf	Iron-free transferrin
CDG	Congenital disorders of glycosylation
CDT	Carbohydrate deficient transferrin
CID	Collision induced dissociation
CRM	Charged residue model
CSF	Cerebrospinal fluid
CZE	Capillary zone electrophoresis
Da	Daltons
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked Immunosorbent Assay
ESI-MS	Electrospray ionization mass spectrometry
ExPasy	Expert protein analysis system
GDP	Guanosine diphosphate
HFBA	Heptafluorobutyric acid
Holo-tf	Iron-laden transferrin
HSA	Human serum albumin
Htf	Human serum transferrin
IEF	Isoelectric focusing
IgA	Immunoglobulin A

IgG	Immunoglobulin G
K_A/K_E	Partitioning coefficient ratio for analyte and electrolyte ions
MALDI	Matrix assisted laser desorption ionization
Mcp	multi-channel plate
PAI-1	Plasminogen activator inhibitor-1
PNGase F	N-Glycosidase F
PMI	Phosphomannose isomerase
PMM	Phosphomannomutase
PSA	Prostate specific antigen
Q-TOF	Quadrupole time-of-flight
RIA	Radioimmunoassay
rtPA	Plasminogen activator
SA	Sialic acid
SIMS	Secondary ion mass spectrometry
sTfr	Serum transferrin receptor
TFAA	Trifluoro acetic acid
TIA	Trisialo-tf
tf	Transferrin
TSAT	Transferrin saturation