

DIPARTIMENTO DI SCIENZE BIOCHIMICHE

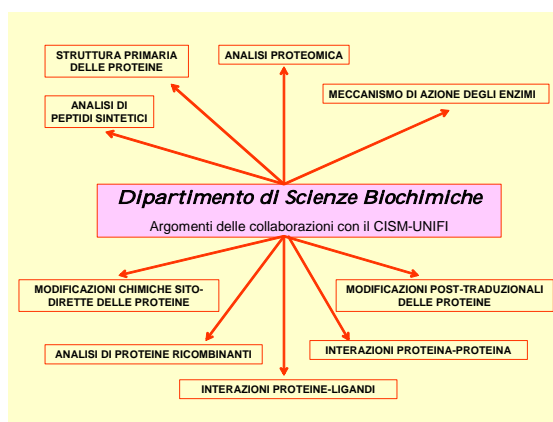
PROGETTO

La spettrometria di massa nelle ricerche del Dipartimento di Scienze Biochimiche

RESPONSABILE SCIENTIFICO: Prof. **Guido Camici**

COLLABORATORI: G. Cappugi, A. Caselli, P. Cirri, F. Magherini, G. Manao, A. Modesti, P. Paoli, L. Pazzagli, G. Ramponi

Fin dalla costituzione negli anni '70 del primo laboratorio di spettrometria di massa



presso l'Istituto di Farmacologia e Tossicologia (oggi Dipartimento di Farmacologia Preclinica e Clinica) dell'Ateneo fiorentino, i docenti/ricercatori dell'Istituto di Chimica Biologica (oggi Dipartimento di Scienze Biochimiche) sono stati sempre convinti sostenitori della validità scientifica dell'iniziativa promossa dal prof. Alberto Giotti. Il Dipartimento di Scienze Biochimiche è stato "socio fondatore" dell'attuale CISM e, come tale, con la

partecipazione alla giornata di studio del 10 marzo 2006 su "La spettrometria di massa e le sue più recenti applicazioni", ha inteso dare piena testimonianza dell'importanza della spettrometria di massa e del CISM nelle ricerche del Dipartimento. Diverse collaborazioni con personale di ricerca del CISM si sono concluse con pubblicazioni su riviste internazionali assai diffuse nella comunità scientifica.

La spettrometria di massa ha avuto ed ha un ruolo essenziale nelle ricerche del Dipartimento: strumenti con sorgenti FAB, elettrospray e MALDI del Centro sono utilizzati da oltre un ventennio per studi sulla struttura primaria delle proteine e sul

meccanismo d'azione degli enzimi (anche con l'uso di isotopi pesanti), per l'analisi delle modificazioni sito-dirette delle proteine, per l'analisi di proteine ricombinanti e dei peptidi sintetici, per l'analisi delle modificazioni post-traduzionali delle proteine e, più recentemente, per l'analisi conformazionale delle proteine ed per l'analisi proteomica. Alcuni gruppi di ricerca del Dipartimento sono attualmente impegnati in studi che si avvalgono delle tecnologie strumentali del

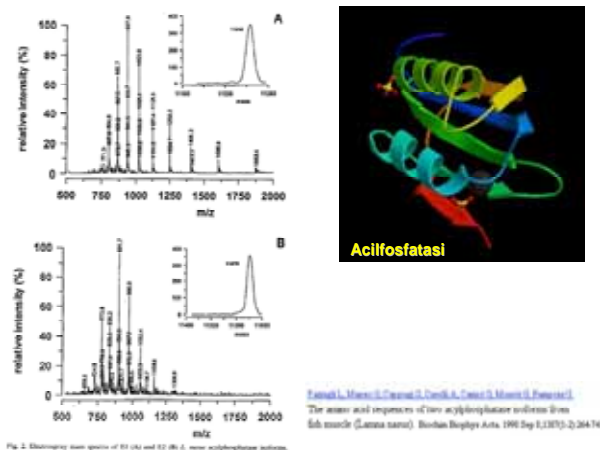


Fig. 2. Electrospray mass spectra of 91 (A) and 92 (B) kD acylphosphatase isoforms.

CISM e del Dipartimento, finanziate dall'Ente Cassa di Risparmio di Firenze a cui, come direttore, esprimo per conto del Dipartimento di Scienze Biochimiche dell'Università degli Studi di Firenze i più sentiti ringraziamenti per il sostegno costantemente dato alle nostre ricerche. A mio avviso, le più recenti acquisizioni strumentali del CISM sono di grande interesse per lo sviluppo delle ricerche in campo biochimico.

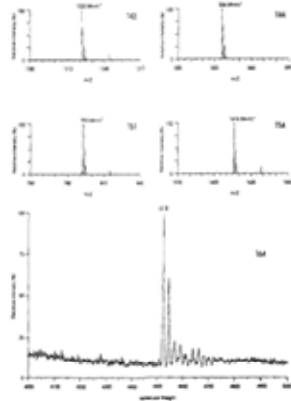


Fig. 2. Mass spectra of the cysteine-containing tryptic peptides from $\text{N}^{\text{EM}}\text{-inactivated}$ and $\alpha\text{-octan-1-ol-yl-modified}$ low M_r PTase from bovine liver. The mass spectra of the peptides T42, T44, T51, and T54 were acquired by FAB mass spectrometry, whereas the peptide T44 was produced through a computer simulation of the spectrum acquired by electrospray mass spectrometry. M^+ indicates the protonated molecular ion.

[Caselli A, Camici G, Manao G, Moneti G, Pazzagli L, Cappugi G, Ramponi G](#)

Nitric oxide causes inactivation of the low molecular weight phosphotyrosine protein phosphatase. *J Biol Chem.* 1994 Oct 7;269(40):24878-82.

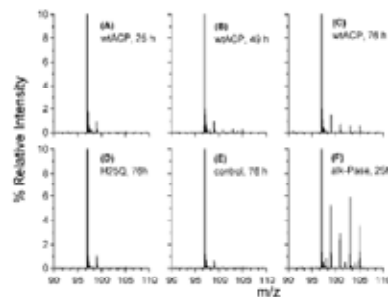
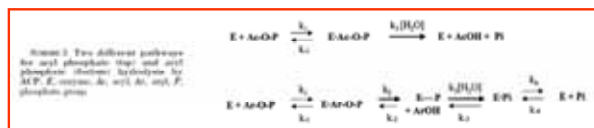
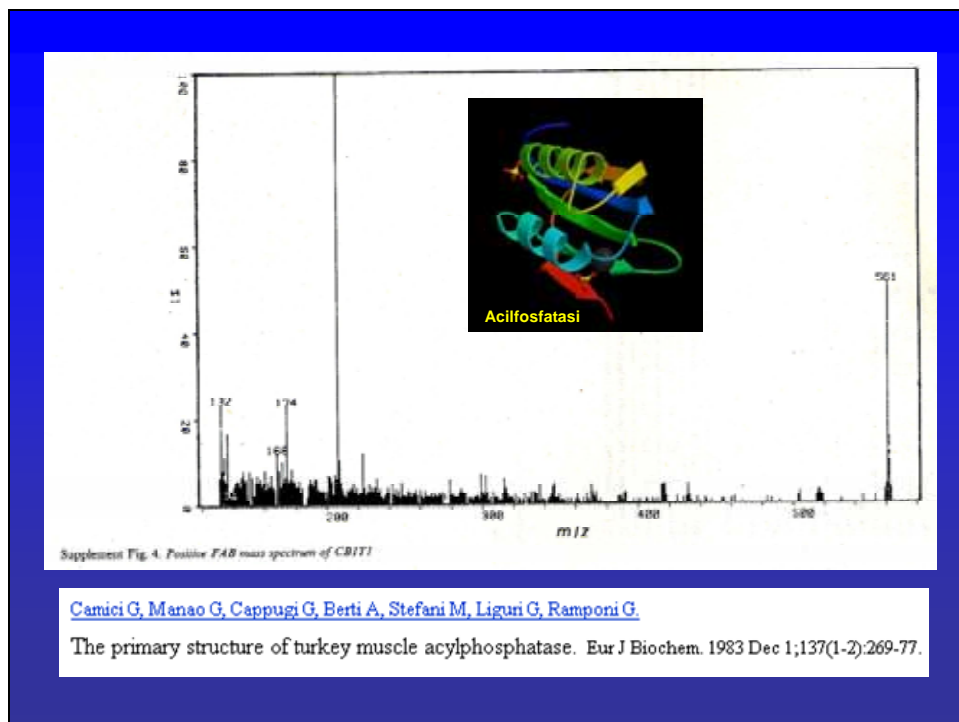
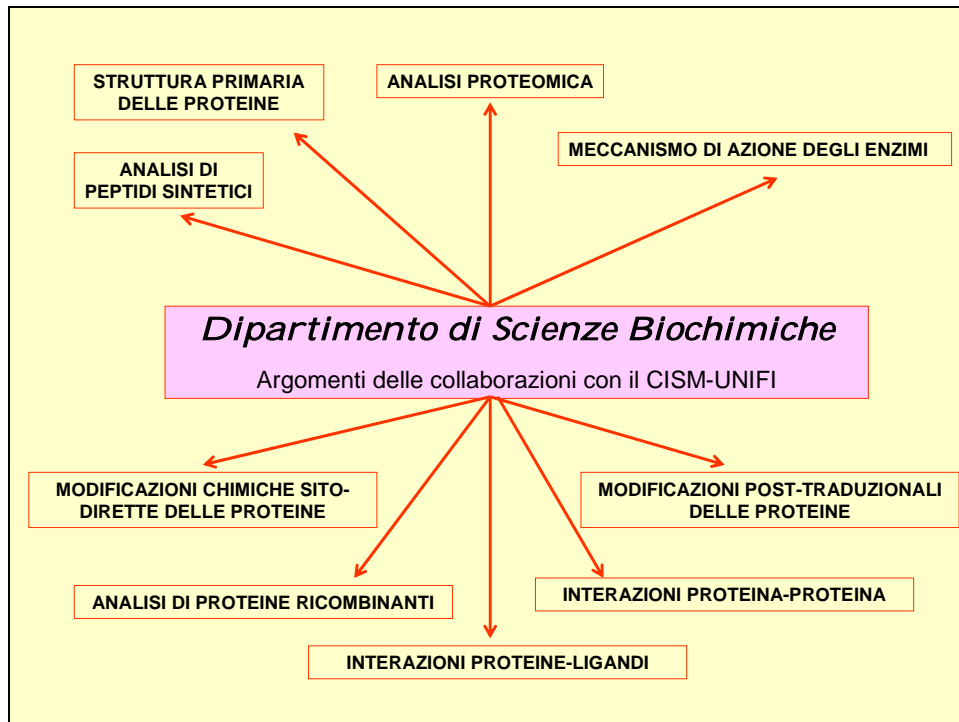


Fig. 2. Electrospray MS-spectra from inorganic phosphate. H_2^{18}O oxygen exchange experiments. The panels show the intensities of ions relative to that of the H_2^{16}O isotope, for which intensity was fixed at 100%. The incubation times are indicated in the panels. wt, wild type.

[Paoli P, Fiaschi T, Cirri P, Caselli A, Manao G, Camici G, Ramponi G](#)

A nucleophilic catalysis step is involved in the hydrolysis of acyl phosphate monoesters by human CT acylphosphatase. *J Biol Chem.* 2003 Jan 3;278(1):194-9. Epub 2002 Oct 29.

- Pantera B., Hoffman D.R., Carresi L., Cappugi G., Turillazzi S., Manao G., Severino M., Spadolini I., Orsomando G., Moneti G., Pazzagli L. "Characterization of the major allergens purified from the venom of the paper wasp *Polistes gallicus*" *Biochim. Biophys. Acta* **1623**, 72-81 (2003)
- Pazzagli L., Cappugi G., Manao G., Camici G., Santini A., Scala A. "Purification, characterization, and amino acid sequence of ceratoplatenin, a new phytotoxic protein from *Ceratocystis fimbriata* f. sp. *Platanii*" *J. Biol. Chem.* **274**, 24959-24964 (1999)
- Pazzagli L., Manao G., Cappugi G., Caselli A., Camici G., Moneti G., Ramponi G. "The amino acid sequences of two acylphosphatase isoforms from fish muscle (*Lamna nasus*)" *Biochim. Biophys. Acta* **1387**, 264-274 (1998)
- Caselli A., Marzocchini R., Camici G., Manao G., Moneti G., Pieraccini G., Ramponi G. "The inactivation mechanism of low molecular weight phosphotyrosine-protein phosphatase by H_2O_2 " *J. Biol. Chem.* **273**, 32554-32560 (1998)
- Paoli P., Fiaschi T., Cirri P., Camici G., Manao G., Cappugi G., Raugei G., Moneti G., Ramponi G. "Mechanism of acylphosphatase inactivation by Woodward's reagent K" *Biochem. J.* 1997 **328**, 855-861
- Paoli P., Cirri P., Camici G., Manao G., Cappugi G., Moneti G., Pieraccini G., Camici G., Ramponi G. "Common-type acylphosphatase: steady-state kinetics and leaving-group dependence" *Biochem. J.* **327**, 177-184 (1997)
- Caselli A., Cirri P., Bonifacio S., Manao G., Camici G., Cappugi G., Moneti G., Ramponi G. "Identity of zinc ion-dependent acid phosphatase from bovine brain and myo-inositol 1-phosphatase" *Biochim. Biophys. Acta* **1290**, 241-249 (1996)
- Caselli A., Camici G., Manao G., Moneti G., Pazzagli L., Cappugi G., Ramponi G. "Nitric oxide causes inactivation of the low molecular weight phosphotyrosine protein phosphatase" *J. Biol. Chem.* **269**, 24878-24882 (1994)



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Characterization of the major allergens purified from the venom of the paper wasp *Polybia pallens*.
Biochim Biophys Acta. 2003 Oct 13;1623(2-3):72-81.

[Pazzagli L, Cappugi G, Manao G, Camici G, Santini A, Scala A](#)

Purification, characterization, and amino acid sequence of cerato-platanin, a new phytotoxic protein from *Ceratocystis fimbriata* f. sp. *platani*.
J Biol Chem. 1999 Aug 27;274(35):24959-64.

[Pazzagli L, Manao G, Cappugi G, Caselli A, Camici G, Moneti G, Ramponi G](#)

The amino acid sequences of two acylphosphatase isoforms from fish muscle (*Lamna nasus*).
Biochim Biophys Acta. 1998 Sep 8;1387(1-2):264-74.

[Caselli A, Marzocchini R, Camici G, Manao G, Moneti G, Pieraccini G, Ramponi G](#)

The inactivation mechanism of low molecular weight phosphotyrosine-protein phosphatase by H₂O₂.
J Biol Chem. 1998 Dec 4;273(49):32554-60.

[Paoli P, Fiaschi T, Ciri P, Camici G, Manao G, Cappugi G, Raugei G, Moneti G, Ramponi G](#)

Mechanism of acylphosphatase inactivation by Woodward's reagent K.
Biochem J. 1997 Dec 15;328 (Pt 3):855-61.

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Common-type acylphosphatase: steady-state kinetics and leaving-group dependence.
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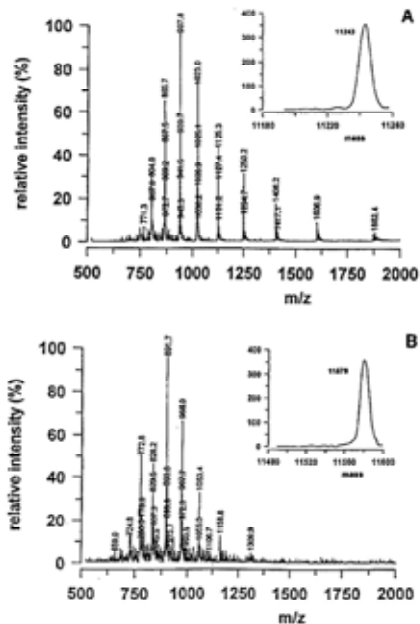


Fig. 2. Electrospray mass spectra of E1 (A) and E2 (B) *L. nasus* acylphosphatase isoforms.



[Pazzagli L, Manao G, Cappugi G, Caselli A, Camici G, Moneti G, Ramponi G](#)

The amino acid sequences of two acylphosphatase isoforms from fish muscle (*Lamna nasus*). *Biochim Biophys Acta*. 1998 Sep 8;1387(1-2):264-74.

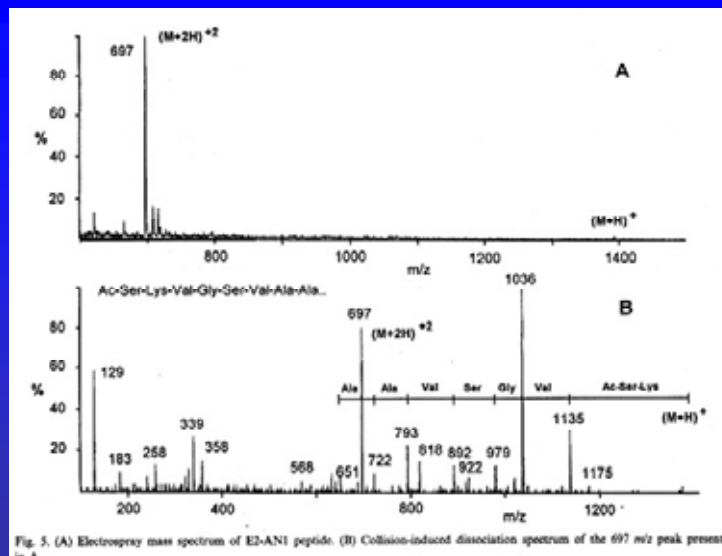


Fig. 5. (A) Electrospray mass spectrum of E2-AN1 peptide. (B) Collision-induced dissociation spectrum of the 697 *m/z* peak present in A.

[Pazzagli L, Manao G, Cappugi G, Caselli A, Camici G, Moneti O, Ramponi O.](#)

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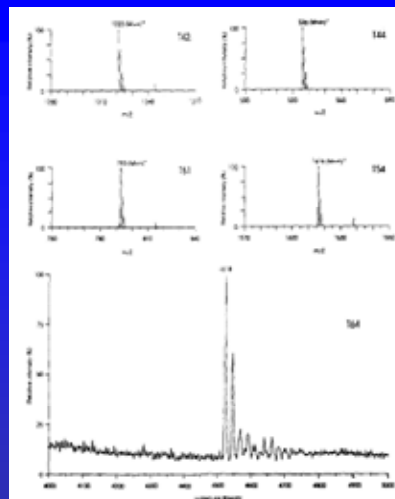
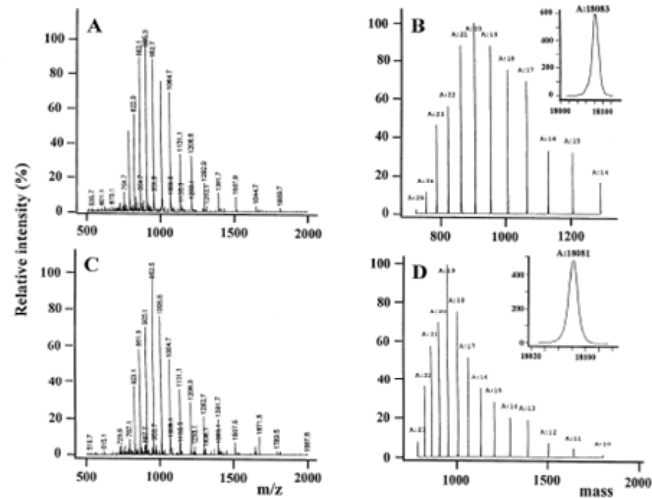


Fig. 2. Mass spectra of the cysteine-containing tryptic peptides from SNP-inactivated and *n*-octane-1-thiol-modified low *M_r* PTPase from bovine liver. The mass spectra of the peptides T42, T44, T46, and T54 were acquired by FAB mass spectrometry, whereas the peptide T64 was produced through a computer elaboration of the spectrum acquired by electrospray mass spectrometry. *(M+H)⁺* indicates the protonated molecular ion.

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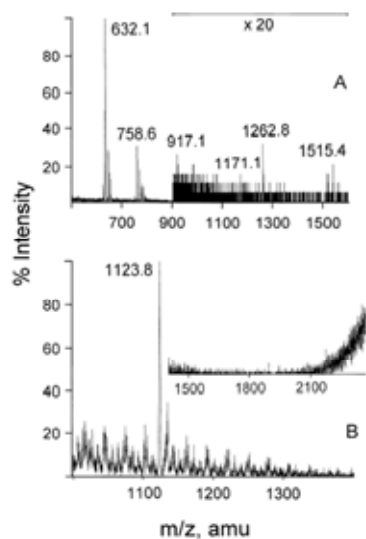
Nitric oxide causes inactivation of the low molecular weight phosphotyrosine protein phosphatase. *J Biol Chem*. 1994 Oct 7;269(40):24878-82.

FIG. 4. Electrospray mass spectra of the unmodified and H₂O₂-modified enzymes. A fraction of the solvent flux from the HPLC column described in Fig. 3 was continuously introduced into the electrospray source of the mass spectrometer, and spectra were acquired. *A*, electrospray ionization mass spectrum of unmodified LMW-PTP IF1 isoform (Fig. 3, left panels, peak I). *B* shows the charge status of a number of mass peaks present in *A*, which were selected by the special mass spectrometry computer program (Hewlett-Packard). *C*, electrospray ionization mass spectrum of LMW-PTP IF1 isoform modified by H₂O₂ (Fig. 3, left panels, peak II). *D* shows the charge status of a number of mass peaks present in *C*, which were selected by the special mass spectrometry computer program (Hewlett-Packard). The same computer program also calculated the reconstructed mass spectra of unmodified (*B*, inset) and modified (*D*, inset) enzymes.



Caelli A, Martocchia R, Canici G, Manao G, Moneti G, Pieraccini G, Rampone G.

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1515.4 m/z ($M^+ + H$)⁺
 1262.8 m/z ($M^+ - WRK$)
 758.6 m/z ($M^+ + 2H$)²⁺
 632.1 m/z [($M^+ - WRK$) + 2H]²⁺

These values fit very well with the WRK adduct of the sequence 20-29 of human CT acylphosphatase

the 1123.8 m/z peak represents the double-charged ($M^+ + 2H$)²⁺ ion of the WRK adduct with residues 13-29 of the acylphosphatase

Figure 5 Mass spectra of WRK-labelled endoprotease Glu-C peptides

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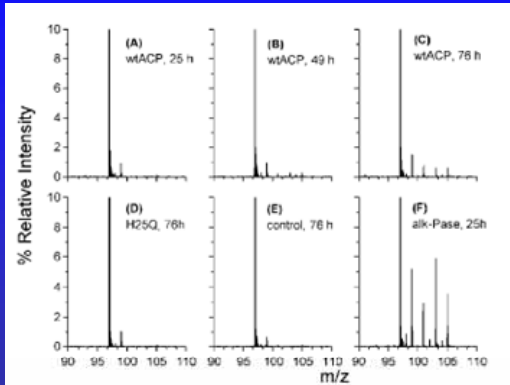
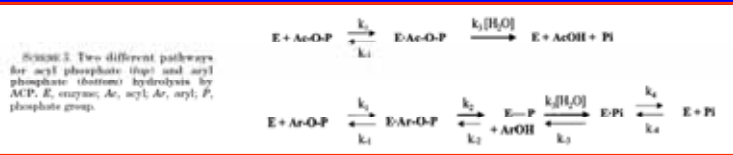


FIG. 2. Electrospray MS-spectra from inorganic phosphate- H_2^{18}O oxygen exchange experiments. The panels show the intensities of ions relative to that of the $\text{H}_2\text{P}^{16}\text{O}_4^-$ isotopomer, for which intensity was fixed at 100%. The incubation times are indicated in the panels. wt, wild type.

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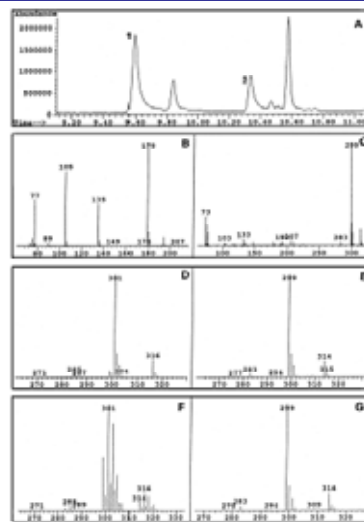
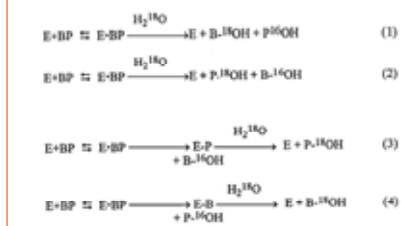


Figure 4. GLC-MS analyses.

Panel A. GC separation of the ^{18}O derivatives of benzoic acid (peak 1) and P (peak 2). The chromatography was performed with an SPB-1 capillary column (30 m \times 0.25 mm ID, 0.25 μm film thickness) before or after use. Single or double peaks are described in the text. M indicates the molecular ion. Panels B and C show the mass spectra of peak 1 (BP = 194.034 M, 192.034 M, 190.034 M).



Paoli P, Cirri P, Camici L, Manao G, Cappugli G, Moneti G, Pieraccini G, Camici G, Ramponi G.

Common-type acylphosphatase: steady-state kinetics and leaving-group dependence. *Biochem J.* 1997 Oct 1;327 (Pt 1):177-84.

Table 3
Correspondence between peptide from the zinc ion-dependent acid phosphatase and myo-inositol 1-phosphatase structure *

Peptide position *	Type of cleavage	Molecular weight		Assignment method							
		calculated *	found								
10-31	CNBr	2334.6	2335	MALDI-TOF/MS							
31-51	EP-GluC	2277.3	2299 *	MALDI-TOF/MS							
35-55	CNBr	2017.4	2018	MALDI-TOF/MS							
37-49	Trypsin	1332.4	1332.4 ± 0.2	HPLC/ESI-MS							
62-78	Trypsin	1807.9	1806.4 ± 1.1	HPLC/ESI-MS							
78-118	EP-GluC	4589.3	4550	MALDI-TOF/MS							
118-130	CNBr	1561.7	1562	MALDI-TOF/MS							
128-152	EP-GluC	2860.2	2860	MALDI-TOF/MS							
131-200	CNBr	7677.8	7678	MALDI-TOF/MS							
148-156	Trypsin	1297.4	1297 ± 0.0	HPLC/ESI-MS							
157-167	Trypsin	1161.3	1160.9 ± 0.4	HPLC/ESI-MS							
181-213	EP-GluC	3520.1	3520	MALDI-TOF/MS							
201-214	CNBr	1475.6	1498 *	MALDI-TOF/MS							
215-246	CNBr	3212.6	3234 *	MALDI-TOF/MS							
231-260	EP-GluC	3075.5	3076	MALDI-TOF/MS							
247-277	CNBr	3566.0	3567	MALDI-TOF/MS							
262-273	Trypsin	1109.3	1108.8 ± 0.1	HPLC/ESI-MS							
Position *	Peptide name *	Sequence results (automatic Edman degradation)									
53-60	sp42	Lys	-Met	-Leu	-Ile	-Thr	-Ser	-Ile	-Lys	-Glu	
		→	→	→	→	→	→	→	→	→	
61-71	sp38	Lys	-Tyr	-Pro	-Ser	-Ile	-Ser	-Phe	-Ile	-Gly	-Glu
		→	→	→	→	→	→	→	→	→	
231-237	sp32	Ala	-Gly	-Gly	-Val	-Leu	-Leu	-Asp			
		→	→	→	→	→	→	→			
266-276	sp48	Ile	-Glu	-Ile	-Ile	-Pro	-Leu	-Glu	-Arg	-Asp	-Asp
		→	→	→	→	→	→	→	→	→	

* From the amino-acid sequence of bovine brain myo-inositol 1-phosphatase [17].
* See Fig. 2.
* Sodium adduct.

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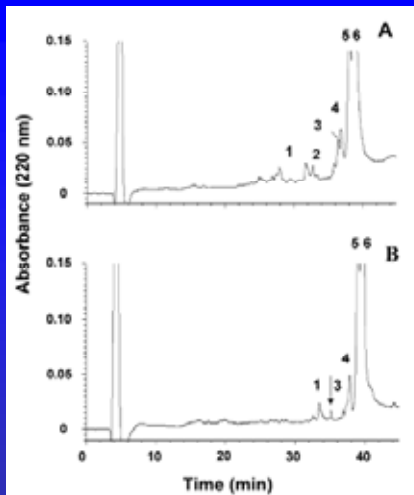


Figure 4. Limited proteolysis experiments of ACP in the amyloidogenic state. HPLC chromatograms of the aliquots withdrawn following 15 minutes of trypsin (A) and chymotrypsin (B) digestions of ACP in the presence of 25% TFE. The indicated fractions were manually collected and the eluted peptides were identified by ESI MS.

Monti M, Garolla di Bard EL, Calloni O, Chiti F, Amorezono A, Ramponi O, Pucci P.

The regions of the sequence most exposed to the solvent within the amyloidogenic state of a protein initiate the aggregation process. *J Mol Biol*. 2004 Feb 6;336(1):253-62.

DIPARTIMENTO DI SCIENZE BIOCHIMICHE

PROGETTO

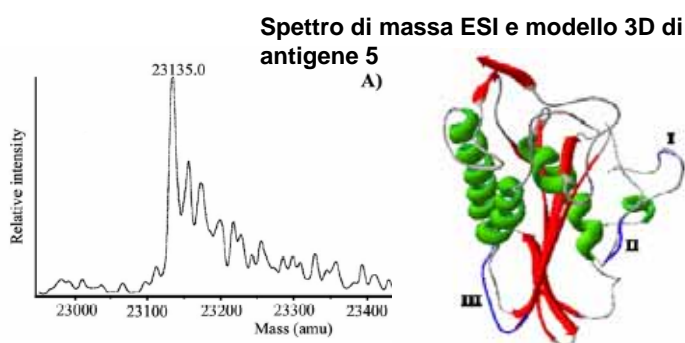
Caratterizzazione di proteine non enzimatiche

RESPONSABILE SCIENTIFICO: Prof. Gianni Cappugi

COLLABORATORI: L. Pazzagli, B. Pantera, G. Zoppi, L. Carresi

In questi ultimi anni la collaborazione di questo gruppo di ricerca con il CISM è stata molto intensa e proficua relativamente alle seguenti linee di ricerca:

1) Caratterizzazione di allergeni contenuti nel veleno di vespe *Polistes gallicus*: nell'ambito di un lavoro volto a caratterizzare il veleno di vespe *Polistes*, da impiegare nella immunoterapia specifica di pazienti allergici, abbiamo purificato e caratterizzato i principali allergeni.



2) Identificazione di uno o più peptidi dotati di attività antibatterica isolati da saliva di vespe *P. dominulus*: la saliva delle larve di *P. dominulus* presenta attività antibatterica sui Gram + e sui Gram -; ai fini di isolare e caratterizzare il principio o i principi attivi, i componenti della saliva sono stati purificati tramite cromatografia e sui picchi attivi è stata fatta la spettrometria di massa