Anticoagulant activity of a natural protein purified from *Hypomesus olidus*

Mengxing Gou\textsuperscript{a1}, Liyan Wang\textsuperscript{a2} & Xuejun Liu\textsuperscript{a*}

\textsuperscript{a}Department of Food Science and Engineering, Jilin Agricultural University, Changchun, China

A novel anticoagulant protein (E-II-1) was separated and purified from *Hypomesus olidus*, a unique freshwater fish in northern China. E-II-1 had a molecular mass of approximately 40 kDa with no subunits. The high contents of hydrophobic amino acids and negatively charged amino acids in E-II-1 demonstrated that the amino acid compositions might contribute to the anticoagulant activity. E-II-1 contained \( \alpha \)-helices 16.75\%, \( \beta \)-sheets 42.67\%, \( \beta \)-turn 25.58\% and random coil 15.00\%. \textit{In vitro} blood coagulation time assay, E-II-1 significantly prolonged the activated partial thrombin time in a dose-dependent manner. Results indicated that E-II-1 acted as anticoagulants through the endogenous pathway with an inhibition of FXa. The specific activity of E-II-1 was 103.50 U/mg at a concentration of 1.00 mg/ml. Therefore, E-II-1 might be one of the promising anticoagulants originated from natural food sources with more safety and less side effects.

**Keywords:** *Hypomesus olidus*; anticoagulant protein; chromatographic column; purification; activated partial thrombin time
1. Experimental Section

Materials

*H.olidus* was obtained from aquatic market in Changchun, China. Electrophoresis Kit, diethylaminoethyl (DEAE)-cellulose-52, Sephadex G-75 and Sepharose CL-6B were purchased from Ding Guo Co., Ltd (China). The activated partial thrombin time (APTT), prothrombin time (PT) and thrombin time (TT) reagents were obtained from Taiyang Biologics Co., Ltd (China). The other chemicals were analytical grade.

Preparation of protein extract

All experiments were done at 4 °C in a chromatography chamber unless stated otherwise. *H.olidus* were homogenized in a tissue homogenizer with 20 mM Tris-HCl buffer (pH 7.60) at a ratio of 1:3 (w/w), and then stood overnight. The homogenate was centrifuged at 10,000 × g for 15 min, and then the supernatant was defatted according to the method (Wang et al. 2013). After evaporating in a rotary evaporator and lyophilised in a freeze dryer, the crude extract proteins were designated as HCP.

Preparation of 40%-60% fraction

The protein extracts of *H.olidus* were precipitated using a two-step salting-out method (Burgess & Deutscher 2011) with 0-40% and 40%-60% ammonium sulphate saturation. The precipitates obtained from the fraction of 40%-60% were dialysed against 10 mM Tris-HCl buffer (pH 7.60). The dialysate was lyophilised and named as F_{40-60}. F_{40-60} was stored at -20 °C as working material for further investigation. All experiments were done at 4 °C in a chromatography chamber unless stated otherwise.

Isolation and purification of anticoagulant protein

F_{40-60} (250.00 mg) was dissolved in 20 mM Tris-HCl buffer (pH 7.60) 5.00 mL and centrifuged at 10,000 × g for 10 min. Then the supernatant was loaded on to a DEAE-cellulose-52 column (2.6 × 30 cm²), equilibrated with the same buffer. The column was eluted with a linear gradient of 0-1 M NaCl in the same buffer at a flow
rate of 0.50 mL/min. The fraction exhibiting the highest anticoagulant activity was collected, desalted and lyophilised. Subsequently, the dissolved powder was applied to a Sepharose CL-6B gel filtration column (1.5 × 75 cm²) previously equilibrated with 20 mM Tris-HCl buffer, at pH 7.60. Elution was performed at a flow rate of 1.00 mL/min and monitored at 280 nm by an automatic UV detector (HUXI®, CBS-B). Fractions with high anticoagulant activities were collected and concentrated. The fraction was further submitted to a Sephadex G-75 (1.0 × 60 cm²) column equilibrated with the same buffer. The flow rate was adjusted to 0.50 mL/min and the anticoagulant activity of per fraction was evaluated.

**Determination of anticoagulant activity**

The platelet poor plasma (PPP) was obtained as follows. Firstly, fresh blood was collected from healthy volunteers in the infirmary of Jilin Agricultural University in Jilin, China. Then the blood was mixed with 0.109 M sodium citrate immediately at a ratio of 9:1 (v/v). Finally, the mixture was centrifuged at 3000 × g for 15 min to prepare the PPP.

Specifically, 100 μL of the mixture G (PPP and sample with a ratio of 4:1, v/v) was added with 100 μL of APTT reagent, and then incubated at 37 °C for 5 min, APTT was immediately recorded after the addition of 100 μL of 25 mM CaCl₂. In the PT assay, 100 μL of the mixture G was incubated at 37 °C for 3 min, after that, 200 μL of PT reagent was added to the mixture G and PT was determined. TT was recorded after adding 200 μL TT reagent to the equal amount of mixture G 20 mM Tris-HCl buffer (pH 7.60) was used as the negative control.

APTT, PT and TT were determined by a semi-automated coagulometer (PUN-2048B, PERLONG®, Beijing) following the instructions of the manufacturers. APTT is a screening test of the coagulation system for endogenous pathway or the common pathway, prothrombin time (PT) is a screening test of exogenous coagulation system, and thrombin time (TT) is used to evaluate the ability to transform fibrinogen into fibrin. One unit of anticoagulant activity was defined as the 1.00 s increase in clotting time compared to the measured clotting time of negative control under the
identical assay conditions.

The inhibition effect of E-II-1 on factor Xa and thrombin (IIa) were determined by a microplate reader (Multiskan FC, Thermo Scientific®, China) according to the method (Li et al. 2011).

**Reversed Phase High Performance Liquid Chromatography (RP-HPLC)**

The active fraction selected by APTT was injected into a C18 HPLC column (Agilent™-ZORBAX 300 SB, USA) to confirm the homogeneity of the purified anticoagulant protein. The column was equilibrated with 0.10% trifluoroacetic acid (TFA) in ultra pure water, and then eluted with a linear gradient of acetonitrile (5-100%) in 0.10% TFA. The elution peak was monitored at 288 nm for 45 min at a flow rate of 0.70 mL/min.

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

The purity and approximate molecular weight of anticoagulant protein were estimated by SDS-PAGE in reducing conditions, according to the method (Laemmli 1970).

**Amino acid composition analysis**

The amino acid composition of samples were determined by ACQUITY UPLC (AccQ-Tag, Waters™, USA), and the operation details were performed by reference to the guidelines.

**Secondary structure analysis**

Fourier transform infrared (FTIR) was used to analyse the secondary structure of protein. And the fitting bands in this paper were ascribed according to the ascriptions (Susi & Byler 1983; Ulrichs et al. 2015).

**Chemical component analysis**

Protein content was determined according to the method (Bradford 1976). Total carbohydrate was quantified by the phenol-sulfuric acid method (Dubois et al. 1956).
**Statistics analysis**

Data were expressed as mean ± standard deviation, with at least three individual replicates. Student’s *t*-test was used to analyse the statistical differences. The value of *p* < 0.05 was considered to indicate significance, *p* < 0.05 (*) and *p* < 0.01 (**).

**2. Results and Discussion**

**Preparation of the crude protein (HCP)**

HCP showed no anticoagulant activity because APTT, PT and TT were not prolonged (data not shown). While the 40%-50% fraction and 50%-60% fraction turned out to have anticoagulant activity considering the APTT (Figure S2). One of the explanations was that the components of HCP were too complicated (Figure S1 (a)), resulting in low content of active fraction. The two active fractions were pooled and named (F_{40-60}).

**Isolation and purification of anticoagulant protein**

Seven fractions (A–G) were separated using the ion exchange chromatograph (Figure S4), and the major peak E exhibited the highest anticoagulant activity (activated partial thrombin time (APTT) was 92.03±3.12 s (Figure S5)). The fraction E was further purified by Sepharose CL-6B column (Figure S6), four major peaks (E-I, E-II, E-III and E-IV) were observed. E-II (APTT was 153.86±7.84 s) (p < 0.01) had the highest activity when compared to others (Figure S7). Then the fraction E-II was fractionated using Sephadex G-75 (Figure S8), the anticoagulant activity of the first peak (E-II-1) had significant difference compared with the control.

**SDS-PAGE**

According to the purification steps described before, the anticoagulant protein E-II-1 showed a single band of approximately 40 kDa (Figure S1 (b)). However, it was amazing to find out that the polysaccharide content of E-II-1 was up to 6.40%. Based on this conclusion, we speculated that E-II-1 was a glycoprotein, yet new problems emerged. Most of the glycoproteins with anticoagulant activity were lectins (Sharon
Lectins (Sharon & Lis 2002) were specific proteins binding with carbohydrates or glycoprotein, and they were widely distributed in natural products (Arcoverde et al. 2014). In addition, a large majority of lectins consisted of subunits. But E-II-1 exhibited no subunits. While we found few anticoagulant proteins (Gao et al. 2011; Silva et al. 2012) had no subunits, which was consistent with the results in this paper. Therefore, the relationship between E-II-1 and lectin and the structure characteristics of E-II-1 would need further researches.
Figure S1: SDS-PAGE of anticoagulant protein purified from *H. olidus* under denaturing conditions.

Figure S2: Anticoagulant activities of different protein fractions precipitated by ammonium sulphate.

Figure S3: The flow diagram for the isolation and purification steps of the anticoagulant protein from *H. olidus*.

Figure S4: The elution profile of F\textsubscript{40-60} on DEAE-cellulose-52 ion exchange column.

Figure S5: Anticoagulant activities of fractions A to G measured by coagulometer with a concentration of 15 mg/ml.

Figure S6: Elution profile of fraction E on Sepharose CL-6B gel chromatography.

Figure S7: Anticoagulant activities of fractions E-I to E-IV with a concentration of 5 mg/ml.

Figure S8: Elution profile of fraction E-II on Sephadex G-75 column.

Figure S9: RP-HPLC analysis of the E-II-1.

Figure S10: The fitting curve of Amide I band.

Figure S11: The fitting results of Amide I band.

Figure S12: Effect of the samples on FXa (a) and FIIa (b) in the presence of anti-thrombin.

Table S1: Purification of anticoagulant protein from *H. olidus*.

Table S2: Amino acid compositions of some anticoagulant fractions from *H. olidus*.

Table S3: The fitting results of amide I band.
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Figure S10: The fitting curve of Amide I band.
Figure S11: The fitting results of Amide I band.

| Parameter | Value            | Error       |
|-----------|------------------|-------------|
| y0        | 0.92746          | ±0.0352     |
| xc1       | 1626.0586        | ±13.48757   |
| w1        | 20.10328         | ±11.47673   |
| A1        | 16.08973         | ±16.9735    |
| xc2       | 1638.43144       | ±5.23227    |
| w2        | 13.95522         | ±12.43316   |
| A2        | 10.16059         | ±27.85901   |
| xc3       | 1650.18157       | ±2.6912     |
| w3        | 11.78733         | ±7.21483    |
| A3        | 11.34789         | ±20.01569   |
| xc4       | 1004.73285       | ±55.3352    |
| w4        | 18.18298         | ±27.97493   |
| A4        | 17.33498         | ±15.77478   |
| xc5       | 1682.06466       | ±11.33519   |
| w5        | 15.44513         | ±22.44318   |
| A5        | 12.14797         | ±35.55904   |
| xc6       | 1055.78924       | ±3.54527    |
| w6        | 10.45768         | ±4.23366    |
| A6        | 4.10557          | ±7.40253    |

The fitting results of Amide I band are shown in the figure. The data fit the Gauss model with the following equation:

\[ y = y_0 + A/(w \sqrt{\pi/2}) \exp(-2((x-xc)/w)^2) \]

The R^2 value is 0.99925, indicating a good fit. The table above lists the fitting parameters with their respective errors.
Figure S12: Effect of the samples on FXa (a) and FIIa (b) in the presence of anti-thrombin.

Table S1. Purification of anticoagulant protein from *H. olidus*.

| Step         | Total protein (mg) | Total anticoagulant activity (U) | Specific activity (U/mg) | Yield (%) | Purification fold |
|--------------|--------------------|----------------------------------|--------------------------|-----------|-------------------|
| F₄₀₋₆₀      | 6061.00            | 6242.83                          | 1.03                     | 100.00    | 1.00              |
| DEAE-52      | 508.00             | 2275.84                          | 4.48                     | 36.46     | 4.35              |
| Sepharose CL-6B | 52.40             | 1355.06                          | 25.86                    | 21.71     | 25.11             |
| Sephadex G-75 | 4.30              | 445.05                           | 103.50                   | 7.13      | 100.44            |
**Table S2.** Amino acid compositions of some anticoagulant fractions from *H. olidus*.

| Amino acids | F_{40-60} (%) | E-II (%) | E-II-1 (%) |
|-------------|---------------|----------|------------|
| His^+       | 2.26±0.00     | 2.14±0.16 | 2.62±0.00  |
| Ser^0       | 5.44±0.06     | 7.02±0.11 | 4.82±0.06  |
| Arg^+       | 6.31±0.30     | 5.76±0.10 | 7.21±0.10  |
| Gly^0       | 5.34±0.20     | 3.44±0.04 | 4.85±0.04  |
| Asp^           | 10.89±0.08    | 10.19±0.92 | 9.10±0.08  |
| Glu^-        | 13.03±0.00    | 13.61±0.08 | 14.60±0.08 |
| Thr^0        | 5.18±0.07     | 4.57±0.07 | 5.05±0.07  |
| Ala^#        | 5.72±0.05     | 5.58±0.05 | 5.65±0.00  |
| Pro^#        | 4.00±0.00     | 3.81±0.07 | 3.55±0.07  |
| Cys^0        | 2.39±0.00     | 4.55±0.24 | 2.12±0.14  |
| Lys^+        | 5.68±0.08     | 6.91±0.15 | 9.34±0.17  |
| Tyr^#        | 4.87±0.69     | 4.04±0.21 | 4.36±0.00  |
| Met^#        | 3.63±0.17     | 3.78±0.15 | 3.85±0.00  |
| Val^#        | 5.58±0.78     | 5.92±0.18 | 5.00±0.07  |
| Ile^#        | 4.89±0.00     | 4.59±0.00 | 4.34±0.08  |
| Leu^#        | 9.00±0.00     | 8.18±0.08 | 8.84±0.08  |
| Phe^#        | 5.79±0.10     | 5.90±0.00 | 4.70±0.10  |
| Hydrophobic amino acid | 43.48±1.79a | 41.80±0.74a | 40.29±0.40a |

Negatively charged amino acid: 23.92±0.08b 23.80±1.00b 23.70±0.16b

Positively charged amino acid: 14.25±0.38c 14.81±0.41c 19.17±0.27bc

^+, positively charged amino acid; ^-, negatively charged amino acid; ^0, neutral amino acid; ^#, hydrophobic amino acid. Different letters indicate that the averages are different at a 0.05 level of significance.

**Table S3.** The fitting results of Amide I band.

| Fitting wavelength (cm\(^{-1}\)) | Area  | Ascription      |
|----------------------------------|-------|-----------------|
| 1626                             | 12.6  | β-sheets        |
| 1638                             | 10.16 | Random coils    |
| 1650                             | 11.35 | α-helices       |
| 1664                             | 17.33 | β-turns         |
| 1682                             | 12.15 | β-sheets        |
| 1695                             | 4.17  | β-sheets        |
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|   | A[Y] | B[Y] | C[Y] | D[Y] | E[Y] | F[Y] |
|---|------|------|------|------|------|------|
| 1 | 0    | 24.56| 3.37 | 12.2 | 10.53| 0.9  |
| 2 | 0.125| 33.07| 5.66 | 12.2 | 12.67| 1.4  |
| 3 | 0.25 | 54.5 | 1.64 | 12.2 | 14.93| 0.803|
| 4 | 0.5  | 74.5 | 2.78 | 12.2 | 17.13| 1.31 |
| 5 | 1    | 103.5| 3.28 | 12.2 | 19.9 | 1.8  |
| 6 | 2    | 105.7| 3.35 | 12.2 | 19.9 | 0.9  |

Figure 1------Original Data

Figure S1------Original Graph (a)
|   | 4ψγ | 5ψγ | CψEr? |
|---|------|------|-------|
| 1 | Control | 24.56 | 3.37 |
| 2 | 20-30% | 23.72 | 1.52 |
| 3 | 30-40% | 32.91 | 2.89 |
| 4 | 40-50% | 49.37 | 1.15 |
| 5 | 50-60% | 52.93 | 3.06 |
| 6 | 60-70% | 33.13 | 2.4 |

Figure S1-----Original Graph (b)

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Figure S2-----Original Data
|   | A(x) | B(y) | C(z) | A(x) | B(y) | C(z) |
|---|------|------|------|------|------|------|
| 1 | 0    | 0.004| 0    | 53   | 104  | 0.3  |
| 2 | 2    | 0.005| 0    | 54   | 106  | 0.28 |
| 3 | 4    | 0.005| 0    | 55   | 108  | 0.15 |
| 4 | 6    | 0    | 0    | 56   | 110  | 0.006|
| 5 | 8    | 0    | 0    | 57   | 112  | 0.002|
| 6 | 10   | 0.006| 0    | 58   | 114  | 1E-3 |
| 7 | 12   | 0    | 0    | 59   | 116  | 0.002|
| 8 | 14   | 0.533| 0    | 60   | 118  | 0.003|
| 9 | 16   | 0.801| 0    | 61   | 120  | 1E-3 |
| 10| 18   | 1.293| 0    | 62   | 120  | 0.003|
| 11| 20   | 0.933| 0    | 63   | 124  | 0.004|
| 12| 22   | 0.266| 0    | 64   | 126  | 1E-3 |
| 13| 24   | 0.112| 0    | 65   | 128  | 0.003|
| 14| 26   | 0.1  | 0    | 66   | 130  | 0.005|
| 15| 28   | 0.06 | 0    | 67   | 132  | 0.07 |
| 16| 30   | 0.053| 0    | 68   | 134  | 0.065|
| 17| 32   | 0.04 | 0    | 69   | 136  | 0.225|
| 18| 34   | 0.024| 0    | 70   | 138  | 0.325|
| 19| 36   | 0.055| 0    | 71   | 140  | 0.065|
| 20| 38   | 0.038| 0    | 72   | 142  | 0.765|
| 21| 40   | 0.032| 0    | 73   | 144  | 0.7  |
| 22| 42   | 0.036| 0    | 74   | 146  | 0.56 |
| 23| 44   | 0.024| 0    | 75   | 148  | 0.45 |
| 24| 46   | 0.013| 0    | 76   | 150  | 0.18 |
| 25| 48   | 0.013| 0    | 77   | 152  | 0.009|
| 26| 50   | 0.01 | 0    | 78   | 154  | 0.004|
| 27| 52   | 0.009| 0    | 79   | 156  | 0.003|
| 28| 54   | 0.004| 0    | 80   | 158  | 1E-3 |
| 29| 56   | 0.003| 0    | 81   | 160  | 0.002|
| 30| 58   | 0    | 0    | 82   | 162  | 0.005|
| 31| 60   | 1E-3 | 0    | 83   | 164  | 0.003|
| 32| 60   | 0.004| 0.2  | 84   | 166  | 0.004|
| 33| 64   | 0.066| 0.2  | 85   | 168  | 0.003|
| 34| 66   | 0.15 | 0.2  | 86   | 170  | 0.002|
| 35| 68   | 0.33 | 0.2  | 87   | 172  | 1E-3 |
| 36| 70   | 0.756| 0.2  | 88   | 174  | 0.002|
| 37| 72   | 0.72 | 0.2  | 89   | 176  | 0.004|
| 38| 74   | 0.67 | 0.2  | 90   | 178  | 1E-3 |
| 39| 76   | 0.59 | 0.2  | 91   | 180  | 0.003|
| 40| 78   | 0.41 | 0.2  | 92   | 180  | 0.005|
| 41| 80   | 0.285| 0.2  | 93   | 184  | 0.004|
| 42| 82   | 0.1  | 0.2  | 94   | 186  | 0.005|
| 43| 84   | 0.006| 0.2  | 95   | 188  | 0.008|
| 44| 86   | 0.005| 0.2  | 96   | 190  | 0.144|
| 45| 88   | 0.003| 0.2  | 97   | 192  | 0.34 |
| 46| 90   | 1E-3 | 0.2  | 98   | 194  | 0.41 |
| 47| 92   | 0.1  | 0.2  | 99   | 196  | 0.49 |
| 48| 94   | 0.31 | 0.2  | 100  | 198  | 0.392|
| 49| 96   | 0.45 | 0.2  | 101  | 200  | 0.329|
| 50| 98   | 0.43 | 0.2  | 102  | 202  | 0.178|
| 51| 100  | 0.37 | 0.2  | 103  | 204  | 0.1  |
| 52| 102  | 0.33 | 0.2  | 104  | 206  | 0.08 |
|   | A[\text{]} | B[\text{]} | C[\text{]} |   | A[\text{]} | B[\text{]} | C[\text{]} |
|---|---|---|---|---|---|---|---|
| 105 | 208 | 0.004 | 0.5 | 105 | 312 | 0.077 | 1 |
| 106 | 210 | 0.006 | 0.5 | 106 | 157 | 0.077 | 1 |
| 107 | 212 | 0.003 | 0.5 | 107 | 158 | 0.077 | 1 |
| 108 | 214 | 1e-3 | 0.5 | 108 | 159 | 0.077 | 1 |
| 109 | 216 | 0.005 | 0.5 | 109 | 160 | 0.077 | 1 |
| 110 | 218 | 0.004 | 0.5 | 110 | 161 | 0.077 | 1 |
| 111 | 220 | 0.006 | 0.5 | 111 | 162 | 0.077 | 1 |
| 112 | 222 | 0.002 | 0.5 | 112 | 163 | 0.077 | 1 |
| 113 | 224 | 0.004 | 0.5 | 113 | 164 | 0.077 | 1 |
| 114 | 226 | 0.003 | 0.5 | 114 | 165 | 0.077 | 1 |
| 115 | 228 | 1e-3 | 0.5 | 115 | 166 | 0.077 | 1 |
| 116 | 230 | 0.002 | 0.5 | 116 | 167 | 0.077 | 1 |
| 117 | 232 | 0.005 | 0.5 | 117 | 168 | 0.077 | 1 |
| 118 | 234 | 1e-3 | 0.5 | 118 | 169 | 0.077 | 1 |
| 119 | 236 | 0.002 | 0.5 | 119 | 170 | 0.077 | 1 |
| 120 | 238 | 0.003 | 0.5 | 120 | 171 | 0.077 | 1 |
| 121 | 240 | 1e-3 | 0.5 | 121 | 172 | 0.077 | 1 |
| 122 | 242 | 0.006 | 0.5 | 122 | 173 | 0.077 | 1 |
| 123 | 244 | 0.004 | 0.5 | 123 | 174 | 0.077 | 1 |
| 124 | 246 | 0.01 | 0.5 | 124 | 175 | 0.077 | 1 |
| 125 | 248 | 0.04 | 0.5 | 125 | 176 | 0.077 | 1 |
| 126 | 250 | 0.08 | 0.5 | 126 | 177 | 0.077 | 1 |
| 127 | 252 | 0.1 | 0.5 | 127 | 178 | 0.077 | 1 |
| 128 | 254 | 0.268 | 0.5 | 128 | 179 | 0.077 | 1 |
| 129 | 256 | 0.272 | 0.5 | 129 | 180 | 0.077 | 1 |
| 130 | 258 | 0.276 | 0.5 | 130 | 181 | 0.077 | 1 |

Figure S4: Original Data
| A[Y] | B[Y]   | C[Y]  |
|------|--------|-------|
| 1    | Control| 24.56 | 3.37 |
| 2    | A      | 20.49 | 1.01 |
| 3    | B      | 30.9  | 1.26 |
| 4    | C      | 41.8  | 3.39 |
| 5    | D      | 50.57 | 1.72 |
| 6    | E      | 92.03 | 3.12 |
| 7    | F      | 43.42 | 2.37 |
| 8    | G      | 20.71 | 1.73 |

**Figure S5------Original Data**

| A[Y] | B[Y]   | C[Y]  |
|------|--------|-------|
| 1    | 0      | 0     | 0    |
| 2    | 2      | 0     | 1E-3 |
| 3    | 3      | 0     | 0.003|
| 4    | 4      | 0     | 0    |
| 5    | 5      | 0     | 1E-3 |
| 6    | 6      | 0     | 0.002|
| 7    | 7      | 1E-3  | 0    |
| 8    | 8      | 0.002 | 1E-3 |
| 9    | 9      | 0.005 | 0.002|
| 10   | 10     | 0.009 | 0    |
| 11   | 11     | 0.01  | 1E-3 |
| 12   | 12     | 0.03  | 0    |
| 13   | 13     | 0.05  | 0.002|
| 14   | 14     | 0.08  | 0.002|
| 15   | 15     | 0.064 | 0.003|
| 16   | 16     | 0.033 | 0.008|
| 17   | 17     | 0.009 | 0.014|
| 18   | 18     | 0.006 | 0.019|
| 19   | 19     | 0.003 | 0.026|
| 20   | 20     | 0     | 0.03 |
| 21   | 21     | 1E-3  | 0.042|
| 22   | 22     | 0.002 | 0.047|
| 23   | 23     | 0     | 0.05 |
| 24   | 24     | 0     | 0.055|
| 25   | 25     | 1E-3  | 0.058|
| 26   | 26     | 1E-3  | 0.056|

| A[Y] | B[Y]   | C[Y]  |
|------|--------|-------|
| 27   | 27     | 0     | 0.051|
| 28   | 28     | 0     | 0.045|
| 29   | 29     | 1E-3  | 0.034|
| 30   | 30     | 0.003 | 0.02 |
| 31   | 31     | 0.01  | 0.015|
| 32   | 32     | 0.035 | 0.01 |
| 33   | 33     | 0.05  | 0.015|
| 34   | 34     | 0.008 | 0.007|
| 35   | 35     | 0.11  | 0.003|
| 36   | 36     | 0.15  | 0    |
| 37   | 37     | 0.12  | 0    |
| 38   | 38     | 0.085 | 0.002|
| 39   | 39     | 0.067 | 1E-3 |
| 40   | 40     | 0.029 | 0.003|
| 41   | 41     | 0.008 | 0.005|
| 42   | 42     | 0     | 0    |
| 43   | 43     | 0     | 0.0017|
| 44   | 44     | 0.002 | 0.002|
| 45   | 45     | 0     | 0.0051|
| 46   | 46     | 0     | 0.0024|
| 47   | 47     | 1E-3  | 0.0015|
| 48   | 48     | 1E-3  | 1E-3 |
| 49   | 49     | 0.002 | 0.002|
| 50   | 50     | 0     | 0    |
| 51   | 51     | 0     | 0    |
| 52   | 52     | 1E-3  | 0    |

| A[Y] | B[Y]   | C[Y]  |
|------|--------|-------|
| 53   | 53     | 0     | 1E-3 |
| 54   | 54     | 0.002 | 0    |
| 55   | 55     | 0.003 | 0    |
| 56   | 56     | 0     | 0    |
| 57   | 57     | 1E-3  | 0    |
| 58   | 58     | 0     | 1E-3 |
| 59   | 59     | 0     | 1E-3 |
| 60   | 60     | 0     | 0.002|
| 61   | 61     | 0.002 | 0    |
| 62   | 62     | 0.006 | 0    |
| 63   | 63     | 0.013 | 0.002|
| 64   | 64     | 0.02  | 0.003|
| 65   | 65     | 0.025 | 1E-3 |
| 66   | 66     | 0.028 | 0    |
| 67   | 67     | 0.03  | 0    |
| 68   | 68     | 0.026 | 0    |
| 69   | 69     | 0.024 | 0.002|
| 70   | 70     | 0.02  | 0.034|
| 71   | 71     | 0.01  | 0.02 |
| 72   | 72     | 0.007 | 0.01 |
| 73   | 73     | 0.004 | 0.007|
| 74   | 74     | 1E-3  | 0.004|
| 75   | 75     | 0     | 1E-3 |
| 76   | 76     | 0     | 0    |
|     | A[γ] | B[γ]  | C[γ]  |
|-----|------|-------|-------|
| 77  | 77   | 0     | 0     |
| 70  | 70   | 0.003 | 0     |
| 79  | 79   | 1E-3  | 0.003 |
| 80  | 80   | 0.004 | 1E-3  |
| 81  | 81   | 0.01  | 0     |
| 82  | 82   | 0.019 | 0.003 |
| 83  | 83   | 0.03  | 0.004 |
| 84  | 84   | 0.035 | 0.002 |
| 85  | 85   | 0.041 | 0.003 |
| 86  | 86   | 0.047 | 0     |
| 87  | 87   | 0.052 | 0     |
| 88  | 88   | 0.055 | 1E-3  |
| 89  | 89   | 0.06  | 0.003 |
| 90  | 90   | 0.058 | 0.002 |
| 91  | 91   | 0.054 | 0     |
| 92  | 92   | 0.05  | 0     |
| 93  | 93   | 0.042 | 0     |
| 94  | 94   | 0.037 | 0.002 |
| 95  | 95   | 0.03  | 0     |
| 96  | 96   | 0.02  | 0     |
| 97  | 97   | 0.016 | 0     |
| 98  | 98   | 0.01  | 0     |
| 99  | 99   | 0.006 | 0     |
| 100 | 100  | 0.004 | 0     |
| 101 | 101  | 0     | 0     |
| 102 | 102  | 0     | 0     |
| 103 | 103  | 0     | 0     |
| 104 | 104  | 0     | 0     |
| 105 | 105  | 1E-3  | 0     |
| 106 | 106  | 0.002 | 0     |
| 107 | 107  | 1E-3  | 0     |
| 108 | 108  | 0.003 | 0     |
| 109 | 109  | 1E-3  | 0     |
| 110 | 110  | 0     | 0     |
| 111 | 111  | 0     | 0     |
| 112 | 112  | 0     | 0     |
| 113 | 113  | 0     | 0     |
| 114 | 114  | 0     | 0     |

Figure S6------Original Data (B Column)
Figure S8------Original Data (C Column)

Note: Figure S6 and Figure S8 have the same A(X).
|   | A(%) | B(%) | C(yEr?) |
|---|------|------|---------|
| 1 | Control | 24.56 | 3.37    |
| 2 | E-I   | 25.4  | 3.04    |
| 3 | E-II  | 153.86| 7.84    |
| 4 | E-III | 40.21 | 3.69    |
| 5 | E-IV  | 36.37 | 1.5     |

Figure S7------Original Data

Figure S9------Original Graph
Figure S10----- Original FTIR spectrum of the E-II-1.
| 名称 | 保留时间 | 面积 | 峰高 | 含量 | 单位 |
|------|--------|------|------|------|------|
| Thr  | 4.959  | 174842 | 77368 | 0.519 | μM   |
| Ala  | 5.374  | 271847 | 118308 | 0.778 | μM   |
| Pro  | 5.986  | 28860  | 14200  | 0.377 | μM   |
| Met  | 6.273  | 2668   | 1766   | 0.377 | μM   |
| Cys2 | 6.717  | 4266   | 2803   | 0.573 | μM   |
| Lyt  | 7.046  | 476446 | 329864 | 0.782 | μM   |
| Tyr  | 7.194  | 115635 | 72247  | 0.295 | μM   |
| Val  | 7.286  | 2848   | 1685   | 0.523 | μM   |
| Met  | 7.331  | 114277 | 69933  | 0.316 | μM   |
| Ile  | 7.449  | 190748 | 123299 | 0.523 | μM   |
| Ala  | 7.586  | 4024   | 3129   | 0.573 | μM   |
| Val  | 7.616  | 11718  | 6272   | 0.316 | μM   |
| Val  | 7.616  | 11718  | 6272   | 0.316 | μM   |
| Thr  | 7.683  | 3272   | 1059   | 0.573 | μM   |
| Thr  | 7.856  | 4746   | 3306   | 0.573 | μM   |
| Thr  | 7.903  | 4704   | 3527   | 0.573 | μM   |
| Thr  | 8.096  | 2313   | 1672   | 0.573 | μM   |
| Val  | 8.087  | 154065 | 96207  | 0.405 | μM   |
| Val  | 8.161  | 308227 | 183396 | 0.826 | μM   |
| Thr  | 8.282  | 131314 | 76547  | 0.348 | μM   |
| Val  | 8.382  | 1469   | 1194   | 0.573 | μM   |
| Thr  | 8.426  | 12741  | 7441   | 0.573 | μM   |
| Thr  | 8.491  | 3547   | 2502   | 0.573 | μM   |

Table S2----The first original results of amino acid compositions of E-II-1.
样品信息

样品名称: 1
样品类型: 未知
编号: 1.2
检测次数: 2
检测样品: 1.00 ul
运行时间: 10.2 Minutes
样品组名称: 乌药片AA测试

采集者: XCPFZ
采集时间: 2016/3/29 12:07:27 CST
处理人: XCPFZ
处理日期: 2016/4/1 18:37:31 CST
处理方法: GMX_AAA
通道名称: PDA Ch1 266 nm@4.8 nm
通道备注: PDA Ch1 266 nm@4.8 nm

色谱峰结果

| 名称 | 保留时间 | 面积 | 峰高 | 含量 | 单位 |
|------|-----------|------|------|------|------|
| AMQ  | 1.429     | 1055640 | 303871 | 0.102 | μM  |
| NH3  | 1.917     | 134306  | 50273  | 0.436 | μM  |
| His  | 2.137     | 5941    | 2198   |       | μM  |
| His  | 2.329     | 69576   | 24847  | 0.207 | μM  |
| Arg  | 2.559     | 53937   | 18242  |       | μM  |
| Ser  | 2.776     | 3970    | 1537   |       | μM  |
| Gly  | 3.030     | 12994   | 5050   |       | μM  |
| Arg  | 3.277     | 18128   | 64135  | 0.563 | μM  |
| Arg  | 3.450     | 164337  | 55358  | 0.508 | μM  |
| Gly  | 3.617     | 255984  | 88050  | 0.792 | μM  |
| Asp  | 4.016     | 271482  | 102623 | 0.839 | μM  |
| Glu  | 4.553     | 399332  | 164071 | 1.217 | μM  |
| 名称 | 保留时间 | 面积   | 峰高   | 含量 | 单位 |
|-----|----------|--------|--------|------|------|
| 13  | 4.765    | 4068   | 1919   |      |      |
| 14  | 4.959    | 175043 | 77310  | 0.520| µM   |
| 15  | 5.374    | 271901 | 117737 | 0.778| µM   |
| 16  | 5.899    | 2865   | 1362   |      |      |
| 17  | 5.968    | 128349 | 63884  | 0.378| µM   |
| 18  | 6.274    | 2865   | 1800   |      |      |
| 19  | 6.719    | 4426   | 2636   |      |      |
| 20  | 6.858    | 151309 | 74309  | 0.578| µM   |
| 21  | 7.017    | 33675  | 17717  | 0.108| µM   |
| 22  | 7.091    | 477461 | 329001 | 0.784| µM   |
| 23  | 7.195    | 115608 | 72398  | 0.295| µM   |
| 24  | 7.286    | 2985   | 1695   |      |      |
| 25  | 7.332    | 114288 | 69006  | 0.316| µM   |
| 26  | 7.448    | 190826 | 123297 | 0.523| µM   |
| 27  | 7.587    | 4124   | 3108   |      |      |
| 28  | 7.617    | 11570  | 6243   |      |      |
| 29  | 7.684    | 3211   | 1073   |      |      |
| 30  | 7.866    | 4924   | 3333   |      |      |
| 31  | 7.903    | 4720   | 3536   |      |      |
| 32  | 8.006    | 2340   | 1660   |      |      |
| 33  | 8.088    | 154022 | 95807  | 0.405| µM   |
| 34  | 8.167    | 303875 | 183380 | 0.827| µM   |
| 35  | 8.282    | 131416 | 76437  | 0.349| µM   |
| 36  | 8.383    | 1463   | 1160   |      |      |
| 37  | 8.426    | 12746  | 7446   |      |      |
| 38  | 8.492    | 3515   | 2922   |      |      |

Table S2-----The second original results of amino acid compositions of E-II-1.
### 样品信息

| 样品名称 | 1 | 采集者 | XCPYFZX |
|----------|---|--------|--------|
| 样品类别 | 未知 | 采集时间 | 2016/3/29 12:18:11 CST |
| 频号 | 1.A,2 | 采集方法 | 动物肉AA |
| 进样次数 | 3 | 处理日期 | 2016/4/1 18:37:54 CST |
| 进样体积 | 1.00 ul | 处理方法 | GMK_AAA |
| 运行时间 | 10.2 Minutes | 通道名称 | PDA On1 268 nm@4.8 nm |
| 样品组名称 | 动物肉AA测试 | 处理通道注释 | PDA On1 268 nm@4.8 nm |

### 色谱峰结果

| 名称 | 保留时间 | 面积 | 峰高 | 含量 | 单位 |
|------|----------|------|------|------|------|
| 1     | AMQ      | 1.424 | 1055904 | 306118 | 0.102 | µM   |
| 2     | NH3      | 1.908 | 134304  | 50563  | 0.436 | µM   |
| 3     |         | 2.128 | 6577    | 2187   |       | µM   |
| 4     | His      | 2.322 | 66936   | 24422  | 0.207 | µM   |
| 5     |          | 2.555 | 53993   | 18222  |       | µM   |
| 6     |          | 2.770 | 3972    | 1535   |       | µM   |
| 7     |          | 3.025 | 12842   | 5039   |       | µM   |
| 8     | Ser      | 3.277 | 186388  | 64213  | 0.563 | µM   |
| 9     | Arg      | 3.445 | 164350  | 55627  | 0.508 | µM   |
| 10    | Gly      | 3.611 | 255455  | 88133  | 0.792 | µM   |
| 11    | Asp      | 4.012 | 271265  | 102470 | 0.838 | µM   |
| 12    | Glu      | 4.550 | 399039  | 163902 | 1.216 | µM   |

报告方法：缺省单个报告
打印时间：18:47:48 PRC
页码：1 (共计2)
Table S2----The third original results of amino acid compositions of E-II-1.

| 名称 | 保留时间 | 面积 | 峰高 | 含量 | 单位 |
|------|----------|------|------|------|------|
| 13   | 4.757    | 4069 | 1026 |      |      |
| 14   | 4.957    | 715074 | 71196 | 0.520 | μM  |
| 15   | 5.373    | 271927 | 117755 | 0.778 | μM  |
| 16   | 5.586    | 26866 | 1352 |      |      |
| 17   | 5.980    | 128123 | 63733 | 0.378 | μM  |
| 18   | 6.274    | 2908 | 1803 |      |      |
| 19   | 6.721    | 4362 | 2607 |      |      |
| 20   | Deriv    | 6.859 | 152102 | 75197 | 0.581 | μM  |
| 21   | Cysx2    | 7.018 | 330865 | 17541 | 0.109 | μM  |
| 22   | LyP      | 7.092 | 477544 | 330494 | 0.784 | μM  |
| 23   | Tyr      | 7.195 | 115818 | 72679 | 0.295 | μM  |
| 24   | 7.286    | 2949 | 1732 |      |      |
| 25   | Met      | 7.331 | 114521 | 69088 | 0.316 | μM  |
| 26   | Val      | 7.449 | 197015 | 123208 | 0.524 | μM  |
| 27   | 7.587    | 4016 | 3134 |      |      |
| 28   | 7.616    | 11773 | 6304 |      |      |
| 29   | 7.883    | 3262 | 1079 |      |      |
| 30   | 7.886    | 4754 | 3303 |      |      |
| 31   | 7.903    | 4722 | 3522 |      |      |
| 32   | 8.006    | 2314 | 1673 |      |      |
| 33   | Ile      | 8.087 | 154875 | 98126 | 0.406 | μM  |
| 34   | Leu      | 8.167 | 308367 | 183596 | 0.827 | μM  |
| 35   | Phe      | 8.282 | 131475 | 78463 | 0.349 | μM  |
| 36   | 8.382    | 1404 | 1184 |      |      |
| 37   | 8.426    | 12722 | 7426 |      |      |
| 38   | 8.491    | 3534 | 2916 |      |      |
### 样品信息

| 样品名称 | 2 | 采集者 | XCPYFZX |
|----------|---|---------|--------|
| 样品类型 | 未知 | 采集时间 | 2016/3/29 12:39:22 CST |
| 编号 | 1, A, 3 | 采集方法 | 姜梦辉 |
| 采样次数 | 1 | 处理方法 | GMX, AAA |
| 采样体积 | 1.00 µl | 处理日期 | 2016/4/18 13:39:10 CST |
| 运行时间 | 10.2 Minutes | 通道名称 | PDA, 265 nm @4.8 nm |
| 样品组名称 | 姜梦辉A, B测试 | 处理温度 | PDA, 265 nm @4.8 nm |

### 色谱峰结果

| 名称 | 保留时间 | 面积 | 峰高 | 含量 | 单位 |
|------|----------|------|------|------|------|
| 1 AMQ | 1.370 | 3431298 | 695193 | 0.331 | µM |
| 2 NH3 | 1.697 | 106819 | 40409 | 0.347 | µM |
| 3 His | 2.303 | 183156 | 6855 | 0.557 | µM |
| 4 | 3.001 | 2850 | 1187 | | |
| 5 Ser | 3.257 | 67518 | 22830 | 0.202 | µM |
| 6 Arg | 3.437 | 45620 | 16458 | 0.141 | µM |
| 7 Gly | 3.604 | 89506 | 31690 | 0.277 | µM |
| 8 Asp | 4.003 | 103980 | 39862 | 0.320 | µM |
| 9 Glu | 4.548 | 113709 | 49123 | 0.347 | µM |
| 10 Thr | 4.952 | 57320 | 25844 | 0.170 | µM |
| 11 | 5.172 | 2038 | 1036 | | |
| 12 | 5.272 | 2441 | 1171 | | |

报告方法：缺省个报告
打印 18:48:17 PRC 2016/4/1
页码：1（共计2）
Table S2-----The first results of amino acid compositions of crude protein F$_{40-60}$.
样品信息

样品名称: A
样品类型: 未知
货架: 1 A, 3
进样次数: 2
进样体积: 1.00 µl
运行时间: 10.2 Minutes
样品组名称: 与CreaA2001测试

采集者: XCPYFZX
采集时间: 2016/3/29 18:00:09 CST
处理日期: 2016/4/1 18:39:34 CST
处理方法: GMX AAA
通道名称: PDA C11 265 nm@4.8 nm
通道通道注释: PDA C11 265 nm@4.8 nm

色谱峰结果

| 名称 | 保留时间 | 面积 | 峰高 | 含量 | 单位 |
|------|----------|------|------|------|------|
| 1    | AMQ      | 3442291 | 692448 | 0.332 | µM  |
| 2    | NH3      | 107435  | 40542 | 0.349 | µM  |
| 3    | His      | 182705  | 6839  | 0.057 | µM  |
| 4    | 3.016    | 2837    | 1157  | 0.005 | µM  |
| 5    | Ser      | 67723   | 22931 | 0.203 | µM  |
| 6    | Arg      | 45789   | 16604 | 0.141 | µM  |
| 7    | Gly      | 89004   | 31945 | 0.278 | µM  |
| 8    | Asp      | 103907  | 40043 | 0.321 | µM  |
| 9    | Glu      | 11358G  | 49234 | 0.347 | µM  |
| 10   | Thr      | 57455   | 25849 | 0.171 | µM  |
| 11   | 5.171    | 2016    | 1034  | 0.005 | µM  |
| 12   | 5.272    | 2442    | 1171  | 0.005 | µM  |

报告方法: 原始数据报告
打印: 18:48:46 FRC  2016/4/1
页码: 1 (共计 2)
Table S2----The second results of amino acid compositions of crude protein F40-60.
样品信息

样品名称: 2
样品类型: 未知
配号: 1-A,3
进样次数: 3
进样体积: 1.00 µl
运行时间: 10.2 Minutes
样品组名称: 品药AA测试

采集者: XCPYFZX
采集时间: 2016/3/29 13:00:56 CST
处理方法: GMK_AAA
处理日期: 2016/4/1 18:40:00 CST
通道名称: PDA Ch1 266 nm@4.8 nm
处理通道注释: PDA Ch1 266 nm@4.8 nm

色谱峰结果

| 名称 | 保留时间 | 面积   | 峰高   | 含量 | 单位 |
|------|----------|--------|--------|------|------|
| 1 AMQ | 1.372    | 3438996 | 697589 | 0.332 | µM  |
| 2 NH3 | 1.659    | 1072356 | 40539  | 0.348 | µM  |
| 3 His | 2.306    | 18465   | 6795   | 0.057 | µM  |
| 4    | 3.007    | 2720    | 1141   |       |      |
| 5 Ser | 3.259    | 67962   | 22989  | 0.203 | µM  |
| 6 Arg | 3.439    | 46551   | 16613  | 0.144 | µM  |
| 7 Gly | 3.604    | 91153   | 31958  | 0.282 | µM  |
| 8 Asp | 4.001    | 103906  | 39835  | 0.321 | µM  |
| 9 Glu | 4.544    | 113061  | 49028  | 0.347 | µM  |
| 10 Thr | 4.949    | 57209   | 25865  | 0.170 | µM  |
| 11    | 5.169    | 2015    | 1031   |      |      |
| 12    | 5.270    | 2435    | 1168   |      |      |
Table S2-----The third results of amino acid compositions of crude protein F\textsubscript{40-60}.

| 名称 | 保留时间 | 面积  | 峰高  | 含量  | 单位 |
|------|----------|-------|-------|-------|------|
| 13   | 5.369    | 87997 | 30761 | 0.252 | μM   |
| 14   | 5.994    | 45186 | 23376 | 0.136 | μM   |
| 15   | 6.072    | 2801  | 1218  |       |      |
| 16   | 6.856    | 187136| 83470 | 0.638 | μM   |
| 17   | 7.015    | 12181 | 5720  | 0.039 | μM   |
| 18   | 7.053    | 1266  | 2020  |       |      |
| 19   | 7.092    | 92753 | 84362 | 0.152 | μM   |
| 20   | 7.192    | 41770 | 24242 | 0.107 | μM   |
| 21   | 7.285    | 1760  | 1111  |       |      |
| 22   | 7.329    | 34041 | 21166 | 0.094 | μM   |
| 23   | 7.447    | 57460 | 44851 | 0.179 | μM   |
| 24   | 7.863    | 2701  | 1875  |       |      |
| 25   | 8.065    | 55703 | 34872 | 0.146 | μM   |
| 26   | 8.185    | 100224| 60531 | 0.269 | μM   |
| 27   | 8.279    | 51574 | 30314 | 0.137 | μM   |
| 28   | 8.421    | 4177  | 2481  |       |      |
样品信息

| 样品名称 | 6 |
|----------|---|
| 样品类别 | 未知 |
| 浓度 | 1A.7 |
| 浓度成分 | 1.00 μl |
| 运行时间 | 10.2 Minutes |
| 运行时间 | 2016/04/1 18:44:10 CST |
| 采样者 | XCPYFZX |
| 采样日期 | 2016/03/29 15:29:54 CST |
| 处理方法 | GMK_AAA |
| 处理时间 | PDA @ 126 nm @ 4.8 nm |
| 处理温度 | PDA @ 126 nm @ 4.8 nm |

色谱峰结果

| 名称 | 保留时间 | 面积 | 峰高 | 含量 | 单位 |
|------|-----------|------|------|------|------|
| 1    | 1.286     | 3195 | 1774 | 51003 | uM  |
| 2    | 1.427     | 2287212 | 51003 | 0.221 | uM  |
| 3    | 1.967     | 162536 | 59022 | 0.527 | uM  |
| 4    | 2.410     | 42727 | 15529 | 0.132 | uM  |
| 5    | 3.356     | 215375 | 73080 | 0.644 | uM  |
| 6    | 3.534     | 103384 | 37528 | 0.319 | uM  |
| 7    | 3.693     | 142623 | 51140 | 0.442 | uM  |
| 8    | 4.084     | 242904 | 91472 | 0.748 | uM  |
| 9    | 4.611     | 292993 | 123777 | 0.893 | uM  |
| 10   | 5.059     | 124575 | 55874 | 0.370 | uM  |
| 11   | 5.419     | 211335 | 93501 | 0.605 | uM  |
| 12   | 6.030     | 108301 | 52448 | 0.319 | uM  |
Table S2-----The first results of amino acid compositions of anticoagulant protein E-II.

| 名称 | 保留时间 | 面积 | 高峰 | 含量 | 单位 |
|------|----------|------|------|------|------|
| 13   | Deriv    | 6.87 | 125389 | 82435 | μM   |
| 14   | Cys2     | 7.029| 57014  | 32966 | μM   |
| 15   |          | 7.067| 4237   | 8457  | μM   |
| 16   | Lyr      | 7.102| 277834 | 193221 | μM   |
| 17   | Tyr      | 7.207| 845264 | 53058  | μM   |
| 18   |          | 7.295| 2461   | 1473  | μM   |
| 19   | Met      | 7.342| 88469  | 54512  | μM   |
| 20   | Val      | 7.458| 183389 | 115352 | μM   |
| 21   |          | 7.622| 8515   | 2967  | μM   |
| 22   |          | 7.690| 3046   | 1444  | μM   |
| 23   |          | 7.731| 2280   | 1861  | μM   |
| 24   |          | 7.773| 1665   | 1010  | μM   |
| 25   |          | 7.870| 8552   | 5408  | μM   |
| 26   |          | 7.966| 3840   | 2830  | μM   |
| 27   |          | 8.009| 2141   | 1551  | μM   |
| 28   | Ile      | 8.093| 129975 | 80256  | μM   |
| 29   | Leu      | 8.172| 224400 | 134181 | μM   |
| 30   | Phe      | 8.287| 129981 | 75450  | μM   |
| 31   |          | 8.426| 3531   | 2117  | μM   |
| 32   |          | 8.492| 4246   | 3573  | μM   |
色谱峰结果

| 名称 | 峰面积 | 峰高 | 含量 | 单位 |
|------|--------|------|------|------|
| 1    | 1.291  | 3221 | 1784 |      |
| 2    | 1.441  | 228843| 510112| 0.221| uM  |
| 3    | 1.974  | 162907| 56532 | 0.528| uM  |
| 4    | 2.426  | 42857 | 15379 | 0.133| uM  |
| 5    | 3.386  | 21567 | 73343 | 0.640| uM  |
| 6    | 3.547  | 103638| 37917 | 0.320| uM  |
| 7    | 3.703  | 142969| 51322 | 0.443| uM  |
| 8    | 4.094  | 233837| 91483 | 0.736| uM  |
| 9    | 4.618  | 293378| 124483| 0.894| uM  |
| 10   | 5.014  | 124815| 56202 | 0.371| uM  |
| 11   | 5.327  | 2106  | 1011  |      |      |
| 12   | 5.422  | 211436| 93782 | 0.605| uM  |
| 名称 | 保留时间 | 面积  | 峰高  | 含量 | 单位 |
|-----|----------|-------|-------|-------|------|
| 13  | Pro      | 6.033 | 108569| 52584 | 0.320 | μM  |
| 14  | Deriv    | 6.877 | 126578| 82885 | 0.483 | μM  |
| 15  | Cys2     | 7.036 | 56736 | 33150 | 0.182 | μM  |
| 16  |          | 7.687 | 5033  | 8051  |       |     |
| 17  | Lyr      | 7.103 | 271586| 193787| 0.457 | μM  |
| 18  | Tyr      | 7.208 | 84442 | 52974 | 0.215 | μM  |
| 19  |          | 7.296 | 2611  | 1400  |       |     |
| 20  | Met      | 7.344 | 88597 | 54587 | 0.245 | μM  |
| 21  | Val      | 7.458 | 183567| 115748| 0.468 | μM  |
| 22  |          | 7.622 | 6562  | 2989  |       |     |
| 23  |          | 7.891 | 3073  | 1457  |       |     |
| 24  |          | 7.731 | 2250  | 1672  |       |     |
| 25  |          | 7.773 | 1659  | 1006  |       |     |
| 26  |          | 7.870 | 8531  | 5506  |       |     |
| 27  |          | 7.965 | 3763  | 2823  |       |     |
| 28  |          | 8.009 | 2178  | 1543  |       |     |
| 29  | Ile      | 8.093 | 129063| 80291 | 0.338 | μM  |
| 30  | Leu      | 8.173 | 224710| 134240| 0.603 | μM  |
| 31  | Phe      | 8.287 | 130090| 75429 | 0.345 | μM  |
| 32  |          | 8.427 | 3529  | 2122  |       |     |
| 33  |          | 8.492 | 4208  | 3569  |       |     |

Table S2-----The second results of amino acid compositions of anticoagulant protein E-II.
Table S2-----The third results of amino acid compositions of anticoagulant protein E-II.

| 名称 | 保留时间 | 面积 | 峰高 | 含量 | 单位 |
|------|----------|------|------|------|------|
| 13 Pro | 6.031 | 109508 | 52588 | 0.320 | μM |
| 14 Deriv | 6.878 | 127014 | 63077 | 0.485 | μM |
| 15 Cys2 | 7.029 | 57376 | 33204 | 0.184 | μM |
| 16 | 7.067 | 4240 | 8447 | | |
| 17 Lyr | 7.102 | 278863 | 194121 | 0.458 | μM |
| 18 Tyr | 7.207 | 84031 | 53169 | 0.217 | μM |
| 19 | 7.295 | 2618 | 1532 | | |
| 20 Met | 7.342 | 89056 | 54641 | 0.246 | μM |
| 21 Val | 7.458 | 184538 | 115990 | 0.490 | μM |
| 22 | 7.621 | 7073 | 3097 | | |
| 23 | 7.898 | 3411 | 1569 | | |
| 24 | 7.730 | 2569 | 1795 | | |
| 25 | 7.772 | 2273 | 1131 | | |
| 26 | 7.389 | 8954 | 5587 | | |
| 27 | 7.964 | 3794 | 2827 | | |
| 28 | 8.007 | 2191 | 1556 | | |
| 29 Ile | 8.091 | 129237 | 80342 | 0.338 | μM |
| 30 Leu | 8.171 | 22492 | 134138 | 0.603 | μM |
| 31 Phe | 8.285 | 130217 | 75662 | 0.345 | μM |
| 32 | 8.426 | 3522 | 2103 | | |
| 33 | 8.491 | 4322 | 3622 | | |
![Figure S12 (a) --- Original data](image1)

| A[X] | B[Y] | C[Y] | D[γEr?] | E[Y] | F[γEr?] |
|------|------|------|---------|------|---------|
| 1    | 0.005| 0.6  | 0.6     | 0.02 | 0.55    | 0.033   |
| 2    | 0.05 | 0.6  | 0.54    | 0.04 | 0.46    | 0.03    |
| 3    | 0.25 | 0.6  | 0.44    | 0.04 | 0.4     | 0.029   |
| 4    | 0.5  | 0.6  | 0.36    | 0.028| 0.32    | 0.022   |
| 5    | 2.5  | 0.6  | 0.23    | 0.017| 0.18    | 0.01    |
| 6    | 5    | 0.6  | 0.11    | 0.009| 0.07    | 0.003   |
| 7    | 50   | 0.6  | 0.06    | 0.004| 0.03    | 1E-3    |

![Figure S12 (b) --- Original data](image2)

| A[X] | B[Y] | C[Y] | D[Y] | E[γEr?] |
|------|------|------|------|---------|
| 1    | 0.05 | 1.3  | 1.25 | 1       | 0.05    |
| 2    | 0.5  | 1.3  | 1.25 | 0.7     | 0.06    |
| 3    | 2.5  | 1.3  | 1.25 | 0.48    | 0.03    |
| 4    | 5    | 1.3  | 1.25 | 0.36    | 0.02    |
| 5    | 50   | 1.3  | 1.25 | 0.11    | 0.01    |
The font information and the source file of the application.

| Name    | Font   | Size     | Source                  |
|---------|--------|----------|-------------------------|
| Figure 1| Arial  | 20       | Origin 7.5/Mac          |
| Figure S1| Arial | 1.67 point | Adobe Photoshop CS5    |
| Figure S2| Arial  | 20       | Origin 7.5/Mac          |
| Figure S3| Arial  | 16       | Origin 7.5/Mac          |
| Figure S4| Arial  | 20       | Origin 7.5/Mac          |
| Figure S5| Arial  | 20       | Origin 7.5/Mac          |
| Figure S6| Arial  | 20       | Origin 7.5/Mac          |
| Figure S7| Arial  | 20       | Origin 7.5/Mac          |
| Figure S8| Arial  | 20       | Origin 7.5/Mac          |
| Figure S9| Arial  | 20       | Origin 7.5/Mac          |
| Figure S10| Arial | 20       | Origin 7.5/Mac         |
| Figure S12| Arial | 20       | Origin 7.5/Mac         |