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Oscillatory reaction of chondroitin sulfate induced by gradual introduction of calcium ion

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Abstract

Rhythm is an important dynamic behavior in biological systems. We have been studying oscillatory reactions of enzymes induced by gradual entry of substances through semipermeable membrane. Not only enzymes but also a few species of substance of living system have been elucidated to cause oscillatory reaction. Here we present the oscillatory reaction by chondroitin sulfate in a system of gradual entry of calcium ion. Introducing calcium ion through dialysis membrane into chondroitin sulfate solution induces an oscillation of free calcium ion concentration in chondroitin sulfate solution. Simultaneously, it is elucidated that oscillation of conformation occurs with permeation of calcium ion. In both measurements, oscillations with 25 h period are obtained. The phases of oscillation, however, differ slightly from each other. From these results, it is suggested that autocatalysis exerts in the contraction of chondroitin sulfate conformation. These phenomena are very intriguing for elucidating oscillation in living system.

Keywords: Chondroitin sulfate; Calcium ion; Oscillatory reaction

One of the characteristic phenomena in living system is rhythm. It occurs at all levels of biological organization, from unicellular to multicellular organisms. Their periods range from the order of second to years. Goldbeter recently reviewed computational approaches to cellular rhythms [3]. In the field of biological chemistry, oscillatory reaction by allosteric effect or autocatalysis is well known [4–6]. We have, however, found oscillatory reaction of enzyme caused by the other factor. Enzymes have essentially a nature of nonlinearity in their reactions. Consequently, slight fluctuation from stationary state has a possibility that causes the oscillatory reaction. With the use of semipermeable membrane or oil/water system, we found that the gradual entry of substrate into enzyme solution caused the oscillatory reaction [1,7,8]. By this method, we revealed that many enzymes, which are neither allosteric nor autocatalytic, caused the oscillatory reaction [2]. Besides underlying cause by gradual entry of the substrate, the elution rate of the product is also an important factor for causing oscillatory reaction. These results suggest a possibility of oscillatory reaction by materials other than enzymes in the system, in which both gradual entry of reactants and elution of products occur simultaneously. For example, we found that the concentration of calcium ion oscillated when toluene solution including phospholipid, which transferred from toluene phase to aqueous phase, was layered on the aqueous solution of calcium ion [8]. The other biomaterials are also considered to cause an oscillatory reaction under the condition of gradual introduction of substance. Here, we show the oscillatory reaction in the interaction of chondroitin sulfate and calcium ion.

Chondroitin sulfates are glycosaminoglycans that are found naturally in the body and are important in maintaining elasticity and integrity of many types of body tissues, including connective tissue and the walls of blood vessels. Hydrocarbon chains of glycosaminoglycan are attached to polypeptide backbone in complex macromolecules known as proteoglycans. Chondroitin sulfate is known to have a conformation of extended hollow helix [9]. In addition, chondroitin sulfate has negative charges

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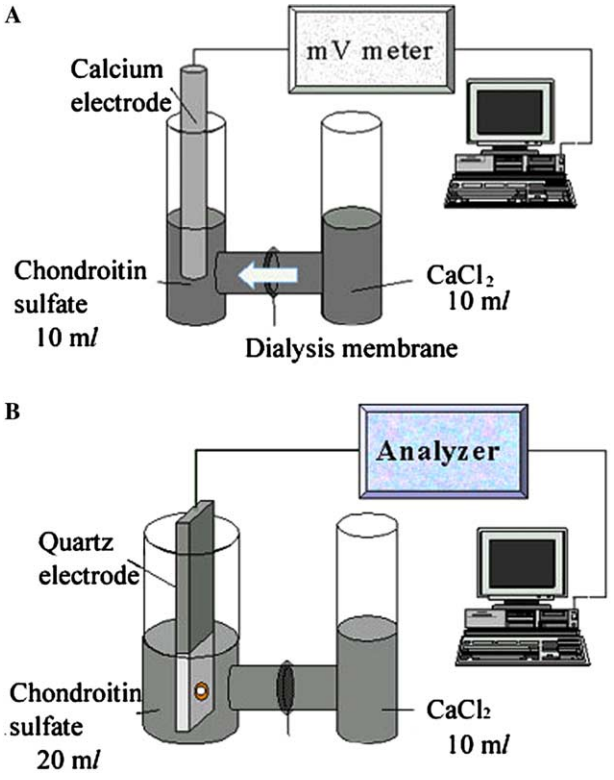

Fig. 1. A schematic illustration of the apparatus used for the experiments. (A) Apparatus for measuring calcium ion concentration. (B) Apparatus for measuring deficit resistance.

of high density due to sulfate groups along the hydrocarbon chain. Negative charges attract water. Hydration maintains the compressibility, elasticity, and fluidity of joint movement characteristic of cartilage.

Materials and methods

Chondroitin sulfate sodium salt, HCl, and calcium carbonate were purchased from Wako Pure Chemical industries. It was used without further purification. Solution of calcium chloride was prepared by adding HCl to calcium carbonate.

Semipermeable membrane [dialysis membrane (Seamless Cellurose Tubing, Viscase Sales)] was put between two glass cells as shown in Fig. 1. Solution of sodium salt of chondroitin sulfate was poured into the one cell and solution of calcium chloride was put into another cell. Calcium electrode (Fig. 1A) or electrode of quartz crystal (Fig. 1B) was inserted into the solution of chondroitin sulfate for measuring the concentration of calcium ion or viscosity of chondroitin sulfate solution.

For measuring [Ca^{2+}], Toa Ion-Meter IM 40-S with Orion Calcium electrode 9300BN was used. For measurement of deficit resistance or viscosity, SEIKO EG&G quartz chemical analyzer QCA917 was used.

The apparatus composed of two cells was put in a water bath of 25 °C. The data were obtained at the interval of 20 s.

Results and discussion

For measuring oscillation, we prepared two glass cells with dialysis membrane sandwiched between them

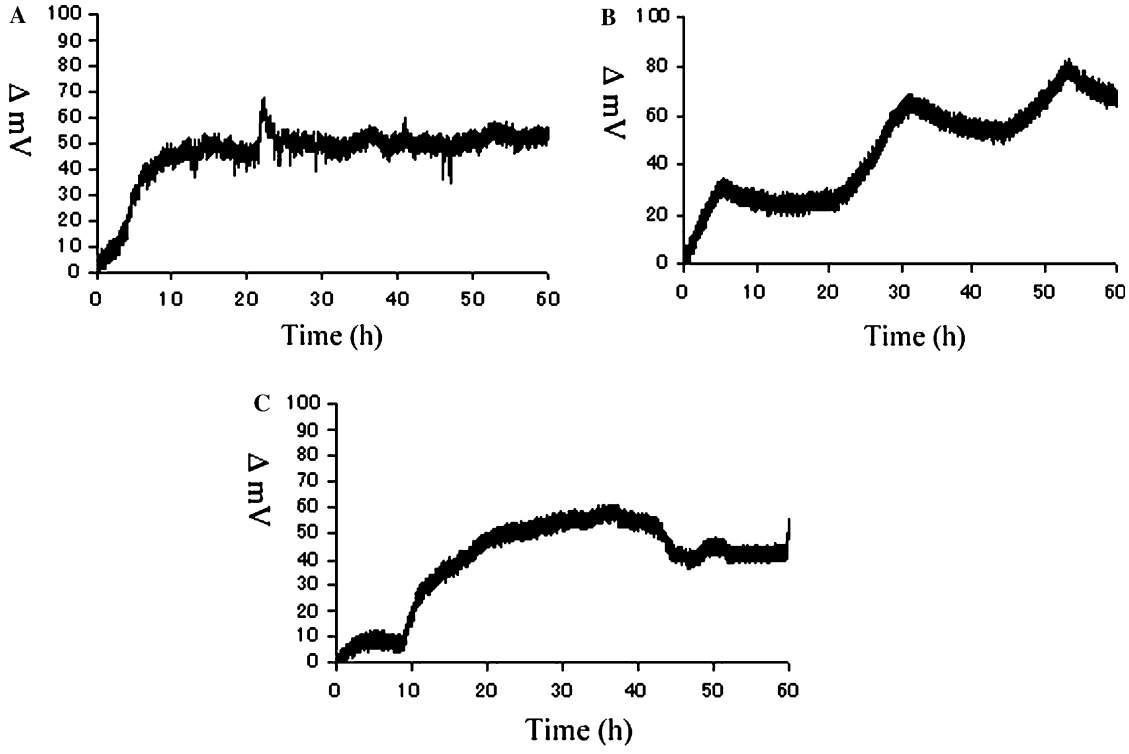

Fig. 2. Time course of electric potential measured by calcium electrode. (A) Each initial concentration is 2.0 mg/ml for sodium salt of chondroitin sulfate and 1 mM for calcium chloride. (B) Each initial concentration is 2.0 mg/ml for sodium salt of chondroitin sulfate and 5 mM for calcium chloride. (C) Each initial concentration is 2.0 mg/ml for sodium salt of chondroitin sulfate and 10 mM for calcium chloride.

(Fig. 1A). One cell dissolved sodium salt of chondroitin sulfate, while the other cell calcium ion. Calcium ion is able to permeate through semipermeable membrane and binds with chondroitin sulfate. In order to measure the free calcium ion concentration in chondroitin sulfate solution, we measured the electric potential with the use of calcium electrode. The electric potential rises as [Ca^{2+}] increases.

As shown in Fig. 2, the electric potential increased accompanying oscillation. When initial concentration of calcium ion was 5 mM, clear oscillation of the period of 25 h with upward peaks was obtained singularly. In the rise in concentration of chondroitin sulfate from 2 mM to 5 mM, oscillation with a period of 25 h remained at a fixed initial concentration of calcium ion (5 mM). The fall in potential means binding of calcium ion with chondroitin sulfate, while the rise in potential reflects the dissociation of binding between calcium ion and sulfate. Therefore, it was found that the repetition of binding and dissociation between calcium ion and sulfate groups of chondroitin sulfate occurred from Fig. 2. To obtain clear oscillation appropriate combination of rate constants (of binding and dissociation) and permeation rate of calcium ion across membrane is thought to be required. Because the oscillation became irregular when membrane with larger pore size, which raised the permeation rate, than dialysis membrane was used.

On the other hand, it is considered that the conformation of chondroitin sulfate changes as the extent of binding varies. The change of conformation should cause the viscosity change of solution. So, in order to investigate the time sequence of viscosity, we measured time course of deficit resistance by using quartz oscillator [10]. The deficit resistance, R, is related to the viscosity as shown in the following equation:

$$R = (2\pi\rho\eta)^{1/2} A\kappa^2,$$

where κ is an electromechanical coupling factor, A is the electrode area, and ρ and η are the density and viscosity of the liquid, respectively. Consequently, the rise in deficit resistance denotes the increase in the viscosity of solution. Fig. 3 shows the time course of deficit resistance. As shown in this figure, it was found that not only calcium oscillation, which is shown in Fig. 2, but also oscillation of viscosity occurred. Clear oscillations of the period of 25 h with downward peaks appeared at 5 mM of initial calcium concentration. The periods were consistent with those measured by calcium electrode. Also, the periods were constant in varying chondroitin sulfate concentration as well as those of calcium electrode.

Results of two methods are gathered in Fig. 4.

Although the periods agree well in both cases, the phases were different by about 5 h. This difference might be interpreted as follows: at first, the binding of chondroitin sulfate with calcium ion begins as calcium ion permeates. As the binding proceeds, the dehydration

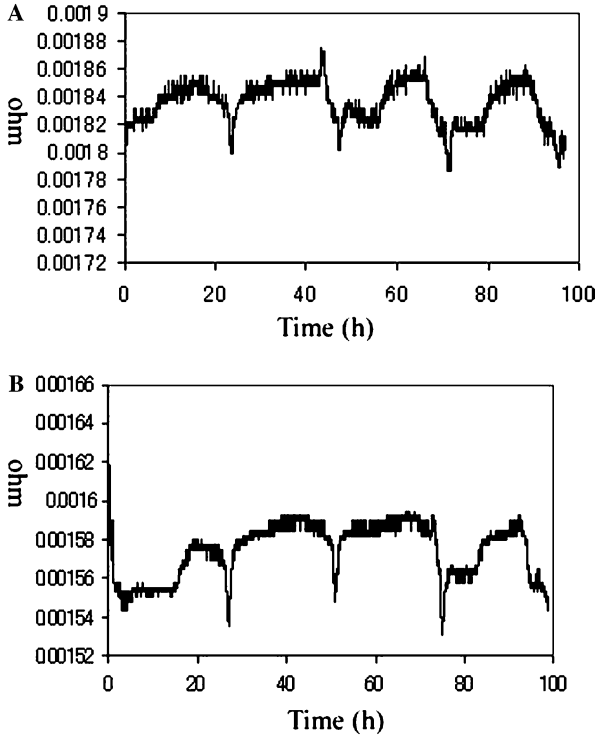

Fig. 3. Time course of deficit resistance measured by quartz oscillator. (A) Each initial concentration is 2.0 mg/ml for sodium salt of chondroitin sulfate and 5 mM for calcium chloride. (B) Each initial concentration is 5.0 mg/ml for sodium salt of chondroitin sulfate and 5 mM for calcium chloride.

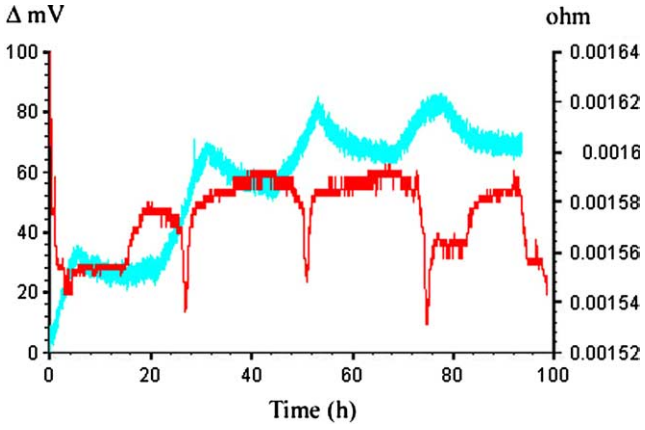

Fig. 4. Time course of electric potential and deficit resistance. Each initial concentration is 2.0 mg/ml for sodium salt of chondroitin sulfate and 5 mM for calcium chloride.

and contraction of chondroitin sulfate conformation takes place. When the binding between Ca^{2+} and chondroitin sulfate and the contraction of conformation occurs to the some extent, further binding and contraction of conformation takes place abruptly. At this point, some autocatalytic mechanisms seem to function. The duration of contraction, however, is very short and release of Ca^{2+} and hydration occurs soon. After the free calcium ion concentration reaches the maximum,

the contraction of the conformation begins also. Thus, the repetition of contraction and extension occurs.

In the present paper, we carried out the study on the basis of an idea that the oscillatory reaction of biopolymers was caused by gradual introduction of a small substance that binds with macromolecule. Our experimental results confirm this hypothesis for chondroitin sulfate. We used a simple model as a model of biomembrane. But, in a more complicated membrane, similar results seem to be gained. It is likely that oscillatory reactions occur generally in everywhere of living system. Phenomena presented here are very interesting for elucidating oscillation of biomaterials, including circadian rhythms.

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