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A LECTIN-LIKE PEPTIDE ISOLATED FROM THE VENOM OF THE CHINESE BIRD SPIDER *SELENOCOSMIA HUWENA*

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S.-P. Liang and X. Pan. A lectin-like peptide isolated from the venom of the Chinese bird spider *Selenocosmia huwena*. *Toxicon* **33,** 875–882, 1995.—A peptide with haemagglutination activity was isolated from the venom of the Chinese bird spider *Selenocosmia huwena* by means of ion-exchange and reverse-phase high-performance liquid chromatography. This peptide, named SHLP-I, agglutinates human and mice erythrocytes at a minimum concentration of 125 μg/ml and 31 μg/ml, respectively. It consists of 32 amino acid residues including 3 Trp and 6 Cys residues, the latter of which form three disulfide bounds. The complete amino acid sequence was determined. The N-terminal and C-terminal residues were Gly and Trp, respectively. SHLP-I shows homology with a fragment of great nettle lectin and with huwentoxin-I from the venom of the same spider.

INTRODUCTION

The venom from the spider *Selenocosmia huwena*, which is distributed in the hilly areas of Yunnan and Guangxi in southern China, contains a mixture of compounds with different types of biological activity (Liang *et al.*, 1993). In our previous work we isolated and characterized a neurotoxin named huwentoxin-I which has 33 amino acid residues and three disulfide bounds (Liang *et al.*, 1993; Zhang and Liang, 1993). Our original interest in studying the *S. huwena* venom was to find some novel neurotoxins. During the separation of the venom we found a peptide component which is relatively abundant in the venom, but has very low toxicity in both mammals and insects. After we partially sequenced this peptide and compared the sequence with those of proteins stored in the data bank, we found that there are some similarities between the sequence of the peptide and that of a fragment of great nettle lectin. This result encouraged us to investigate further to elucidate the biological activity of this peptide. In this paper we describe a procedure involving a combination of ion-exchange and reverse-phase high-performance liquid chromatography (HPLC) that was used to isolate the *Selenocosmia huwena* lectin-like peptide-I (SHLP-I) from the venom of the spider *S. huwena*. In addition, some biological properties and the complete amino acid sequence of the peptide are presented.

MATERIALS AND METHODS

Venom of S. huwena

The venom of the adult female *Selenocosmia huwena* was obtained by the method described in our previous work (Liang *et al.*, 1993).

Chemicals

TPCK-trypsin and sequencing grade phenylisothiocyanate (PITC) and trifluoroacetic acid (TFA) were from Sigma (St Louis, MO, U.S.A.); aminophenyl glass beads were from CPG Inc.; 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), dithiothreitol and iodoacetic acid were from Aldrich; methanesulfonic acid (MSA) and tryptamine were from Pierce; bovine serum albumin (BSA), and concanavalin A were from Dongfeng Chemicals, Shanghai. All other reagents were of analytical grade.

Venom fractionation

Five milligrams of lyophilized venom dissolved in 500 μl of double-distilled water was first subjected to ion-exchange HPLC on a Shim-pak WCX-1 column (5 × 0.4 cm). The column was previously equilibrated with 0.02 M sodium phosphate buffer, pH 6.6 (buffer A). Elution was performed with a linear gradient of 0 – 40% buffer B (1 M sodium acetate, 0.02 M phosphate buffer, pH 7.0) over 30 min at a flow rate of 0.8 ml/min. The fractions from the WCX-1 column were further subjected to reverse-phase HPLC on a YWG-pak C18 column (0.39 cm × 30 cm). Elution was performed with a linear gradient of 0–45% acetonitrile containing 0.1% TFA over 30 min at a flow rate of 0.7 ml/min. All the HPLC were performed using a Waters 600E HPLC separation system with a 490 model UV detector.

Capillary electrophoresis

Capillary electrophoresis of the peptide samples was performed on a Waters Quanta 4000E capillary electrophoresis system using an uncoated capillary tube of 0.75 μm × 65 cm. The electrophoresis was conducted with a constant voltage of 15 kV in a buffer of 50 mM phosphoric acid, pH 2.0.

Haemagglutination assay

Haemagglutination assays were performed on microagglutination slides using serial dilutions of 25 μl of a lectin solution in physiological saline (0.9% NaCl pH 7.5). Slides were placed on a minishaker and to each dilution was added 25 μl of a four times washed 2% suspension of human or mice erythrocytes suspension. The slides were shaken for 1 min after addition of the erythrocytes. The slides were subsequently incubated for 1 hr at 4°C. Agglutination was read macroscopically and recorded semiquantitatively using a 0 to 4+ scale (e.g. no agglutination to total agglutination).

Determination of covalently bound sugar

Assays of carbohydrates covalently bound to the peptides were performed using the phenol–sulfuric acid reaction as described by Ashwell (1966). Preparations of 50 μg peptide samples were compared with control solutions containing 2–20 μg glucose.

Amino acid analysis

Native or S-carboxymethylated polypeptide (0.1 mg) was dissolved in 100 μl of constant boiling HCl, containing 0.02% phenol. The glass tubes were flushed with N_2 and heat sealed *in vacuo*. The hydrolysates were dried and derivatized with phenylisothiocyanate, and the resulting PTC-amino acids were analysed by HPLC using a YWG-pak C18 column. Polypeptides were also analysed after hydrolysis with 4 M methanesulfonic acid (MSA) containing 0.2% (w/v) tryptamine.

Caboxymethylation of the peptide

The native peptide was caboxymethylated using the method described in our previous work (Zhang and Liang, 1993).

Amino acid sequence analysis

Peptides were sequenced by solid-phase Edman degradation on prototypes of MilliGen/Biosearch Model 6600 ProSequencer (Laursen *et al.*, 1989) using capillary columns prepacked with aminophenyl glass beads for immobilization and sequencing (Liang and Laursen, 1990).

Tryptic digestion

A sample (0.5 mg) of carboxymethylated peptide was digested at 37°C for 5 hr with 100 μg of TPCK-trypsin in 600 μl of 0.1 M *N*-methylmopholine buffer, pH 8.0. Peptide fragments were purified by reverse-phase HPLC using a Nova-pak C_{18} column (0.46 cm × 15 cm) and a linear gradient of 0–50% acetonitrile containing 0.1% TFA over 25 min.

Titration of thiol groups

Thiol groups were estimated by titration with DTNB (Anderson and Wetlaufer, 1975). A 20 nmole sample of native peptide was dissolved in 2 ml of 0.1 M phosphate buffer (pH 7.3) containing 6 M guanidinium chloride and 1 mM EDTA. The difference in absorbance at 412 nm between the sample and water reference sample was measured. Then 100 μl of 3 mM DTNB in 0.1 M phosphate buffer was added to the sample and reference solutions, which were mixed thoroughly. The reactions were monitored at 412 nm, and absorption difference was measured when the absorbance stopped increasing. The concentration of thiols was calculated using an extinction coefficient at 412 nm of 13,700 M^{-1} cm^{-1}.

RESULTS

Dissolved crude venom was applied first to the WCX-1 ion-exchange column. Figure 1 is a typical WCX-1 elution profile. There are about eight major peaks of absorption at 254 nm. Peak C, with a retention time of 16.1 min, was found to have haemagglutination activity. The fractions comprising peak 3 were then applied directly to a YWG-pak C18 reverse-phase column (0.39 cm × 30 cm) equilibrated with 0.1% TFA. The sample was eluted using a linear gradient of 0–45% acetonitrile (containing 0.1% TFA). Figure 2 shows the elution profile from reverse-phase HPLC. Five major peaks absorbing at 225 nm were observed; these were then collected and freeze-dried.

The largest peak, with a retention time 16.02 min (Fig. 2), was found to have haemagglutination activity and appeared to be homogeneous by capillary electrophoresis (Fig. 3) and by N-terminal amino acid analysis. We named this peptide *Selenocosmia huwena* lectin-like peptide-I (SHLP-I). Three types (A, B, and O) of human erythrocytes and mouse erythrocytes were used for the haemagglutination assay. Figure 4 showed that this peptide agglutinates human types A, B and C erythrocytes equally well. The initial concentration of SHLP-I in the haemagglutination assays was 2 mg/ml. The minimal concentration of peptide required to produce an evident haemagglutination was 125 μg/ml, and the titre was 32 for all three types of the human red blood cells. Two kinds of control were used simultaneously in parallel assays. In control 1, only physiological saline and erythrocytes were added without SHLP- I (rows 1, 2 and 3 in Fig. 3). In control 2, SHLP-I was replaced by an equal concentration of bovine serum albumin (row 7 in Fig. 4). In the haemagglutination assay using mouse erythrocytes we found that the minimal haemagglutination concentration of SHLP-I was 31μg/ml and the titre was 128.

The results of amino acid analysis of S-carboxymethylated SHLP-I after hydrolysis with 6 N HCl or 4 N mercaptoethanesulfonic acid (MSA) are given in Table 1. Among the 32 amino acid residues per molecule of peptide are 6 Cys, 5 Gly and 3 Trp. In order to determine the complete amino acid sequence of SHLP-I, two samples of 20 μg (5 nmoles) of native peptide were immobilized on aminophenyl glass capillary columns (Liang and Laursen, 1990). One of the immobilized samples was then further subjected to *in situ* reduction and S-carboxymethylation according to the method of Zhang and Liang (1993). The samples were then individually placed in the column holder in the MilliGen/Biosearch model 6600 Prosequencer. A programme of 35 Edman degradation cycles plus three precycles for phenylthiohydatoin (PTH) standards was set and then performed. The complete amino acid sequence of SHLP-I was determined for both the S-carboxymethylated and the intact peptides. The N-terminal amino acid residue of SHLP-I was

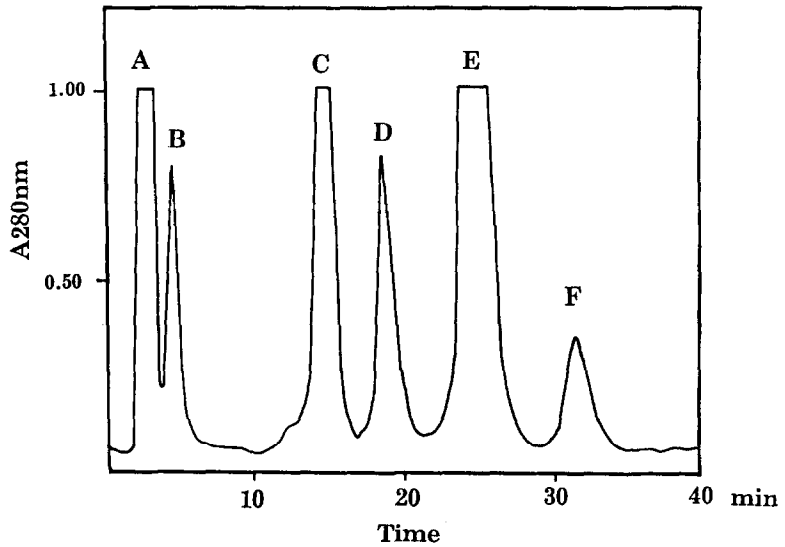

Fig. 1. Ion-exchange HPLC of *S. huwena* venom on WCX-1 column.
Two milligrams of crude venom from *S. huwena* was applied to a WCX-1 ion-exchange column (0.4 cm × 25 cm), which had previously been equilibrated with 0.02 M sodium phosphate buffer, pH 6.6. The elution was performed with a linear gradient of 0–50% 1 M NaAC in a constant 0.02 M phosphate buffer, pH 6.6, over 30 min, with a flow rate of 0.8 ml/min at room temperature.

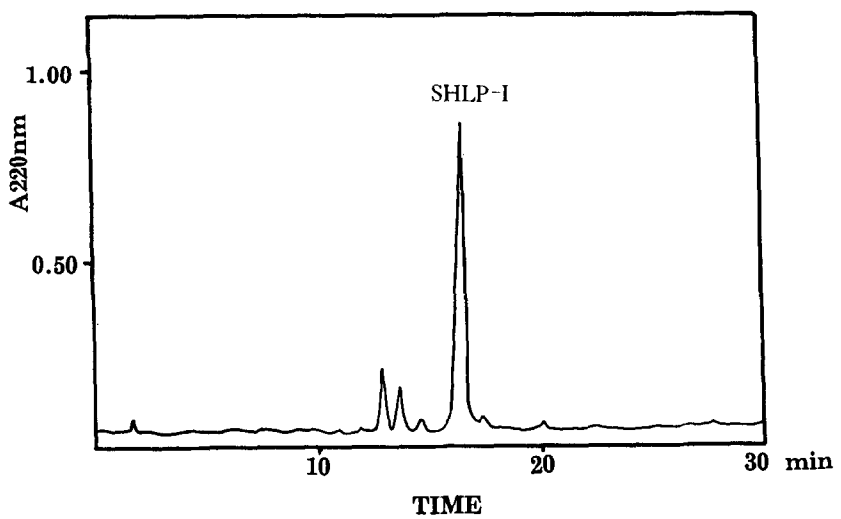

Fig. 2. Reverse-phase HPLC of partially purified SHLP-I.
Approximately 0.5 mg of material eluted from fraction C (Fig. 1) by ion-exchange HPLC was applied to a YWG-pak C18 column (0.39 cm × 30 cm). Elution was performed with a linear gradient of 0–45% acetonitrile containing 0.1% TFA over 30 min with a flow rate of 0.7 ml/min at 45°C.

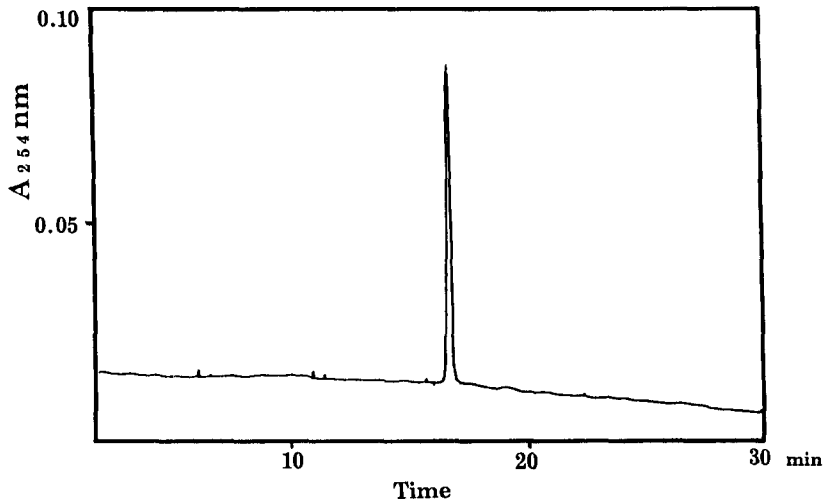

Fig. 3. Capillary zone electrophoresis of purified SHLP-I.
Conditions: Capillary: 60 cm × 70 μm, uncoated; buffer: 50 mM phosphoric acid, pH 2.0; injection: 10 sec hydrostatic loading; sample concentration: 1 μg/μl; run conditions: 15 kV, constant voltage; detection: UV, 254 nm.

determined to be Gly. After cycle 32 (Trp), four blank cycles were produced, indicating that Trp 32 is the C-terminal residue of the peptide. The complete amino acid sequence of SHLP-I is: NH_2-G C L G D K C D Y N N G C C S G Y V C S R T W K W C V L A G P W-COOH. In order to confirm the sequence analysis of the peptide, 1 mg of the

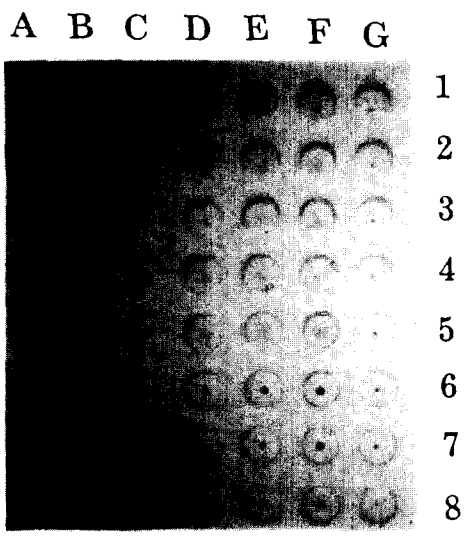

Fig. 4. Haemagglutination assay of SHLP-I.
The assay was performed on a microagglutination slide using serial dilutions (lines 1–8) of 25 μl of a SHLP-I solution in physiological saline (0.9% NaCl, pH 7.5). The initial concentration of SHLP-I (line 1) was 2 mg/ml. To each dilution was added 25 μl of a four times washed 2% human erythrocyte suspension. Columns A–C are controls without SHLP-I, and contain type A, B and O human erythrocytes, respectively. Column D–F contain SHLP-I and type A, B and O human erythrocytes, respectively. Column G is a control in which SHLP-I was replaced by BSA of same concentration and containing type A human erythrocytes.

S-carboxymethylated SHLP-I was subjected to TPCK-trypsin hydrolysis. Figure 5 shows the tryptic map of S-carboxymethylated SHLP-I by reverse-phase HPLC. Three peptide fragments were isolated and subjected to sequence analysis. The sequence of fragment TP1 was determined to be T-W-K. TP2 is the N-terminal peptide with the sequence G-C-L-G-D-K-C-D-Y-N-N-G-C-C-S-G-Y-V-C-S. TP3 is an octapeptide with the sequence W-C-V-L-A-G-P-W, and is the C-terminal peptide of SHLP-I.

Sugar analysis using the phenol–sulfuric acid reaction revealed that no carbohydrates were covalently bonded to SHLP-I. Titration of possible thiol groups in a 20 nmole sample of SHLP-I using Ellman's reagent (DTNB) showed liberation of only 1.6 nmole of nitrothiobenzoate ion out of an expected 40, if one pair of the six Cys residues was in the reduced form. In a control experiment with 50 nmoles of DTT, 100 nmoles of thiol was detected. Therefore we conclude that there are three disulfide bounds in the molecule of SHLP-I.

DISCUSSION

Lectins or agglutinins are naturally occurring proteins, usually found in plants, that have antibody-like activity in that they cause red blood cell agglutination. Their activities are due to their ability to bind certain carbohydrates, including those in glycoproteins. There have recently been several reports of lectins extracted from animal venoms such as from snakes (Gartner *et al.*, 1980; Ogilvie *et al.*, 1986; Bruno *et al.*, 1990; Liang *et al.*, 1993). Most of the lectins have mol. wts higher than 20,000. No lectin with a mol. wt less than 5000 has yet been sequenced and reported.

At the beginning of our study, when we first isolated the SHLP-I, we were perplexed as to its biological activity because this peptide is abundantly present in the venom, but has very low toxicity to both mammals and insects. After we partially sequenced the peptide and scanned for homologies with proteins in the U.S. National Biomedical Research Foundation 1990 Data Bank, we found that there is sequence similarity between SHLP-I and a fragment of great nettle lectin, as follows:

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SHLP-I                GCLGDKCDYN--NGCCSGYVCS RTWKWCVLAGPW

                      .:.  .  .:::..  :.  :  ::  .  :

great nettle lectin:  QRCGS QGGGGTCPALRCCS I WGWCGAS SPYC
(fragment)
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Here the double dots imply the identical amino acids between the two sequences and the single dots imply the amino acids which have similar properties. Subsequent haemagglutination assay demonstrated that this peptide does have the ability to haemagglutonate human and mouse erythrocytes. An interesting point is that no haemagglutination activity has been found in the crude venom of the spider *S. huwena* using the same assay, suggesting the presence of some kind of antagonistic factor in the venom, which inhibits the haemagglutination activity of SHLP-I.

Table 1. Amino acid composition of reduced and carboxymethylated SHLP-I from *S. huwena*

Amino acid	Composition (residues/molecule) from hydrolysis with 6 M HCl	4 M MSA	from sequence
Asx	3.9 (4)	4.3 (4)	4
Glx	0.1 (0)	0.0 (0)	0
Cys	6.2 (6)	5.9 (6)	6
Ser	2.2 (2)	1.6 (2)	2
Gly	5.1 (5)	5.0 (5)	5
His	0.0 (0)	0.0 (0)	0
Arg	0.6 (1)	0.9 (1)	1
Thr	0.8 (1)	1.0 (1)	1
Ala	1.0 (1)	0.6 (1)	1
Pro	1.2 (1)	1.0 (1)	1
Tyr	2.1 (2)	2.3 (2)	2
Met	0.0 (0)	0.0 (0)	0
Ile	0.1 (0)	0.0 (0)	0
Leu	2.0 (2)	2.2 (2)	2
Phe	0.4 (0)	0.1 (0)	0
Trp	ND	2.8 (3)	3
Lys	1.4 (2)	1.9 (2)	2
Total	(29)	(32)	32

Values are given for SHLP-I after 24 hr hydrolysis by either 6 M HCl or 4 M methanesulfonic acid (MSA) containing 0.2% (w/v) tryptamine. The hydroylsates were derivatized with PITC and analyzed by RP-HPLC.
ND, Not determined.

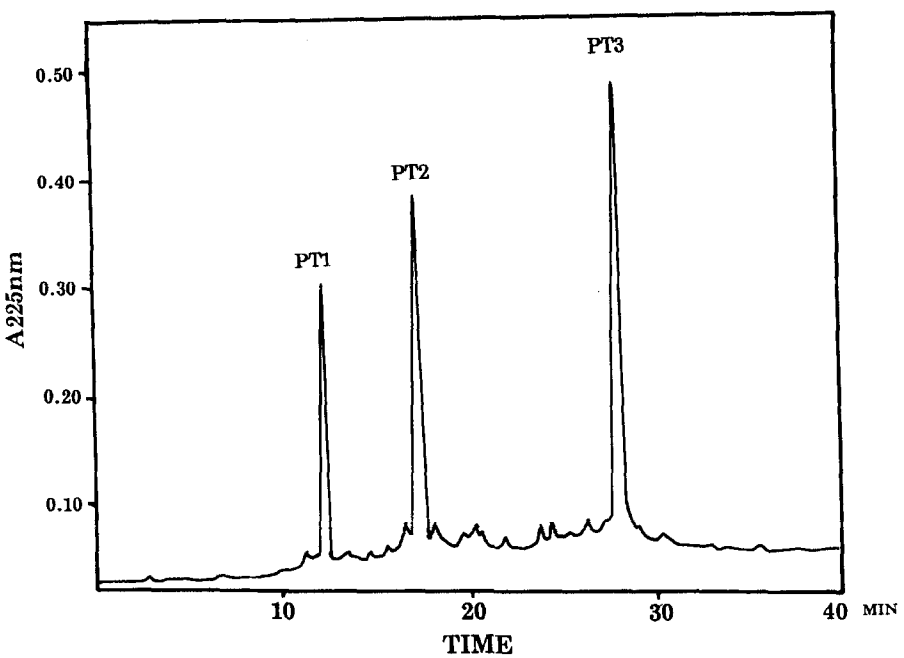

Fig. 5. Tryptic peptide map of SHLP-I by reverse-phase HPLC.
Tryptic hydrolysate of SHLP-I was separated by reverse-phase HPLC using a YWG-pac C18 column (0.46 cm × 15 cm) and a linear gradient of 0–50% acetonitrile containing 0.1% TFA over 30 min.

Comparing the sequence of SHLP-I with that of huwentoxin-I (HWTX-I) (Liang *et al.*, 1993) we found that there are also similarities between these two peptides:

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SHLP-I:   GCL GDK——CD—YNNGCCSGYVCSRT WKWCVL AGPW
           . :  :       :    :  : . : : . .   : : :      : : :
HWTX-I:   ACKGVFDACTP GLNECCPNRVCSDKH KWCKWKL
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Huwentoxin-I is a neurotoxin in mammals. The intraperitoneal and intracisternal LD_{50} values of huwentoxin-I in mice were 0.70 mg/kg and 9.40 μg/kg, respectively. However, SHLP-I has almost no toxicity in mice. No symptoms of poisoning were observed even when 1.5 mg of SHLP-I was injected i.p. into a mouse.

So far, the function of the snake venom lectins is unknown and remains a subject of some speculation. Similarly, the function of SHLP-I in spider venom is not known. We believe that this lectin-like peptide may be an ideal model molecule to study the relationship between structure and function in haemagglutination. Further studies are in progress to characterize the sugar binding specificity and the location of disulfide bonds in SHLP-I.

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