โครงสร้างของพันธะไดซัลไฟด์ของโปรตีนในมูกคอปากมดลูก

โกสุม จันทร์ศิริ, Ph.D. (Biochemistry)*

บทคัดย่อ หลังจากทำลายพันธะไดซัลไฟด์ของโปรตีนในมูกคอปากมดลูกโดยใช้ β-mercaptoethanol พบว่า โปรตีนที่ได้ถูกแบ่งออกเป็น 3 กลุ่ม คือ กลุ่มแรกได้แก่โปรตีนที่มีขนาดเล็กลงหลังจากที่พันธะไดซัลไฟด์ถูกทำลาย กลุ่มที่สองได้แก่โปรตีนที่มีขนาดเพิ่มขึ้นหลังจากที่ทำปฏิกิริยากับ β-mercaptoethanol กลุ่มสุดท้ายได้แก่โปรตีนที่มีขนาดไม่เปลี่ยนแปลงผลการทดลองชี้ให้เห็นว่า โปรตีนในมูกคอปากมดลูกถูกเชื่อมกันด้วยพันธะไดซัลไฟด์ 2 ชนิด คือ ชนิดที่เกิดภายในโปรตีน และชนิดที่เกิดระหว่างโปรตีน พันธะไดซัลไฟด์ทั้งสองชนิดนี้เป็นพันธะที่สำคัญในการยึดเหนี่ยวและการคงสภาพของโครงสร้างของโปรตีนในมูกคอปากมดลูก

Abstract **The infrastructure of disulfide bonds in cervical mucus**

Kosum Chansiri, Ph.D. (Biochemistry)*

After disrupting disulfide bridges in the cervical mucus by reduction with β-mercaptoethanol, the reduced protein products of three catagories were generated. The first group represented the proteins that decreased in size after reduction. The second group represented the proteins whose the size increased after incubating with reducing agent. The third group was the protein bands that remained unchange. The results indicate the existence of both intramolecular and intermolecular disulfide bonds in the infrastructure of cervical mucus. The integrity of the disulfide bonds are essential in the formation and stabilization of the cervical mucus structure. (SM J 1994; 2 : 1-5)

Introduction

Cervical mucus in known to be composed of the insoluble mucin[1] and the soluble proteins.[2] The peptide chains of both mucin and soluble proteins contain a certain number of sulfur-containing amino acids (Cysteine and Methionine).[3] The sulfur contents can establish the disulfide bridges for the structural stabilization of the mucus. This protein disulfide bond could be cleaved by reducing agents resulting in the abolishment of mucus viscosity.[4]

This study was designed to gain the insight arrangement of disulfide bonds in the infrastucture of cervical mucus. The reslults obtained could provide the basic knowledge of the formation and stablization of the mucus structure.

* ภาควิชาชีวเคมี คณะแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ ประสานมิตร กรุงเทพฯ
Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand.

Materials and methods

Samples

Cervical mucus samples were collected from normal, healthy and fertile women. These samples were aspirated by using the hollow plastic rod which was connected to the bulb followed by transferring to the clean plastic tubes and kept on ice. Samples contaminated with blood were discarded.

Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was the discontinuous slab gel performed according to a modified method of Laemmi's (1970).[5] All slab gels were cast in an exponential-linear gradient fashion from 10-15% of acrylamide concentration. The stacking gel 4.65% acrylamide were used. Electrophoresis was carried out in tris-glycine electrode buffer, pH 8.3 at 150 Volts. The samples were stained with Coomassie Brilliant Blue R-250 and visualized by destaining in 5% methanol and 7% acetic acid.

Sample preparation for SDS-PAGE

Cervical mucus during menstrual cycle were sonicated to decrease viscosity. The cervical mucal proteins were determined by Lowry assay. The mucus was dissolved in 2.3% SDS, 10% glycerol, 0.0625 M Tris HCl pH 6.8. The mixture were boiled for a few minutes before subjecting to 5-15% SDS-PAGE.

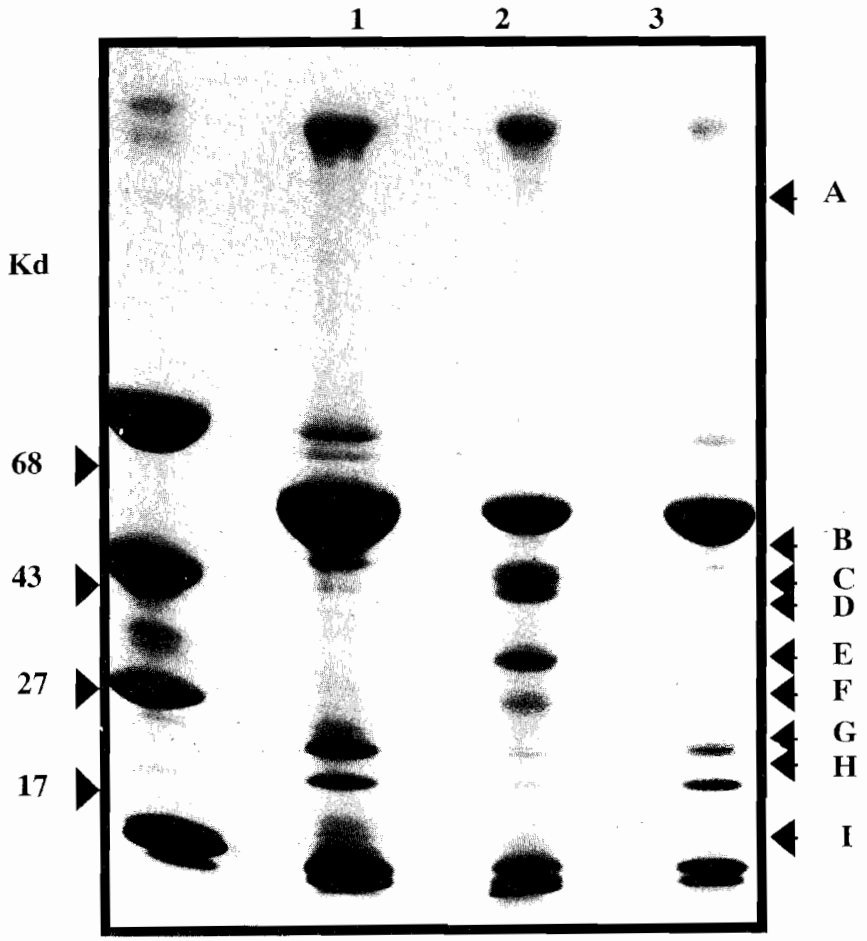

Fig. 1 SDS-PAGE pattern of cervical mucal proteins during menstrual cycle treated with 2.3% SDS, 10% glycerol and 0.0625 N Tris HCl pH 6.8 in the absence of β-mercaptoethanol. Lanes 1-3 represent the cervical mucus obtained from preovulatory, midcycle and postovulatory periods respectively. Bands A, B, C, D, E, F, G, H and I are the proteins of the molecular weight 160, 56, 47, 43, 33, 25, 23, 20 and 17-11 Kd.

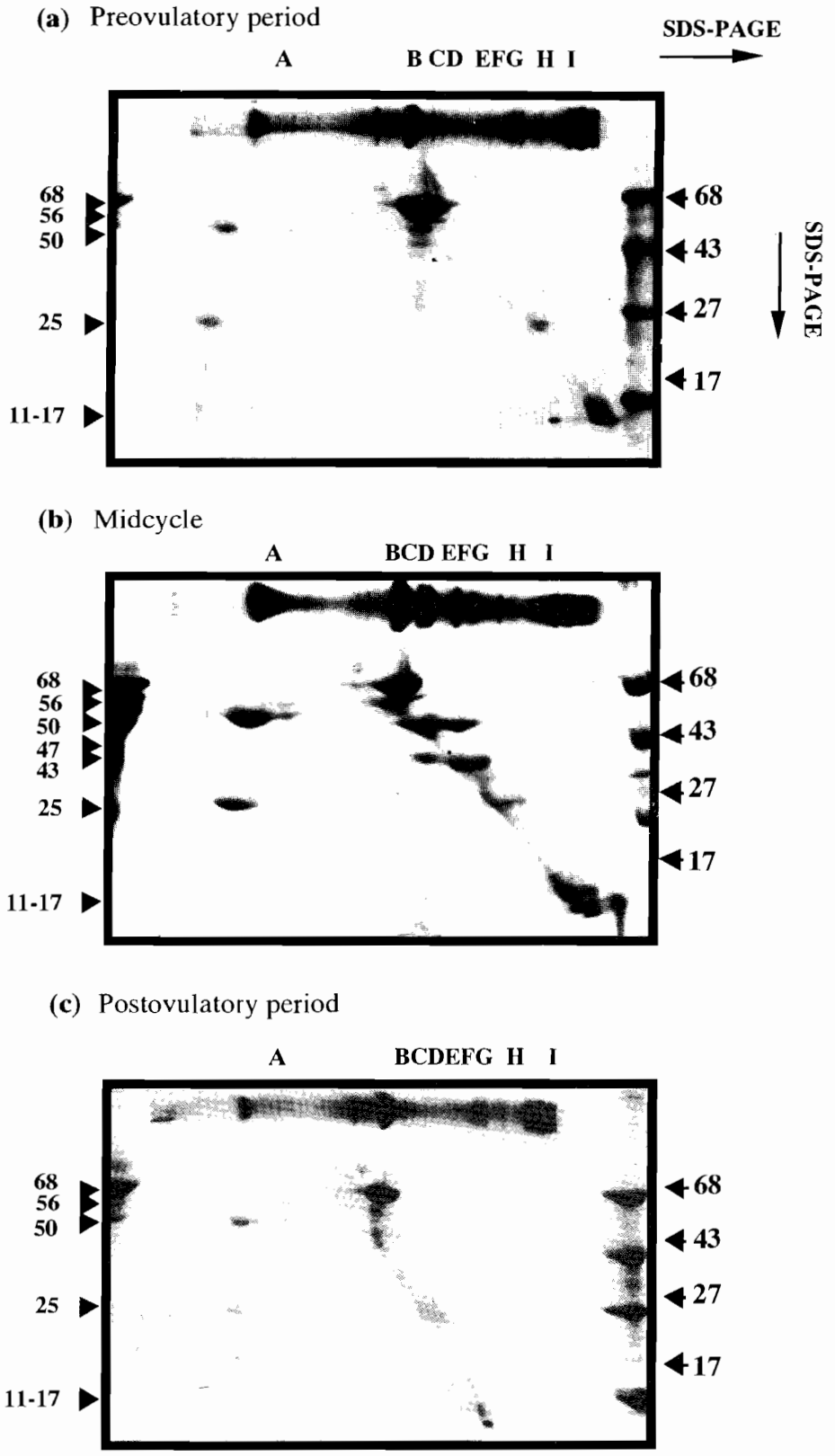

Fig. 2 Two-dimensional SDS-PAGE protein pattern of cervical mucus during menstrual cycle. First dimension was performed in the absence of β-mercaptoethanol and a duplicate of the original tube gel (first dimension) has been placed on the top of the gel after equilibrating with 2.3% SDS, 10% glycerol, 0.0625 M Tris HCl and 100 mM β-mercaptoethanol for at least one hour and electrophoresed in the second dimension.

Results

Protein disulfide bonds were known to serve as structural elements and stabilize the fold integrity of the mucus. These bonds could be destroyed by mild chemical reductants such as β-mercaptoethanol and dithiothreitol. In order to test for the intermolecular and intramolecular disulfide bonds in the cervical mucal proteins, two-dimensional SDS-PAGE patterns were investigated. Figure 1 demonstrated the first dimension (SDS-PAGE) of cervical mucal proteins during menstrual cycle treated with buffer in the absence of β-mercaptoethanol as indicated in Materials and Methods section. The distribution of the protein products were 160 (A), 56 (B), 47 (C), 43 (D), 33 (E), 25 (F), 23 (G), 20 (H) and 11-17 Kd (I). The proteins C, D, E and F were prominent at midcycle. Second dimension (SDS-PAGE) of these proteins was done in the presence of β-mercaptoethanal. Figure 2 revealed that the reduced protein products were separated into three catagories. The first group represented the proteins that decreased in size after incubating with reducing agent. In this group, there was one major protein of the molecular weight 160 Kd (A). This band was cleaved to yield the smaller molecular weight of 50 and 25 Kd. The second group represented the proteins which increased in size after reduction, for example, the bands that have molecular weight 56 (B), 33 (E), 25 (F), 23 (G) and 20 Kd (H). The last group was the protein bands that remained unchange. The proteins in this group were the bands of the molecular weight 47 (C) and 43 (D) Kd.

Discussion

One of factors involved in maintaining the structure of cervical mucus was disulfide linkage. Reduction of the mucus with β-mercaptoethanol under denaturing condition caused the destruction of the structure. This study shows that the cervical mucus contained both intermolecular and intramolecular disulfide bonds in the infrastructure. The 68 Kd protein was revealed the presence of intramolecular disulfide bond whereas the 50 and 25 Kd proteins were the cleavage products of the intermolecular disulfide bridge in 160 Kd protein. These linkages were reported to involve in the structural maintainance of other biochemical substances e.g. immunoglobulins[6] and corticotropin[4] as well as the other mucus secreted from respiratory organ[7] and small intestine.[8] It could be summerized that the native structure of the cervical mucus (without treating by sulfhydryl reagent) was complicated due to the existence of both types of disulfide linkages.

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