Available online at www.sciencedirect.com
SCIENCE DIRECT
Protein Expression and Purification 47 (2006) 398–404
Protein Expression & Purification
www.elsevier.com/locate/yprep

Gene synthesis, expression, purification, and characterization of human Jagged-1 intracellular region

Matija Popovic [a], Maristella Coglievina [a], Corrado Guarnaccia [a], Giuliana Verdone [b], Gennaro Esposito [b], Alessandro Pintar [a,*], Sándor Pongor [a,*]

[a] *International Centre for Genetic Engineering and Biotechnology (ICGEB), Protein Structure and Bioinformatics Group, AREA Science Park, Padriciano 99, I-34012 Trieste, Italy*
[b] *Dipartimento di Scienze e Tecnologie Biomediche, MATI Centro di Eccellenza, Università di Udine, P.le Kolbe 4, I-33100 Udine, Italy*

Received 26 September 2005, and in revised form 25 November 2005
Available online 28 December 2005

Abstract

Notch signaling plays a key role in cell differentiation and is very well conserved from *Drosophila* to humans. Ligands of Notch receptors are type I, membrane spanning proteins composed of a large extracellular region and a 100–150 residue cytoplasmic tail. We report here, for the first time, the expression, purification, and characterization of the intracellular region of a Notch ligand. Starting from a set of synthetic oligonucleotides, we assembled a synthetic gene optimized for *Escherichia coli* codon usage and encoding the cytoplasmic region of human Jagged-1 (residues 1094–1218). The protein containing a N-terminal His_6-tag was over-expressed in *E. coli*, and purified by affinity and reversed phase chromatography. After cleavage of the His_6-tag by a dipeptidyl aminopeptidase, the protein was purified to homogeneity and characterized by spectroscopic techniques. Far-UV circular dichroism, fluorescence emission spectra, fluorescence anisotropy measurements, and ^1H nuclear magnetic resonance spectra, taken together, suggest that the cytoplasmic tail of human Jagged-1 behaves as an intrinsically unstructured domain in solution. This result was confirmed by the high susceptibility of the recombinant protein to proteolytic cleavage. The significance of this finding is discussed in relation to the recently proposed role of the intracellular region of Notch ligands in bi-directional signaling.

Keywords: Notch signaling; Synthetic gene; Intrinsically unstructured proteins; Circular dichroism; Tryptophan fluorescence; NMR; Limited proteolysis

The type I membrane-spanning proteins Jagged (Jagged-1 and -2) and Delta (Delta-1, -3 and -4) are the human ligands of Notch receptors, which mediate key signaling events in cell differentiation and morphogenesis [1]. Binding of Jagged and Delta proteins to Notch receptors triggers a series of proteolytic cleavages eventually leading to the release of the intracellular part of the receptor from the membrane, its translocation to the nucleus, and the activation of transcription factors [2,3]. ADAM (a disintregrin and metalloprotease) and presenilin/γ-secretase, the proteases involved in Notch receptor processing, have been recently shown to be involved also in the proteolytic cleavage of Jagged and Delta [4–6], with the consequent release of signaling fragments from the membrane [4,6,7]. Furthermore, Delta-1, Delta-4, and Jagged-1 (but not Delta-3 and Jagged-2) contain a PDZ binding motif at the C-terminus of their cytoplasmic tail [7,8], suggesting their involvement in Notch-independent signaling, as well as a possible role of their intracellular region in the cross-talk between Notch signaling and other signal transduction pathways. The cytoplasmic tail of Jagged-1 has been shown to bind the PDZ domain of AF-6 (Afadin) [7], a protein belonging to the cell adhesion system and containing two Ras association domains. To address the structural grounds of the interaction between the cytoplasmic region of human Jagged-1 and its partner proteins, we

* Corresponding authors. Fax: +39 040 226555.
E-mail addresses: pintar@icgeb.org (A. Pintar), pongor@icgeb.org (S. Pongor).

1046-5928/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pep.2005.11.027

assembled a synthetic gene encoding the 125 residue, intracellular region of human Jagged-1 (JAG1IC) and expressed it in *Escherichia coli*. The recombinant protein was purified and characterized by circular dichroism, tryptophan fluorescence, NMR, and limited proteolysis. Spectroscopic and biochemical data show that JAG1IC behaves in solution as a natively unfolded protein, and the biological relevance of this scenario is discussed in the context of Notch signaling.

Materials and methods

Gene synthesis

The synthetic gene encoding JAG1IC was obtained in a two-step PCR (assembly and amplification). The oligonucleotides used for the gene assembly were designed using DNAWorks v2.3 [9]. Briefly, the amino acid sequence of the human Jagged-1 cytoplasmic region (JAG1IC, corresponding to residues 1094–1218 of JAG1_HUMAN) was back-translated using the *Escherichia coli* Class II codon usage [10] and the resulting DNA sequence was split into 16 partially overlapping oligonucleotides by DNAWorks. Synthetic oligonucleotides were purchased from Sigma-Genosys (0.05 μmol scale, desalted), dissolved in equimolar concentration and assembled by PCR using the *Pfu* polymerase (Promega). PCR conditions were: 5 min at 95 °C during which *Pfu* was added (hot start), 25 cycles of amplification (30 s denaturation at 95 °C, 30 s annealing at 58 °C, 90 s elongation at 72 °C), 10 min at 72 °C for the final elongation. Amplification of the assembled gene was achieved using the following forward and reverse primers (Sigma-Genosys, 0.05 μmol scale, purified by polyacrylamide gel electrophoresis): 5′-TAA TAG TAG CAT ATG AAA CAC CAT CAC CAT CAC CAT CGT AAA CGT CGT AAA CCG GGT AGC and 5′-TAG TAG GGA TCC TCA TTA AAC GAT GTA TTC CAT ACG GTT CAG GCT. The forward primer contains (underlined): a *Nde*I restriction site, encoding the start methionine, a AAA triplet encoding lysine, and a six histidine tag. The synthetic gene was digested with *Nde*I and *Bam*HI and directionally cloned into the pET-11a vector (Novagen). Positive clones were obtained by transformation in DH5α *E. coli* cells grown on LB plates in the presence of 50 μg/mL ampicillin. Plasmid constructs containing the synthetic gene were sequenced and the correct one was used for transformation of BL21(DE3) *E. coli* (Novagen).

Expression and purification

Typically, 100 mL of LB containing 50 μg/mL ampicillin were inoculated with the transformed BL21(DE3) clone, cells were grown at 37 °C to a density of ~1 OD units, and induced with IPTG (1 mM) for 3 h. Cells were harvested by centrifugation and pellets frozen at −80 °C. Cells were resuspended in the lysis buffer (20 mM sodium phosphate buffer, 0.5 M NaCl, 50 mM Chaps, 2% Tween 20, 1 mM DTT,[1] 10 mM imidazole, pH 7.4, containing one protease inhibitor cocktail tablet (Roche)) and sonicated. After centrifugation, the supernatant was loaded on a Ni^{2+}–Sepharose HisTrap HP column (1 mL, Amersham Biosciences), the column washed with 20 mM sodium phosphate buffer, 0.5 M NaCl, 1 mM DTT, 10 mM imidazole, pH 7.4 and the protein eluted with a 10–500 mM imidazole gradient. The crude material was purified by RP-HPLC on a Zorbax 300SB-CN column (9.4 × 250 mm, 5 μm, Agilent) using a 0–50% gradient of 0.1% TFA in H_2O and 0.1% TFA in acetonitrile, and freeze-dried. The His_6-tag was removed with the TAGzyme (Qiagen) for 1 h at 37 °C following the manufacturer's protocol. The digested protein was purified on a HisTrap HP column (1 mL) with a 10–100 mM imidazole gradient. After a final RP-HPLC purification step, the protein was analyzed by LC–MS on a Gilson HPLC system coupled to a ESI-MS single quadrupole mass spectrometer (Applied Biosystems API-150EX), using a Zorbax 300SB-CN column (2.1 × 150 mm, 5 μm, Agilent) and a 0–50% gradient of 0.1% TFA in H_2O and 0.1% TFA in acetonitrile. Deconvolution of the multicharge ion spectrum was carried out using the BioMultiView software (Applied Biosystems). The purified protein was freeze-dried and used for spectroscopic studies. The final yield was ~8 mg of purified material from 1 L culture.

Circular dichroism

Samples for CD spectroscopy were prepared dissolving the freeze-dried protein in 5 mM Mops buffer, pH 7.4. Protein concentration (8.2 μM) was determined by UV absorbance at 280 nm using the calculated ε value of 9530 $M^{-1}\,cm^{-1}$. CD spectra were recorded at room temperature on a Jasco J-810 spectropolarimeter in the range 190–250 and 250–350 nm using quartz cuvettes of 0.1 and 1 cm pathlengths, respectively. For each spectrum, five scans were acquired at 10 nm/min, and the mean residue ellipticity (MRE, deg $cm^2\,dmol^{-1}\,residue^{-1}$) was calculated from the baseline-corrected spectrum. A quantitative estimation of secondary structure content was carried out using SELCON3, CONTINLL, and CDSSTR, all run from the DichroWeb server [11].

Fluorescence spectroscopy

Samples prepared for CD were also used for fluorescence spectroscopy. Fluorescence spectra were recorded at 298 K on a Jobin-Yvon FluoroMax-3 spectrofluorimeter equipped with a Peltier temperature control apparatus using 1 × 0.2 cm pathlength quartz cuvettes. Excitation was set at 295 nm and spectra were recorded between 300 and 450 nm. Fluorescence anisotropy was measured at the

[1] *Abbreviations used:* DSS, 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt; DTT, 1,4-dithiothreitol; LC–MS, liquid chromatography coupled to mass spectrometry; PDZ, domain present in PSD-95, Dlg and ZO-1/2; RP-HPLC, reverse phase high performance liquid chromatography.

maximum of emission (355 nm) using the same excitation wavelength. Measurements were corrected for the background and averaged.

NMR spectroscopy

The sample for NMR spectroscopy was prepared dissolving the freeze-dried material in H_2O/D_2O (90/10, v/v) containing 2 mM EDTA-d_{16} and 20 μM DSS and adjusting the pH to 6.6 with small aliquots of 0.1 N KOH, for a final sample concentration of ~0.9 mM. Spectra were recorded at 298 K on a Bruker Avance 500 operating at a ^1H frequency of 500.13 MHz and equipped with a three-axis gradient probe. A 2D TOCSY spectrum was acquired using a 50 ms mixing time, with solvent suppression obtained by excitation sculpting [12]. The acquisition was performed over a spectral width of 7002.8 Hz in both dimensions, with matrix size of 2048 points in t_2 and 512 points in t_1. Data were transformed using X-WinNMR (Bruker). Chemical shifts were referenced to internal DSS.

Limited proteolysis

The freeze-dried material (~150 μg) was dissolved in Tris buffer (0.1 M, pH 7.4) containing $CaCl_2$ (10 mM) and subjected to proteolysis at room temperature by addition of chymotrypsin (sequencing grade, Roche, 1:100 w/w) dissolved in Tris buffer [13]. Aliquots of the reaction mixture were quenched with HCOOH, and analyzed by LC–MS on a Gilson HPLC system coupled to a ESI-MS single quadrupole mass spectrometer (Applied Biosystems API-150EX). Separation of peptide fragments was achieved with a C18 column (150 × 1 mm, 5 μm, Phenomenex) using a 0–40% gradient of 0.1% HCOOH in H_2O and 0.1% HCOOH in acetonitrile at a flow of 35 μL/min. Comparison between the fragments expected from chymotrypsin cleavage and experimentally found fragments was carried out using the BioMultiView software (Applied Biosystems). The identity of candidate peptide fragments was confirmed by deconvolution of the multicharge ion spectra.

Results and discussion

Gene synthesis

The synthetic gene encoding JAG1IC and optimized for *E. coli* codon usage [10,14] was assembled from 16 oligonucleotides designed by DNAWorks [9] (Fig. 1). The synthetic oligonucleotides had a maximum length of 40 bases, a calculated annealing temperature (T_m) of 64 °C with a T_m range of 1.8 °C, a minimal overlap of 13 bases, and no repeats that could potentially lead to hairpin formation or to mispriming in the PCR. As a result of codon optimization, no codon with an abundance <20% was used, and ~80% of the protein residues were encoded by >50% abundant codons, according to Class II genes codon usage [10] (Fig. 2). After the assembly PCR (Fig. 3A), the gene was amplified in a second PCR (Fig. 3B) using two primers

```
5'   CGTAAACGTCGTAAACCGGGTAGCCACACCCACTCC       aagataacaccaccaac
3'                         CGGTGTGGGTGAGGCGTAGGCTTCTATTGTGGTGGTTG
1094  R  K  R  R  K  P  G  S  H  T  H  S  A  S  E  D  N  T  T  N

      aacgttcgtgagcagctgaacca        ACCCGATCGAAAAACACGGTGCTAACACC
      TT      actcgtcgacttggtctagtttttgggctagctttttgtg       TGTGG
       N  V  R  E  Q  L  N  Q  I  K  N  P  I  E  K  H  G  A  N  T

      GTTCCGATCAA   ttacgaaaacaaaaacagcaaaatgtccaaaatccgtacc  CAAC
      CAAGGCTAGTTTCTAATGCTTTTGTTTTTGTCGTT  acaggttttaggcatgggtgttg
       V  P  I  K  D  Y  E  N  K  N  S  K  M  S  K  I  R  T  H  N

      TCTGAAGTTGAAGAAGACGACATGGACAAACACCAG       cgcgtttcgctaaacag
      agacttcaacttcttct  TGTACCTGTTTGTGGTCGTCTTTCGCGCAAAGCGATTTGT
       S  E  V  E  E  D  D  M  D  K  H  Q  Q  K  A  R  F  A  K  Q

      ccggcgtacactctggttgaccg          CCGCCGAACGGTACCCCGACTAAACAC
           atgtgagaccaactggcacttctttttggcggcttgccat           GTG
       P  A  Y  T  L  V  D  R  E  E  K  P  P  N  G  T  P  T  K  H

      CCGAACTGGACTA         acaaccgtgacctggaatccgcgcagagcctgaaccgt
      GGCTTGACCTGATTGTTTGTCCTGTTGGCACTGGACC         tctcggacttggca
       P  N  W  T  N  K  Q  D  N  R  D  L  E  S  A  Q  S  L  N  R

      at                    3'
      taccttatgtagcaaatt     5'
       M  E  Y  I  V  X   1218
```

Fig. 1. DNA and amino acid sequence of human JAG1IC. Codon optimized synthetic oligonucleotides used for the gene assembly (first and second lines, alternatively in capital and small letters) and amino acid sequence of JAG1IC (third line, X = stop codon).

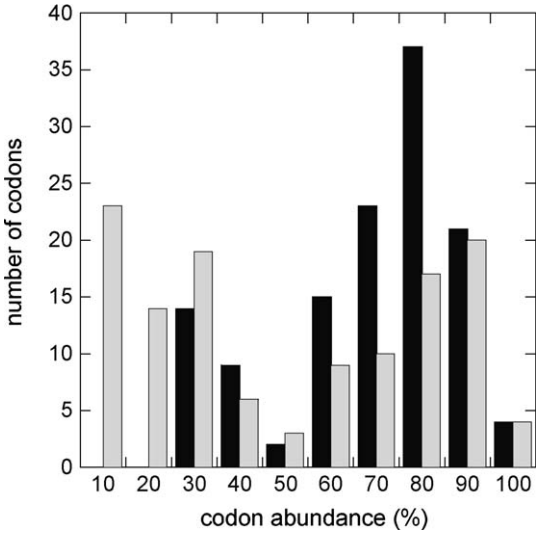

Fig. 2. Codon usage. Number of codons used in the human cDNA (grey) and in the synthetic gene (black) of JAG1IC partitioned according to their relative abundance in *E. coli* Class II genes.

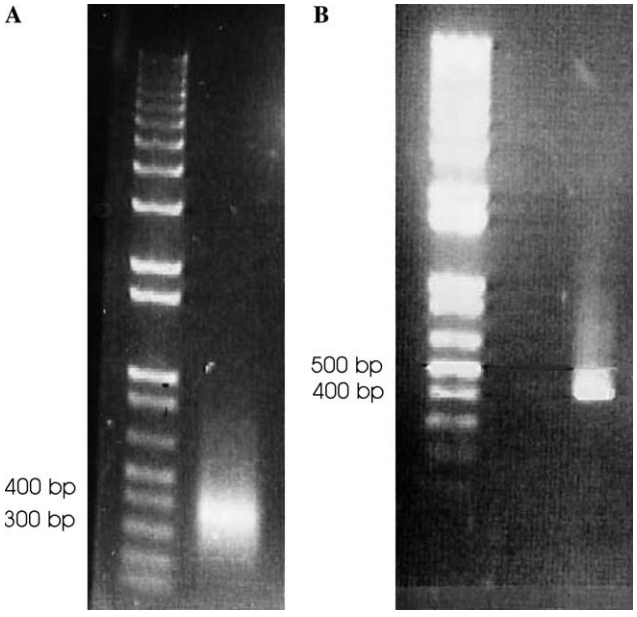

Fig. 3. Gene synthesis. Agarose gel (1%) of (A) oligonucleotide assembly and (B) gene amplification PCR mixtures.

containing the chosen restriction sites. Both gene assembly and amplification were straightforward. Automated DNA sequencing of clones lead to the identification of the correct one (1/12), which was used for protein expression.

Protein expression and purification

Codon optimization tailored on the expression host has proved to be an effective way to increase heterologous protein expression, especially in *E. coli* [15]. High expression levels could be achieved in standard *E. coli* strains from the combination of a codon-optimized synthetic gene and a T7 RNA polymerase-based vector (Fig. 4). Total gene synthesis cannot be considered of general use because of the cost of the oligonucleotides and the errors introduced both in the oligonucleotide synthesis and in the gene assembly. It is particularly attractive, on the other hand, when the target protein is relatively small and high expression levels are needed, as is the case of JAG1IC. The protein was recovered from the soluble fraction of the bacterial lysate, and the use of detergents increased the recovery yield, suggesting that the target protein can interact with other cellular components. The general strategy for protein purification was based on immobilized metal ion affinity chromatography (IMAC) followed by removal of the N-terminal His_6-tag (Fig. 5A). However, an additional purification step was necessary between the IMAC and the His_6-tag removal because of partial proteolytic degradation of the target protein, despite the use of protease inhibitors. The His_6-tag was removed using a recombinant dipeptidyl aminopeptidase I (DAPase) containing a C-terminal His-tag (TAGZyme, Qiagen). DAPase cleaves two amino acid residues at a time, starting from the N-terminus of the polypeptide chain, and stops when it finds a KX, RX, XP, or XXP sequence (X = any residue). It is particularly convenient to use when, as in the case of JAG1IC, the target sequence starts with one of these residue combinations. On the other hand, the start codons should be carefully designed to avoid out-of-frame digestion that would lead to protein degradation, and reaction time should be optimized to avoid overdigestion. Removal of the enzyme, of partially digested products,

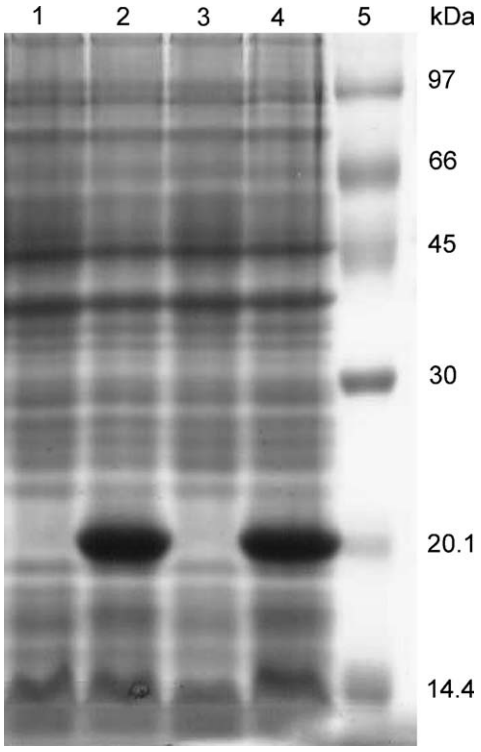

Fig. 4. Protein expression. Total cell SDS–PAGE (15%) of *E. coli* cultures stained with Coomassie blue. Lanes 1 and 3, not induced; lane 2, 3 h induction (1 mM IPTG); lane 4, 5 h induction (1 mM IPTG); lane 5, LMW markers.

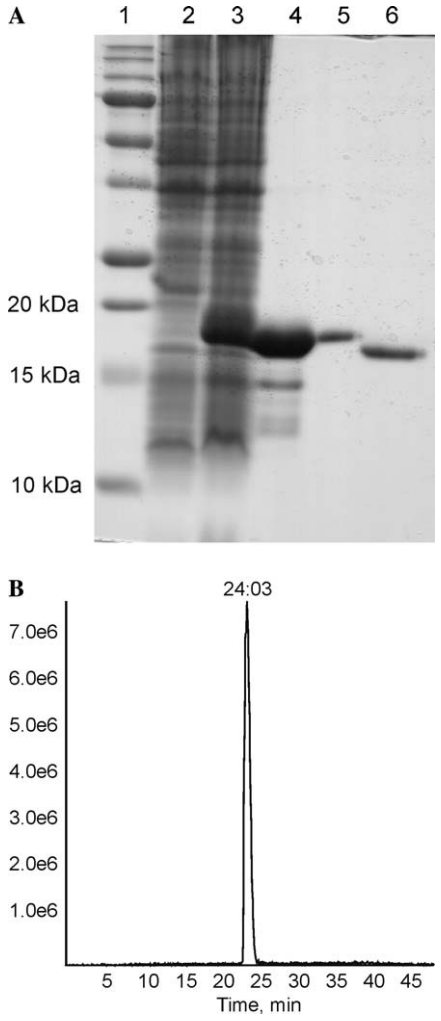

Fig. 5. Protein purification. SDS–PAGE (A) at the different purification steps (lane 1, LMW markers; lane 2, total cell lysate before induction; lane 3, total cell lysate after 3 h induction; lane 4, IMAC-purified; lane 5, RP-HPLC-purified; lane 6, after removal of the His_6-tag, subtractive IMAC and RP-HPLC) and LC–MS chromatogram (B) of the final material.

and of cleaved His_2 dipeptides was achieved through an additional IMAC step (Fig. 5A). After a final RP-HPLC purification step (Fig. 5A), JAG1IC was analyzed and identified by LC–MS (Fig. 5B) (M_r calculated: 14,588; found: 14,591). An alternative purification protocol, based on IMAC, desalting by gel filtration, and ion-exchange chromatography on a strong cation exchanger resin (see Supplementary material) was also developed in order to confirm that the biophysical properties of the target protein are not dependent on the purification scheme used.

Structural characterization

Far-UV CD spectra Fig. 6 show a strong minimum at ~200 nm, which is typical of random coil polypeptides or unfolded proteins. A quantitative estimation of the secondary structure content was achieved through deconvolution of the CD spectrum in the range 190–240 nm using several methods [11]. The best fit between the experimental and calculated spectrum was obtained from CDSSTR using a set of reference spectra that include unfolded proteins [16] (normalized RMSD = 0.014). Results suggest a very low content of secondary structure: 4% helix, 17% strand, 12% turn and 65% coil and similar values were found also by other methods [17] (SELCON3, CONTINLL). The mainly disordered nature of JAG1IC in solution was confirmed by the lack of any detectable optical activity in the aromatic region (~280 nm) (data not shown), by the position of the maximum in the tryptophan fluorescence spectra (355 nm) (Fig. 7) and by the very low value of the tryptophan fluorescence anisotropy (0.06). ^{1}H NMR spectra (Fig. 8) show very little dispersion in the chemical shift of both the backbone NH and the Hα protons, most of them being clustered in the 8.0–8.5 and 3.9–4.9 ppm range, respectively. Methyl resonances from valine, leucine and isoleucine residues are also clustered in

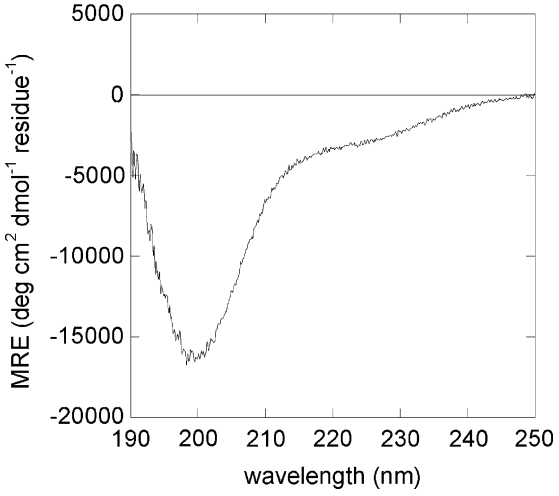

Fig. 6. Circular dichroism. Far-UV CD spectrum of JAG1IC (8.2 μM) in 5 mM Mops, pH 7.4.

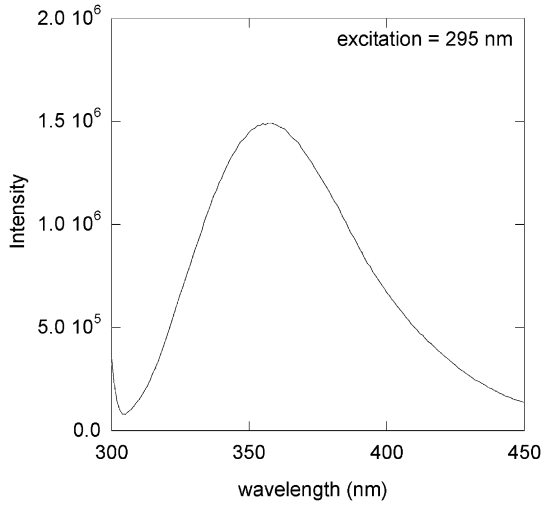

Fig. 7. Fluorescence spectroscopy. Fluorescence spectrum of JAG1IC (8.2 μM) in 5 mM Mops, pH 7.4. Excitation wavelength was at 295 nm and the raw spectrum was corrected for the contribution of the buffer alone.

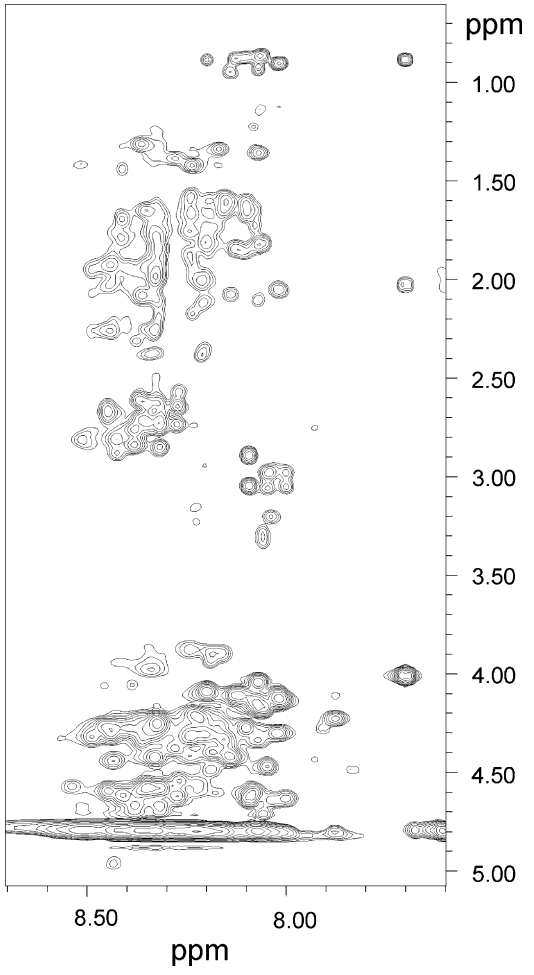

Fig. 8. NMR spectroscopy. The NH/aliphatic region of a 2 D TOCSY spectrum recorded at 298 K with a 50 ms mixing time; solvent: H_2O/D_2O (90/10 v/v), pH 6.6.

a small range (0.8–1.0 ppm) with no methyl resonance below 0.8 ppm. Aromatic signals were tentatively assigned and are all close to the random coil values, histidines: 7.9–8.0 ($H^{\varepsilon 1}$)/7.0–7.1 ($H^{\delta 2}$) ppm, phenylalanine: 7.3–7.4 ppm, tyrosines: 6.8 (H^{ε})/7.1 (H^{δ}) ppm, tryptophan: 10.2 ($H^{\varepsilon 1}$)/7.2 ($H^{\delta 1}$)/7.6 ($H^{\varepsilon 3}$)/7.4 ($H^{\zeta 2}$)/7.2 ($H^{\eta 2}$)/7.1 ($H^{\zeta 3}$) ppm.

Overall, CD, fluorescence and NMR data are consistent with the lack of tertiary structure and the presence of very little or no secondary structure, thus suggesting that in the experimental conditions tested JAG1IC behaves as a mainly disordered protein in solution [18].

To confirm the results obtained from these spectroscopic techniques, JAG1IC was subjected to controlled proteolysis using chymotrypsin. Chymotrypsin was selected because it has a higher specificity towards aromatic and bulky hydrophobic residues (F, Y, W, L, and M). These amino acid types are expected to be buried in the hydrophobic core of the protein, hence less accessible to protease cleavage, when a globular structure is present. JAG1IC, however, was very rapidly degraded by chymotrypsin. After 15 min, no native protein could be detected, and more than 15 different fragments were separated. Under the same conditions, small globular proteins such as myoglobin and ribonuclease A are not digested to an appreciable extent [13,19]. The time course of the reaction and the identification of many of the fragments by LC–MS also show that no particular region in JAG1IC is protected against proteolytic cleavage (Fig. 9). These results thus confirm that JAG1IC is essentially unstructured in solution.

Finally, the anomalous SDS–PAGE mobility displayed by JAG1IC (Figs. 4 and 5) is an additional, although indirect evidence of its disordered nature [20].

Regions that are predicted to be intrinsically disordered are quite common, especially in eukaryota [21]. These regions, or entire proteins, often play an important

1 26 46 53 59 68 71 77 83 103 118 123

RKRRKPGSHTHSASEDNTTNNVREQLNQIKNPIEKHGANTVPIKDYENKNSKMSKIRTHNSEVEEDDMDKHQQKARFAKQPAYTLVDREEKPPNGTPTKHPNWTNKQDNRDLESAQSLNRMEYIV

121

pep.	res.	Mass, calc. (Da)	Mass, found (Da)	t (min)	sequence
1	1-26	3019.25	3019.9	17.8	<RKRRKPGSHTHSASEDNTTNNVREQL>N
2	1-46	5280.81	5281.6	23.3	<RKRRKPGSHTHSASEDNTTNNVREQLNQIKNPIEKHGANTVPIKDY>E
3	1-53	6112.76	6114.4	22.6	<RKRRKPGSHTHSASEDNTTNNVREQLNQIKNPIEKHGANTVPIKDYENKNSKM>S
4	27-53	3111.53	3112.2	20.9	L<NQIKNPIEKHGANTVPIKDYENKNSKM>S
5	47-71	3002.25	3001.9	17.8	Y<ENKNSKMSKIRTHNSEVEEDDMDKH>Q
6	47-83	4419.88	4418.9	22.1	Y<ENKNSKMSKIRTHNSEVEEDDMDKHQQKARFAKQPAY>T
7	54-71	2170.30	2170.2	17.3	M<SKIRTHNSEVEEDDMDKH>Q
8	54-77	2929.18	2930.0	18.3	M<SKIRTHNSEVEEDDMDKHQQKARF>A
9	54-83	3587.94	3587.4	19.5	M<SKIRTHNSEVEEDDMDKHQQKARFAKQPAY>T
10	60-77	2206.34	2207.1	19.4	H<NSEVEEDDMDKHQQKARF>A
11	69-103	4114.60	4114.3	24.5	M<DKHQQKARFAKQPAYTLVDREEKPPNGTPTKHPNW>T
12	78-83	676.77	676.5	17.0	F<AKQPAY>T
13	78-118	4676.10	4676.2	22.6	F<AKQPAYTLVDREEKPPNGTPTKHPNWTNKQDNRDLESAQSL>N
14	84-103	2316.56	2316.8	21.1	Y<TLVDREEKPPNGTPTKHPNW>T
15	84-118	4017.34	4018.3	21.9	Y<TLVDREEKPPNGTPTKHPNWTNKQDNRDLESAQSL>N
16	84-123	4711.12	4712.5	22.8	Y<TLVDREEKPPNGTPTKHPNWTNKQDNRDLESAQSLNRMEY>I
17	122-125	522.60	521.5	14.1	M<EYIV>

Fig. 9. Limited proteolysis. Peptide fragment identification (fragment identification number, residue number, expected and found masses, RP-HPLC retention time, and sequence) in the chymotrypsin cleavage mixture after 30 min reaction. The amino acid sequence of JAG1IC and the cleavage sites are also shown on top of the table.

role in protein–protein interactions, especially in signaling networks [22]. Intrinsically unstructured regions can play as docking stations hosting several protein interactions motifs, potentially offer a very large interaction surface compared to their volume, can bind their target molecules with high specificity but low affinity, and by coupling folding to binding they can work as flexible, adaptable partners in molecular recognition [23–25]. It cannot be ruled out that post-translational modifications such as phosphorylation and monoubiquitinylation, or the particular environment given by the interface between the inner face of the plasma membrane and the cytoplasm might induce some conformational change in JAG1IC. However, the most plausible hypothesis at this stage is that the entire cytoplasmic tail of Jagged-1, and not only its C-terminal tetrapeptide, might contribute to the specific recognition and binding of its PDZ containing partner, providing at the same time a spacer between the membrane and the bulky PDZ containing protein.

This first report on the expression and purification of the intracellular region of Jagged-1 opens up the way to further studies aimed at the structural characterization of the interaction between the cytoplasmic tail of Notch ligands and their protein targets containing PDZ domains.

Acknowledgment

We thank Doriano Lamba (CNR-ELETTRA, Trieste) for use of the CD spectropolarimeter.

Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pep.2005.11.027.

References

[1] S. Artavanis-Tsakonas, M.D. Rand, R.J. Lake, Notch signaling: cell fate control and signal integration in development, Science 284 (1999) 770–776.
[2] G. Weinmaster, Notch signal transduction: a real rip and more, Curr. Opin. Genet. Dev. 10 (2000) 363–369.
[3] T. Kadesch, Notch signaling: the demise of elegant simplicity, Curr. Opin. Genet. Dev. 14 (2004) 506–512.
[4] T. Ikeuchi, S.S. Sisodia, The Notch ligands, Delta1 and Jagged2, are substrates for presenilin-dependent "gamma-secretase" cleavage, J. Biol. Chem. 278 (2003) 7751–7754.
[5] E. Six, D. Ndiaye, Y. Laabi, C. Brou, N. Gupta-Rossi, A. Israel, F. Logeat, The Notch ligand Delta1 is sequentially cleaved by an ADAM protease and gamma-secretase, Proc. Natl. Acad. Sci. USA 100 (2003) 7638–7643.
[6] M.J. LaVoie, D.J. Selkoe, The Notch ligands, Jagged and Delta, are sequentially processed by alpha-secretase and presenilin/gamma-secretase and release signaling fragments, J. Biol. Chem. 278 (2003) 34427–34437.
[7] J.M. Ascano, L.J. Beverly, A.J. Capobianco, The C-terminal PDZ-ligand of JAGGED1 is essential for cellular transformation, J. Biol. Chem. 278 (2003) 8771–8779.
[8] E.M. Six, D. Ndiaye, G. Sauer, Y. Laabi, R. Athman, A. Cumano, C. Brou, A. Israel, F. Logeat, The notch ligand Delta1 recruits Dlg1 at cell–cell contacts and regulates cell migration, J. Biol. Chem. 279 (2004) 55818–55826.
[9] D.M. Hoover, J. Lubkowski, DNAWorks: an automated method for designing oligonucleotides for PCR-based gene synthesis, Nucleic Acids Res. 30 (2002) e43.
[10] C. Medigue, T. Rouxel, P. Vigier, A. Henaut, A. Danchin, Evidence for horizontal gene transfer in *Escherichia coli* speciation, J. Mol. Biol. 222 (1991) 851–856.
[11] A. Lobley, L. Whitmore, B.A. Wallace, DICHROWEB: an interactive website for the analysis of protein secondary structure from circular dichroism spectra, Bioinformatics 18 (2002) 211–212.
[12] T.L. Hwang, A.J. Shaka, Water suppression that works. Excitation sculpting using arbitrary waveforms and pulsed field gradients, J. Magn. Reson. 112 (1995) 275–279.
[13] A. Fontana, M. Zambonin, P. Polverino de Laureto, V. De Filippis, A. Clementi, E. Scaramella, Probing the conformational state of apomyoglobin by limited proteolysis, J. Mol. Biol. 266 (1997) 223–230.
[14] S.P. Zhang, G. Zubay, E. Goldman, Low-usage codons in *Escherichia coli*, yeast, fruit fly and primates, Gene 105 (1991) 61–72.
[15] C. Gustafsson, S. Govindarajan, J. Minshull, Codon bias and heterologous protein expression, Trends Biotechnol. 22 (2004) 346–353.
[16] N. Sreerama, S.Y. Venyaminov, R.W. Woody, Estimation of protein secondary structure from circular dichroism spectra: inclusion of denatured proteins with native proteins in the analysis, Anal. Biochem. 287 (2000) 243–251.
[17] N. Sreerama, R.W. Woody, Estimation of protein secondary structure from circular dichroism spectra: comparison of CONTIN, SELCON, and CDSSTR methods with an expanded reference set, Anal. Biochem. 287 (2000) 252–260.
[18] V.N. Uversky, Natively unfolded proteins: a point where biology waits for physics, Protein Sci. 11 (2002) 739–756.
[19] U. Arnold, K.P. Rucknagel, A. Schierhorn, R. Ulbrich-Hofmann, Thermal unfolding and proteolytic susceptibility of ribonuclease A, Eur. J. Biochem. 237 (1996) 862–869.
[20] P. Tompa, Intrinsically unstructured proteins, Trends Biochem. Sci. 27 (2002) 527–533.
[21] A.K. Dunker, J.D. Lawson, C.J. Brown, R.M. Williams, P. Romero, J.S. Oh, C.J. Oldfield, A.M. Campen, C.M. Ratliff, K.W. Hipps, J. Ausio, M.S. Nissen, R. Reeves, C. Kang, C.R. Kissinger, R.W. Bailey, M.D. Griswold, W. Chiu, E.C. Garner, Z. Obradovic, Intrinsically disordered protein, J. Mol. Graph. Model. 19 (2001) 26–59.
[22] L.M. Iakoucheva, C.J. Brown, J.D. Lawson, Z. Obradovic, A.K. Dunker, Intrinsic disorder in cell-signaling and cancer-associated proteins, J. Mol. Biol. 323 (2002) 573–584.
[23] P. Tompa, The interplay between structure and function in intrinsically unstructured proteins, FEBS Lett. 579 (2005) 3346–3354.
[24] H.J. Dyson, P.E. Wright, Intrinsically unstructured proteins and their functions, Nat. Rev. Mol. Cell Biol. 6 (2005) 197–208.
[25] P. Tompa, C. Szasz, L. Buday, Structural disorder throws new light on moonlighting, Trends Biochem. Sci. 30 (2005) 484–489.