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References

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Chaperone Activity of α B-Crystallin Is Responsible for Its Incorrect Assignment as an Autoantigen in Multiple Sclerosis

Jonathan B. Rothbard,* Xiaoyan Zhao,*‡ Orr Sharpe,*‡ Michael J. Strohman,‡ Michael Kurnellas,‡ Elizabeth D. Mellins,‡ William H. Robinson,*‡ and Lawrence Steinman‡

For 15 years, α B-crystallin (heat shock protein [Hsp] B5) with multiple sclerosis (MS) has been labeled an autoantigen in multiple sclerosis (MS) based on humoral and cellular responses found in humans and animal models. However, there have been several scientific inconsistencies with this assignment, ranging from studies demonstrating small differences in anticrystallin responses between patients and healthy individuals to the inability of crystallin-specific T cells to induce symptoms of experimental allergic encephalomyelitis in animal models. Experiments in this article demonstrate that the putative anti-HspB5 Abs from 23 MS patients cross-react with 7 other proteins. This work was supported by National Institutes of Health Grants R01NS55997 (to L.S. and W.H.R.), R21DK079163 (to E.D.M. and M.J.S.), and 5-T32AI07290 to M.J.S.; the Endriz Fund (to W.H.R.); National Multiple Sclerosis Society (to J.B.R., M.K., and L.S.); and Lawrence Steinman and the National Multiple Sclerosis Society (to J.B.R., M.K., and L.S.). E-mail address: steinman@stanford.edu

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The online version of this article contains supplemental material.

Abbreviations used in this article: EAE, experimental autoimmune encephalomyelitis; Hsp, heat shock protein; MS, multiple sclerosis; sHsp, small heat shock protein.
dynamic oligomers with different stoichiometries, but in all cases, the unit cell was a dimer with the monomer folding as a conserved β barrel with amino- and carboxyl-terminal extensions (19). The extensions are integral in the formation and stabilization dimers and aggregates of dimers (20). Recent resolution of the β barrel of human HspB5 has shown the dimer contacts are between the edge strands (20, 21). A groove, which is postulated to be one of the principal hydrophobic binding sites, is formed between the two subunits because the register of the strands is not symmetrical. A conserved structural feature of the groove is the presence of salt bridges at both ends formed between a conserved arginine at residue 120 of one domain with a conserved aspartic acid at residue 110 in the adjacent subunit (21). A naturally occurring mutation of this arginine to a glycine has been shown to compromise the structural integrity of the groove and the chaperone activity of the protein (22). Most importantly, the mutation has been shown to be physiologically relevant being linked to a human desmin myopathy (23, 24).

HspB5 is one of a family of 10 sHsps in humans, all of which are closely related in structure and function (12). They are molecular chaperones, capable of binding partially unfolded proteins principally through hydrophobic contacts limiting the formation of aggregates (25). Functional studies of the family have established they have multiple binding sites, and most importantly, the chaperone activity increases with temperature. The recent crystal structures have provided a model for this effect in which the C terminus of a subunit occupies the principle groove at ambient temperature but dissociates at temperatures greater than 37°C, exposing both the groove and the conserved hydrophobic residues of the terminus (20, 21). Such a model would be consistent with greater binding capacity of the molecules with increasing temperature and the observed dynamic nature of the molecular aggregate.

Abs are prone to aggregate by forming extended β pleated sheets at high local concentrations, leading to problems in both their handling in the laboratory and, more importantly, in vivo pathology leading to systemic amyloidosis (26, 27). Clearly, Abs specific for HspB5 will present a significant complication if the protein were to be used as a human therapeutic. Because HspB5 was therapeutically effective in animals with EAE, the Abs either were directed at determinants that did not inhibit the anti-inflammatory activity of the protein or there was an alternative explanation for the presence of the humoral response. The alternative we propose in this article is that as molecular chaperones, the sHsps bind the Igs at multiple sites, which could help explain the varying experimental inconsistencies in the story in the literature.

Materials and Methods

Cloning, expression, and purification of T7-human shspB1-8

The full-length clones of human shspB1-8 were obtained from Open Biosystems. An EcorI, an ATG site, a HindIII, and stop site were introduced into the gene encoding each of the shsps using PCR. The resulting shsp PCR fragments were ligated into the EcoRI-HindIII restriction site of pET21b (+) (Novagen) in-frame with the amino-terminal T7-tag. One-shot TOP10 cells (Invitrogen) were transformed with the resulting colonies and DNA was amplified by PCR. The resulting colonies were selected, expanded, and the insertions were verified by restriction digest with EcoR1 and HindIII, and sequencing.

Several of the resulting colonies were selected, expanded, and the insertion was confirmed by gel electrophoresis on agarose gels, and the fragments were sequenced by mass spectrometry. The quaternary structure was established using gel filtration on Sephacryl S-300 and by dynamic light scattering.

Enzyme-linked immunosassays

Solutions of recombinant T7-shspB1-8 in PBS pH 7.4 were coated on 96-well ELISA plates (MaxiSorp, Nunc, Rochester, NY) overnight at 4°C at a concentration of 20 µg/ml. Subsequent incubations and washes were performed at room temperature. The plates were blocked with 3% BSA in PBS with 0.05% Tween 20 (Sigma, St. Louis, MO) for 1 h, washed, and incubated for 2 h with serum samples diluted 1:200 from either patients with active MS or healthy individuals. The plates were washed, and bound Abs in sera were detected using HRP-conjugated secondary reagents specific for human IgG (H chain) Abs diluted to 1:10,000 (Jackson ImmunoResearch, West Grove, PA). The colorimetric reaction was developed with 3,3′,5,5′-tetramethylbenzidine (1-step Ultra TMB; Thermo Scientific), stopped with 1 M sulfuric acid, and quantitated using a SpectraMax M5 (Molecular Devices).

Biolayer interferometry

The Octet QK biosensor (Fortebio, Menlo Park, CA) uses biolayer interferometry, a label-free, optical-based technology capable of measuring interactions between sensor-immobilized and solution-phase compounds. Each of the small Hsps was titrated from 10 µM to 123 nM in PBS pH 7.2 containing 0.1% BSA and 0.002% Tween 20 in black 96-well polystyrene plates (E&K Scientific Products, Santa Clara, CA). Kinetic analyses of the binding of the sHsps to commercially available biosensors coated with anti-human IgG Fc were performed on an Octet QK system (Fortebio, Menlo Park, CA) under orbital shaking conditions (1000 rpm) following details recommended by the manufacturer at 40, 37, or 23°C depending on the assay.

The association and dissociation curves were fit using a single-phase exponential model, with the individual rate constants being calculated using Origin software provided by the manufacturer (Fortebio). Data from maximum wavelength (nm) shift under equilibrium binding conditions at increasing sHsp concentrations were plotted and fit to a single exponential association curve, 
\[ Y = Y_D + A \left(1 - \exp\left(-k_{on} t\right)\right), \]
where \( k_{on} \) is [sHsp]. \( Y_0 \) is wavelength shift at \( t = 0 \) s, \( A \) is maximum wavelength shift (time dependent), and \( k_{on} \) is observed first-order rate constant. Maximum wavelength shift at each concentration was determined by the summation of \( Y_0 \) and \( A \). Maximum wavelength shift at a each concentration of sHsp was plotted and fitted to one site binding model (hyperbola), 
\[ S = \frac{M \times [sHsp]}{K_s + [sHsp]}, \]
where \( S \) is wavelength shift at equilibrium, \( M \) is maximum wavelength shift, and [sHsp] is [sHsp].

Mass spectral analyses

T7-HspB5, 10 µg, was added to 300 µl MS plasma or normal plasma and incubated at 42°C for 2 h, after which 50 µl anti-T7 Sepharose beads was added and the mixture was incubated an additional 2 h at 42°C. The resin was separated from the plasma by centrifugation, washed multiple times with PBS pH 7.4, and eluted with 100 µl of 100 mM glycine pH 1.8. Aliquots of samples were separated on 12% Tris PAGE, and the gel was stained with Coomassie brilliant blue. Gel pieces corresponding to the five prominent bands were subjected to trypsin (Sigma) digestion and identified by liquid chromatography tandem mass spectrometry using the Agilent 1100 LC system and the Agilent XCT plus Ion Trap (Agilent Technologies, Santa Clara, CA) as previously described (28). The tandem mass spectra were further searched against the SwissProt database using the SpectrumMill software (Agilent); a minimum of two peptides was required for protein identification, with \( p = 0.05 \) for each peptide identified.

\[ \alpha \text{ B-CRYSTALLIN BINDS Igs} \]
Results

Interactions between sHsps and Igs when measured using solid-phase assays

To confirm that MS patient sera contain Abs specific for HspB5 and to explore whether there was reactivity to any of the other sHsps, we coated 96-well plates with HspB1-8 and treated them with serial dilutions of plasma from 23 different MS patients. The amount of Ig bound was quantified by treating the wells with an HRP-conjugated mouse anti-human Ig, washing, adding a colorimetric HRP substrate, and measuring the OD of each well. Surprisingly, strong signals were seen for each sHsp, with HspB5 not even being the strongest signal (Fig. 1A). A possible explanation was that the high homology among the family members led to highly cross-reactive Ab specificity. However, if the Abs were generated against HspB5, the relatively low response to HspB5 was inconsistent with this hypothesis. An alternative explanation became apparent when sera from 17 healthy individuals, together with sera from 4 additional MS patients, were assayed (Fig. 1B). As with the patients’ sera, strong signals were seen for each of the sHsps, but equivalent pattern of reactivity, within error, was observed for both normal and patient sera. Clearly, these data are inconsistent with the patients having a humoral response against HspB5, but does argue there is a biologically significant interaction between the sHsps and Igs. The sHsps appear to be binding the Igs, not the reverse.

![Figure 1](http://www.jimmunol.org/)

**FIGURE 1.** Average binding of Igs in MS patients’ and normal sera to each of the recombinant human sHsps. (A) Differential binding of Igs to the family of human sHsps from sera of 23 MS patients; (B) comparison of the relative signals from sera from 13 healthy individuals with that of sera from an additional 4 MS patients. Error bars represent SD of both the variation between the individual samples and the small differences from four separate readings.

Biolayer interferometry

Support for this hypothesis came from biolayer interferometry, in which the rate of binding and dissociation of the eight sHsps to a biotinylated polyclonal murine anti-human Fc Ig attached to a sensor on a surface of a tip compatible with a 96-well plate is measured (Fig. 2A). The system was chosen both because of its ease and simplicity, and because the attached Ab configuration was commercially available and well studied. The design is used to attach a second Ab of desired specificity and measure its binding kinetics to the relevant Ag. For this study, the murine anti-human Fc represents an Ig with known specificity completely unrelated to sHsps. In interferometry, binding of sHsp in solution to a sensor-immobilized Ab changes the interference pattern of white light reflected from the sensor surface relative to a reference surface. The change in interference pattern is a direct measure of the change in thickness of the biological layer. The binding signal, measured in units of nanometer shift, is proportional to the number of sHsp molecules bound to the Ab.

Ab binding to an Ag through a single combining site with high specificity exhibits defined rates of association and dissociation that do not differ significantly with temperature or the concentration of the ligand. In contrast, because sHsps have multiple binding sites, each with different affinities for the ligand, the binding will be temperature dependent, and because of the multiple binding sites, the rates of association and dissociation might vary with the concentration of the sHsp (25). When HspB5 was incubated with the Ab-coated probe, biologically relevant rates of association were observed with varying concentrations of the sHsp from 10 μM to 13 nM, with significantly lower rates of dissociation, consistent with tight binding between the Hsp to an Ab with an irrelevant specificity (kinetic details are provided in the Supplemental Material). Supporting that the sHsps bound the Ab and not the reverse was that both the rate and amount of complex formation was temperature dependent (Fig. 2B). After 4000 s, there was a clear and significant difference in the amount of complexes formed as the temperature increased from 23 to 40°C (Fig. 2B). Increasing the temperature from 23 to 37°C resulted in a 36% increase in complex formation, whereas increasing the temperature to 40°C increased binding an additional 42%. Such a pattern of temperature dependence is consistent with the expected behavior of a sHsp and is not characteristic of Ig–Ag interactions.

At a constant temperature, 40°C, analysis of the binding data revealed another characteristic consistent with the Hsp binding the Ig. The rate of association (k\(_a\)) increased as the concentration of the sHsp decreased, whereas the rate of dissociation (k\(_d\)) remained approximately constant (Supplemental Tables I, II). Consequently, the observed rate constant (k\(_o\)) and the calculated K\(_D\) decreased over two orders of magnitude as the concentration of the sHsp decreased (Fig. 2C, Table I). Variations of K\(_D\) with the concentration of an Ag are not characteristic of Ab–Ag interactions. Although there is not a published precedent for kinetic analysis of sHsp binding clients under relatively native conditions as in this assay, the pattern can be rationalized for the sHsp binding the Ab on the sensor. A relatively conserved, slow rate of dissociation, which does not significantly vary with concentration, would be expected from an oligomer, in which multiple binding sites in close proximity would limit the rate of dissociation. An increase in the rate of association with decreasing concentration of the “ligand” would be consistent with a protein with multiple binding sites, each with a different K\(_D\). At low concentrations, relative to the Ab concentration on the sensor, the majority of the binding will be at the highest affinity sites. As the relative concentration of the Hsp increases, the rate of association becomes an average of...
the rate of binding to multiple sites, with the high-affinity sites being less important and the association rates decreasing. The observed temperature-dependent interaction between HspB5 and an irrelevant Ab supports the hypothesis that the sHsp is binding the Ab. The observed variation of the association rates with the concentration of the Hsp provides even stronger evidence supporting the direction of the interaction.

A similar pattern of variation in the rates of association, and in the dissociation constants, was observed between the Ab-coated probe and seven other human sHsp family members (Fig. 2D, Supplemental Table II), consistent with the earlier ELISA result that each of the proteins bound the Ig. The different sHsps bound the polyclonal Ab with a range of $K_D$ values from approximately micromolar to high nanomolar, which was consistent with a specific and biologically relevant interaction. The only caveat of this interpretation of the results is that the murine anti-Fc Ab attached to the tip was polyclonal. Consequently, a similar argument using the varying affinities of the Abs can be made to rationalize the observed variation in the association rates, but such an argument does not explain how an anti-Fc Ab binds not just one, but eight sHsps with apparent equivalent affinity. Such an interaction also would not be expected to exhibit the observed temperature depen-

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<th>Molar Concentration (M)</th>
<th>$k_{obs}$ (1/s)</th>
<th>Error in $k_{obs}$</th>
<th>$k_a$ (1/s)</th>
<th>Error in $k_a$</th>
<th>$k_1$ (1/Ms)$^a$</th>
<th>$K_D$ (M)$^a$</th>
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$^a$Increases occur in the measured rates of association, and consequently a decrease in the calculated $K_D$ values, as the concentration of the sHsp is reduced.
dence. More likely, the polyclonality of the Ig contributes to the complexity of the binding kinetics of the sHsps. **Immunoprecipitation and identification of interactions using mass spectrometry**

If sHsps bind Igs with micromolar-nanomolar affinity/avidity in vitro, the interaction should be detected in human serum. To test this hypothesis, we incubated 5 µg HspB5 with 300 µl aliquots of sera from MS patients and healthy individuals at 42°C for 4 h, after which time we added anti–T7-resin (recognizing the HspB5 epitope tag) and incubated the mixture at 42°C for another 4 h. The beads were separated from the sera by centrifugation, and the HspB5 and the mixture of bound proteins were eluted with glycine buffer pH 1.8. There was approximately three times more protein eluted from patients than normal sera at this step based on absorption at 280 nm. The eluted proteins were concentrated, and the resultant client proteins and HspB5 were separated by SDS-PAGE. The Coomassie-stained bands were excised, the proteins lysed with trypsin, and the resultant peptides analyzed by mass spectrometry.

The principal bands precipitated from MS sera in addition to HspB5 corresponded to IgM, IgG1, and IgG4 H chains, and albumin, whereas albumin, IgG1 H chain, and L chains and albumin, whereas albumin, IgG1 H chain, and L chains and albumin, were observed in samples from normal sera (see Supplemental Material). The ability of HspB5 to precipitate Ig from patients’ and normal sera is the third independent confirmation that sHsps can bind Igs.

**Discussion**

Capturing Abs from patients’ sera using microtiter plates or microchips coated with HspB5 was the basis for assigning the protein as a serological autoantigen in each literature citation we were able to identify (3–7, 29). In this article, the interaction between HspB5 and Igs was confirmed using this assay; however, by extending the investigation to multiple members of the sHsp family and by studying the kinetics of binding, the sHsps were shown to be receptors of, and not Ags for, Igs. By demonstrating that sHsps bind Igs, the puzzle of how the sHsps were historically assigned as autoantigens, even though they were therapeutically beneficial, can be understood. Because the sHsps can bind Abs, the standard method of analyzing whether Abs in plasma can bind the putative Ag when adhered to a solid support leads to problems of interpretation because the assay is fundamentally ambiguous. Whether there is a humoral response to HspB5 in MS or other neurologic disorders will be difficult to establish because the design of most immunomasays relies on the specificity of the Ab–Ag interaction and does not consider the possibility of an Ag binding the Ab.

The experiments in this report also revealed a number of important features of sHsps, previously unappreciated. The first was that the entire family of sHsps is able to bind Igs in a temperature-dependent fashion because of their shared function as chaperones. The binding of Igs in vivo also could be a factor in the therapeutic role of sHsp in reducing inflammation (6). The doses of the protein used in the therapeutic experiments were too low to modulate anti-melanin–specific Igs from sera (6), but sHsps could modulate Ig concentration in cerebrospinal fluid. Reduction of Abs to myelin correlated with a positive therapeutic outcome in experiments on Ag-specific tolerance to myelin proteins (30). The temperature dependence of binding was expected from an Hsp; nevertheless, the differences in the amount of protein bound were impressive. What was unexpected was the rate of association of sHsps with an Ig as their concentration decreased without significant changes in the rate of dissociation, resulting in significant lower dissociation constants. Such a result can be rationalized because of the multiple, divergent binding sites on the Hsp, but nevertheless was remarkable and could explain how a “nonspecific” Hsp could exhibit selectivity in binding proteins at sites of inflammation and not act systemically. This important hypothesis is being explored further in a separate study (J.B. Rothbard, M. Kurnellas, C. Addams, L. Su, C.G. Fathman, and L. Steinman, manuscript in preparation).

Where on the Ig molecule the sHsps bind remains to be determined, but as chaperones, they likely will interact with the edge strands, which are known to be susceptible to partial unfolding and responsible for the formation of extended β-pleated sheets characteristic of L chain amyloid formation (26, 27, 31). Reduced folding stability appears to be a unifying property of amyloidogenic L chains (27).

The assignment that α B-crystallin was an autoantigen was primarily based on the presence of specific Abs against the protein. Data in this article establish that the association arises from the Hsp binding the Ab and not the reverse, which also is consistent with similar apparent responses between MS patients and healthy control subjects. When the lack of clear data that there is a humoral response to the protein is taken together with the inability of T cells specific for HspB5 to transfer symptoms of EAE (9, 32), the argument that this protein is a pathogenic autoantigen is weak and perhaps without foundation.

**Disclosures**

The authors have no financial conflicts of interest.

**References**


Supplemental data for Rothbard et al.

Supplemental Table I. Observed rate constants and the calculated $k_s$ and $K_p$ for each of eight small heat shock proteins binding to a murine polyclonal anti human Fc at 40°C at varying concentrations, which were plotted in Figure 2D. The calculated rate constants for the interaction of HspB5 and the IgG at 23 and 37°C are also included.
Supplemental Table II. Summary of mass spectral data of proteins identified from HspB5 precipitation from sera from a patient with multiple sclerosis (A.) and normal plasma (B.) Proteins were eluted from Coomassie stained bands from a 12% SDS polyacrylamide gel as described in the Materials and Methods section. The sequences in bold type correspond to peptides identified in the mass spectral analyses, with the grey residues chemically modified either in the alkylation step, or by oxidation.

A.

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<th>Protein</th>
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<td>PI</td>
<td>P100745872.2 Tax Id=9606 Gene Symbol=ALB Isoform 1 of Serum albumin</td>
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| gll|117385 (100%), 20,159.5 Da Alpha crystallin B chain (Alpha-B--crystallin) (Rosenthal fiber component) |
| 13 unique peptides, 25 unique spectra, 294 total spectra, 121/175 amino acids (69% coverage) | |
| MDIAIHHPPW | RRPFPPFPFHPSP | SLRFDQFFGE | HLESDDLFTP |
| STLSLPFSL | PPFSFLFAPSW | FDGLSMRL | EKRDFSVPNLD |
| VKHFSPEELK | VKVLQDQIEV | HEGHKEERQDE | HGFISREFHR |
| KRYIPADVDP | LTISSLSSSL | GVLTVNGPRK | QVSGPERTIP |
| iTREEKPAVT | AAPKK | |

| gll|125145 (100%), 11,608.6 Da Ig kappa chain C region |
| 8 unique peptides, 15 unique spectra, 109 total spectra, 91/106 amino acids (86% coverage) | |
| TVAAPSVFIF | PPSDEQLKSG | TASSVCELLNN | FYPREAKVQW |
| KVDAQLNQGQ | SQSVEQEQDS | KDSSTYSLT | LTLSDMADYK |
| HKVYACEVTHG | OGLSSPVTKS | FNRGE | |

| gll|125946 (100%), 11,236.1 Da Ig lambda chain C regions |
| 5 unique peptides, 9 unique spectra, 61 total spectra, 69/105 amino acids (66% coverage) | |
| QPKAAPSSTL | FPSSSELEQA | NKATLVLCLIS | DFYPGAVTVA |
| WKADSSPSVKA | GVTETPSQK | SNNKYYASSY | LSLTPEQWK5 |
| HRYSQCQVTH | EGSTVEKTPA | PTECS | |

| gll|121047 (100%), 35,939.5 Da Ig gamma-4 chain C region |
| 3 unique peptides, 6 unique spectra, 48 total spectra, 158/327 amino acids (48% coverage) | |
| ASTKGPSVF | LAPSRSCE | STAALGLC | DYEPEPVTVS |
| WNNSGALTSGV | HTFPAVOLQS | GLLSSV | VPSSSLGTK |
| YTCNVDHP | NTKVDRKES | KYGPPCPSC | APEFLCGPSV |
| FLFPKPPK | LMSRTPEV | CYYVDVOSQED | PEVFQNYVWD |
| GVEVHNAKT | PREEQFNSTY | RUVSVLTVLH | QDWNLNCKEYK |
| CKVSNKLPS | SIEKTISSAK | GOPREPQYV | LPPSQQEMTK |
| NQQSLTCVLV | GYPSPDIAV | WESNGQPENN | YKKTPVLIDS |
| DGSNFSLYSL | TVDSRQWQG | NVFSCSCVMHE | ALHNHYQTOKS |
| LLSLGLK | | | |
B.

IP00745872 (100%), 69,366.9 Da
IP00745872.2 Tax Id=9606 Gene Symbol=ALB Isoform 1 of Serum albumin
13 unique peptides, 18 unique spectra, 34 total spectra, 202/454 amino acids (44% coverage)

MKWVTFLSSL LF FSSAY SRL VFR RDAH KSE VAHR FKDL GE ENEFKALV LS AQ ILQ CDP FED HV KL VNEV TEFA TECD EASA ECDKL SHT LFGDKLCT VAT LRETYGE MADCA KQEP ERNEF LQHK DDP NLPLPRL VR PEVDVMCTA FHD BBELTFLK RHLY CIARKF FYFAP ELEQ FARKY KAET EGQQADA KCC KKDLELR DKGASSAKQ RLKCALQKF GERA FKA WAVAR LSRIFRPKA EFAEVSKLVT DLTKVHTEC HGDLLECADD RADLAKY ICEQ NDS ISSK LK ECEC KKLP LEK SHECAEV ND EMP ADEPSLAI ADFVESKDVC KNYAEAKDFVMFL EYAR KPDVS SLL VLK LAK IYIE LKCCAADP HELYAYKVDL F KPLVEEPO LQK QELKE QLGEYKQFNA LLVRYTKVQ PQRPTLV ESSNLG KVR CK HK PEA KRM PAKADLYL SV LNQLC EUL VHEK TPSV DRVTK CTCELSVNRPR CFSA LEVD E YVPKF NAE Q E F FHDACLTL SEKEROIKKO TALVEVLHKPK KATK KEQLKA VMDDFAAFVE KCC KADKDKET CFBAEEGK LVAASQ AALGL
IPI00448925 (100%), 60.101.4 Da

13 unique peptides, 15 unique spectra, 132 total spectra, 181/544 amino acids (33% coverage)

MEFLGW VLLL VVQQ ELVQ QGSS GSV VV VQPS LSLR L
ASVGFRF INY CYNMH NQY APGKGL GLEW AF SYDESKY
PSWYNSWFL VFTD SASTK PGA SPSLAPP S
KTS GGTAAL GELVK YFPE PTVSVNSGAA LTSQHFPAP
VLQSGLYSL SSVTYPPSSS LTA ICGNHKPSNVT
KKEVPSDCDK TCHCCP CAP PSSLGGPSVFL FPKPKDTL M
ISRTPEVTECV VVDHSVHEDPE VVKNWVDGV EVHNAKT PR
EEQNYSTYRV VSVLTVLHQD WNLGKEYKCK VSNKAPAP I
EKTISSACQ PEPQVVTLPSRDELTKQVSTLCGLKGF
YPSDIAVEWE NSGQPENNYKT TTPPVLDSDG SFFLYSKLT V
DKSRWQQGNV SCSVMHEAL HNYTQKSLLS LSEPQLLEES
CAEAQDGELD GLWTTITIFI TLFLLSSVCSY ATVTFFFKVKW
IFS VDLPQ TIPDYRNMIGQGA

IPI00430820 (100%), 25.765.2 Da

7 unique peptides, 11 unique spectra, 111 total spectra, 103/235 amino acids (44% coverage)

MEAPAQLL LLLLP DSGTIGI VMTQS PAT LSVSPGERA T
LSCRASQIS NNLWYQRQP GQAPRLIIYGA SRSRVTGIPG
RFSGSSTEITLSSQ DAFYFCQO YNDWLYTFG
QGTLKIKRT VAAPSFVFIP SDFQILKSGT ASVCLNNF
YPREAKVOWK VDNALQGNS QESVTEDQSK DTSYSSLSTL
TLSKADYKH KVA LTHVQ GLSSPVTKS NRFEC

IPI00719373 (74%), 23.062.9 Da

13 unique peptides, 2 unique spectra, 5 total spectra, 69/214 amino acids (32% coverage)

MRP KTGGVCG ETPEELPGPG RQRWPLLLL LAMVAGHLL R
PMVAP QSSDP DPGAVSGSSR SLSRLSFWGL LLOPSPORAD
PRCWRFGWS EPQSLCYFEG GTKTVLVQG PAKNPPVTTLF
PSSSEELQAN KATLKEI SD FYPGAVTVAW KADGSVYKAG
VETTHPSKOS NKYASSYL SLTPEQWKSH RYSQCQVTHE
GST VEKTVAP TECS