Contents

1 Introduction **8**
- 1.1 Signalling of cyclic adenosine monophosphate 9
- 1.2 Protein kinase A (PKA) 11
- 1.3 A kinase anchoring proteins (AKAPs) 13
  - 1.3.1 Subcellular targeting of PKA by AKAPs 15
  - 1.3.2 Interaction of AKAPs and PKA type II 16
  - 1.3.3 AKAPs as scaffolding proteins 18
- 1.4 Peptides as disruptors of PKA-anchoring 21

2 Materials and Experimental procedures **24**
- 2.1 Material 24
  - 2.1.1 Chemicals, buffers 24
  - 2.1.2 Antibodies 27
  - 2.1.3 Peptides and proteins 27
  - 2.1.4 Bacterial hosts, eucaryotic cells, animals 28
  - 2.1.5 Apparatus and software 29
- 2.2 Experimental procedures 32
  - 2.2.1 Molecular biology techniques 32
  - 2.2.2 Immunoblotting 34
  - 2.2.3 Immunoprecipitation 35
  - 2.2.4 Vesicle isolation and PKA activity measurements . . . 35
  - 2.2.5 RII overlay 36
  - 2.2.6 Peptide synthesis 37
  - 2.2.7 Cell culture 37
  - 2.2.8 Patch-clamp 40
  - 2.2.9 Laser Scanning Confocal Microscopy 41
  - 2.2.10 Circular dichroism measurements 42
  - 2.2.11 Surface plasmon resonance measurements 42
- 2.3 Algorithms and bioinformatics 43

3 Results **48**
- 3.1 AKAP7δ-derived peptides bind RII subunits with high affinity 48

3.2 Determination of the secondary structure and of the RIIα-binding affinity of the AKAP7δ-derived peptides. 52
3.3 AKAP7δ-derived peptides inhibit PKA-anchoring in a competitive manner 55
3.4 AKAP7δ-derived peptides function as AKAP-PKA disruptors *in vivo* 59
3.5 Determinants of high affinity PKA-anchoring 63
3.6 A bioinformatics-based approach to identify new AKAPs . . . 70
3.7 The CN129 protein functions as AKAP *in vitro* 76
3.8 Subcellular distribution of CN129 81
3.9 The CN129 protein is highly conserved 83
3.10 The structure of the CN129 protein 85

4 Discussion **88**
4.1 AKAP7δ-derived peptides are potent disruptors of PKA-RII subunit anchoring 88
4.2 Determinants of high affinity PKA-anchoring 91
4.3 Combination of bioinformatics and peptide spot-synthesis to identify new AKAPs 93
4.4 Characterisation of the AKAP function of the CN129 protein . 96

5 Summary **100**

6 Publications **102**

Appendix **113**

A Vector map **114**

B Program source codes **115**

C Database derived peptides probed for RIIα binding **124**

D Acknowledgements **160**

List of Figures

1.1 Model of an AKAP. 14
1.2 AKAPs target PKA to specific subcellular compartments. . . 16
1.3 Molecular basis of the AKAP-PKA interaction. 18
1.4 Involvement of AKAP7α in the regulation of the L-type calcium channel. 20
1.5 Peptidic disruptors of the AKAP-PKA interaction mimic the RII-binding domain of AKAPs. 22

2.1 Scheme of the filter-algorithm. 47

3.1 Substitution array of the AKAP7δ RII-binding domain. 51
3.2 AKAP7δ-derived peptides display high affinity RII binding and α-helical secondary structure. 54
3.3 AKAP7δ-derived peptides act as PKA-anchoring disruptors *in vitro*. 57
3.4 AKAP7δ-derived peptides detach PKA activity from AQP2-bearing vesicles. 58
3.5 AKAP7δ-derived peptides act as PKA-anchoring disruptors *in vivo* abolishing β-adrenoceptor induced L-type Ca^{2+}-channel currents. 61
3.6 AKAP7δ-L314E peptide acts as PKA-anchoring disruptor *in vivo* preventing AQP2 redistribution in IMCD-cells. 62
3.7 Multiple sequence alignment of RII-binding domains of the indicated AKAPs. 64
3.8 Model of the interaction between the RIIα dimer and AKAP-derived peptides. 67
3.9 Influence of amino acids flanking the RII-binding domain of AKAP7δ. 68
3.10 Contribution of amino acid residues in non-conserved positions to the AKAP-RII binding. 69
3.11 A bioinformatics approach to identify new AKAPs. 73
3.12 All 829 of the 2572 peptides that bound RIIα in the RII overlay assay. 74

3.13 Peptide assay for competitive RII-binding. 75
3.14 Mapping the RII-binding domain of CN129. 78
3.15 Substitution array of the CN129 RII-binding domain. 79
3.16 CN129 functions as AKAP *in vitro*. 80
3.17 Subcellular distribution of CN129-CFP fusion protein in different cell lines. 82
3.18 The amino acid sequence of human CN129 is highly conserved. 84
3.19 The NMR structure of the AKAP CN129. 87

A.1 Vector map of human full length CN129-CFP. 114

C.1 Database-derived peptides probed for RII-binding. 159

Abbreviations

AC	adenylyl cyclase
AKAP	A kinase anchoring protein
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AQP2	aquaporin-2
ATP	adenosine trisphosphate
AVP	arginine-vasopressin
Ca^{2+}/CaM	calcium/calmodulin
cAMP	cyclic 3′-5′ adenosine monophosphate
CFP	cyan fluorescent protein
CRE	cAMP response element
CREB	cAMP response element binding protein
cDNA	complementary DNA
cGMP	cyclic 3′-5′ guanosine monophosphate
dbcAMP	dibutyryl cAMP
DMEM	Dulbecco′s modified eagle′s medium
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide trisphosphate
DTT	dithiothreitol
EDTA	ethylenediamine tetra-acetic acid
EGTA	ethylene glycol tetra-acetic acid
Epac	exchange protein directly activated by cAMP
FCS	foetal calf serum
FMP	Leibniz-Institut für Molekulare Pharmakologie
GEF	guanine nucleotide exchange factor
GFP	green fluorescent protein
GPCR	G-protein coupled receptor
G-protein	guanine nucleotide binding protein
GTP	guanosine triphosphate

HEPES	N-2-Hydroxyethylpiperazine-N′-2-ethanesulfonic acid
IBMX	isobutylmethylxanthine
IB	immuno-blot
Km	Michealis-Menton constant
kb	kilo base
K_d	equilibrium dissociation constant
kDa	kilo Dalton
KO	knockout
LB	lysogeny broth also Luria broth or Luria-Bertani
LR	linker region
M	molar
mRNA	messenger RNA
msa	multiple sequence alignment
NMR	nuclear magnetic resonance
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PDB	protein database
PDE	phosphodiesterase
PKA	protein kinase A
PKC	protein kinase C
PLB	phospholamban
PVDF	polyvinylidene fluoride
RNA	ribonucleic acid
rpm	revolutions per minute
RT	room temperature
SDS	sodium dodecylsulfate
TAE	tris/acetate/EDTA
TBS	tris-buffered saline
TE	tris/EDTA
TLC	thin-layer cellulose
Ub	ubiquitin
UCR	upstream conserved region
V2R	vasopressin 2 receptor
YFP	yellow fluorescent protein

Physical units were abbreviated according to the SI-system or derived units thereof. Amino acids are abbreviated by their single letter code. Patterns are described by regular expressions where X represents any amino acid, square brackets enclose alternatives and sub-pattern might be separated by dashes for better visualisation. Swissprot identifiers are abbreviated by their prefix as the suffix indicates the organism which is '_HUMAN' (*Homo sapiens*) throughout this work.

Aim of this work

Protein kinase A (PKA) is tethered to subcellular compartments by direct interaction of its regulatory subunits (RI or RII) with A kinase anchoring proteins (AKAPs). AKAPs preferentially bind RII subunits *via* their RII-binding domains. RII-binding domains form structurally conserved amphipathic helices with poor sequence homology. Their binding affinities for RII subunits differ greatly within the AKAP family. Amongst the AKAPs that bind RIIα subunits with high affinity is AKAP7δ (K_d value of 31 nM). The aim of this study was the development of peptides with high affinity to RII subunits of PKA as tools to disrupt AKAP-RII interactions and thus help to investigate the functional relevance of PKA compartmentalisation. A further aim of this work was to evaluate the determinants of the high affinity AKAP7δ-RII binding and utilisation of the results for a rational approach to identify new AKAPs.