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Short communication

Determination of glutathione-*S*-transferase traces in preparations of p53 C-terminal domain (aa320–393)

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Abstract

Tumor suppressor protein p53 is often expressed as a fusion protein with glutathione-*S*-transferase (GST). The sensitive determination of GST in p53 samples is thus necessary. We propose a method for the determination of traces of GST in the p53 C-terminus based on the constant current chronopotentiometric stripping analysis (CPSA) with hanging mercury drop electrode (HMDE). GST produces a catalytic signal in cobalt-containing solutions due to cysteine residues. A large excess of the C-terminus does interfere with the determination because of the lack of cysteines in the molecule. This method is simple and very sensitive and is capable of detecting $<1\%$ GST in the p53 sample.

Keywords: p53; Glutathione-*S*-transferase determination; Constant current chronopotentiometry; Hanging mercury drop electrode

1. Introduction

Peptides and proteins (depending on their amino acid composition) produce electrochemical signals at mercury or carbon electrodes [1–3]. Recently, we have shown that using the constant current chronopotentiometric stripping analysis (CPSA), remarkably low levels of DNA, RNA, peptides and proteins can be determined [1–5]. At the carbon electrodes, CPSA of the proteins is based on the oxidation of tyrosine and tryptophan residues in the protein molecule. Subnanogram amounts of bioactive peptides containing these amino acids can be determined [1–3]. At the mercury electrodes, the peptides and proteins containing cysteine/cystine residues produce catalytic signals in cobalt-containing solutions and "peak H" (not requiring cobalt ions) [3] at highly negative potentials (close to -1.7 V against SCE). In difference to the polarographic presodium wave [6], the CPSA "peak H" is well developed and can be used for the determination of peptides and proteins at nanomolar concentrations. Nucleic acid- and protein-modified electrodes can be utilised in molecular recognition studies including DNA hybridization and DNA–protein interactions [5,7].

To our knowledge, electrochemical methods have not been so far used in the research of the p53 protein. The wild-type human p53 is a 393-amino acid phosphoprotein functioning as a stress-activated transcription factor. It can drive transcription by the binding of the central portion of the protein to specific DNA elements in the promoters or regulatory regions of genes and can induce growth arrest or apoptosis [8]. The C-terminal basic part (aa320–393) assembles stable tetramers and binds DNA non-specifically [9,10]. This part of the molecule is responsible for the interaction with single-stranded DNA ends and DNA which is mismatched or damaged enzymatically or by ionising radiation [11]. The central p53 portion contains 10 cysteine residues, while the C-terminal domain contains a tyrosine residue (but no cysteine). Such a distribution of the electroactive amino acids is advantageous for the electrochemical analysis of the individual protein domains. Full-length p53 yields catalytic currents at the mercury electrode in the presence of cobalt ions [12].

To obtain more stable and soluble proteins which can be isolated at a high degree of purity, many proteins including p53, and particularly, its deletion mutants are expressed as fusion proteins with glutathione-*S*-transferase (GST) molecules [13]. At the end of the procedure, the GST protein (containing four cysteine residues) is usually split off by thrombin from the fusion protein to obtain the desired protein molecule in a highly purified state. Traces of the

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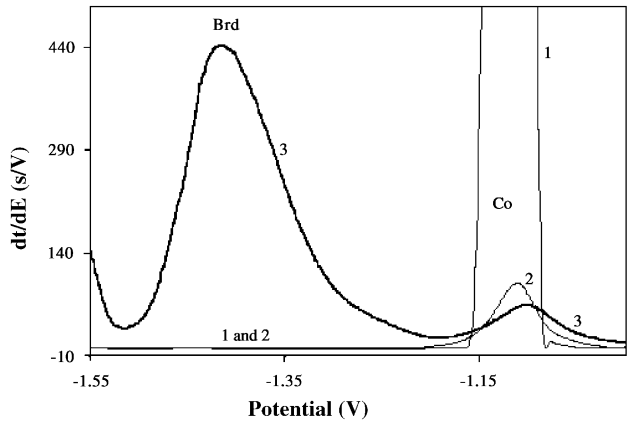

Fig. 1. CPSA signals of the background electrolyte (1), 1 μM p53 C-terminal domain (aa320–393) (2) and 200 nM glutathione-*S*-transferase (GST) (3): the peak of cobalt (Co) and the catalytic peak Brd yielded by the protein in the presence of cobalt ions (Brdička's reaction). E_A (accumulation potential) = −0.10 V, t_A (accumulation time) = 90 s, I_{str} (stripping current) = −3 μA. Solution: 0.1 M ($NH_4OH + NH_4Cl$) and 1 mM $[Co(NH_3)_6]Cl_3$, pH = 9.5.

GST protein can contaminate the p53 sample, and a simple and sensitive method of determining traces of the GST protein is therefore needed.

2. Experimental

2.1. Proteins

C-terminal domain (aa320–393) was expressed in *E.coli* strain BL21 as fusion protein GSTp53(aa320–393) [14]. Soluble lysate was loaded onto a HiTrap Heparin column and eluted with 0.05–1 M KCl gradient (about 700 mM KCl). C-terminal domain and GST alone were purified by exchange chromatography after thrombin cleavage of the GST tag domain from the fusion protein. The sample was loaded into a MonoS HR10/10 (column volume: 1 ml) column (Pharmacia, Sweden) in 25 mM HEPES, pH 7.6, 10% glycerol, and 40 ml NaCl gradient (0–1 M) was used for the elution-bound C-terminal domain (about 600 mM NaCl, 24 min of gradient). The GST tag protein was purified in the same way by a HiTrapQ column (column volume: 5 ml) (Pharmacia, Sweden). The GST protein was eluted as single peak by about 250 mM NaCl in 20 min of 40-ml gradient (0–1 M NaCl). Purified proteins were analysed by SDS-PAGE as described by Laemmli [15] and stained with Coomassie blue. C-terminal domain and GST were obtained in >99.5% purity by this technique. The concentration of the proteins was estimated from Coomassie blue-stained gels with BSA as standard. The

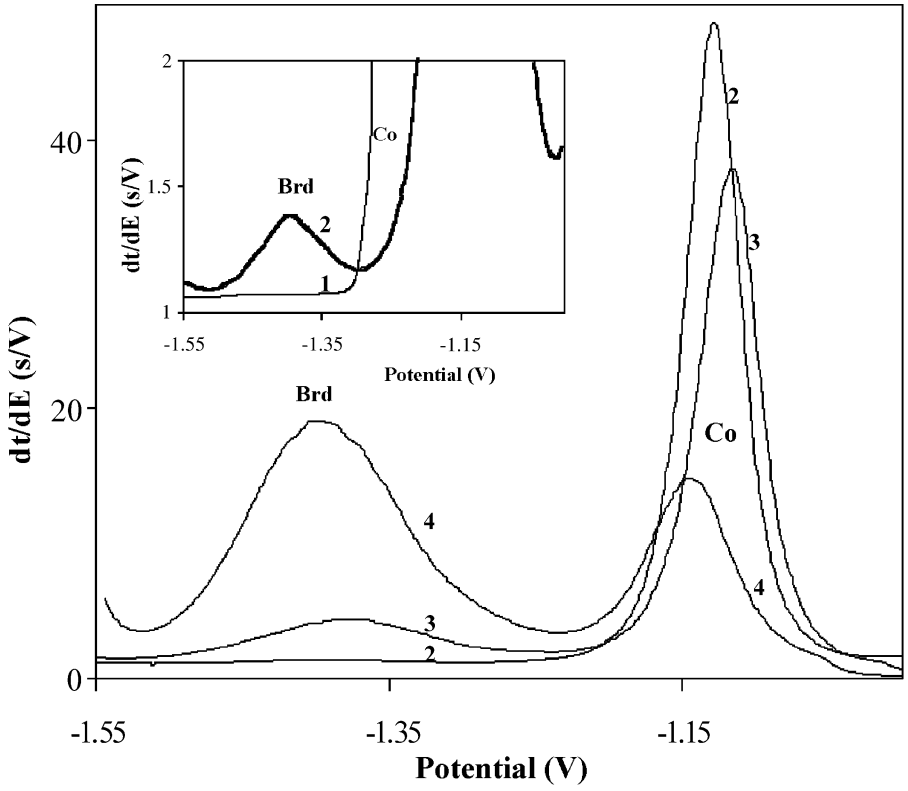

Fig. 2. CPSA signals of GST added to 1 μM sample of the p53 C-terminal domain (aa320–393) (for details, see Fig. 1). (1) Background electrolyte, (2) addition of 6, (3) 60 and (4) 200 nM GST. Inset: detail of curves (1) and (2).

FPLC chromatography system and columns were supplied by Pharmacia, Sweden.

2.2. Apparatus and procedures

Electrochemical measurements were performed by an AUTOLAB analyzer (EcoChemie, The Netherlands) in connection with VA-Stand 663 (Metrohm, Zurich, Switzerland). The standard cell with three electrodes was used. The working electrode was the hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2. The reference electrode was the Ag/AgCl/3 M KCl electrode, and the platinum electrode was used as the auxiliary electrode. All experiments were carried out at 25 °C.

3. Results and discussion

Almost 70 years ago, it was shown by Brdička [16,17] that cystine/cysteine containing proteins produced a characteristic D.C. polarographic double wave in cobalt-containing solutions due to the catalytic hydrogen evolution. Later, we proposed a more sensitive variant of this method based on the differential pulse polarography [18]. Here, we used the medium applied by Brdička, but instead of D.C. polarography, we applied chronopotentiometry in a stripping mode in combination with HMDE. Using this method, we obtained no signal with the highly purified 1 μM p53 C-terminal domain (because of the lack of cysteine in its molecule) (Fig. 1). On the other hand, the GST protein at much lower concentrations produced the typical ''Brdička'' catalytic signal(s) (Fig. 1, peak Brd). Similar signals were obtained if small amounts of GST protein were added to the p53 C-terminal domain (Fig. 2). GST (6 nM) was easily detectable in the 1 μM p53 C-terminal domain, corresponding to 0.6 mol% contamination of p53 with GST. Such a small contamination is not detectable by the polyacrylamide gel electrophoresis with Commasie blue staining that is usually used to establish the degree of the protein purity. Considering the fact that by using the adsorptive transfer CPSA (AdTCPSA), the analysis can be easily performed with 5 μl of the analyte and 0.75 ng of GST can be determined by this new method of ultramicroanalysis of the GST protein contamination in the p53 C-terminal domain. The presence of GST in p53 samples can reduce the DNA-binding activity of the p53 protein and should be therefore carefully controlled. Compared to gel electrophoresis, this method is simpler and faster. More details will be published elsewhere.

4. Conclusion

We proposed a CPSA method which is based on the catalytic signal produced by GST and the absence of this signal in the p53 C-terminus. This method is simple and very sensitive and is capable of detecting < 1% GST in the p53 sample. It can be expected that the principles of the method described in this paper will find their use in the determination of the traces of various cysteine-containing proteins and peptides in histones, protamines, etc. (not containing cysteine).

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