Ph. D. Thesis

Ph. D. Program: Fundamental Chemistry,
Organic Chemistry, 1997-1999.

Surface plasmon resonance as a tool in the functional analysis of an immunodominant site in foot-and-mouth disease virus

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to apply for the degree of Doctor in Chemical Sciences

at the University of Barcelona

and revised by

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En primer lugar, me gustaría agradecer al Dr. David Andreu, supervisor de la presente tesis, y al Dr. Ernest Giralt por la calurosa acogida en su laboratorio, dándome la gran oportunidad de formar parte de su grupo de investigación. También quisiera agradecerles las fructuosas discusiones y el constante apoyo siempre que me sentía perdida entre "biacores" y péptidos...

A Jaume y Chary, de los SCT, porque siempre han traído un rayo de Sol a mis días de autismo cuando tocaba hacer biacore (al final, la "unidad" paula se puede desacoplar del BIACORE 1000!)

Estoy igualmente en deuda con Wendy Fernández, Dr. Núria Verdaguer y Dr. Ignacio Fita por presentarme el guapísimo mundo de la cristalografía de proteínas y por toda la paciencia que tuvieron guiando mis pasitos infantiles en este campo de investigación.

A todo el maravilloso "grup 10", mi casa y mi familia en estos cuatro años en que Barcelona ha sido mi ciudad adoptiva...quisiera agradecer muy especialmente a los compañeros y compañeras que han conquistado un rincón especial en mi corazón: Judit(h) "Peter Pan", Lorena (la dulce), Meritxell (alias, Cielitos), Albert(it)o Adeva, Melenita, Marta Molina, Mac… y jamás olvidaré a gente como Oscar Millet, genio por profesión… y ornitólogo por devoción… y a Paul, por su formidable personalidad.

Antes de mais devo agradecer à Prof. Doutora Maria Joaquina Amaral Trigo, não só por me apoiar e acreditar em mim profissionalmente, mas também pela sua valiosa amizade.

Agradeço à Fundação Calouste Gulbenkian (Lisboa, Portugal) a bolsa de doutoramento que me foi concedida, o apoio financeiro para assistir a congressos internacionais e a gentileza com que sempre fui tratada.

À Lia, catalã por motivos geográfico-sentimentais, portuguesa pelo idioma (si beim qui teim um cerrto sôtaqui dá cidádji dji São Paulo, né?) e com a bondade e a doçura de brasileira que é. És daquelas jóias raras com quem se pode sempre contar.

Aos meus amigos de sempre, Helena, Carla e Alfredo, por continuarmos a ser uma espécie de D'Artagnan e os, ou melhor, as Três Mosqueteiras..."voltar" não seria o mesmo sem vocês e nunca me cansarei de agradecer a vossa amizade!

Aos meus pais, Tina e Quim, e ao Richard, por
tudo o que são para mim, dedico a presente Tese.

General index

Abstract

A fast and direct surface plasmon resonance (SPR) method for the kinetic analysis of the interactions between peptide antigens and immobilised monoclonal antibodies (mAb) has been established. Protocols have been developed to overcome the problems posed by the small size of the analytes (< 1600 Da). The interactions were well described by a simple 1:1 bimolecular interaction and the rate constants were self-consistent and reproducible. The key features for the accuracy of the kinetic constants measured were high buffer flow rates, medium antibody surface densities and high peptide concentrations. The method was applied to an extensive analysis of over 40 peptide analogues towards two distinct anti-FMDV antibodies, providing data in total agreement with previous competition ELISA experiments.

Eleven linear 15-residue synthetic peptides, reproducing all possible combinations of the four replacements found in foot-and-mouth disease virus (FMDV) field isolate C-S30, were evaluated. The direct kinetic SPR analysis of the interactions between these peptides and three anti-site A mAbs suggested additivity in all combinations of the four relevant mutations, which was confirmed by parallel ELISA analysis. The four-point mutant peptide (A15S30) reproducing site A from the C-S30 strain was the least antigenic of the set, in disagreement with previously reported studies with the virus isolate. Increasing peptide size from 15 to 21 residues did not significantly improve antigenicity. Overnight incubation of A15S30 with mAb 4C4 in solution showed a marked increase in peptide antigenicity not observed for other peptide analogues, suggesting that conformational rearrangement could lead to a stable peptide-antibody complex. In fact, peptide cyclization clearly improved antigenicity, confirming an antigenic reversion in a multiply substituted peptide. Solution NMR studies of both linear and cyclic versions of the antigenic loop of FMDV C-S30 showed that structural features previously correlated with antigenicity were more pronounced in the cyclic peptide.

Twenty-six synthetic peptides, corresponding to all possible combinations of five single-point antigenicity-enhancing replacements in the GH loop of FMDV C-S8c1, were also studied. SPR kinetic screening of these peptides was not possible due to problems mainly related to the high mAb affinities displayed by these synthetic antigens. Solution affinity SPR analysis was employed and affinities displayed were generally comparable to or even higher than those corresponding to the C-S8c1 reference peptide A15. The NMR characterisation of one of these multiple mutants in solution showed that it had a conformational behaviour quite similar to that of the native sequence A15 and the X-ray diffraction crystallographic analysis of the peptide – mAb 4C4 complex showed paratope – epitope interactions identical to all FMDV peptide – mAb complexes studied so far. Key residues for these interactions are those directly involved in epitope – paratope contacts (^{141}Arg, ^{143}Asp, ^{146}His) as well as residues able to stabilise a particular peptide global folding. A quasi-cyclic conformation is held up by a hydrophobic cavity defined by residues 138, 144 and 147 and by other key intrapeptide hydrogen bonds, delineating an open turn at positions 141, 142 and 143 (corresponding to the Arg-Gly-Asp motif).

Resumen

Se diseñó un método rápido y sencillo para el análisis cinético por resonancia de plasmón superficial (RPS) de las interacciones entre antígenos peptídicos de bajo peso molecular (< 1600 Da) y anticuerpos monoclonales (AM) inmovilizados en la superficie de un chip sensor. Dichas interacciones se ajustaron a un modelo de interacción bimolecular 1:1 y las constantes cinéticas obtenidas resultaron fiables y reproducibles. Los parámetros clave para la calidad de las constantes cinéticas medidas fueron un flujo de tampón elevado, una densidad superficial de AM intermedia y una elevada concentración de péptido. El método se extendió a más de 40 análogos peptídicos frente a dos AM contra el virus de la fiebre aftosa (VFA), obteniéndose total correlación con datos anteriores de ELISA competitivo.

Se sintetizaron once pentadecapéptidos con todas las combinaciones posibles de las cuatro mutaciones que caracterizan el bucle GH del aislado C-S30 del VFA respecto a la secuencia de referencia C-S8c1. Los resultados del análisis cinético directo, por RPS, de la antigenicidad de estos péptidos frente a tres AM sugirieron que dichas combinaciones eran aditivas, observación que fué confirmada por ELISA competitivo. Así, el tetramutante (A15S30) que mimetiza el bucle GH de C-S30 resultó ser el peor antígeno de la serie, en contraste con resultados anteriores con este aislado. Aumentando el tamaño del tetramutante de 15 a 21 aminoácidos no afectó significativamente su antigenicidad. En cambio, una incubación prolongada con el AM llevó a un aumento de reactividad no observado para otros análogos. Posiblemente una reordenación conformacional del péptido pudo conllevar a la formación de un complejo estable con el anticuerpo. Experimentos de RPS con un análogo cíclico del péptido A15S30 confirmaron una reversión en la antigenicidad del tetramutante inducible a través de restricciones conformacionales. Estudios de ambos péptidos, lineal y cíclico, por resonancia magnética nuclear (RMN) mostraron que características estructurales anteriormente correlacionadas con la antigenicidad eran más pronunciadas en el análogo cíclico.

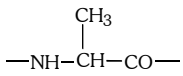
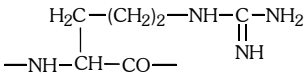
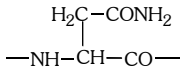
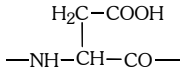
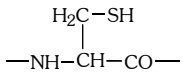
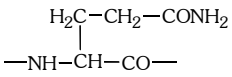
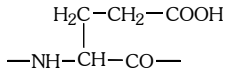
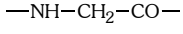
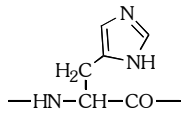
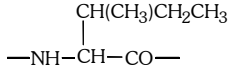
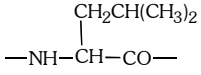
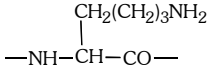
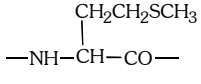
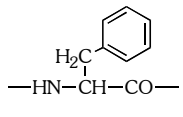
Se prepararon veintiseis péptidos con todas las posibles combinaciones de cinco sustituciones específicas en el bucle GH del VFA C-S8c1. Dichas sustituciones individuales habían sido objeto de estudios anteriores, obteniéndose una elevada antigenicidad para los correspondientes péptidos mutantes frente a AM anti-VFA. No se pudo sistematizar el análisis cinético por RPS de los nuevos mutantes multiples, debido a problemas tanto en la determinación de las constantes cinéticas de disociación, como en la regeneración de las superficies de AM. Se utilizó así la RPS para la determinación de la afinidad péptido – AM en solución, obteniéndose antigenicidades comparables o incluso superiores a las del péptido nativo A15 (VFA C-S8c1). Se estudió uno de los mutantes multiples (A15FPS) por RMN, observándose una conformación identica a la del péptido nativo. El estudio del complejo cristalino entre el péptido A15FPS y el AM 4C4 por difracción de RX mostró que las interacciones parátopo – epítopo eran similares a las observadas con el péptido nativo. Se concluyió que los residuos clave para el reconocimiento son tanto aquellos involucrados en contactos directos (^{141}Arg, ^{143}Asp, ^{146}His) como aquellos que estabilizan el plegamiento adecuado del péptido. Así, una conformación casi cíclica es soportada por una cavidad hidrofóbica definida por los residuos 138, 144 y 147 y por puentes de hidrógeno intra-peptídicos clave, diseñándose un bucle abierto centrado en las posiciones 141, 142 and 143 (triplete Arg-Gly-Asp).

Abbreviations

AA	Amino acid
AAA	Amino acid analysis
AcOH	Acetic acid
AM	2-[4-aminomethyl-(2,4-dimethoxyphenyl)phenoxy]acetic acid
APS	Ammonium persulphate
ATR	Attenuated total reflection
BSA	Bovine serum albumin
CDR	Complementarity determining region
Da	Dalton
DCM	Dichloromethane
DIEA	Diisopropylethylamine
DIP	Diisopropylcarbodiimide
DMF	dimethylformamide
EDC	N-ethyl-N'-(dimethylaminopropyl)carbodiimide
EDTA	Ethylenediaminotetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
eq	equivalent
ESI	Electro-spray ionisation
Fab	Fragment, antigen-binding
Fc	Fragment, crystallisable
FMD	Foot-and-mouth disease
FMDV	Foot-and-mouth disease virus
FT-IR	Fourier-transform infrared spectroscopy
HBcAg	Hepatitis B core antigen
HCA	Human carbonic anhydrase
HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulphonic acid
HOBt	1-Hydroxybenzotriazole
HPLC	High performance liquid chromatography
HRV	Human rhino virus
HS	Heparan sulphate
IC_{50}	Antigen concentration giving 50% inhibition
IFC	Integrated fluidic cartridge
Ig	Immunoglobulin
k_a	Association rate constant / $M^{-1}s^{-1}$
K_A	Affinity constant (association) / M^{-1}
k_d	Dissociation rate constant / s^{-1}
K_D	Affinity constant (dissociation) / M
KLH	Keyhole limpet hemocyanin
k_s	Apparent/global rate constant / $M^{-1}s^{-1}$
LED	Light-emitting diode

mAb	Monoclonal antibody
MALDI-TOF	Matrix-assisted laser desorption ionisation – time-of-flight
MAP	Multiple antigenic peptide
MBHA	*p*-methylbenzhydrylamine resin
MBS	*m*-maleimidobenzoyl-N-hydroxysuccinimide
MeCN	acetonitrile
MeOH	methanol
MPLC	Medium-pressure liquid chromatography
MS	Mass spectrometry
MW	Molecular weight
NHS	N-hydroxysuccinimide
NMM	N-methylmorpholine
NMP	N-methylpyrrolidone
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser effect spectroscopy
OD	Optical density
PBS	Phosphate buffer saline
PEG	Polyethylene glycol
PS	Polystyrene
PVC	Polyvinyl chloride
R	Response
$\boldsymbol{R_{eq}}$	Response at equilibrium
RI	Refractive index
$\boldsymbol{R_{max}}$	Maximal response
RNA	Ribonucleic acid
$\boldsymbol{R_{tot}}$	Total response
RU	Resonance unit
SD	Standard deviation
SDS	Sodium dodecylsulphate
SDS-PAGE	Sodium dodecylsulphate – polyacrylamide gel electrophoresis
SPPS	Solid-phase peptide synthesis
SPR	Surface plasmon resonance
SPW	Surface plasmon wave
VP	Viral protein
TBTU	N-[(1H-benzotriazol-1-yl)dimethylaminomethylene]-N-methylmethaneaminium N-oxide tetrafluoroborate
TEMED	N,N,N',N'-tetramethylethylenediamine
TFA	Trifluoroacetic acid
TFE	2,2,2-trifluoroethanol
TIR	Total internal reflection
TOCSY	Total correlation spectroscopy
UV - Vis	Ultraviolet – visible spectroscopy

Amino acids

Three-letter code	*One-letter code*	*Name*	*Formula*
Ala	A	Alanine	
Arg	R	Arginine	
Asn	N	Asparagine	
Asp	D	Aspartic acid	
Cys	C	Cysteine	
Gln	Q	Glutamine	
Glu	E	Glutamic acid	
Gly	G	Glycine	
His	H	Histidine	
Ile	I	Isoleucine	
Leu	L	Leucine	
Lys	K	Lysine	
Met	M	Methionine	
Phe	F	Phenylalanine	

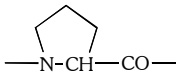
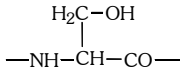
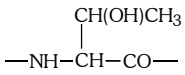
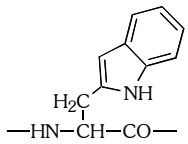
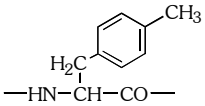
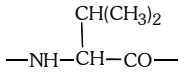
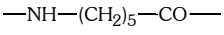
Three-letter code	***One-letter code***	***Name***	***Formula***
Pro	P	Proline	
Ser	S	Serine	
Thr	T	Threonine	
Trp	W	Triptophan	
Tyr	Y	Tyrosine	
Val	V	Valine	
Ahx	*	6-aminohexanoic acid	

Table I Abbreviations used for amino acid residues according to the Biochemistry Nomenclature Committee of the IUPAC-IUB [specified in *Eur. J. Biochem.* ***138***, 9-37 (1984) and *J. Biol. Chem.* ***264***, 633-673 (1989)]. α carbon side chains are presented in the non-ionic form for the twenty coded amino acids; All amino-acid residues employed corresponded to the natural L-configuration.

** Ahx is a non-coded amino acid residue used in this work.*

Amino acid protecting groups

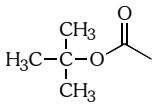
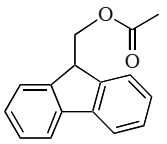
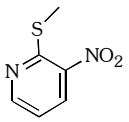
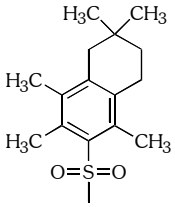
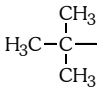
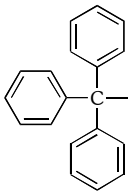
Abbreviation	***Name***	***Stability***	***Formula***
Boc	*t*-butyloxycarbonyl	Stable to bases, labile to TFA	
Fmoc	9-fluorenylmethyloxycarbonyl	Stable to acids and labile to bases	
Npys	3-nitro-2-pyridylsulphenyl	Stable to acids and bases, labile to nucleophiles	
Pmc	2,2,5,7,8-pentamethylchromane-6-sulphonyl	Stable to bases, labile to TFA	
^{t}Bu	*t*-butyl	Stable to bases, labile to TFA	
Trt	Triphenylmethyl (trityl)	Stable to bases, labile to 1% TFA	

Table II Amino acid protecting groups employed in this work.

Resins, handles and coupling reagents

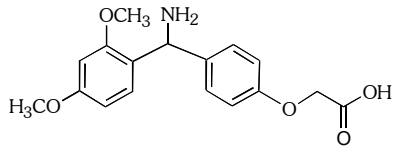
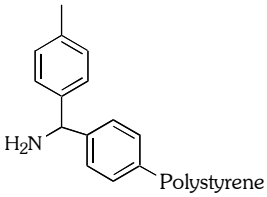
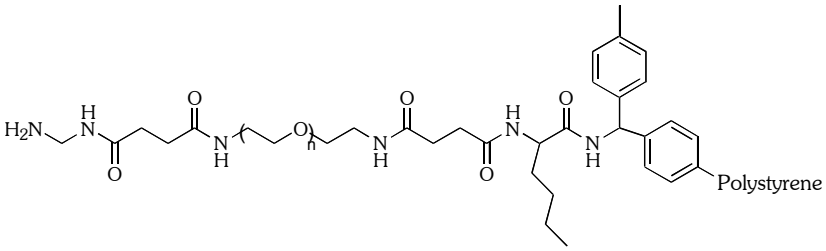
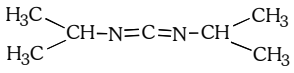
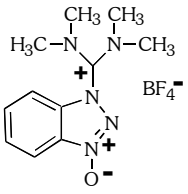
Abbreviation	*Structure*
AM	
MBHA	
PEG-PS	
DIP	
TBTU	

Table III Resins, handles and coupling reagents used in this work.