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2005

## The effect of pulsed electromagnetic fields on protein unfolding

Yoke Berry

*University of Wollongong, yoke@uow.edu.au*

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# THE EFFECT OF PULSED ELECTROMAGNETIC FIELDS ON PROTEIN UNFOLDING

YOKE (*J.C.M.*) BERRY, *B. Sc. (HONS)*

Submitted in fulfilment of the requirements  
for the Degree of Doctor of Philosophy



DEPARTMENT OF CHEMISTRY  
WOLLONGONG  
AUSTRALIA

JUNE 2005

Printed on 80% Australian recycled paper

**DECLARATION OF AUTHENTICITY**

This thesis is submitted in accordance with the regulations of the University of Wollongong in fulfilment of the degree of Doctor of Philosophy. It does not include any material previously published by another person except where due reference is made in the text. The experimental work described in this thesis is original and has not been submitted for a degree to any other University.

Johanna Cornelia Maria (Yoke) Berry

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In the course of collating data and results, it became apparent that, although insight has been gained from this PhD study, I have to humbly admit that there is so much still to learn.

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5 June 2005

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**LIST OF ABBREVIATIONS USED**

A	absorbance
AC	alternating current
ADH	alcohol dehydrogenase
ANS	8-anilino-1-naphthalene sulfonate
ARPANSA	Australian Radiation Protection and Nuclear Safety Agency
ATP	adenosine triphosphate
BSA	bovine serum albumin
CD	circular dichroism
CO <sub>2</sub>	carbon dioxide
CS	citrate synthase
CW	continuous wave
Da	dalton
DC	direct current
D,L	dextrorotatory, levorotatory
DNA	deoxyribonucleic acid
D <sub>2</sub> O	deuterated water
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
EHF	extremely high frequency
ELF	extremely low frequency
EM	electromagnetic
ESI-MS	electrospray mass spectrometry
G	gauss
GHz	giga hertz
HMW	high molecular weight
Hsp	heat shock protein
ICNIRP	International Commission on Non-Ionizing Radiation Protection
Hz	hertz
kHz	kilo hertz
kg	kilogram
km	kilometre
kV	kilo volts
LCZ	electrospray ionization single quadrupole platform
$\alpha$ -La	alpha lactalbumin
$\mu$ l	micro litre
M	molar

mHz	mega hertz
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
ms	millisecond
mRNA	messenger ribonucleic acid
$\mu$ M	micro molar
NaCl	sodium chloride
NaN <sub>3</sub>	sodium azide
NHMRC	National Health and Medical Research Council (Australia)
nm	nanometre
NSW	New South Wales
P	probability
PDB	Protein Data Bank
RF	radio frequency
RNA	ribonucleic acid
S	second
SAR	specific absorption rate
SDS	sodium dodecyl sulphate
SHF	super high frequency
sHsp	small heat shock protein
$\mu$ s	micro second
$\mu$ S	micro Siemens
TEM	transverse electromagnetic mode
TRIS	trisaminomethane
tRNA	transport ribonucleic acid
UHF	ultra high frequency
UV	ultraviolet
V	volts
W	watts
Zn	zinc

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## ABSTRACT

This thesis describes a spectroscopic investigation of the effects of pulsed electromagnetic radiation on the conformation, unfolding, aggregation and precipitation of a variety of proteins. Initial experiments required the calibration of a microwave exposure system thus the temperature of different buffer solutions was studied in detail. The exposure system comprised of an incubator and a rebuilt domestic microwave oven that delivered pulses every six minutes to give a plot of the temperature before, during and after a pulse period of microwaves. All solutions returned to their baseline temperature prior to the next pulse period.

The effect of exposure to microwave pulses of several seconds duration on the aggregation rate of six stressed target proteins (alcohol dehydrogenase, bovine serum albumin, catalase, citrate synthase, insulin and ovotransferrin) was examined in solution. The hypothesis tested was that the initial rate of precipitation of samples exposed to microwave pulses once every six minutes was significantly higher than that of a control held at the same average temperature. The results show statistical significance to confirm the hypothesis in all cases except for insulin, bovine serum albumin and citrate synthase when the latter two proteins were maintained at an average temperature of 45°C and 37°C respectively. In these exceptional cases, the microwave induced temperature excursion was not expected to induce a change in the precipitation rate on the basis of previous knowledge of the unfolding behaviour of the proteins. The second hypothesis tested was that the molecular chaperone,  $\alpha$ -crystallin prevents the aggregation and precipitation of target proteins under the above regimes. It was found that  $\alpha$ -crystallin suppressed precipitation but it was not as effective when proteins were also exposed to pulsed microwaves.

The effect of exposure to 50 Hz DC and AC electric field was examined on stressed alcohol dehydrogenase and citrate synthase. The hypothesis tested was that the initial rate of precipitation of samples exposed to microwave pulses once every six minutes was significantly higher than that of a control held at the same average temperature. This was not detected. When a difference could be detected, it was only observed in a increase or decrease in precipitation, well after exposure.

The results of this thesis are relevant to the setting of standards for microwave exposure as they show that a six-minute averaging period of temperature is not appropriate in the prediction of protein unfolding.