A study on the interactions of synthetic IGF-II analogues with the type 1 IGF and insulin receptors

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Abstract

Insulin-like growth factor II (IGF-II) is a unique regulatory peptide containing 67 residues and three disulfide bonds. It binds with high affinity to three receptors, the insulin receptor (IR), the type 1 insulin-like growth factor receptor (IGF-1R) and the type 2 insulin-like growth factor receptor (IGF-2R). Binding of IGF-II to these receptors signals mitogenic responses, such as cell proliferation, differentiation and migration. The interactions of IGF-II with the IR and IGF-1R have recently been identified as potential therapeutic targets for the treatment of cancer. Thus, an increased understanding of the interactions of IGF-II with the IGF-1R and the IR-A is required for the improved design and development of potential anticancer therapeutics.

A crystal structure of IGF-II bound to either the IGF-1R or the IR-A has not been reported. Thus, the precise location of IGF-II within the receptor binding pocket remains undefined. A fluorescence resonance energy transfer (FRET) approach was proposed to investigate the binding location and orientation of IGF-II within the IGF-1R. Two fluorescent IGF-II analogues, the F19Cou IGF-II and F28Cou IGF-II proteins, were synthesised for use in the desired FRET studies.

These FRET experiments first required the synthesis of an appropriate coumarin-based probe for incorporation into IGF-II. The synthesis of a range of fluorescent coumaryl amino acids is described in Chapter 2, and an analysis of the spectroscopic properties of these coumaryl amino acids is also detailed.

Site-specific incorporation of the coumarin-based probe into IGF-II was then undertaken. Three complementary methods were used for the preparation of the desired fluorescent IGF-II analogues. Chapter 3 describes the use of the nonsense suppression methodology for the expression of the novel F19Cou IGF-II protein. This was followed by an improved chemical synthesis of the F19Cou IGF-II protein using a linear solid phase peptide synthesis (SPPS) approach and is detailed in Chapter 4. A robust native chemical ligation approach was developed in Chapter 5, which allowed for the facile incorporation of the coumarin-based

probe at various locations within the IGF-II protein. Chapter 5 also details the synthesis of the native IGF-II, F19Cou IGF-II and F28Cou IGF-II proteins. The biological activity of the resultant IGF-II analogues was evaluated by competition binding assays. The fluorescent IGF-II analogues bind with low nanomolar affinity to the IR and IGF-1R, and as such were deemed suitable for use in the desired FRET-based experiments.

The FRET-based investigation into the binding interactions of the native IGF-II, F19Cou IGF-II and F28Cou IGF-II proteins to the IGF-1R is described in Chapter 6. FRET interactions were observed for both the F19Cou IGF-II and F28Cou IGF-II proteins. The results show the fluorophore binds in close proximity to Trp residues within the IGF-1R receptor and suggest the location of IGF-II binding within the IGF-1R is consistent with what is proposed in the literature. These experiments provide a basis for further investigations for determining the precise binding location and orientation of IGF-II within the IGF-1R.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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Publications

Work in this thesis has appeared in the following publication:

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Abbreviations

$[\alpha]^{23}_{D}$	specific rotation at the sodium D line (589 nm) at 23 °C
2-Br-Z	*2*-bromobenzyloxycarbonyl
2-Cl-Z	*2*-chlorobenzyloxycarbonyl
2-HED	2-hydroxyethyl disulfide
2xYT	2x yeast extract and tryptone
4-MeBzl	4-methyl benzyl
aaRS	aminoacyl-tRNA synthetase
Acm	acetamidomethyl
AIM	auto-inducing medium
Ala (A)	alanine
Amp	ampicillin
approx.	approximately
Ar	aromatic
Arg (R)	arginine
Asn (N)	asparagine
Asp (D)	aspartic acid
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Br	broad (spectroscopy)
Bz	benzyl
calcd.	calculated
Cbz	benzyloxycarbonyl
CDI	1,1-carbonyldiimidazole
cDNA	coding DNA
CH_2N_2	diazomethane
conc.	concentrated
Cou	coumarin fluorophore **2.2**
CR	cysteine-rich domain
CT	C-terminal domain
Cys (C)	cysteine
DCM	dichloromethane
DIC	*N*,*N*′-diisopropylcarbodiimide
DIPEA	*N*,*N*-diisopropylethylamine
Dmb	*N*-α-(2,4-dimethoxybenzyl)
DMF	*N*,*N*-dimethylformamide
DNA	deoxyribonucleic acid
Dnp	2,4-dinitrophenyl
DODT	3,6-dioxa-1,8-octanedithiol
DTT	dithiothreitol
E	efficiency of the energy transfer (in FRET)
EDTA	ethylenediaminetetraacetic acid
equiv.	equivalent
ESI-MS	electrospray ionisation mass spectrometry
Et_2O	diethyl ether
EtOAc	ethyl acetate
EuIGF-II	europium labelled IGF-II
Ex11	exon 11
Fmoc	9-fluorenylmethyloxycarbonyl
FnIII	fibronectin type III domain
FRET	fluorescence resonance energy transfer

Gln (Q)	glutamine
Glu (E)	glutamic acid
Gly (G)	glycine
GnHCl	guanidine hydrochloride
h	hour(s)
HATU	2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HBTU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HCTU	2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate
HEPES	*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid
HF	hydrogen fluoride
His (H)	histidine
HOBt	*N*-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
Hz	hertz (in NMR)
IB	inclusion bodies
ID	insert domain
IGF	insulin-like growth factor
IGF-1R	type 1 insulin-like growth factor receptor
IGF-2R	type 2 insulin-like growth factor receptor
IGFBP	insulin-like growth factor binding Protein
IGF-I	insulin-like growth factor I
IGF-II	insulin-like growth factor II
Ile (I)	isoleucine
IPTG	isopropyl-β-D-thiogalactoside
IR	insulin receptor
IR-A	insulin receptor isoform A
IR-B	insulin receptor isoform B
JM	juxatamembrane
kan	kanamycin
L1	large domain 1
L2	large domain 2
LB	Luria Bertani
LCMS	liquid chromatography mass spectrometry
Leu (L)	leucine
lit.	literature value
Lys (K)	lysine
m/z	mass to charge ratio
Me	methyl
$MeONH_2$ •HCl	methoxyamine hydrochloride
Met (M)	methionine

MHz	megahertz (in NMR)
MIN	minimal medium
min	minute(s)
mp	melting point
MPAA	mercaptophenylacetic acid
mRNA	messenger RNA
NCL	native chemical ligation
NIM	non-inducing medium
NIR	near infrared
NMM	*N*-methylmorpholine
NMP	*N*-methylpyrrolidine
NMR	nuclear magnetic resonance
O-2-Ada	2-adamantyl
o-aaRS	orthogonal aminoacyl-tRNA synthetase
OcHx	cyclohexyl ester
OD_{600nm}	optical density at 600 nm
o-tRNA	orthogonal tRNA
PAL	5-[3,5-dimethoxy-4-(fmoc-aminomethyl)phenoxy]pentanoic acid
PAM	4-hydroxymethylphenylacetamidomethyl
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
PDB	protein data bank
PEG	polyethylene glycol
pet. spirit	petroleum spirit
PG	unspecified protecting group
Phe (F)	phenylalanine
ppm	parts per million
Pro (P)	proline
r	distance between the donor and acceptor (in FRET)
RF1	release factor 1
RNA	ribonucleic acid
R_0	Förster distance
RP-HPLC	reverse phase high performance liquid chromatography
rt	room temperature
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
semi-prep	semi-preparative
Ser (S)	serine
SPE	solid phase extraction
SPPS	solid phase peptide synthesis
*t*Bu	*tert*-butyl
TCEP	tris(2-carboxyethyl)phosphine hydrochloride
TEMED	*N,N.N,N'N'*-tetramethylethylenediamine
tet	tetracycline
TFA	trifluoroacetic acid

THF	tetrahydrofuran
Thr (T)	threonine
TIPS	triisopropylsilane
TK	tyrosine-kinase domain
TLC	thin-layer chromatography
TM	transmembrane domain
TNBSA	2,4,6-trinitrobenzene sulfonic acid
Tos	tosyl
Tris	tris(hydroxymethyl)aminomethane
tRNA	transfer RNA
Trp (W)	tryptophan
Trt	trityl
Tyr (Y)	tyrosine
Uaa	unnatural amino acid
UV	ultraviolet
v/v	volume per unit volume
Val (V)	valine
w/v	mass per unit volume
Xaa	amino acid
Xan	xanthyl
αCT	C-terminal region of the α-subunit
λ_{Em}	emission maximum
λ_{Ex}	excitation maximum