Chemistry of Natural Compounds, Vol. 31, No. 1, 1995

ISOLATION FROM THE WORM *Eisenia foetida* OF PROTEINS AND PEPTIDES POSSESSING ENZYMATIC AND MEMBRANOTROPIC ACTIVITIES

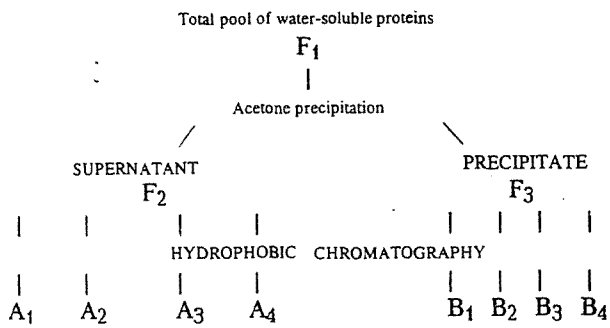
Yu. V. Krasikova, S. O. Kolusheva, O. N. Veshkurova, A. A. Takanaev, and Sh. I. Salikhov

UDC 547.993.577.11

Protein-peptide fractions possessing enzymatic and membranotropic activities have been isolated from the biomass of Eisenia foetida *by fractional precipitation and hydrophobic chromatography.*

Vermicultivation serves as a source of two ecologically unobjectionable products: a humous organic fertilizer and the biomass of *Eisenia foetida*. According to the literature the latter contains a number of biologically active substances. The presence in the biomass of a number of enzymes of interest for modern biotechnological processes is also assumed [1, 2].

We have isolated from this biomass a number of proteins and peptides and have investigated their enzymatic and membranotropic activities. The total scheme of separation of the proteins is given below.



The greatest enzymatic activity was possessed by fraction F_2, which was separated by hydrophobic chromatography into four subfractions, A_1-A_4. Results of enzymatic activity are given in Table 1. Cellulase activity was most pronounced in fraction A_1, alkaline phosphatase activity in fraction A_2, and proteolytic activity in fractions A_3 and A_4.

We have shown previously that the total water-soluble proteins from *Eisenia foetida* possess a fairly pronounced cytotoxic effect [3]. Since the latter is usually linked with a disturbance of the physicochemical properties of biological membranes, we investigated the influence of the fractions isolated on the thermotropic behavior of multilamellar phosphatidylcholine dispersions (Table 2).

The addition of the total fraction F_1 led to a shift of the temperatures of the pretransition peaks and of the main phase transitions of lipids in the high-temperature direction. At the same time a slight increase in $\Delta T_{1/2}$ of the pretransition and of the main phase transition took place, which showed a disturbance of the cooperativity of the transition. The value of ΔH, showing the total enthalpy, scarcely changed, which indicated the participation of all the lipids in the melting process.

In an investigation of fraction F_2, on the addition of 100 γ of the preparation the pretransition peak disappeared and the temperature of the peak of the main phase transition shifted in the higher-temperature direction; the cooperativity of the transition did not change but ΔH fell considerably, which showed a withdrawal of 15% of the lipids from the melting process. These effects were most probably connected with the presence of phospholipids, such presence being confirmed by biochemical methods.

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the the Republic of Uzbekistan, Tashkent, fax (3712) 62 70 71. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 132-135, January-February, 1995. Original article submitted October 17, 1994.

0009-3130/95/3101-0108$12.50

TABLE 1. Enzymatic Activities of Protein Fractions from the Biomass of *Eisenia foetida*

Material	Activity		
	proteolytic, sp. units	cellulase, sp. units	alkaline phosphatase, nM/min/mg
F_1	33.0	0.552	0.50
F_2	50.0	0.998	0.45
F_3	12.0	0.262	–
A_1	–	0.900	–
A_2	–	–	0.66
A_3	65.0	–	–
A_4	40.0		–

TABLE 2. Membranotropic Activities of Protein Fractions from the Biomass of *Eisenia foetida*

Material	$\frac{\Delta H_{п/п}}{\Delta H_{п/п^c}}$	$\frac{\Delta H_п}{\Delta H_п{}^\circ}$	$\frac{\Delta T_{1/2}п \,/\, п}{\Delta T_{1/2}п^\circ \,/\, п.}$	$\frac{\Delta T_{1/2}п}{\Delta T_{1/2}п^\circ}$
F_1	1.75	1.005	2.00	1.35
F_2	0.35	0.852	0.66	1.00
F_3	0.80	0.918	1.60	2.83
B_1	1.80	1.076	1.10	1.35
B_2	–	1.360	–	1.50
B_3	–	1.200	–	1.50
B_4		0.730	–	2.00

Fraction F_3 caused pronounced changes in the thermograms. The temperature of the peak of the main transition shifted sharply — to 26.2°C, and a new high-temperature phase of the lipids was formed which melted at 28.3°C. These processes were accompanied by a sharp increase in $\Delta T_{1/2}$ and a fall in ΔH. In view of the facts that fraction F_3 was the most active in relation to the thermotropic behavior of the lipids and that the influence of fraction F_2 was due to the presence of a phospholipase, the further investigations were conducted with fractions obtained from F_3: B_1, B_2, B_3, and B_4.

Fraction B_1 caused very weak temperature effects revealed by shifts of the pretransition and main phase transition temperatures in the high-temperature direction. Fraction B_2 led to the complete disappearance of the pretransition and to a shift of the temperature of the main phase transition to 24.7°C. The magnitude $\Delta T_{1/2}$ increased 1.5-fold, but the amount of lipids participating in the melting process did not change. In the case of fraction B_3 the pretransition was absent, the temperature [of the peak of the main phase transition (?) — Translator] shifted in the low-temperature direction to 22.8°C, the enthalpy ΔH did not change, and $\Delta T_{1/2}$ increased 1.5-fold. With fraction B_4 there was no pretransition, the peak of the main phase transition shifted to 23.5°C, and a lipid phase with a transition temperature of 24.75°C was formed. The cooperativity of the transition doubled, and 30% of the lipids withdrew from the melting process.

Fraction B_4 influenced the thermotropic behavior of the lipids most actively. This is probably connected with the fact that it was the most hydrophobic and, correspondingly, possessed a high affinity for the model membrane. Fraction B_1 was the least hydrophobic and membranoactive. Fractions B_2 and B_3 were similar in their effects on a membrane, since they caused no change in ΔH, increased $\Delta T_{1/2}$ 1.5-fold and caused the disappearance of the pretransition. They differed with respect to their shift of the temperature of the main phase transition: higher in the case of fraction B_2, and lower in the case of B_3.

The experiments showed a clear difference of the components of the biomass of *Eisenia foetida* with respect to biological activity.

Thus, a vermiculture of *Eisenia foetida* contains valuable biologically active substances from which new enzyme and pharmacological preparations can be created.

EXPERIMENTAL

Eisenia foetida worms were obtained from the firm Apeks. The reagents used were of KhCh ["chemically pure"] or ChDA ["pure for analysis"] grades.

Isolation of the Protein and Peptide Fractions. The biomass of *Eisenia foetida* (100 g) was washed with distilled water to free it from biohumus and was kept in 1 liter of 0.05 M citrate buffer, pH 6.0, at 10°C for 3 h, washed with distilled water, and homogenized, first in a tissue grinder, and then in a Potter homogenizer. The homogenate was treated with 0.025 M ammonium acetate buffer, pH 6.5, in a ratio of 1:2. The mixture was centrifuged at 6000 rpm for 30 min. The supernatant was treated with cooled acetone in a ratio of 1:1, and the resulting mixture was kept at 4°C for 10 h. For a sharper separation, the fractions obtained were centrifuged at 600 rpm for 30 min. The resulting fractions were freed from acetone in a rotary evaporator and lyophilized.

The supernatant and the precipitate were fractionated by hydrophobic chromatography on the sorbent Polikhrom-1 in a 1.5 × 50 cm column with a stepwise concentration gradient of isopropanol (20, 40, 60, and 80%). Rate of flow 60 ml/h, absorption at 280 nm.

Proteolytic activity was determined by Kunitz's method [4], cellulase activity viscosimetrically as in [5], and alkaline phosphatase activity spectrophotometrically [6]. Calorimetric investigations were conducted with the aid of a DASM-4 microcalorimeter (USSR). The suspension of multilamellar liposomes for the calorimetric measurements were prepared from phosphatidylcholine by the method of [7].

REFERENCES

1. US Patent No. 5,024,844 (1987).
2. Yu. Kholodova, The Bioconversion of Organic Wastes for Obtaining Biohumus, Biogas, and Protein Substances and for Protecting the Environment. Proceedings of the First Congress [in Russian], Kiev (1991), p. 104.
3. Zh. F. Ziyavitdinov, A. A. Takanaev, O. N. Veshkurova, N. Zh. Sagdiev, Sh. Ya. Mirzaakhmedov, N. A. Evgrafova, and Sh. I. Salikhov, Khim. Prir. Soedin., 118 (1994).
4. M. Koida and R. Valter, J. Biol. Chem., **254**, 7340 (1976).
5. A. V. Bolobova, A. A. Klesov, and M. L. Rabinovich, Prikl. Biokhim. Mikrobiol., **21**, 807 (1985).
6. M. Kunitz, J. Biol. Chem., **164**, 563 (1946).
7. S. É. Gasanov, F. G. Kamaev, and I. A. Rozenshtein, in: Problems and Prospects of the Development of the Chemistry of Natural and Physiologically Active Substances [in Russian], Fan, Tashkent (1988), p. 360.