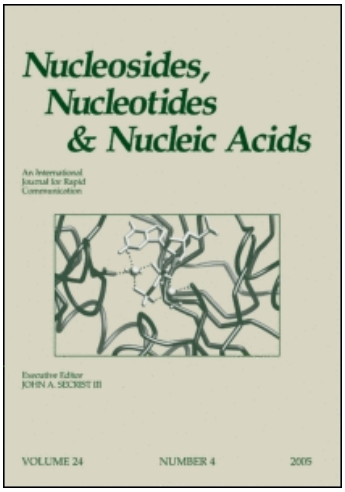

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Comparison of Enzymatic Properties of DNA-Abzymes and Human DNAses

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ABSTRACT

DNA-hydrolyzing antibodies (DNA-abzymes, Abz) were shown to be good biochemical markers of some autoimmune diseases such as systemic lupus erythematosus (SLE) and multiple sclerosis (MS). To better understand mechanisms of abzyme generation, one needs to know optimal conditions for DNA hydrolysis by DNA-abzymes, as well as their enzymatic properties in comparison with those of enzymes possessing the same activity. In contrast to human urine deoxyribonucleases, DNA-hydrolyzing antibodies efficiently digested both single- and double-strand DNA. It was shown that polyclonal antibodies (Abs) in MS may contain up to several types of DNase activities, either activated by metal ions or not.

Key Words: Multiple sclerosis; DNase activity; Catalytic IgG; Substrate specificity.

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INTRODUCTION

The field of natural catalytically active antibodies has been reviewed recently.[1] Multiple sclerosis is a chronic inflammatory demyelinating pathology of the central nervous system presenting a serious medical and social problem. Enhanced synthesis of immunoglobulins (usually IgG), their free light chains, and polyspecific DNA-binding Abs interacting with phospholipids is observed in MS patients.[2] Recently we have shown that homogeneous IgGs from the sera and cerebrospinal fluid of MS patients are active in DNA hydrolysis.[3]

MATERIALS AND METHODS

Homogenous preparations of IgGs from sera of MS patients were obtained by three chromatographic steps as describe in Ref. [3]. The preparations of alkaline and acid DNases were isolated from human urine. All other chemicals were from Sigma or ICN. The reaction mixture (20 μl) for analysis of DNAse activity of IgGs, containing 20 μg/ml supercoiled pBluescript DNA (or 10^{-8} M oligodeoxynucleotides, ONs), 5 mM $MgCl_2$, 25 mM Tris-HCl (pH 7.5), and 10–50 μg/ml Abs unless indicating otherwise, was incubated for 2 h at 37°C. The cleavage products of plasmid DNA and ONs were analyzed by electrophoresis in 1.2% agarose gel or 25% polyacrylamide gel with 7 M urea, respectively.

RESULTS AND DISCUSSION

Human DNase I and DNase II at optimal conditions predominantly generated single-strand breaks in both strands of helical DNA. This initially resulted in an

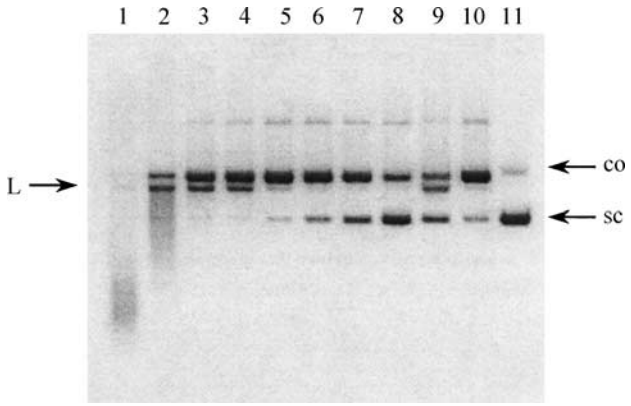

Figure 1. The character of supercoiled (sc) plasmid DNA hydrolysis to circular open (co) and linear (L) forms. Lane 1—5 ng/ml DNase 1 from human urine; lanes 2–8—stepwise twofold dilutions of 5 ng/ml DNase; lane 9, 10—preparations of IgG from the sera of MS patients; lane 11— control incubation in absence of enzymes.

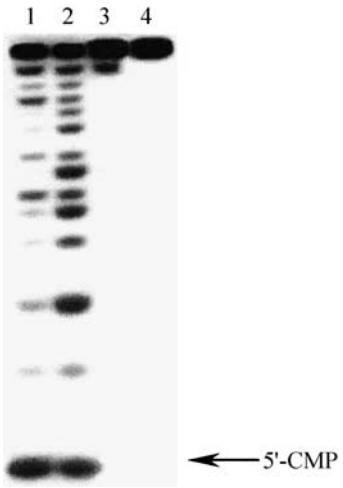

Figure 2. Me^{2+}-dependence of [5′-^{32}P]dCCAGTCACGACGTT hydrolysis by IgG from the serum of MS patient. Lane 1—5 mM $MgCl_2$ + 0.2 mM EGTA; lane 2—5 mM $MgCl_2$ + 0.2 mM $CaCl_2$; lane 3—5 mM EDTA; lane 4—control incubation in absence of enzymes.

accumulation of the circular open form of the plasmid, and later, in its transition to the linear form and low molecular weight products (Fig. 1, lanes 1–8). In most cases, the same pattern of plasmid hydrolysis has been observed among one hundred samples of DNA-abzymes isolated from sera of different MS patients (for example, lane 10). Some preparations showed a quick appearance of the linear form (lane 9) indicative of a high frequency of double-strand breaks or the processive type of hydrolysis or specificity to parts of DNA containing single-strand breaks. For DNase 1, the highest activating effect is observed for Mg^{2+}+ Ca^{2+} or Mn^{2+} ions, whereas DNase II did not require metal ions for activity. Analysis of the patterns of cleavage of ONs reveals that the preparations of DNA-hydrolyzing Abs consist of several subfractions possessing different metal dependencies. Figure 2 shows three types of activity: one dependent on Mg^{2+} only (lane 1), Mg^{2+}+ Ca^{2+}-dependent (lane 2) and one not requiring metal activators (lane 3). It should be noted that polyclonal MS-Abs effectively hydrolyzed both single- and double-stranded DNA whereas DNase I and II digested ss ONs very poorly. Our data clearly show that polyclonal catalytic antibodies of each MS patient may contain extremely different repertories of DNA-hydrolyzing IgG subfractions; some of these have no analogues among other human DNases.

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