研究快报

Cinphorin: a Novel Type Ⅱ Ribosome-inactivating Protein With Miniature Active A-chain*

HOU Fa-Jian, LIU Ren-Shui, LIU Wang-Yi**
(State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, The Chinese Academy of Sciences, Shanghai 200031, China)

Abstract Cinnamomin is a type Ⅱ ribosome-inactivating protein (RIP) isolated from the seed of camphor tree (*Cinnamomum camphora*). Recently, a small RIP named as cinphorin was isolated from the seed of camphor tree. The reduced cinphorin exhibited the RNA N-glycosidase activity and inhibitory activity to protein synthesis *in vitro* like the reduced cinnamomin did. Cinphorin B-chain exhibited the same N-terminal 10 amino acid sequence and molecular mass as the B-chain of cinnamomin. Its A-chain exhibited the same 10 N-terminal amino acid sequence and owned a C-terminal cysteine residue linked to the B-chain, but the molecular mass of cinphorin A-chain was only half of cinnamomin A-chain. RT-PCR and Northern blotting revealed that there was no corresponding mRNA of cinphorin. Cinphorin is probably produced from cinnamomin by protein splicing.

Key words cinnamomin, cinphorin, protein splicing, ribosome-inactivating protein

Plant ribosome-inactivating proteins (RIPs) are a group of toxic proteins that can inactivate the ribosome and thus inhibit protein biosynthesis. Due to the diversity in primary structure of these proteins, they can be divided into three types, of which type Ⅱ RIP is composed of two chains (A- and B- chain)[1]. Cinnamomin is a novel type Ⅱ RIP isolated in our laboratory from the seeds of *Cinnamomum camphora*. Its A-chain (31 ku) that exhibits the RNA N-glycosidase activity is linked by a disulfide bridge to the B-chain (34 ku) with the lectin activity[2]. The structure, function, gene pattern and physiological role of cinnamomin have been intensively studied[3~5]. Recently, an interesting result we obtained is that a small protein was co-bound with cinnamomin on the acid-treated sepharose 4B column and eluted together with cinnamomin by lactose from the column. This small protein was named as cinphorin (46 ku), much less than that of cinnamomin (64 ku). It could be separated well from cinnamomin by gel eletrophoresis (Figure 1). The reduced cinphorin exhibited the RNA N-glycosidase activity and strong inhibitory activity to protein synthesis *in vitro* with the IC_{50} of 1.2 nmol/L, which is comparable to that of the reduced cinnamomin (1.0 nmol/L).

Like cinnamomin, cinphorin consists of two chains connected by the disulfide bond since it could be reduced with 2-mercaptoethanol to produce a miniature A-chain and a normal B-chain (Figure 1). Our results showed that the N-terminal 10 amino acid sequence and molecular mass of cinphorin B-chain are the same compared with the normal cinnamomin B-chain. This indicated that the B-chain of cinphorin is identical to that of cinnamomin. The miniature A-chain owns the entirely identical N-terminal 10 amino acid sequence with that of cinnamomin A-chain. In addition, cinphorin A-chain is linked to its B-chian by the disulfide bond, demonstrating that the C-terminus of cinphorin A-chain owns a cysteine residue like cinnamomin A-chain. These data showed that cinphorin A-chain contains the same N- and C-terminal sequence compared to cinnamomin A-chain. However, cinphorin A-chain owns a molecular mass of 16 ku, only half of that of cinnamomin A-chain (31 ku). How does the cinphorin A-chain produce *in vivo* and how does such a small cinphorin A-chain account for its N-glycosidase activity? These were quite intriguing.

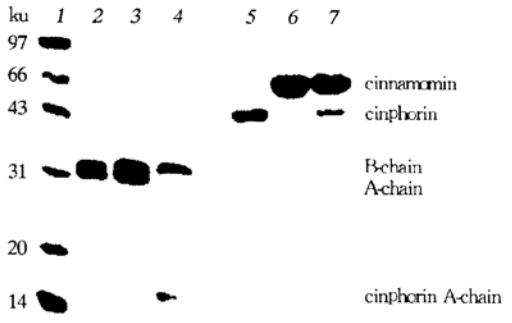

Fig. 1 Cinnamomin (A- and B-chain) and cinphorin (A- and B-chain) revealed by SDS-PAGE (12%)

The protein samples were reduced (2 ~ 4) with 100 mmol/L β-mercaptoethanol or unreduced (5 ~ 7). *1*: protein molecular mass marker; *7*: crude cinnamomin preparation (containing cinnamomin and cinphorin); *2*, *3* and *6*: pure cinnamomin; *4* and *5*: the pure cinphorin.

* This work was supported by a grant from The Chinese Academy of Sciences (KSCX2-02-04) and a grant from The National Natural Science Foundation of China(39970163).

** Corresponding author.
Tel: 86-21-64374430-5238, E-mail: liuwy@sunm.shcnc.ac.cn

Received: June 25, 2002 Accepted: July 12, 2002

The existence of a miniature type Ⅱ RIP related to cinnamomin was further confirmed by the Western blotting, by which it was indicated that another protein of about 45 ku with immune-reaction against the antibody of cinnamomin appears in the blotting membrane[4]. However, RT-PCR did not reveal the mRNA or gene of cinphorin *in vivo*[3, 5]. Northern blotting also showed that the mRNA of cinnamomin only exists as one form in the cotyledons of seeds of *Cinnamomum camphora*[4,5]. It has been known that the tertiary structure and the five active amino acids of cinnamomin A-chain are very similar to that of ricin A-chain. Mutation experiments on ricin A-chain showed that among its 267 amino acids, 222 could be deleted from one another of 74 mutants without loss of the RNA N-glycosidase activity[6]. Based on these facts, we propose that cinphorin A-chain contains all essential amino acids that could form the active site cleft from the N-extein and C-extein of cinnamomin A-chain.

So far, proteins with splicing activity have been found only in unicellular organisms. These proteins were very large (at least 60 ku) and exhibited no enzymatic activity before the splicing[7, 8]. In our case, cinnamomin was isolated from the multicellular organism. Its A-chain is much smaller (31 ku) than other protein precursor with splicing activity. Moreover, cinnamomin A-chain demonstrated the same enzymatic activity before/after the splicing process. All these distinct properties make cinnamomin A-chain a very proper model to study the protein splicing and the molecular evolution of RIP.

References

1 Nielsen K, Boston R S. Ribosome-inactivating proteins: a plant perspective. Annu Rev Plant Mol Biol, 2001, **52** (1): 785~816

2 Ling J, Liu W Y, Wang T P. Simultaneous existence of two types o ribosome-inactivating proteins in the seeds of *Cinnamonum camphora*: characterization of the enzymatic activities of these cytotoxic proteins. Biochim Biophys Acta, 1995, **1252** (1): 15~22

3 Xie L, Wang B Z, Hu R G, *et al*. Structural and functional studies of cinnamomin, a new type Ⅱ ribosome-inactivating protein isolated from the seeds of the camphor tree. Eur J Biochem, 2001, **268** (22): 5723~5733

4 Liu R S, Wei G Q, Yang Q, *et al*. Cinnamomin, a type Ⅱ ribosome-inactivating protein, is a storage protein in the seed of the camphor tree (*Cinnamomum camphora*). Biochem J, 2002, **362** (3): 659~663

5 Yang Q, Liu R S, Gong Z Z, *et al*. Studies of three genes encoding Cinnamomin (a type Ⅱ RIP) isolated from the seeds of camphor tree and their expression patterns. Gene, 2002, **284** (1-2): 215~223

6 Morris K N, Wool I G. Determination by systematic deletion of the amino acids essential for catalysis by ricin A chain. Proc Natl Acad Sci USA, 1992, **89** (11): 4869~4873

7 Paulus H. Protein splicing and related forms of protein autoprocessing. Annu Rev Biochem, 2000, **69** (1): 447~496

8 Perler F B. Protein splicing of inteins and hedgehog autoproteolysis: structure, function and evolution. Cell, 1998, **92** (1): 1~4

新丰毒蛋白*
——一种新的具有活性小A链的核糖体失活蛋白

侯法健 刘仁水 刘望夷**

(中国科学院生物化学与细胞生物学研究所, 上海 200031)

摘要 辛纳毒蛋白是从香樟种子中分离的一种Ⅱ核糖体失活蛋白. 最近, 从香樟种子中还分离到另一种微型双链核糖体失活蛋白, 命名为新丰毒蛋白. 还原的新丰毒蛋白表现出与还原的辛纳毒蛋白同样的RNA N-糖苷酶和体外对抑制蛋白质翻译的活力. 新丰毒蛋白的B链与辛纳毒蛋白的B链具有同样的分子质量和相同的N端10个氨基酸序列. 它的A链N端10个氨基酸序列也与辛纳毒蛋白的A链完全一致, 并且C端与辛纳毒蛋白的A链一样具有半胱氨酸, 但是它的分子质量却只有辛纳毒蛋白A链的一半. RT-PCR和RNA印迹结果表明体内不存在新丰毒蛋白的mRNA. 推测新丰毒蛋白是从辛纳毒蛋白通过蛋白质剪接而产生的, 是一种研究蛋白质剪接的好材料.

关键词 辛纳毒蛋白, 新丰毒蛋白, 蛋白质剪接, 核糖体失活蛋白

学科分类号 Q51

* 中国科学院基金 (KSCX2-2-04) 和国家自然科学基金 (39970163) 资助项目.

** 通讯联系人. Tel: 021-64374430-5238, E-mail: liuwy213@sunm.shcnc.ac.cn

收稿日期: 2002-06-25, 接受日期: 2002-07-12