SUPPLEMENTARY MATERIAL

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

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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 α -helices 16.75%, β -sheets 42.67%, β -turn 25.58% and random coil 15.00%. *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 $10,000 \times g$ 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 F₄₀₋₆₀. F₄₀₋₆₀ 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

F₄₀₋₆₀ (250.00 mg) was dissolved in 20 mM Tris-HCl buffer (pH 7.60) 5.00 mL and centrifuged at $10,000 \times g$ for 10 min. Then the supernatant was loaded on to a DEAE-cellulose-52 column ($2.6 \times 30 \text{ cm}^2$), 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 ($1.5 \times 75 \text{ cm}^2$) 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 ($1.0 \times 60 \text{ cm}^2$) 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 $3000 \times g$ 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 CaCl_2 . 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 $\pm$ 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 (F₄₀₋₆₀).

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

& Lis 1972; Sun et al. 2007; Sudhakar & Vincent 2014). 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 F₄₀₋₆₀ 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.

Data: Data9_D
Model: Gauss
Equation: $y=y_0 + (A/(w*\sqrt{PI/2}))*\exp(-2*((x-xc)/w)^2)$
Weighting:
y No weighting

Chi^2/DoF = 0.00027
R^2 = 0.99925

y0	0.92746	±0.0352
xc1	1626.0586	±13.48757
w1	20.10328	±11.47673
A1	12.59873	±18.9735
xc2	1638.43744	±5.23227
w2	13.85522	±12.43316
A2	10.16059	±27.83901
xc3	1650.38157	±2.6212
w3	11.76733	±7.21483
A3	11.34786	±28.01589
xc4	1664.73285	±5.53352
w4	16.48298	±27.97493
A4	17.33498	±45.77478
xc5	1682.06466	±11.33519
w5	15.44513	±22.44318
A5	12.14797	±35.55804
xc6	1695.78924	±3.54527
w6	10.45768	±4.23366
A6	4.16557	±7.40253

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*.

Step	Total protein (mg)	Total anticoagulant activity (U)	Specific activity (U/mg)	Yield (%)	Purification fold
F ₄₀₋₆₀	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[X]	B[Y]	C[yEr?	D[Y]	E[Y]	F[yEr?
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)

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

	A[X]	B[Y]	C[yEr?
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	3.86
6	60-70%	33.13	2.4

Figure S2-----Original Data

	A[X]	B[Y]	C[Y]		A[X]	B[Y]	C[Y]
1	0	0.004	0	53	104	0.3	0.2
2	2	0.006	0	54	106	0.28	0.2
3	4	0.005	0	55	108	0.15	0.2
4	6	0	0	56	110	0.006	0.2
5	8	0	0	57	112	0.002	0.2
6	10	0.006	0	58	114	1E-3	0.2
7	12	0	0	59	116	0.002	0.2
8	14	0.533	0	60	118	0.003	0.2
9	16	0.801	0	61	120	1E-3	0.2
10	18	1.283	0	62	120	0.003	0.3
11	20	0.933	0	63	124	0.004	0.3
12	22	0.266	0	64	126	1E-3	0.3
13	24	0.112	0	65	128	0.003	0.3
14	26	0.1	0	66	130	0.005	0.3
15	28	0.06	0	67	132	0.07	0.3
16	30	0.053	0	68	134	0.065	0.3
17	32	0.04	0	69	136	0.225	0.3
18	34	0.024	0	70	138	0.325	0.3
19	36	0.055	0	71	140	0.685	0.3
20	38	0.038	0	72	142	0.765	0.3
21	40	0.032	0	73	144	0.7	0.3
22	42	0.036	0	74	146	0.56	0.3
23	44	0.024	0	75	148	0.45	0.3
24	46	0.013	0	76	150	0.18	0.3
25	48	0.013	0	77	152	0.009	0.3
26	50	0.01	0	78	154	0.004	0.3
27	52	0.008	0	79	156	0.003	0.3
28	54	0.004	0	80	158	1E-3	0.3
29	56	0.003	0	81	160	0.002	0.3
30	58	0	0	82	162	0.005	0.3
31	60	1E-3	0	83	164	0.003	0.3
32	60	0.004	0.2	84	166	0.004	0.3
33	64	0.066	0.2	85	168	0.003	0.3
34	66	0.15	0.2	86	170	0.002	0.3
35	68	0.33	0.2	87	172	1E-3	0.3
36	70	0.756	0.2	88	174	0.002	0.3
37	72	0.72	0.2	89	176	0.004	0.3
38	74	0.67	0.2	90	178	1E-3	0.3
39	76	0.59	0.2	91	180	0.003	0.3
40	78	0.41	0.2	92	180	0.005	0.5
41	80	0.285	0.2	93	184	0.004	0.5
42	82	0.1	0.2	94	186	0.005	0.5
43	84	0.006	0.2	95	188	0.008	0.5
44	86	0.005	0.2	96	190	0.144	0.5
45	88	0.003	0.2	97	192	0.34	0.5
46	90	1E-3	0.2	98	194	0.41	0.5
47	92	0.18	0.2	99	196	0.49	0.5
48	94	0.31	0.2	100	198	0.392	0.5
49	96	0.45	0.2	101	200	0.328	0.5
50	98	0.43	0.2	102	202	0.178	0.5
51	100	0.37	0.2	103	204	0.1	0.5
52	102	0.33	0.2	104	206	0.08	0.5

	A[X]	B[Y]	C[Y]		A[X]	B[Y]	C[Y]
105	208	0.004	0.5	157	312	0.077	1
106	210	0.006	0.5	158	314	0.047	1
107	212	0.003	0.5	159	316	0.031	1
108	214	1E-3	0.5	160	318	0.015	1
109	216	0.005	0.5	161	320	0.011	1
110	218	0.004	0.5	162	322	0.016	1
111	220	0.006	0.5	163	324	0.008	1
112	222	0.002	0.5	164	326	0.012	1
113	224	0.004	0.5	165	328	0.009	1
114	226	0.003	0.5	166	330	0.002	1
115	228	1E-3	0.5	167	332	1E-3	1
116	230	0.002	0.5	168	334	0.003	1
117	232	0.006	0.5	169	336	0.002	1
118	234	1E-3	0.5	170	338	1E-3	1
119	236	0.002	0.5	171	340	0.004	1
120	238	0.003	0.5	172	342	0.003	1
121	240	1E-3	0.5	173	344	1E-3	1
122	240	0.006	0.8	174	346	0.002	1
123	244	0.004	0.8	175	348	0.003	1
124	246	0.01	0.8	176	350	0.005	1
125	248	0.04	0.8	177	352	1E-3	1
126	250	0.08	0.8	178	354	0.003	1
127	252	0.1	0.8	179	356	0.004	1
128	254	0.268	0.8	180	358	0.002	1
129	256	0.272	0.8	181	360	1E-3	1
130	258	0.216	0.8				
131	260	0.096	0.8				
132	262	0.092	0.8				
133	264	0.02	0.8				
134	266	0.004	0.8				
135	268	0.003	0.8				
136	270	0.004	0.8				
137	272	0.006	0.8				
138	274	0.002	0.8				
139	276	0.004	0.8				
140	278	0.003	0.8				
141	280	1E-3	0.8				
142	282	0.002	0.8				
143	284	0.006	0.8				
144	286	1E-3	0.8				
145	288	0.002	0.8				
146	290	0.003	0.8				
147	292	1E-3	0.8				
148	294	0.005	0.8				
149	296	0.007	0.8				
150	298	0.006	0.8				
151	300	0.004	0.8				
152	300	0.008	1				
153	304	0.016	1				
154	306	0.018	1				
155	308	0.056	1				
156	310	0.082	1				

Figure S4-----Original Data

	A[X]	B[Y]	C[yEr?
1	Control	24.56	3.37
2	A	28.49	1.81
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[X]	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	0.008	0
11	11	0.01	1E-3
12	12	0.03	0
13	13	0.06	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[X]	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	0.06	0.015
34	34	0.08	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

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[X]	B[Y]	C[Y]
77	77	0	0
78	78	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[X]	B[Y]	C[yEr?
1	Control	24.56	3.37
2	E-I	25.4	3.84
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.

样品信息

样品名称: 1
样品类型: 未知
瓶号: 1:A,2
进样次数: 1
进样体积: 1.00 ul
运行时间: 10.2 Minutes
样品组名称: 苟梦星AA测试

采集者: XCPYFZX
采集时间: 2016/3/29 11:56:40 CST
采集方法组: 苟梦星AA
处理日期: 2016/4/1 18:36:53 CST
处理方法: GMX_AAA
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	1.424	1054104	304723	0.102	uM
2	NH3	1.912	132260	50122	0.429	uM
3		2.134	6049	2126		
4	His	2.330	66764	24403	0.207	uM
5		2.562	53770	18276		
6		2.779	3832	1539		
7		3.031	12859	5053		
8	Ser	3.279	187984	64124	0.562	uM
9	Arg	3.452	164183	55749	0.507	uM
10	Gly	3.618	255323	88272	0.791	uM
11	Asp	4.018	271138	102895	0.838	uM
12	Glu	4.553	398993	164484	1.216	uM

用户名称: XCPYFZX

项目名称: 氨基酸2015

色谱峰结果

	名称	保留时间	面积	峰高	含量	单位
13		4.760	4023	1913		
14	Thr	4.959	174842	77368	0.519	uM
15	Ala	5.374	271647	118308	0.778	uM
16		5.898	2890	1430		
17	Pro	5.997	128033	63778	0.377	uM
18		6.273	2888	1796		
19		6.717	4260	2603		
20	Deriv	6.857	150150	73948	0.573	uM
21	Cys2	7.016	33464	17613	0.108	uM
22	Lyr	7.090	476446	329864	0.782	uM
23	Tyr	7.194	115635	72247	0.295	uM
24		7.286	2848	1685		
25	Met	7.331	114277	69933	0.316	uM
26	Val	7.449	196748	123299	0.523	uM
27		7.586	4024	3129		
28		7.616	11719	6272		
29		7.683	3272	1069		
30		7.865	4746	3306		
31		7.963	4704	3527		
32		8.006	2313	1672		
33	Ile	8.087	154665	96207	0.405	uM
34	Leu	8.167	308027	183396	0.826	uM
35	Phe	8.282	131314	76547	0.348	uM
36		8.382	1499	1194		
37		8.425	12741	7441		
38		8.491	3547	2928		

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
瓶号: 1:A,2
进样次数: 2
进样体积: 1.00 ul
运行时间: 10.2 Minutes
样品组名称: 苟梦星AA测试

采集者: XCPYFZX
采集时间: 2016/3/29 12:07:27 CST
采集方法组: 苟梦星AA
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
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	1.429	1055640	303871	0.102	uM
2 NH3	1.917	134306	50273	0.436	uM
3	2.137	6541	2196		
4 His	2.329	66876	24647	0.207	uM
5	2.559	53937	18242		
6	2.776	3870	1537		
7	3.030	12864	5050		
8 Ser	3.277	188128	64135	0.563	uM
9 Arg	3.450	164337	55358	0.508	uM
10 Gly	3.617	255584	88050	0.792	uM
11 Asp	4.016	271482	102623	0.839	uM
12 Glu	4.553	399332	164071	1.217	uM

用户名称: XCPYFZX

项目名称: 氨基酸2015

色谱峰结果

	名称	保留时间	面积	峰高	含量	单位
13		4.760	4068	1919		
14	Thr	4.959	175043	77310	0.520	uM
15	Ala	5.374	271901	117737	0.778	uM
16		5.899	2805	1392		
17	Pro	5.998	128349	63884	0.378	uM
18		6.274	2885	1800		
19		6.719	4426	2636		
20	Deriv	6.858	151309	74399	0.578	uM
21	Cys2	7.017	33675	17717	0.108	uM
22	Lyr	7.091	477461	329001	0.784	uM
23	Tyr	7.195	115609	72398	0.295	uM
24		7.286	2985	1695		
25	Met	7.332	114285	69906	0.316	uM
26	Val	7.449	196826	123297	0.523	uM
27		7.587	4124	3108		
28		7.617	11570	6243		
29		7.684	3211	1073		
30		7.866	4934	3333		
31		7.963	4720	3539		
32		8.006	2340	1680		
33	Ile	8.088	154822	95897	0.405	uM
34	Leu	8.167	308375	183380	0.827	uM
35	Phe	8.282	131416	76437	0.349	uM
36		8.383	1463	1180		
37		8.425	12746	7445		
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
样品类型: 未知
瓶号: 1:A,2
进样次数: 3
进样体积: 1.00 ul
运行时间: 10.2 Minutes
样品组名称: 苟梦星AA测试

采集者: XCPYFZX
采集时间: 2016/3/29 12:18:11 CST
采集方法组: 苟梦星AA
处理日期: 2016/4/1 18:37:54 CST
处理方法: GMX_AAA
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	1.424	1055904	306118	0.102	uM
2	NH3	1.908	134304	50563	0.436	uM
3		2.128	6577	2187		
4	His	2.322	66839	24422	0.207	uM
5		2.553	53893	18222		
6		2.770	3872	1535		
7		3.025	12842	5039		
8	Ser	3.272	188088	64213	0.563	uM
9	Arg	3.445	164350	55627	0.508	uM
10	Gly	3.611	255455	88133	0.792	uM
11	Asp	4.012	271265	102470	0.838	uM
12	Glu	4.550	399039	163902	1.216	uM

用户名称: XCPYFZX

项目名称: 氨基酸2015

色谱峰结果

	名称	保留时间	面积	峰高	含量	单位
13		4.757	4099	1926		
14	Thr	4.957	175074	77196	0.520	uM
15	Ala	5.373	271927	117755	0.778	uM
16		5.898	2695	1352		
17	Pro	5.998	128123	63733	0.378	uM
18		6.274	2908	1803		
19		6.721	4362	2607		
20	Deriv	6.859	152192	75197	0.581	uM
21	Cys2	7.018	33895	17841	0.109	uM
22	Lyr	7.092	477544	330494	0.784	uM
23	Tyr	7.195	115819	72679	0.295	uM
24		7.286	2949	1732		
25	Met	7.331	114521	69988	0.316	uM
26	Val	7.449	197015	123208	0.524	uM
27		7.587	4015	3134		
28		7.616	11773	6304		
29		7.683	3292	1079		
30		7.865	4754	3303		
31		7.963	4722	3522		
32		8.006	2314	1673		
33	Ile	8.087	154875	96126	0.406	uM
34	Leu	8.167	308367	183596	0.827	uM
35	Phe	8.282	131475	76463	0.349	uM
36		8.382	1494	1184		
37		8.425	12722	7426		
38		8.491	3534	2918		

Table S2-----The third original results of amino acid compositions of E-II-1.

样品信息

样品名称: 2
样品类型: 未知
瓶号: 1:A,3
进样次数: 1
进样体积: 1.00 ul
运行时间: 10.2 Minutes
样品组名称: 苟梦星AA测试

采集者: XCPY/FZX
采集时间: 2016/3/29 12:39:22 CST
采集方法组: 荷梦星AA
处理日期: 2016/4/1 18:39:10 CST
处理方法: GMX_AAA
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	1.370	3431258	695193	0.331	uM
2	NH3	1.897	106818	40409	0.347	uM
3	His	2.303	18316	6855	0.057	uM
4		3.001	2850	1167		
5	Ser	3.257	67518	22830	0.202	uM
6	Arg	3.437	45629	16458	0.141	uM
7	Gly	3.604	89506	31690	0.277	uM
8	Asp	4.003	103680	39802	0.320	uM
9	Glu	4.548	113709	49123	0.347	uM
10	Thr	4.952	57320	25844	0.170	uM
11		5.172	2008	1036		
12		5.272	2441	1171

用户名称: XCPYFZX

项目名称: 氨基酸2015

色谱峰结果

	名称	保留时间	面积	峰高	含量	单位
13	Ala	5.372	87773	39759	0.251	uM
14	Pro	5.996	46095	23279	0.136	uM
15		6.073	2765	1219		
16	Deriv	6.857	166147	82994	0.634	uM
17	Cys2	7.016	12280	5749	0.039	uM
18		7.053	1381	2201		
19	Lyr	7.093	92846	64154	0.152	uM
20		7.165	2656	2546		
21	Tyr	7.194	39692	24316	0.101	uM
22		7.285	2028	1210		
23	Met	7.330	34650	21264	0.096	uM
24	Val	7.448	71563	45084	0.190	uM
25		7.864	2810	1895		
26	Ile	8.087	55663	34803	0.146	uM
27	Leu	8.167	100387	60549	0.269	uM
28	Phe	8.280	52191	30367	0.138	uM
29		8.422	4143	2478		

Table S2-----The first 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
瓶号: 1:A,3
进样次数: 2
进样体积: 1.00 ul
运行时间: 10.2 Minutes
样品组名称: 苟梦星AA测试

采集者: XCPYFZX
采集时间: 2016/3/29 12:50:09 CST
采集方法组: 苟梦星AA
处理日期: 2016/4/1 18:39:34 CST
处理方法: GMX_AAA
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	1.374	3442061	692448	0.332	uM
2	NH3	1.903	107435	40542	0.349	uM
3	His	2.311	18273	6839	0.057	uM
4		3.010	2837	1157		
5	Ser	3.264	67733	22931	0.203	uM
6	Arg	3.445	45784	16604	0.141	uM
7	Gly	3.609	89804	31945	0.278	uM
8	Asp	4.004	103907	40043	0.321	uM
9	Glu	4.547	113962	49234	0.347	uM
10	Thr	4.952	57455	25849	0.171	uM
11		5.171	2016	1034		
12		5.272	2442	1171

用户名称: XCPYFZX

项目名称: 氨基酸2015

色谱峰结果

	名称	保留时间	面积	峰高	含量	单位
13	Ala	5.371	87995	39736	0.252	uM
14	Pro	5.995	46231	23299	0.136	uM
15		6.072	2755	1221		
16	Deriv	6.858	168330	83880	0.643	uM
17	Cys2	7.018	12048	5754	0.039	uM
18		7.053	1750	2442		
19	Lyr	7.095	93072	64679	0.153	uM
20	Tyr	7.195	42473	24429	0.108	uM
21		7.286	1991	1224		
22	Met	7.331	34844	21342	0.096	uM
23	Val	7.449	71850	45222	0.191	uM
24		7.865	2783	1900		
25	Ile	8.089	55725	34831	0.146	uM
26	Leu	8.169	100300	60601	0.269	uM
27	Phe	8.282	51622	30277	0.137	uM
28		8.425	4163	2500		

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
瓶号: 1:A,3
进样次数: 3
进样体积: 1.00 ul
运行时间: 10.2 Minutes
样品组名称: 苟梦星AA测试

采集者: XCPYFZX
采集时间: 2016/3/29 13:00:56 CST
采集方法组: 苟梦星AA
处理日期: 2016/4/1 18:40:00 CST
处理方法: GMX_AAA
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	3438986	697589	0.332	uM
2	NH3	1.899	107256	40538	0.348	uM
3	His	2.306	18465	6795	0.057	uM
4		3.007	2720	1141		
5	Ser	3.259	67962	22989	0.203	uM
6	Arg	3.439	46551	16613	0.144	uM
7	Gly	3.604	91153	31956	0.282	uM
8	Asp	4.001	103806	39835	0.321	uM
9	Glu	4.544	113961	49028	0.347	uM
10	Thr	4.949	57269	25865	0.170	uM
11		5.169	2018	1031		
12		5.270	2438	1168

用户名称: XCPYFZX

项目名称: 氨基酸2015

色谱峰结果

	名称	保留时间	面积	峰高	含量	单位
13	Ala	5.369	87997	39761	0.252	uM
14	Pro	5.994	46189	23278	0.136	uM
15		6.072	2801	1218		
16	Deriv	6.856	167136	83470	0.638	uM
17	Cys2	7.015	12181	5720	0.039	uM
18		7.053	1266	2020		
19	Lyr	7.092	92793	64392	0.152	uM
20	Tyr	7.192	41770	24242	0.107	uM
21		7.285	1760	1118		
22	Met	7.329	34041	21166	0.094	uM
23	Val	7.447	67450	44861	0.179	uM
24		7.863	2701	1875		
25	Ile	8.085	55703	34872	0.146	uM
26	Leu	8.165	100224	60531	0.269	uM
27	Phe	8.279	51574	30314	0.137	uM
28		8.421	4177	2481		

Table S2-----The third results of amino acid compositions of crude protein F₄₀₋₆₀.

样品信息

样品名称: 6
样品类型: 未知
瓶号: 1:A,7
进样次数: 1
进样体积: 1.00 ul
运行时间: 10.2 Minutes
样品组名称: 苟梦星AA测试

采集者: XCPYFZX
采集时间: 2016/3/29 15:29:54 CST
采集方法组: 苟梦星AA
处理日期: 2016/4/1 18:44:10 CST
处理方法: GMX_AAA
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	1.286	3195	1774		
2 AMQ	1.437	2287212	510003	0.221	uM
3 NH3	1.967	162538	59022	0.527	uM
4 His	2.410	42727	15529	0.132	uM
5 Ser	3.356	215375	73060	0.644	uM
6 Arg	3.534	103364	37526	0.319	uM
7 Gly	3.693	142623	51146	0.442	uM
8 Asp	4.084	242064	91472	0.748	uM
9 Glu	4.611	292993	123777	0.893	uM
10 Thr	5.009	124575	55874	0.370	uM
11 Ala	5.419	211335	93501	0.605	uM
12 Pro	6.030	108301	52448	0.319	uM

用户名称: XCPYFZX

项目名称: 氨基酸2015

色谱峰结果

	名称	保留时间	面积	峰高	含量	单位
13	Deriv	6.876	125389	62435	0.479	uM
14	Cys2	7.029	57014	32996	0.183	uM
15		7.067	4237	8457		
16	Lyr	7.102	277834	193221	0.456	uM
17	Tyr	7.207	84294	53058	0.215	uM
18		7.295	2491	1473		
19	Met	7.342	88469	54512	0.244	uM
20	Val	7.458	183389	115352	0.487	uM
21		7.622	6515	2967		
22		7.690	3046	1444		
23		7.731	2280	1661		
24		7.773	1652	1010		
25		7.870	8532	5495		
26		7.966	3840	2830		
27		8.009	2141	1551		
28	Ile	8.093	128975	80256	0.338	uM
29	Leu	8.172	224400	134181	0.602	uM
30	Phe	8.287	129981	75450	0.345	uM
31		8.426	3531	2117		
32		8.492	4246	3573		

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

样品信息

样品名称: 6
样品类型: 未知
瓶号: 1:A,7
进样次数: 2
进样体积: 1.00 ul
运行时间: 10.2 Minutes
样品组名称: 苟梦星AA测试

采集者: XCPYFZX
采集时间: 2016/3/29 15:40:38 CST
采集方法组: 苟梦星AA
处理日期: 2016/4/1 18:44:37 CST
处理方法: GMX_AAA
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	1.291	3221	1784		
2	AMQ	1.441	2288843	510112	0.221 uM
3	NH3	1.974	162607	58532	0.528 uM
4	His	2.428	42857	15376	0.133 uM
5	Ser	3.368	215676	73343	0.645 uM
6	Arg	3.547	103638	37917	0.320 uM
7	Gly	3.703	142969	51322	0.443 uM
8	Asp	4.094	238237	91483	0.736 uM
9	Glu	4.618	293378	124463	0.894 uM
10	Thr	5.014	124815	56202	0.371 uM
11		5.327	2106	1011	
12	Ala	5.422	211438	93782	0.605 uM

用户名称: XCPYFZX

项目名称: 氨基酸2015

色谱峰结果

	名称	保留时间	面积	峰高	含量	单位
13	Pro	6.033	108569	52564	0.320	uM
14	Deriv	6.877	126578	62895	0.483	uM
15	Cys2	7.030	56736	33150	0.182	uM
16		7.067	4523	9051		
17	Lyr	7.103	278596	193787	0.457	uM
18	Tyr	7.208	84442	52974	0.215	uM
19		7.296	2611	1490		
20	Met	7.344	88597	54567	0.245	uM
21	Val	7.459	183567	115748	0.488	uM
22		7.622	6562	2989		
23		7.691	3073	1457		
24		7.731	2293	1672		
25		7.773	1659	1006		
26		7.870	8531	5508		
27		7.965	3793	2823		
28		8.009	2178	1543		
29	Ile	8.093	129093	80291	0.338	uM
30	Leu	8.173	224710	134240	0.603	uM
31	Phe	8.287	130090	75429	0.345	uM
32		8.427	3529	2122		
33		8.492	4208	3569		

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

样品信息

样品名称: 6
样品类型: 未知
瓶号: 1:A,7
进样次数: 3
进样体积: 1.00 ul
运行时间: 10.2 Minutes
样品组名称: 苟梦星AA测试

采集者: XCPYFZX
采集时间: 2016/3/29 15:51:22 CST
采集方法组: 苟梦星AA
处理日期: 2016/4/1 18:45:03 CST
处理方法: GMX_AAA
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	1.293	3169	1767		
2	AMQ	1.442	2292415	509977	0.221 uM
3	NH3	1.974	162900	58878	0.529 uM
4	His	2.422	43309	15612	0.134 uM
5	Ser	3.364	215819	72820	0.646 uM
6	Arg	3.543	103695	37789	0.320 uM
7	Gly	3.700	143106	51480	0.443 uM
8	Asp	4.090	238215	91311	0.736 uM
9	Glu	4.615	293495	124077	0.894 uM
10	Thr	5.011	124833	56147	0.371 uM
11		5.324	2096	1003	
12	Ala	5.419	211776	93727	0.606 uM

用户名称: XCPYFZX

项目名称: 氨基酸2015

色谱峰结果

	名称	保留时间	面积	峰高	含量	单位
13	Pro	6.031	108508	52588	0.320	uM
14	Deriv	6.876	127014	63077	0.485	uM
15	Cys2	7.029	57375	33204	0.184	uM
16		7.067	4243	8447		
17	Lyr	7.102	278863	194121	0.458	uM
18	Tyr	7.207	84931	53169	0.217	uM
19		7.295	2618	1532		
20	Met	7.342	89055	54641	0.246	uM
21	Val	7.458	184538	115990	0.490	uM
22		7.621	7073	3097		
23		7.689	3411	1569		
24		7.730	2569	1795		
25		7.772	2273	1131		
26		7.869	8934	5597		
27		7.964	3794	2827		
28		8.007	2191	1556		
29	Ile	8.091	129237	80242	0.338	uM
30	Leu	8.171	224902	134138	0.603	uM
31	Phe	8.285	130217	75662	0.345	uM
32		8.425	3522	2103		
33		8.491	4322	3602		

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

	A[X]	B[Y]	C[Y]	D[yEr?	E[Y]	F[yEr?
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	0.028
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 (a) ----- Original data

	A[X]	B[Y]	C[Y]	D[Y]	E[yEr?
1	0.05	1.3	1.25	1	0.06
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

Figure S12 (b) ----- Original data

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