

Renaturation of telomere-binding proteins after the fractionation by SDS-polyacrylamide gel electrophoresis

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ABSTRACT

A simple method for identification and characterization of telomere-binding proteins is described in this article. After Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE), proteins are eluted, renatured and used for retardation analysis with labelled oligonucleotides corresponding to human and plant of telomeric sequences. We show here that this method is efficient to recover sequence-specific DNA-binding abilities of putative telomere-binding proteins.

Keywords: method; renaturation; telomere-binding proteins; telomeres

Telomeres are the physical ends of eukaryotic chromosomes. These nucleoprotein complexes protect chromosomes from degradation and end-to-end fusion and are essential in solving the end replication problem. Telomere-binding proteins are necessary building blocks of telomere structure. These proteins participate in localization of chromosomes in cell nucleus and they are involved in regulation of telomere length as well as in protective function of telomeres. Telomeric DNA is characterized by tandem repeats with small differences across phylogenetic spectrum. In most of the studied plants (TTTAGGG)_n repeats were found, but there are some exceptions. One of these exceptions is the order *Asparagales*. Sýkorová et al. (2003) found that in several families of this order, the “typical” (*A. thaliana*-like) telomeres are partially or fully replaced by alternative telomere sequences. Consistent with this change, our previous results show changes in telomeric chromatin structure of these plants (Rotková et al. 2004). The present method of rapid renaturation of DNA-binding proteins after their separation by SDS-polyacrylamide gel electrophoresis

can further elucidate changes to cope with the telomere DNA sequence change, which happens at the proteomic level.

MATERIAL AND METHODS

Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) is an efficient method for separation of proteins according to their size. DNA-binding abilities of proteins fractionated by this simple method can be found by elution of these proteins from polyacrylamide slices followed by retardation analysis (Fulnečková 2000).

A procedure was published (Hager and Burgess 1980), in which proteins are eluted by elution buffer (0.1% SDS, 0.05M Tris-HCl pH 7.9, 0.1mM EDTA, 5mM DTT, 0.1 mg/ml BSA, and 0.15 or 0.20M NaCl) from the SDS-PAGE gel, precipitated with acetone to remove SDS, then denatured with 6M guanidium hydrochloride (Gd-HCl) and renatured by the removal (diluting) of Gd-HCl with dilution buffer (0.05M Tris HCl, pH 7.9, 20% glycerol, 0.1 mg/ml BSA, 0.15M NaCl, 1mM DTT and

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0.1mM EDTA). This denaturation/renaturation method usually results in low recoveries of active DNA-binding proteins, and becomes impractical if large number of gel slices have to be handled.

However, there is a simpler method, described by Ossipow et al. (1993), which is based on the observation that mild non-ionic detergents, such as Triton X-100, remove SDS from protein-SDS complexes and sequester it into micelles that do not interfere with DNA binding. This method is not time-consuming and enables to analyse DNA-binding abilities of SDS-PAGE-fractionated proteins. It can also reveal if proteins bind their target sequences as monomers, homo-multimers or hetero-multimers. We show in this paper that the method is applicable for a more convenient analysis of plant telomere-binding proteins.

Chemicals and instrumentation

Chemicals – SDS, acrylamide, bisacrylamide, TEMED, ammoniumpersulphate, marker protein ladder, Tris-HCl, glycine, Hepes, EDTA, NaCl, Triton X-100, BSA, DTT, PMSF, glycerol, bromophenol blue.

Instrumentation – equipment for polyacrylamide gel electrophoresis, thermal incubator or thermocycler, microfuge.

Technique

Nuclear proteins were extracted from plant tissue as described in Espinas and Carballo (1993) and protein concentration was determined according to Bradford (1976). Nuclear protein extracts (30 µg of total protein) are heated for 10 min at 37°C in SDS-PAGE loading buffer (2× concentration 0.125mM Tris-Cl, pH 6.8, 4% SDS, 20% glycerol, 0.02% bromophenol blue, 0.2M DTT) and then separated in 12% SDS-polyacrylamide gel. After electrophoresis, bands of fractionated proteins are cut out. Gel pieces are homogenized in three volumes of elution-renaturation buffer (20mM Hepes pH 7.6, 1mM EDTA, 100mM NaCl, 1% Triton X-100, 5 mg/ml BSA, 2mM DTT, 0.1mM PMSF) and incubated at 37°C for 3 to 4 h. The polyacrylamide gel residues are centrifuged in a microfuge for 10 min. Supernatants can be used directly in further analyses, e.g. in retardation assay. If starting volume of total protein is 30 µg, then the concentration of each eluted protein varies about 1 µg/µl.

Retardation analysis is an easy method to use, if studied proteins have DNA-binding abilities. To reduce non-specific protein-DNA interactions, we have pre-incubated 5 µg of protein with 25 pmol of non-telomeric oligodeoxynucleotides. The end-labelled telomeric oligodeoxynucleotides (2.5 pmol) were then added to the reaction and incubated with proteins on ice. Afterwards the electrophoresis is carried out to resolve the DNA-protein complexes.

RESULTS

Retardation analysis with renatured proteins

The first step is SDS-polyacrylamide gel electrophoresis (Figure 1) and renaturation of fractionated proteins. Products of this separation are proteins eluted in elution-renaturation buffer, which can be used in further downstream reactions. In this particular case, renatured proteins were used in retardation analysis. Since it was expected that

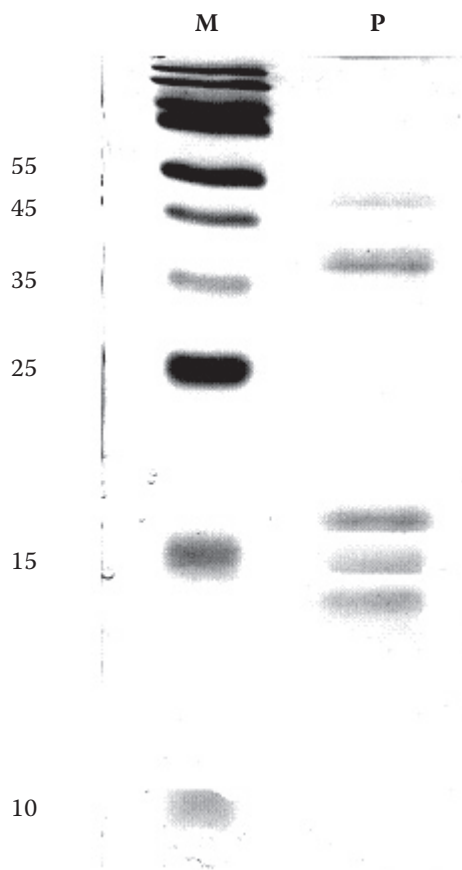


Figure 1. SDS-polyacrylamide gel electrophoresis (M – prestained protein ladder, P – protein nuclear extract)

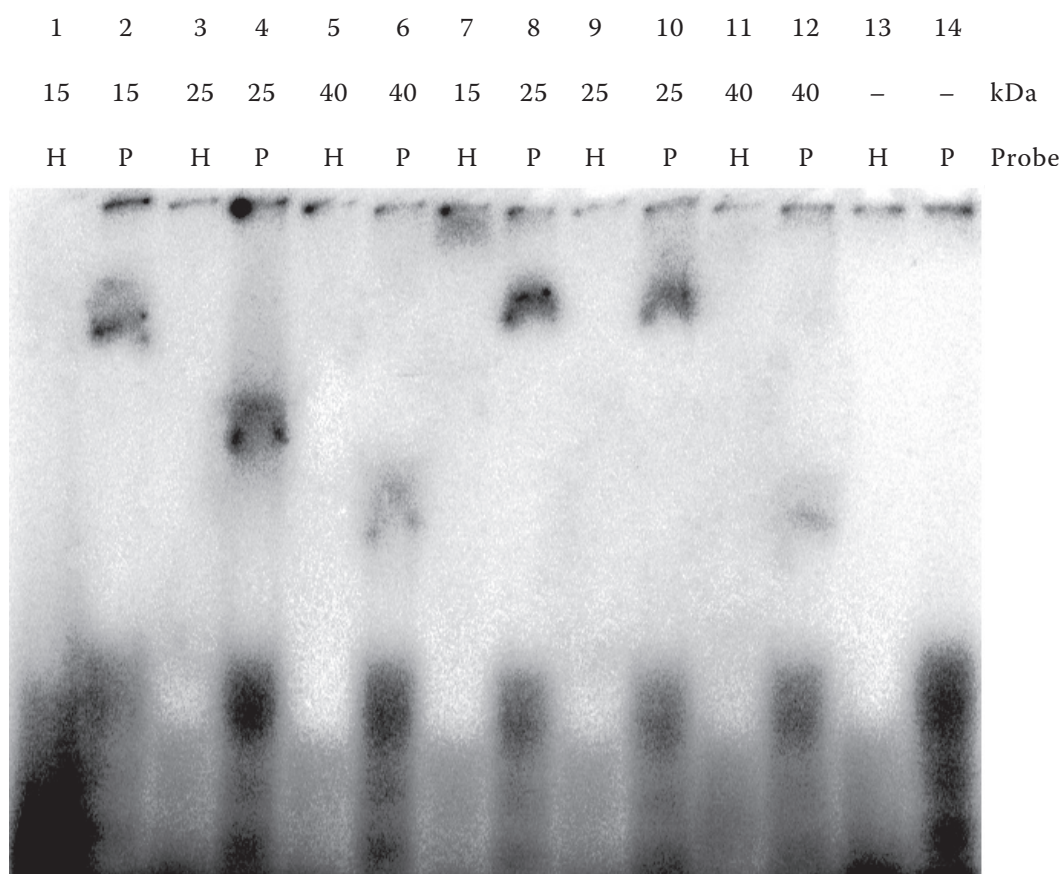


Figure 2. Gel retardation assays with SDS-polyacrylamide gel-fractionated proteins; lanes 1–6 show interactions between *Muscari* proteins and HutlG (H) or PltelG (P); lanes 7–12 show interactions between *Scilla* proteins and HutlG (H) or PltelG (P); lanes 13 and 14 contain free probe

the surveyed plants possess both plant and human type of telomeres, two types of probes (PltelG – [TTTAGGG]₆-3' and HutlG – 5'-[TTAGGG]₆-3') were used. It was found that telomere-binding abilities were recovered for proteins that were supposed as telomere-binding according to previous experiments. As shown in Figure 2, when fractionated and subsequently renatured proteins from *Muscari armeniacum* plants (Asparagales) were used, retardation analysis revealed a binding activity of the plant telomeric probe to 15, 25 and 40 kDa proteins. When the same analysis was applied to *Scilla peruviana* (Asparagales) nuclear extract, similar results were obtained. It is also obvious that there are proteins in nuclear protein extract, which lack telomere-binding abilities.

Classification of the method

This method is timesaving, and requires only basic equipment that is available in most molecular biology or biochemical laboratories.

The only difficulty to be mentioned is that using of three volumes of elution-renaturing buffer can result in dilution of renatured proteins to undesirably low concentrations. A subsequent concentration step might be needed.

A major advantage of the method is obtaining a relatively “purified” protein; it makes subsequent analyses easier and more informative than analyses with whole protein extracts, containing complex mixtures of proteins, sugars and other products of cell metabolism.

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