알츠하이머병의 아밀로이드 펩타이드 섬유화 원인 밝힌다

• 교신저자 : 김선희(서울서부)

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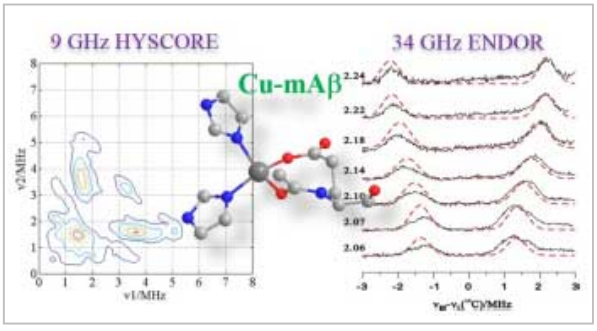
연구내용

전자상 자기공명(EPR; Electron Paramagnetic Resonance)법을 이용하여 쥐의 아밀로이드 펩타이드(Murine Amyloid b Peptide)와 구리의 결합구조를 규명하였음.

뇌 내 아밀로이드 섬유 침착 현상은 뇌 단백질의 일종인 아밀로이드 펩타이드가 집적(aggregation) 되어 섬유조직(fibrils)을 형성, 이 섬유조직이 모여 플라크(Plaques)를 형성하는 것으로, 아밀로이드 펩타이드와 금속이온(구리)의 결합에 밀접한 관련 있는 것으로 알려져 있음. 인간과 달리 치매에 걸 리지 않는 쥐의 경우 아밀로이드 펩타이드가 금속 이온과 결합하는 과정에서 섬유화가 일어나지 않 아 이 원인을 밝히려는 연구가 이어져 왔는데, 이 번 연구를 통해 쥐의 아밀로이드 펩타이드와 금속 이온을 결합 해주는 아미노산이 인간과 다름을 밝혀냄.
이번 연구는 다중주파수 (9/34 GHz)의 전자상자기공명장치의 펄스기법 '이차원 전자스핀메아리변조법'의 일종인 'HYSCORE'와 '전자-핵 이중공명'등 다중기법의 전자상자기공명법을 통해 이뤄졌음. 자기공명법의 하나인 전자상자기공명은 상자기성 을 띠는 물질을 연구하는데 있어서, 다른 분광학적 방법으로 얻을 수 없는 중요한 구조적 정보를 얻을 수 있는 방법으로, 이번 연구에 사용 된 CW/Pulse EPR 시스템은 국내에서 유일하게 KBSI가보유하고 있음.



[그림 1] 이차원 전자스핀메아리변조 스펙트럼(왼쪽), 전자-핵 이중공명 스펙트럼(오른쪽), 금속이온인 구리와 쥐(murine) 아밀로이드 펩타이드의 결합구조(가운데)

기대효과

알츠하이머병을 유발하는 주원인으로 알려진 뇌내 아밀로이드 섬유 침착(沈 着) 현상 관련, 쥐의 아 밀로이드 펩타이드와 금속이온의 결합구조가 분자 수준에서 규명됨에 따라 향후 알츠하이머병 치료 제의 개발에 한 걸음 더 다가서게 될 전망임. 인간과 쥐의 차이점 연구를 통해 향후 알츠하이머 치료제 개발에 적극 활용할 수 있을 것으로 기대됨.

Angewandte

Binding Modes in Peptides

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Multi-Frequency, Multi-Technique Pulsed EPR Investigation of the Copper Binding Site of Murine Amyloid β Peptide**

*Donghun Kim, Jeong Kyu Bang, and Sun Hee Kim**

Abstract: *Copper-amyloid peptides are proposed to be the cause of Alzheimer's disease, presumably by oxidative stress. However, mice do not produce amyloid plaques and thus do not suffer from Alzheimer's disease. Although much effort has been focused on the structural characterizations of the copper-human amyloid peptides, little is known regarding the copper-binding mode in murine amyloid peptides. Thus, we investigated the structure of copper-murine amyloid peptides through multi-frequency, multi-technique pulsed EPR spectroscopy in conjunction with specific isotope labeling. Based on our pulsed EPR results, we found that Ala2, Glu3, His6, and His14 are directly coordinated with the copper ion in murine amyloid β peptides at pH 8.5. This is the first detailed structural characterization of the copper-binding mode in murine amyloid β peptides. This work may advance the knowledge required for developing inhibitors of Alzheimer's disease.*

Alzheimer's disease (AD) is a well-known neurodegenerative disease that has received considerable attention as the population of patients suffering from this disease grows rapidly.[1] The most common pathological feature of AD is the extracellular deposits of amyloid β-peptide (Aβ) plaques, and interestingly, the concentration of metal ions such as copper in the plaques is high.[2] This finding leads to the hypothesis of metal-induced amyloid fibrillation by oxidative stress owing to the reaction between redox-active metal ions and oxygen.[3] Because a detailed knowledge of the coordination environment of copper is required to understand the molecular mechanism of the amyloid fibrillation event, extensive efforts have been focused on elucidating the coordination of copper ions in human amyloid β peptides (hAβ).[4–7]

Murine amyloid β peptides (mAβ), however, differ from hAβ in three amino acids: R5G, Y10F, and H13R. It has been shown that these three different amino acid residues make mAβ soluble, without peptide fibrillation, and thus, Aβ deposition is not encountered in rats.[8] Furthermore, copper has long been known to ligate to the mAβ, and the coordination of Cu^{II} in mAβ has been proposed to be different from that in hAβ.[7,9] However, the detailed structure of Cu^{II}-mAβ has not been thoroughly studied.[9,10] To better understand the AD mechanism, investigations involving mouse animal models are essential. Therefore, it is of particular importance to elucidate the structure of the copper-coordinated murine amyloid peptide (Cu-mAβ), which may provide insight into the aggregation mechanism of amyloid peptides.

In this work, we employed multi-frequency, multi-technique pulsed electron paramagnetic resonance (EPR) spectroscopy to explore the details of the copper coordination of mAβ. A combination of 9 GHz hyperfine sublevel correlation (HYSCORE) and 34 GHz electron nuclear double resonance (ENDOR) spectroscopy along with specific isotope labeling is transcendently useful for identifying the coordination environment of the copper binding site in mAβ. A set of specifically isotope-labeled mAβ peptides consisting of uniformly ^{13}C- and ^{15}N-labeled Ala2, ^{13}C- and ^{15}N-labeled Gly3, ^{15}N-labeled His6, and ^{15}N-labeled His14 was used.

The CW-EPR of Cu-mAβ at pH 7.4 exhibits two distinct sets of hyperfine peaks of the copper ion. This EPR spectrum can be effectively reproduced in simulations involving two different copper species (Supporting Information, Figure S1). At the pH was raised to 8.5, the CW-EPR of Cu-mAβ showed only one species, which is comparable to the results observed for Cu-mAβ.[10] The spectrum at pH 8.5 can be well simulated using $g = [2.23, 2.06, 2.06]$ and $A = [154, 14, 14]$ G (Supporting Information, Figure S1). Thus, owing to the complexity of the interpretation, we will exclusively focus on examining the coordination environment of the Cu-mAβ at pH 8.5. Furthermore, no effect of isotope labeling on the EPR spectra was observed (Supporting Information, Figure S2). Thus, high-resolution pulsed EPR techniques were used to detect hyperfine interactions not observed in the CW-EPR.

To probe the detailed coordination environment of the copper site in mAβ, 9 GHz HYSCORE experiments were conducted. A two-dimensional HYSCORE spectrum consists of cross-peaks that represent correlations between the nuclear frequencies of one electron spin manifold and the nuclear frequencies of the other electron spin manifold, simplifying the analysis of spectra.[11] Figure 1a displays the HYSCORE spectrum of natural-abundance Cu-mAβ, which shows the cross-peaks near (4.0, 1.6) and (1.6, 4.9) MHz. These peaks have been assigned to the double-quantum of the amino nitrogen nucleus of histidine (His) directly bound to

[+] D. Kim, Dr. S. H. Kim
Western Seoul Center, Korea Basic Science Institute
Seoul 120-140 (Korea)
E-mail: shkim@kbsi.re.kr
Dr. J. K. Bang
Division of Magnetic Resonance, Korea Basic Science Institute, Ochang
Chungbuk 363-883 (Korea)

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