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Plant Physiology and Biochemistry 43 (2005) 309–313
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

DNA mismatch binding activities in *Chlorella pyrenoidosa* extracts and affinity isolation of G-T mismatch binding proteins

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Received 27 September 2004; accepted 9 February 2005

Available online 17 March 2005

Abstract

DNA mismatch recognition proteins contained in the extracts of unicellular alga *Chlorella pyrenoidosa* were isolated by affinity adsorption and 2-D gel electrophoresis. Incubation of the algal extracts with a 38-mer duplex oligonucleotide carrying a single DNA simple mispair generated a few gel retardation complexes. G-T mispair was recognized significantly better than C-T, G-G, G-A, and C-C mispairs by the algal extracts and these extracts bound very weakly to G-A and C-C mispairs, displaying a universal trend of mismatch binding efficiency. The levels of mismatch recognition complexes were slightly increased in the presence of 1 mM ATP. Two 13-kDa G-T binding polypeptides possessing p*I*s of 5.3 and 5.5 were isolated after resolving affinity-captured proteins by 2-D gel electrophoresis and the two factors were found to bind 5.5- and 2.8-fold stronger to heteroduplex than to homoduplex DNA, respectively. No proteins significantly homologous to the two algal G-T binding proteins were found by peptide mass fingerprinting (PMF). The sequence of a peptide generated from trypsin-cleavage of one G-T binding factor (p*I* 5.5) could be aligned with the amino acid sequences that form the C-terminal active sites of human and mouse mismatch-specific uracil/thymine-DNA glycosylases, suggesting the possibility of this factor as an algae- or a *Chlorella*-specific DNA mismatch glycosylase.

Keywords: Affinity adsorption; Binding proteins; *Chlorella pyrenoidosa*; DNA mismatch; G-T mispair

1. Introduction

Non-complementary base pairing errors may arise due to DNA polymerase misincorporation, spontaneous deamination of 5-methylcytosine or induced base damage like O^6-methylguanine-C pairing after treatment of cells with alkylating agents [1,2]. Loops of extra bases generated from DNA polymerase slippage during replication of microsattelite DNA or misalignment produced in homologous recombination also appear in duplex DNA [2,3].

In *Escherichia coli*, mismatched nucleotides are bound by a MutS homodimer [4]. The MutS dimer interacts with the MutL protein, and an ATP hydrolysis-dependent translocation of this complex to a hemimethylated GATC site that is bound by the MutH protein. The activated MutH endonuclease produces a nick at the unmethylated strand, directing exonucleolytic digestion of mismatched nucleotides on the newly replicated strand, and correct base pairs are restored after DNA synthesis [5,6]. In human cells, mismatch recognition is carried out by complexes of MutS homologs (MSH) consisting of either hMSH2 and hMSH6 (hMutSα) or hMSH2 and hMSH3 (hMutSβ). The hMutSα complex recognizes simple base-base mispairs and small insertion-deletion loops, whereas the hMutSβ complex binds preferentially to loops of extra bases [7,8]. Mismatched nucleotides in human cells are removed by a nick-directed pathway as revealed by in vitro repair assay [9,10]. The nicks that fre-

Abbreviations: CHAPS, (3-[(3-cholaminedopropyl)dimethylamino]-1-propanesulfonate); DTT, dithiothreitol; EMSA, electrophoretic mobility shift assay; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight; MS/MS, tendam mass spectrometry; p*I*, isoelectric point; PMF, peptide mass fingerprinting; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; 2-D, two-dimensional.

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doi:10.1016/j.plaphy.2005.02.008

quently appear on the ends of Okazaki fragments on the lagging strand during DNA replication may provide the strand discrimination signal [11]. Although genes encoding proteins homologous to eukaryotic MSHs have been isolated from higher plants like *Arabidopsis thaliana* and *Zea mays* [12,13], some MSHs such as MSH7 in *A. thaliana* and its ortholog in *Z. mays* called Mus2 might be plant-specific as their corresponding genes were placed by phylogenetic analysis to a subgroup of MSH genes [13]. Cell-free extracts of the unicellular alga *Chlorella pyrenoidosa* were prepared in this study to search for mismatch recognition proteins in lower eukaryotic plants. Differential binding of algal extract proteins to simple mispairs were detected by a band shift assay. Two 13-kDa G-T mismatch binding factors possessing weak acidic p*I*s were isolated by affinity adsorption and 2-D gel electrophoresis. One binding protein was suspected to be a uracil/thymine-DNA glycosylase after mass spectrometric analysis and amino acid comparison.

2. Results and discussion

2.1. Mismatch binding activities in C. pyrenoidosa extracts detected by EMSA

EMSA indicated that the levels of G-T binding complexes produced by *C. pyrenoidosa* extracts were significantly higher than those of G-G, G-A, C-T, and C-C binding complexes. *C. pyrenoidosa* extracts generated C-T and G-G mismatch binding complexes with intermediate band intensities and very little G-A and C-C mismatch-dependent binding. No gel shifting could be produced in the absence of extract proteins (Fig. 1A).

Because of the importance of ATP hydrolysis in translocating mismatch recognition proteins [1,7], the effects of ATP on the binding of algal extracts to heteroduplex DNA were also studied. Mismatch-dependent binding produced in ATP-free reaction mixtures were not affected after the addition of 1 mM ATP except that the intensities of C-T, G-G, and G-A binding complexes were slightly increased (Fig. 1B). A portion of the oligonucleotide carrying a single or no DNA mismatch was highly shifted to the gel well in the presence of ATP (Fig. 1B). As the shifting of protein–DNA complexes to gel wells was mismatch-independent, DNA binding proteins unrelated to mismatch recognition might be activated by ATP to cause the formation of these high-shifting complexes.

The pattern of differential binding of *C. pyrenoidosa* extracts to simple DNA mispairs resembles the trends produced by mismatch recognition activities in bacteria, yeast, and mammalian cell extracts [1]. The efficient binding of mismatch recognition proteins in different living organisms to G-T mispair indicates the importance of mismatch repair systems to deal with G-T mispair that may frequently appear in specific DNA sequences due to the spontaneous deamination of 5-methylcytosine paired with guanine [1]. The oligonucleotide containing a G-T mispair was therefore employed as

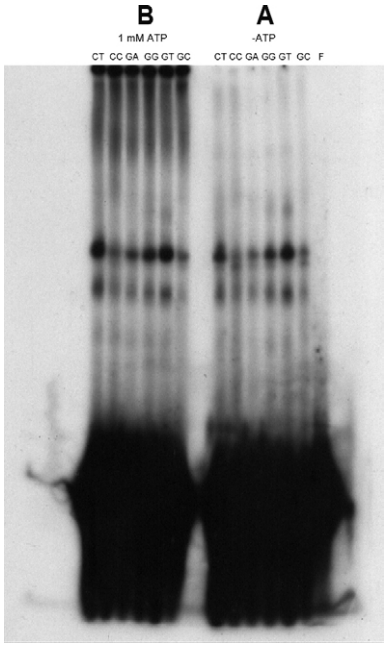

Fig. 1. Detection of DNA mismatch binding activities in *C. pyrenoidosa* extracts in the absence and presence of ATP by EMSA. (A) A ^{32}P-labeled heteroduplex probe carrying the indicated type of mispair or a homoduplex probe (G-C probe) was incubated at 30°C for 20 min with *C. pyrenoidosa* extracts containing 30 μg extract proteins in the absence of ATP and the reaction mixture was electrophoresed on a 6% non-denaturing polyacrylamide gel. The formation of gel retardation complexes was detected by autoradiography. F indicates the free G-T probe that was incubated in a protein-free reaction mixture. (B) Each heteroduplex probe and the homoduplex probe was incubated with algal extract proteins in the presence of 1 mM ATP under the same EMSA condition as described above.

the representative probe for the isolation of mismatch binding proteins by affinity adsorption and this experiment was performed under an ATP-free condition to avoid capturing mismatch-independent DNA binding proteins.

2.2. Isolation of G-T binding proteins by affinity adsorption followed by 2-D gel electrophoresis and quantitative analysis of mismatch-dependent binding

When algal proteins adsorbed onto the immobilized G-T probe and the homoduplex G-C probe were separated by 2-D gel electrophoresis in a 3–10 pH gradient, the preferential capture of a 13-kDa polypeptide having a p*I* about 5 by the heteroduplex probe was observed after fluorescence staining of affinity-captured proteins in our preliminary 2-D separations (data not shown). Further 2-D experiments using a narrow-range 4–7 pH gradient for isoelectrofocusing detected the preferential binding of two 12–13-kDa polypeptides having p*I*s at 5.3 (spot A) and 5.5 (spot B) to the immobilized G-T probe (Fig. 2). Both the homoduplex and the heteroduplex probes were repeatedly found to pull down a very similar level of a 36-kDa polypeptide (spot C) from the algal extracts. Our previous affinity adsorption experiments showed that no DNA damage binding proteins could be captured by ligand-free agarose beads [14]. Based on image analysis of 2-D gels, G-T binding factors A (p*I* 5.3) and B (p*I* 5.5) were