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Evaluation of Natural Antioxidants

Jingli Zhang

Thesis submitted for the requirement for the degree of Doctor of Philosophy

The University of Auckland
December 2004
Abstract

This thesis relates the physicochemical properties of phenolic compounds to their antioxidant activities. It focuses on the partitioning of phenolic compounds between hydrophilic and lipophilic environments and the relevance this has to their in vivo health effects.

Data in the literature was lacking so the phase partition coefficients (log P) of 53 phenolic antioxidants were measured by reversed-phase HPLC and calculated by log P prediction software. There was a very strong linear correlation between measured and calculated values (r = 0.91).

The importance of log P in determining antioxidant assay values was then tested by developing an assay system capable of measuring activities of both hydrophilic and lipophilic antioxidants. This Lipid Peroxidation Inhibition Capacity Assay (LPIC), based on using liposomes to simulate a cell membrane environment, was then used to measure the activity of antioxidants with a broad range of structures. The activities were correlated against log P, the difference of heat of formation (ΔH_f) and half-wave potential (E_{p/2}) and used to derive a predictive model to calculate the LPIC activity. There was a highly significant linear correlation between the calculated and measured values. The LPIC activities also correlated well to published LDL inhibition activities but not to measured ORAC activities.

These findings suggested that behaviours of antioxidants in the small unilamellar vesicles of the LPIC assay were similar to that in the LDL assay but not to the aqueous phase based ORAC assay. The LPIC assay may therefore be a better indicator of potential health benefits of antioxidants in the human body than the ORAC assay. The possible mechanistic reasons are that it may better reflect ability to prevent the oxidation of LDL blood stream particles that leads to cardiovascular disease and also takes into account the importance of membrane solubility which can raise the cellular concentration and thus potential to protect cells from oxidative damage.

KEYWORDS:

LPIC, LDL; Antioxidant; Phytochemical; Polyphenolic; Phenolic acid; Flavonoids; log P; Partition Coefficient; Liposome; Lipid bilayer; Lipid Membrane; ORAC; Comet assay; Flow Cytometry.
Dedication

This thesis is dedicated to my wife,

Jessie Huifang Jiang
and my son,
Matthew Yifan Zhang
with love.
Acknowledgements

There are many people to whom I am very grateful for their help and encouragement while undertaking the work described in this thesis.

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I would like to express my sincere gratitude to:

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- Dr. Roger A. Stanley, Food Sector, HortResearch, Mt Albert Research Center, Auckland, New Zealand.

for supervising this project, providing a great deal of technical support and careful and constructive reviewing of the report.

I would like to give my special thanks to Dorian N. Scott who made the completion of this thesis possible. I am indebted Dr. Browen Smith, Dr. Paul Kilmartin, Dr. Margot Skinner and Dr. Andrew Allen for advice and help.

To all my friends and colleagues at HortResearch and the University of Auckland I had the great fortune to work with, thank you for making the last three years memorable and all the best for the future.

Finally, I wish to thank to my family for all their invaluable love, encouragement and support.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAPH</td>
<td>2,2'-Azobis(2-amidinopropane) dihydrochloride</td>
</tr>
<tr>
<td>ABTS</td>
<td>2,2'-Azinobis(3-ethylbenzothiazoline 6-sulfonic acid)</td>
</tr>
<tr>
<td>AM1</td>
<td>Austin Model 1</td>
</tr>
<tr>
<td>AMVN</td>
<td>2,2'-Azobis(2,4-dimethyl-valeronitrile)</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BDE</td>
<td>Bond dissociation energy</td>
</tr>
<tr>
<td>BHA</td>
<td>Butylated hydroxyanisole</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated hydroxytoluene</td>
</tr>
<tr>
<td>BODIPY</td>
<td>Bora-4,4-difluoro-4-bora-3a, 4a-diaza-s-indacene</td>
</tr>
<tr>
<td>C\textsubscript{11}-BODIPY</td>
<td>4,4-Difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge-coupled devices</td>
</tr>
<tr>
<td>DAPI</td>
<td>4',6-Diamidino-2-phenylindole</td>
</tr>
<tr>
<td>DFT</td>
<td>Density functional theory</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNP</td>
<td>Dinitrophenylhydrazine</td>
</tr>
<tr>
<td>DNPH</td>
<td>2,4-Dinitrophenyl hydrazine</td>
</tr>
<tr>
<td>DOPC</td>
<td>1,2-Dioleoyl-sn-glycero-3-phosphocholine</td>
</tr>
<tr>
<td>DPPH</td>
<td>1,1-Diphenyl-2-picrylhydrazyl</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>dUTP</td>
<td>2'-Deoxyuridine-5'-triphosphate, sodium salt</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FRAP</td>
<td>Ferric-reducing ability of plasma</td>
</tr>
<tr>
<td>FSC</td>
<td>Forward scatter</td>
</tr>
<tr>
<td>gly-CPG</td>
<td>Glyceryl-coated controlled-pore glass</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HBAB</td>
<td>Hydrogen bond acceptor basicity</td>
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</table>
HBDA: Hydrogen bond donor acidity
IC\textsubscript{50}: Induction concentration (50%)
INDO: Intermediate neglect of differential overlap
IP: Ionization potential
LDL: Low-density lipoprotein
LMPA: Low melting point agarose
log k': logarithmic capacity factor
log P: Logarithmic partition coefficient
log P\textsubscript{C}: log P calculated by computer program
log P\textsubscript{M}: log P measured by reversed-phase HPLC
LPIC: Lipid peroxidation inhibition capacity
LPIC\textsubscript{Inco}: Lipid peroxidation inhibition capacity (Incorporation)
LPIC\textsubscript{Mixed}: Lipid peroxidation inhibition capacity (Mixed)
LSER: Linear solvation energy relationship
LUV: Large unilamellar vesicle
MLR: Multiple linear regression
MLV: Multilamellar vesicle
MNDO: Modified neglect of diatomic overlap
MW: Molecular weight
NBD: 7-Nitro-2,1,3-benzoxadiazol-4-yl
ORAC: Oxygen radical absorbance capacity
ODS: Octadecylsilica
PBS: Phosphate buffered saline
PC: Phosphocholine
PD: Photodiode array
PE: Phycoerythrin
PI: Propidium iodide
PM3: Parameterization method 3
PMT: Photomultiplier tube
QSAR: Quantitative structure-activity relationships
RHF: Restricted Hartree-Fock
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
RP-HPLC: Reversed-phase high-performance liquid chromatography
SAR: Structure-activity relationships
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>SCE</td>
<td>Saturated calomel reference electrode</td>
</tr>
<tr>
<td>SSC</td>
<td>Side scatter</td>
</tr>
<tr>
<td>SUV</td>
<td>Small unilamellar vesicle</td>
</tr>
<tr>
<td>TBA</td>
<td>2-Thiobarbituric acid</td>
</tr>
<tr>
<td>TBHQ</td>
<td>t-Butylhydroquinone</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid-reactive substances</td>
</tr>
<tr>
<td>TEAC</td>
<td>Trolox equivalent antioxidant capacity</td>
</tr>
<tr>
<td>TdT</td>
<td>Terminal deoxynucleotidyl transferase</td>
</tr>
<tr>
<td>TOSC</td>
<td>Total oxyradical scavenging capacity</td>
</tr>
<tr>
<td>TPTZ</td>
<td>2,4,6-Tris(2-pyridyl)-1,3,5-triazine</td>
</tr>
<tr>
<td>TRAP</td>
<td>Total (peroxyl) radical-trapping antioxidant parameter</td>
</tr>
<tr>
<td>Trolox</td>
<td>6-Hydroxy-2,5,7,8-terramethylchroman-2-carboxylic acid</td>
</tr>
<tr>
<td>TUNEL</td>
<td>Terminal deoxynucleotidyl transferase-mediated dUTP Nick End-Labeling</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low-density lipoprotein</td>
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