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Capillary zone electrophoresis characterization of low molecular weight heparin binding to interleukin 2

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Abstract

A method based on capillary zone electrophoresis (CZE) was used to study the interaction between low molecular weight heparin (LMWH) and interleukin 2 (IL-2). The results showed that the increase of the concentration of LMWH led to the decrease of the peak height and the increase of the peak width of IL-2, but the peak areas were kept constant. The binding constant of IL-2 with LMWH was calculated as $1.2 \times 10^6\ M^{-1}$ by Scatchard analysis, which is in good agreement with the results found in the references using enzyme-linked immunosorbent assay (ELISA). The results demonstrated that the interaction between IL-2 and LMWH is of fast on-and-off kinetic binding reaction. CZE might be used to study not only slow on-and-off rates interactions, but also fast on-and-off rates ones. The binding constant can be calculated easily, and the method can be applied to study a wide range of heparin–protein interactions.

Keywords: Low molecular weight heparin; Interleukin 2; Capillary zone electrophoresis; Interaction

1. Introduction

An increasing number of proteins are found to bind not only to high affinity cell surface receptors, but also to glycosaminoglycans, particularly the heparin/heparin sulfate (HS) family [1]. These polysaccharides are linear, with highly sulfated chains and extremely heterogeneous in structure [2]. Heparin, a highly sulfated variant, composes of repeating disaccharide units consisting of hexuronic acids linked to glucosamine units by $\alpha(1 \rightarrow 4)$ bonds [3]. It is well known that heparin interacts with many biologically important proteins such as proteases inhibitors, extracellular signaling molecules, lipid- or membrane-binding proteins and adhesion proteins. Some of the interactions are mediated by specific intra-chain sequence. Such specificities are assumed to be indicative of significant biological relevance and these are indeed the cases for AT III, basic and acidic fibroblast growth factors, which are activated by binding to appropriate sequences in HS [4]. The binding of heparin to protein may have highly diverse functional roles such as the control of homeostasis, the regulation of protease, cell behavior and metabolism, etc. [5].

Many methods have been used to study the interactions between heparins and proteins such as isothermal titration calorimetry [6–11], surface plasmon resonance spectrometry (SPR) [5,6,8,12,13], affinity chromatography (AC) [6,8–10,14,15], nuclear magnetic resonance spectrometry [5,6,16], X-ray [5,10,17,18], mass spectrometry [19], circular dichroism [11,14], Fourier transform infrared spectrometry [7], polyacrylamide gel electrophoresis [5], fluorescence [20], equilibrium dialysis [9,21] and capillary electrophoresis (CE) [19,22,23]. Among these methods, SPR and AC are widely used, but they require immobilization of either of the involved substances to supporting material and this poses a problem of steric hindrance [24,25]. Except for the advan-

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tages of high speed, high resolution, low sample consumption, reproducibility, flexibility and capable of using a wide range of buffers, CE can also be used to study the interactions of individual components in a mixture, and to determine binding parameters and stoichiometry in one step [22]. To this end, CE is a preferred method to study heparin–protein interactions. Affinity capillary electrophoresis (ACE) and capillary zone electrophoresis (CZE) are two of five CE methods (ACE, CZE, Frontal analysis (FA), Hummel–Dreyer and Vacancy peak) available to study interactions. Though Heegaard and Gunnarsson et al. have used ACE to study the heparin–peptide [19,22,23] and heparin–protein interactions [26], little work has been done on heparin–protein interactions using CZE [27]. Previously, the slow on-and-off kinetic bindings of granulocyte colony stimulating factor (G-CSF) to heparin were studied using CZE [28]. However, the fast kinetic systems in heparin–protein interactions were never studied by CZE.

To exploit the applicability of the assay in fast on-and-off interactions and investigate the binding kinetics of the interaction between recombinant human interleukin 2 (IL-2) and low molecular weight heparin (LMWH), we investigated the sensitivity and specificity of the assay employing IL-2 as a heparin binding protein. The interaction between IL-2 and heparin has been investigated by an enzyme-linked immunosorbent assay (ELISA) [1,29]. IL-2 binds to heparin in a dose-dependent manner. The interaction is dependent on the heparin chain length and the chains as small as 5 kDa retain the ability to bind to IL-2. However, to the best of our knowledge, the interaction has never been studied by CZE, the binding kinetics has not been investigated, and the CZE assay has never been used to study fast on-and-off kinetic systems in protein–heparin interactions. In this study, the effects of the addition of LMWH to the injection volume of a sample and the negative control of the interaction between epidermal growth factor (EGF) and heparin were investigated by CZE. The binding kinetics of the interaction between LMWH and IL-2 was represented and the binding constant was determined. The applicability of the CZE assay in both slow and fast on-and-off interactions was also discussed.

2. Experimental

2.1. Materials

Recombinant human interleukin 2 (Purity: 97% by SDS-PAGE and HPLC analyses) with a molecular weight of 15.4 kDa and with the concentration of 1 mg/mL in 100 mM acetic acid was purchased from USBiological (Swampscott, Massachusetts, USA). EGF (solid, MW: 6216) was given by Prof. Ren-Bao Gan (Institute of Biochemistry and Cell Biology, Shanghai Academy of Life Science, Chinese Academy of Sciences, Shanghai, China). Heparin (powder, MW: 15 kDa) and LMWH (powder, MW: 5 kDa) were kindly provided by Qilu Pharmaceutical Factory (Jinan, Shandong, China). Mannitol was purchased from Beijing Jingke Company (Beijing, China). Other chemicals were all analytical grade. Redistilled water was used throughout this work. Microcon YM-3 and 10 were purchased from Millipore (Billerica, MA, USA).

2.2. Sample preparation

A 10 μL of recombinant human IL-2 (1 mg/mL in 100 mM acetic acid) was diluted with water to 200 μL and ultracentrifuged by Millipore microcon YM-3 to obtain 20 μL stock solution. The stock solution was diluted with 10 mM acetic acid to various concentrations in the subsequent experiments. Benzoic acid and EGF were all dissolved and diluted with water. LMWH was dissolved and diluted using 10 mM acetic acid and water in the (IL-2)–LMWH and (benzoic acid)–LMWH interactions, respectively. Heparin was dissolved and diluted with water.

2.3. Capillary zone electrophoresis

A Beckman apparatus consists of a P/ACE MDQ system (Beckman, Fullerton, CA, USA) with a photodiode array detector was used to all experiments. A fused-silica capillary (31.2/21 cm × 50 μm i.d.) was obtained from Yongnian Optical Fibre Corp (Hebei, China). Data were collected and processed with the Beckman System software. In the present study, the temperatures of the cartridge and sample room were 25 and 20 °C, respectively. Before each measurement, the capillary was rinsed with 50 mM phosphate buffer, pH 9.0 for 3.0 min at 137.895 kPa. Samples containing mixtures of protein and LMWH were injected at the anodic end using a pressure injection mode with 3.447 kPa for 4 s and detected at the cathodic end at the wavelength of 201 nm. The running voltage was 8 kV. After each run, the capillary was flushed consecutively with 1 mol/L HCl for 2.0 min, water for 3.0 min, 1 mol/L NaOH for 2.0 min, and finally with water again for 3.0 min at 137.895 kPa. Duplicate for each sample was performed.

2.4. Quantitative model of the binding study

Binding studies often involve a proof of bindings, the number of binding sites and an estimation of the quantitative parameters [30]. Binding constant and stoichiometry are important parameters to be determined. Scatchard analysis is a common way to linearize the binding data, and the model can be expressed in the following equation:

$$\frac{r}{C_f} = -Kr + nK \quad (1)$$

where r is the ratio of the concentration of the bound ligand (or receptor) to the total receptor (or ligand) and C_f is the unbound ligand (or receptor) concentration. K is the apparent binding constant and n is the number of binding sites [31]. In this study, r is the concentration ratio of the bound protein to the total LMWH and C_f is the unbound protein concentration.