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The periodicity in the structure of native neurofilaments studied with scanning tunneling microscopy

Jie Liu [a,b,*], Xiangjun Tong [a,b], Shijin Pang [a,b], Zhonghe Zhai [a,b]

[a] *Beijing Laboratory of Vacuum Physics, Center for Condensed Matter Physics, Inst. Physics, Chinese Academy of Sciences, P.O. Box 2724, Beijing 100080, China*

[b] *College of Life Sciences, Peking University, Beijing 100871, China*

Abstract

Neurofilaments (NFs) are composed of neurofilament triplet proteins (NFTPs), NF-L, NF-M and NF-H. The structure of neurofilaments, isolated from bovine spinal cords, was studied with scanning tunneling microscope (STM). STM images of NFs with relatively higher resolution were obtained for the first time. The results showed that NFs revealed as a kind of periodic structure, composed of a long core filament (9–11 nm in diameter) and lateral sidearms projecting from the core filament every 10 nm space. There were undulant structures on the surface of core filament. The periodicity along the core filament was obtained from cross-section analysis. The periods along the axis and two sides of the core filament were uniform to be about 10 nm. In some regions, there was also 20 nm periodicity, indicating the distance between two adjacent long sidearms in the topography, which coincided with the periodic distribution of sidearms observed by metal shadowing electron microscopy. However, the previous half-staggered model could not explain the 10-nm periodicity. According to the results from STM experiments, it could be deduced that three-quarter-staggered organization of subunits must take place during the assembly of native NFs.

Keywords: Neurofilaments; Scanning tunneling microscopy; Periodicity

1. Introduction

Neurofilament (NF) is one of the major components of cytoskeleton in neural system. Its abnormal expression can play a causative role in motor neuron disease [1]. Compared with other types of intermediate filaments (IF), the distinguishing feature of NF lies in that it is constituted by three kinds of subunits —neurofilament triplet proteins (NFTPs): NF-L, NF-M and NF-H [2–4]. NFTPs are all composed of three segments: α-helical rod domain (about 310 amino acids 47 nm in length), non-α-helical NH_2-terminal head domain and COOH-terminal tail domain. NFTPs co-assemble to form core filament 10 nm in diameter, which projects thinner 'sidearms' every 22 nm space according to the observation by metal shadowing electron microscopy [5]. The rod domains of NFTPs are responsible for the formation of core filament. During the assembly process in vitro, NF-L can self-assemble into the 10 nm filament while NF-M and NF-H can only co-assemble with NF-L. They are not able to self-assemble in vitro and their COOH-terminal tail domains are found to contribute to the formation of sidearms [6–11].

* Corresponding author. Tel.: +86-10-62568306; Fax: +86-10-62556598; E-mail: pang@image.blem.ac.cn

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Here scanning tunneling microscopy (STM) was used to study the superstructure of native NFs isolated from bovine spinal cords. Some periodicity characters were found in STM images. It could be deduced from STM images that 3/4-staggered organization happened between rod domains of NFTPs in the assembly process.

2. Materials and methods

NFs were purified from bovine spinal cords according to the method of Hisanaga and Hirokawa [5]. The sample solution was directly dropped onto the surface of newly cleaved HOPG (highly oriented pyrolytic graphite). The STM experiments were performed in ambient environment at constant current mode using domestic STM setup CSPM-930a (manufactured by Institute of Chemistry, Chinese Academy of Sciences). The tungsten tips were made using the method of electrochemical etching. STM image presented here was raw data image without any smoothing or filtering. Measurements of the distances and cross-section analysis were carried out with the image processor.

3. Results and discussion

From the STM image of Fig. 1, three fish-bone-like structures with 9–11 nm in diameter are found. There were periodic protrusions on the surface of backbones while thorn-like thin sidearms laterally spread from the backbones along their entire lengths. The distance between each two adjacent sidearms was approximately 10 nm. The periodicity along the axis and two sides of the middle core filament were revealed by cross-section analysis in Fig. 2.

In Fig. 2, the periodic spaces along the axis and two sides of core filament are found to be uniform. There were peaks nearly every 10 nm along the three curves. Particularly, there were some big leaps every 20 nm space along the upper side of the core filament (curve C) where the 10 nm periodicity was covered up to be indistinct. From the topography in Fig. 1, the relatively long sidearms (indicated by

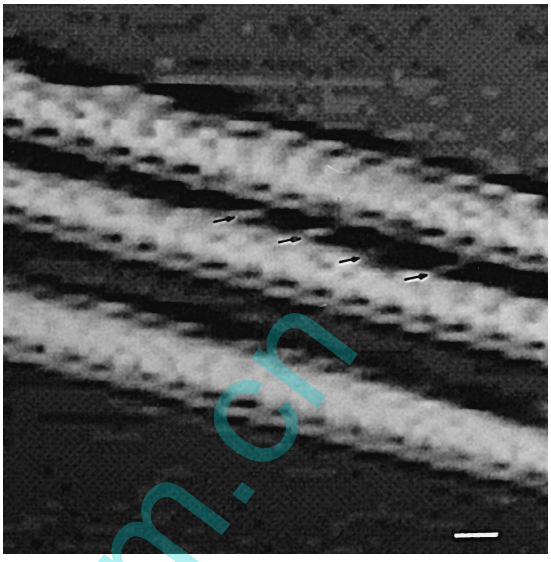

Fig. 1. STM image of native NFs isolated from bovine spinal cords. $I_{ref} = -0.10$ nA; $V_{bias} = 479$ mV; the arrows indicated the long sidearms; bar: 10 nm.

arrows), whose interval is 20 nm are found in the relevant region. And there are short sidearms between the long ones. Moreover, the STM image indicated that the ends of long sidearms were stuck on the proximal upper filament, just like the cross-bridges connecting NFs in vivo [9]. No long sidearms were found in the other regions, where the sidearms regularly arrayed on their own.

During the sampling processes, the sidearms of NFs were very easy to shrink and collapse onto the core filaments, which made it difficult to detect [5]. So it was very difficult to determine the real length of sidearms, but two kinds of sidearms could still be distinguished in the STM image. Only those unshrunk sidearms revealed long, whose ends were fixed onto the other filament while the others all looked shorter, which was deduced as the result of shrinkage. That is to say, it was the influence from the upper filament that prevented the long sidearms from shrinking. Taking it into account that the free longer sidearms might shrink and collapse onto the surface of the core filaments in the course of air drying, some apparently shorter sidearms should be formed from the shrunk longer ones. Because the space between two adjacent longer sidearms was 20 nm and the space between each two adjacent sidearms

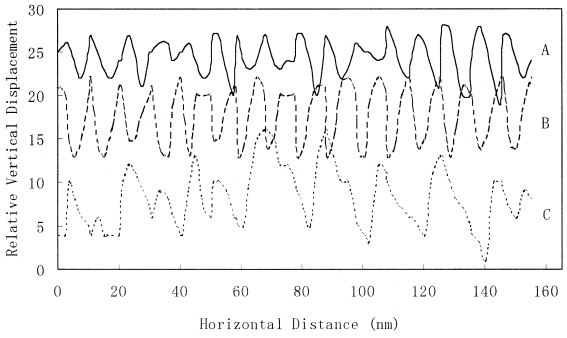

Fig. 2. The periodicity along the middle core filament in Fig. 1 analyzed by cross-section. (A) Along the axis; (B) along the lower side; (C) along the upper side.

was 10 nm, it was reasonable to predict that the longer sidearms and the shorter ones arrayed along the core filaments one by one.

Since there is little difference between NF-M and NF-H, the longer sidearms should be composed of the carboxy-terminal tail domains of NF-H and NF-M, while the shorter ones should be composed of those of NF-L. The carboxy-terminal tail domain of NF-L is very short (142 amino acids) and low phosphorylated. But it was inferred to form 15–20 nm long projection, which might be too short to be detected in most cases [5] but sometimes could be detected by metal-shadowing electron microscopy [7]. Only the long sidearms composed of the tail domains of NF-M and NF-H were long enough to be detected constantly. Due to the high resolution of STM, two kinds of sidearms were distinguished by length.

In the previous studies, some molecular models for NF architecture have been proposed basing on 22-nm periodicity in the observation by metal shadowing electron microscopy and the results from NF-L assembly studies [10,12,13]. According to these models, the 10 nm filament is formed step by step, from monomer to dimer, to tetramer (protofilament), to octamer (protofibril), to 10 nm filament. Half-staggered organization takes place between rod domains of NFTPs, which leads to 22 nm periodicity. The COOH-terminal tail domains of NF-M and NF-H project from the 10 nm core filament as sidearms. However, the detailed assembly mechanism is not clear so far. In addition, the models above mainly result from the studies of the assembly of NF-L in vitro. It has not been determined whether the assembly model in vitro is the same as that in vivo and whether the architecture of filament constructed by NF-L alone is the same as that of native NF, a composite of NF-L, NF-M and NF-H.

In any case, half-staggered model can not explain 10 nm periodicity resulted from our STM observation of native NFs. It is easily deduced that 3/4-staggered (or 1/4-staggered) organization and half-staggered organization of NFTPs must take place simultaneously during the assembly process so as to produce 10 nm periodicity (as indicated in Fig. 3). In other words, 10 nm periodicity would emerge if only one kind of subunits align in 3/4 staggered fashion

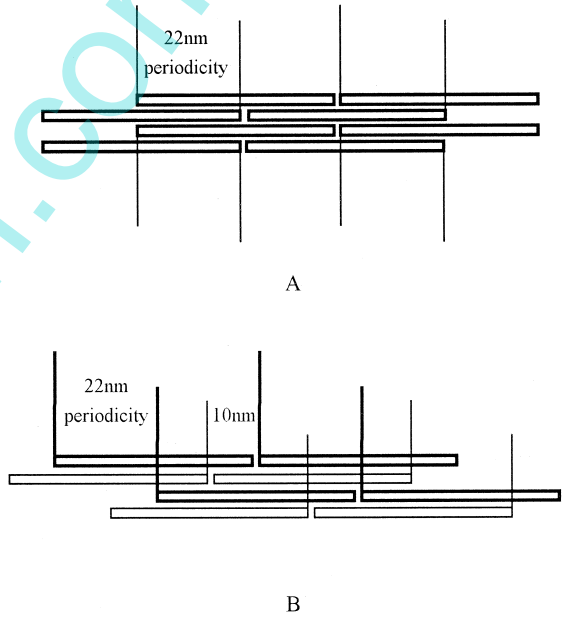

Fig. 3. The schematic representation of NF architecture. (A) 1/2-staggered model; (B) 3/4-staggered model. The open bar represents a kind of subunit: dimer, tetramer, or octamer. One straight line is used to indicate the sidearm projected from the relative subunit, in which monomers align in register and parallel and all their tail domains twist together to form one sidearm. In (B), the brunet bar represents the subunit containing NF-L and NF-M or NF-H, which produces long sidearm. Then the bar with light color represents the subunit only containing NF-L, which produces short sidearm. In this scheme, antiparallel is accompanied by 3/4-staggered organization. The coincidence of antiparallel and half-staggered organization will lead to the same result.

and another kind of subunits align in half-staggered. In fact, 3/4 staggered organization will undoubtedly lead to half-staggered organization between two higher-level subunits. According to the previous studies, there are only three kinds of dimers: NF-L/NF-L, NF-L/NF-M, and NF-L/NF-H [7,14]. So each subunit contains NF-L. In view of the existence of two kinds of sidearms, there must be two kinds of subunits, one containing NF-M and NF-H with long tail domains besides NF-L, the other only containing NF-L with short tail domains. Because the long sidearms and short ones array along the core filaments one by one, 3/4-staggered organization must happen to the different type of subunits (hetero-subunits) while 1/2-staggered organization happens to the same subunits (homo-subunits). So there is only 1/2-staggered arrangement of subunits in the NFs assembled from NF-L alone. In order to avoid polarity, some kind of subunits must align in antiparallel. So far, it can not be determined in which stages 3/4-staggered and antiparallel organizations happen. Further evidences are needed to confirm and supplement this 3/4-staggered organization of NF subunits.

Acknowledgements

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