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Immunology Letters 96 (2005) 63–71
Immunology Letters
www.elsevier.com/locate/

Investigation of active form of catalytic antibody light chain 41S-2-L

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Received 21 May 2004; received in revised form 21 July 2004; accepted 29 July 2004
Available online 25 August 2004

Abstract

We have raised a monoclonal antibody (41S-2) against the conserved sequence, RGPDRPEGIEEEGGERDRD, of human immunodeficiency virus type1 (HIV-1) envelope gp41. That antibody light chain (41S-2-L) cleaves gp41-derived peptide (TPRGPDRPEGIEEEGGERDRD; TP41-1) with a characteristic biphasic profile composed of induction and active phases. It is considered that the conformation of 41S-2-L is changed, by such as induced fitting, to move to active phase to decompose the antigenic peptide during the induction phase.

In order to investigate what happens to 41S-2-L in the induction and active phase, the cleavage reaction of the peptide by 41S-2-L was examined in detail from the viewpoint of kinetic and spectroscopic analysis. The kinetic data showed that the preferable conformational transition of 41S-2-L took place by the unimolecular reaction of 41S-2-L in the induction phase.

UV–vis and fluorescence spectroscopic analysis suggested that the conformational transition leads to the generation of aggregates of 41S-2-L in the reacting solution, which causes the huge enhancement of the catalytic activity of 41S-2-L. The nuclei of the aggregates may be formed in the induction phase. The aggregates and soluble 41S-2-L are considered to be in chemical equilibrium during the cleavage reaction of the antigen.

Keywords: Catalytic antibody; Serine protease; Catalytic triad; Fluorescence; Aggregate

1. Introduction

Many natural catalytic antibodies have been prepared in this decade. Some were found from humoral samples such as urine and serum. Paul et al. found a natural catalytic antibody capable of cleaving vasoactive intestinal peptide (VIP) [1]. Gabibov et al. [2] and Nevinsky et al. [3] prepared a catalytic antibody as a DNAase. Matsuura et al. [4,5] and Paul et al. [6] found that Bence Jones Proteins (human antibody light chains) could have peptidase activity. A natural catalytic antibody against factor VIII was isolated by Kaveri et al. [7]. On the other hand, Paul et al. [8] and Uda et al. [9–12] have succeeded in producing some natural catalytic antibodies by immunizing a ground-state polypeptide or protein into mice. Former natural catalytic antibody could cleave the antigenic peptide, vasoactive intestinal peptide, by its antibody light chain [13]. In the latter cases, Uda et al. obtained a light chain of a 41S-2 mAb-cleaving HIV-1 gp41 molecule. They also succeeded in the production of natural catalytic antibody light and/or heavy chains such as i41-7 [14], i41SL1-2 [15] and ECL2B mAbs [16].

In the cleavage reaction using those natural catalytic antibodies against the antigenic peptide, a biphasic reaction profile is frequently observed. After mixing an antigen and a catalytic antibody subunit (light or heavy chain), a very slow cleavage reaction lasts for several tens of hours and then the reaction is accelerated about 20 times faster than that of the slow cleavage reaction. The cleavage of the antigenic peptide

Abbreviations: mAb, monoclonal antibody; PB, phosphate buffer; PBS, phosphate-buffered saline; KLH, keyhole limpet haemocyanine; *env* gp41, envelope glycoprotein of HIV-1gp41; BSA, bovine serum albumin

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doi:10.1016/j.imlet.2004.07.018

proceeds with two kinds of reaction phases, namely induction phase and active phase. Many natural catalytic antibodies prepared by Uda et al. show this characteristic feature. Pillet et al. also showed the idiotypic catalytic antibody cleaved BSA with induction phase [19]. Hence, in this study, in order to clarify the mechanism of the two reaction phases, detailed analysis with UV–vis and fluorescence spectroscopy were conducted by using a light chain of 41S-2 mAb (41S-2-L).

2. Materials and methods

2.1. Production of antibody

Details of the production of the monoclonal antibody, 41S-2, were described in a former paper [12]. Briefly, a 19-meric peptide (gp41 peptide, RGPDRPEGIEEEGGERDRD) conjugated with KLH was immunized into balb/c mice, followed by cell fusion with myeloma cells, SP2/0-Ag14. For screening of the fused clones, conjugates coupled with BSA at the N- and C-terminus of the peptide were employed. After cloning twice, a monoclonal antibody (mAb)-secreting hybridoma was established as the cell line designated 41S-2.

2.2. Peptides

The gp41 peptide and TP41-1, 21-meric peptide (TPRGPDRPEGIEEEGGERDRD), were synthesized by the F-moc solid-phase method using an automated peptide synthesizer (Applied Biosystems 431A, CA, USA). After deprotection of the synthesized peptide from resin, the peptide was purified using reversed-phase HPLC (RP-HPLC; Waters 490E, Waters μBONDASPHERE C_{18} column; Waters, NY, USA) and the purity was confirmed at >99% by HPLC. The peptide was identified using an ion-spray type mass spectrometer (API-III, Perkin-Elmer Sciex, Ont., Canada).

2.3. Purification of the antibody and isolation of the light chain

41S-2 mAb was purified according to the purification instructions in the Bio-Rad Protein A MAPS-II kit (Nippon BIO-RAD, Tokyo, Japan). The affinity-purified antibody was ultrafiltered three times by using Centriprep 10 (Amicon, MA, USA). The purified mAb was dissolved in 2.7 ml of a buffer (pH 8.0) comprised of 50 mM of Tris–HCl and 0.15 M NaCl. Into this solution, 0.3 ml of 2 M 2-mercaptethanol (final concentration: 0.2 M) was added and reacted for 3 h at 15 °C to reduce 41S-2 mAb. The solution was adjusted to pH 8.0 with 2 M Tris after the addition of 3 ml of 0.6 M iodoacetamide (final concentration: 0.3 M) and then the solution was incubated for 15 min at 15 °C. The resultant solution was concentrated to 0.4 ml using Centriprep 10. A half-volume of the sample was injected into a size-exclusion chromatography column (column; Protein-Pak 300, 7.8 × 300 mm, Nippon Waters, Tokyo, Japan) at a flow rate of 0.15 ml/min by using 6 M guanidine hydrochloride (pH 6.5) as an eluent buffer. The corresponding fractional light chain was collected and diluted with 6 M guanidine hydrochloride two- to five-fold and then the diluted fraction was dialyzed against PBS (pH 7.4) by replacing the solution seven times and further dialyzed against PB (pH 6.5) twice at 4 °C to accomplish the refolding of the light chain. After dialyzation, the light chain was harvested from the dialysis bag in the safety cabinet. The concentration of the light chain was determined by a DC protein assay kit (BIO-RAD, Tokyo, Japan). This procedure is the same as the method described by Hifumi et al. [12].

2.4. Catalytic reaction by the light chain

Prior to carrying out the catalytic reaction between the light chain (41S-2-L) and antigenic peptide (TP41-1), most of the glassware, plasticware and buffer used in this experiment were sterilized by heating (180 °C, 2 h) or autoclaving (121 °C, 15 min). All experimental manipulations were performed in a safety cabinet to avoid air contamination into the reaction solution. The TP41-1 peptide was dissolved in 15 mM PB (pH 6.5), followed by passing through a 0.20-μm sterilized filter. The degradation reaction was carried out in 15 mM PB (pH 6.5) at 25 °C.

To monitor the degradation of TP41-1, an aliquot of 20 μl of the reaction solution was injected into the reversed phase HPLC (RP-HPLC; PU980, UV975, Jasco, Tokyo, Japan, Column; Waters Puresil C18 column of 4.6 mm × 150 mm, Waters, USA) at a flow rate of 0.5 ml/min under an isocratic condition (0.07% TFA and 13% acetonitrile) with a column temperature of 40 °C. The purified and reacted 41S-2-L was subjected to SDS–PAGE analysis with silver staining.

2.5. UV–vis spectroscopy

During the degradation of TP41-1, UV–vis spectroscopic analysis (V-530, Jasco, Tokyo, Japan) was carried out at room temperature. One milliliter of sample solution (in 15 mM PB, pH 6.5) in a quartz cell was scanned from 900 nm to 200 nm with a scan speed of 400 nm/min.

2.6. Fluorescence spectroscopy

Three hundred microliter of the sample solution in a quartz cell was subjected to fluorescence spectroscopic measurement (FP-777, Jasco) at an excitation wavelength of 228 nm (λ_{ex}) and we scanned the emission spectra from 260 nm to 430 nm with a scan speed of 100 nm/min.

2.7. Separation of aggregates and supernatants

Some samples, to analyze the character of aggregates and supernatants, were prepared as follows. (A) After the cleavage reaction was completed (Sample 1), a portion of the reaction solution (0.6 ml) in a sterilized micro tube (1.5 mL Microcentrifuge tube; Quality Scientific Plastics, CA, USA)