Communication

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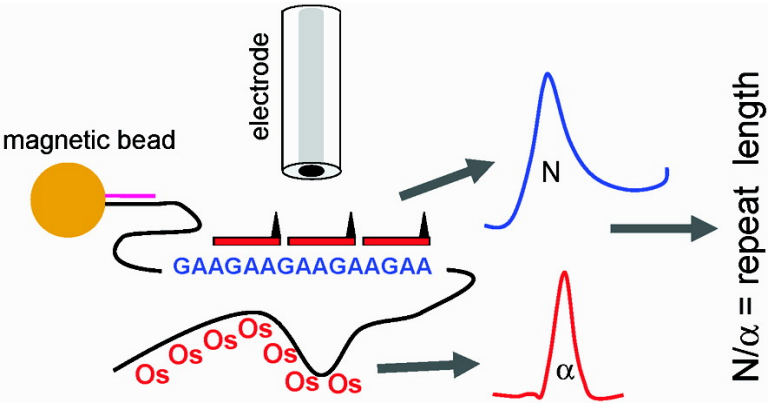

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Electrochemical Detection of DNA Triplet Repeat Expansion

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Genomic expansion of trinucleotide repetitive sequences is connected with inherited neurodegenerative diseases such as fragile X syndrome (CGG·CCG triplet), Huntington's disease (CAG·CTG), myotonic dystrophy (CTG·CAG), or Friedreich ataxia (FRDA; GAA·TTC triplet).[1,2] Diagnostics of these diseases at the DNA level is important for confirming clinical diagnosis, for genetic screening of families at risk, and for early recognition of heterozygous carriers. The current methods of detection of triplet repeat expansion involve Southern blotting or PCR amplification of DNA regions spanning the repeat followed by determination of the amplicon length using gel electrophoresis.[2−4]

DNA arrays and biosensors are increasingly used for sequencing DNA by hybridization. Recently, it has been shown that optical detection usually used in these devices[5] can be complemented by electrochemical methods, offering low-cost, simple design, small dimensions, and low power requirements.[6−9] Double-surface techniques (DST) have been proposed, in which DNA hybridization is performed on one surface (H), and electrochemical detection on another surface, the detection electrode (DE).[11−13] Using magnetic beads as the surface H and solid amalgam or carbon as DE's,[12−15] great increases in specificity and sensitivity were obtained, particularly in combination with nanoparticles.[8,16] Magnetic beads can be incorporated into microfluidic platforms and can work in an array format,[17] offering parallel DNA hybridization analysis.

We propose a new electrochemical detection of the length of the triplet expansion based on multiple hybridization of the DNA repetitive sequence with a short labeled reporter probe (RP) (Figure 1). To determine lengths of guanine (G)-containing triplet repeats, Thorp et al.[10] combined electrochemistry with radioactive labeling. In contrast to their approach,[10] our DST is not limited to G-containing sequences and offers improved separation and specific enrichment of the target DNA (tDNA). In this paper, we propose a method for detection of the $(GAA)_n$ repeat expansion in FRDA. PCR-amplified tDNA fragments were denatured and modified by osmium tetroxide 2,2′-bipyridine (Os,bipy) (Figure 1A) known to react with pyrimidine but not with purine bases.[18] To capture the $(GAA)_n$-containing DNA strand at the magnetic beads with covalently attached oligo$(dT)_{25}$ (B_T), serving as surface H,[11−14] we utilized the $(dA)_{18}$ stretch that occurs naturally next to $(GAA)_n$ in the genomic locus of human frataxin gene[2] (Figure 1B). Because there are no pyrimidine residues in GAA, the number of pyrimidines in the captured tDNA strand was independent of the $(GAA)_n$ length and the electrochemical signal of Os,bipy-modified pyrimidines in the tDNA (Figure 1C) was thus related to the number of DNA molecules captured. Biotin-labeled RP $(CTT)_{12}$ (RP-biot) was hybridized to the captured tDNA to obtain a signal characterizing the $(GAA)_n$ length (Figure 1D,E). The RP-biot was detected via an electrochemical enzyme-linked assay involving binding of streptavidin-alkaline phosphatase conjugate to the RP-biot and conversion of electroinactive 1-naphthyl phosphate to electroactive 1-naphthol which was determined at carbon electrodes using an anodic peak N.[12,15] The relative length of the triplet expansion was calculated from the ratio of the electrochemical signal intensities of hybridized RP-biot (enzyme-linked assay)/tDNA (Os,bipy label).

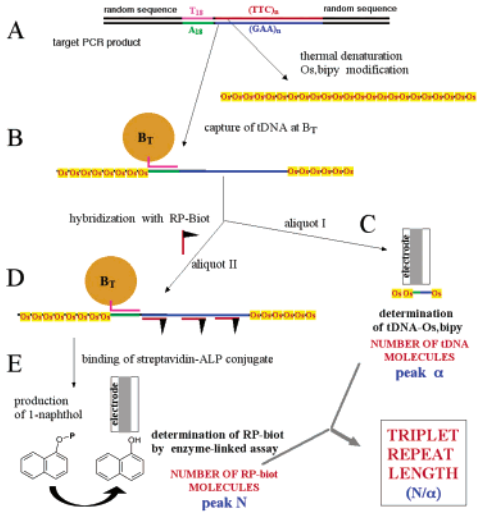

Figure 1. Scheme of the detection of the $(GAA)_n$ repeat expansion in PCR products by means of the electrochemical B_T hybridization assay using biotinylated $(TTC)_{12}$ reporter probe (RP-biot). (A) The target DNA (tDNA) is thermally denatured and modified with Os,bipy. (B) tDNA strand containing the $(GAA)_n$ sequence is captured at the B_T via its stretch of A_{18} residues. After being washed, the B_T suspension is split into two aliquots. (C) Aliquot I: the tDNA-Os,bipy is released from the B_T and determined using osmium peak α at the pyrolytic graphite electrode (PGE). The number of pyrimidine residues modified by Os,bipy in the GAA-strand of the tDNA (reflected by peak α) is constant and independent of the length of the repeat tract. (D, E) Aliquot II: RP-biot is hybridized with the target $(GAA)_n$ sequence at the beads followed by binding of streptavidin-alkaline phosphatase conjugate (SALP). The beads are then transferred into a solution of 1-naphthyl phosphate which is enzymatically converted to electroactive 1-naphthol yielding anodic peak N at the PGE. The resulting 1-naphthol concentration depends on the number of RP-biot molecules captured at the B_T which is directly proportional to the $(GAA)_n$ length. The triplet repeat expansion length is estimated from the peak height ratio N/α.

Figure 2A shows the effects of tDNA concentration on peak N arising from the RP-biot enzyme-linked hybridization assay and on the peak α[14] of Os,bipy-labeled tDNA (tDNA-Os,bipy), both measured at a pyrolytic graphite electrode (PGE). Two amplicons differing in the $(GAA{\cdot}TTC)_n$ repeat length, RW59 (59 triplets) and RW150 (150 triplets), were used as the tDNAs. The intensities of peak α were similar for both amplicons and increased almost linearly with their concentration between 0.5 and 10 ng μL^{-1} (i.e., 10−200 ng of DNA per sample). Peak N also increased linearly with the amplicon concentration (Figure 2A), but the slope of the dependence was about 3 times higher for RW150, as compared to RW59. Signals of the $(GAA)_n$ repeats normalized to the captured tDNA amounts, that is, the N/α ratios calculated for each individual sample (Figure 2B), were practically constant for tDNA concentra-

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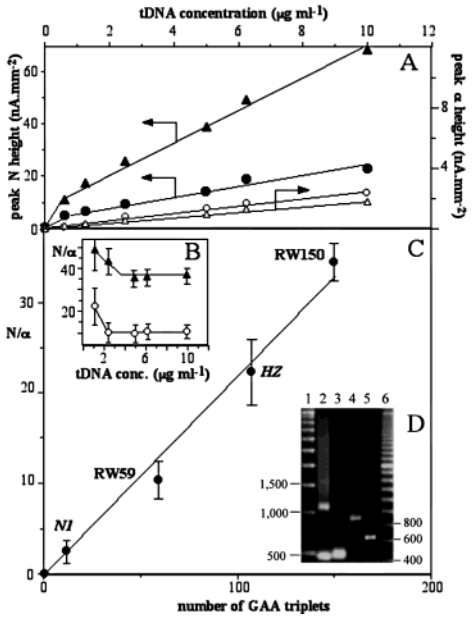

Figure 2. (A) Dependence of the heights of tDNA-Os,bipy peak α and RP-biot peak N on the tDNA amplicon concentrations: (○) α for RW59; (△) N for RW59; (●) α for RW150; (▲) N for RW150. (B) Dependence of the peak height ratio N/α on the tDNA concentration: (○) RW59; (▲) RW150. (C) Dependence of the normalized signal (N/α) on the length of the GAA·TTC triplet repeat in amplicons RW59, RW150 (prepared on recombinant templates), HZ, and NI (amplified from patients' whole DNA). For HZ, an average length value calculated from the normal and the expanded alleles was used; the zero signal corresponds to a tDNA lacking the triplet repeat. (D) Agarose gel electrophoresis showing the analyzed PCR products: lanes 1 and 6, length markers; lane 2, HZ; lane 3, NI; lane 4, RW150; lane 5, RW59.

tions between 2 and 10 ng μL^{-1} (RW59) or between 5 and 10 ng μL^{-1} (RW150). N/α values were about 3.3 times higher for the RW150 PCR product, as compared to the RW59, exhibiting a correlation with the lengths of the repeats in the amplicons (150/59 = 2.5). When the tDNA-Os,bipy was determined at a mercury electrode instead of the PGE (using a catalytic osmium peak Os[18,19] that offers a higher sensitivity), constant values of the normalized signal (N/Os) were obtained for RW150 concentrations down to 175 pg μL^{-1}.

In addition to the RW59 and RW150 amplicons (prepared on templates of frataxin gene fragments cloned into plasmids[20]), we also prepared PCR-amplified tDNAs from clinical samples. PCR products were prepared from whole blood DNA of a normal individual (NI) and of a heterozygous FRDA carrier (HZ). By means of agarose gel electrophoresis (Figure 2D), one PCR product was identified for NI (~500 bp, i.e., about 15 triplet units[2,3]). For the HZ sample, two distinct products (~500 and ~1100 bp) and a remarkable amount of material smeared within higher molecular mass region (most likely due to heteroduplexes formed by the expanded allele[3]) were observed. The length of the larger product corresponded to about 210 triplets. The same samples were analyzed by the B_T hybridization assay. Plotting of normalized N/α signals obtained for PCR products RW59, RW150, NI, and HZ against the corresponding triplet repeat lengths gave a straight line (Figure 2C; for the HZ, an average value calculated from the lengths of the normal and the expanded alleles, i.e., 110 triplet units, was used).

In this Communication, we show for the first time that a long repetitive sequence can be detected electrochemically without any nonelectrochemical labeling. To obtain a reliable signal depending on the triplet repeat length, only 1−2 ng of tDNA per assay is required when mercury or amalgam electrodes are used for tDNA-Os,bipy determination. Nonradioactive electrophoretic techniques usually require at least 5−10 ng of DNA per band, but their sensitivity may be considerably lowered, for example, due to band smearing. Linearity of the signal versus triplet expansion dependence (Figure 2C) makes it possible to differentiate between small normal, large normal, premutated, and mutated alleles with an accuracy comparable to the electrophoretic assay (reported as ~30 triplets for expanded alleles[4]). As compared to electrophoresis, the electrochemical analysis is faster, it determines just the length of the repetitive sequence regardless of the length of the flanking sequences, and the results are less affected by the formation of heteroduplexes of the expanded alleles.[3] Although the electrochemical method yields average repeat lengths (not providing specific signals for individual alleles), our results demonstrate that the presence of an expanded allele in a heterozygote can be easily recognized, offering a potential application in preventive screening of FRDA carriers. The principles of this method can be applied for the determination of the triplet repeat expansion in other neurodegenerative diseases. Low-cost systems can be developed, and their use for screening large numbers of people could help to diagnose the incidence of neurodegenerative diseases in populations.

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Supporting Information Available: Details of the B_T hybridization assay, electrochemical measurements, and optimization of the technique. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Paulson, H. L.; Fischbeck, K. H. *Annu. Rev. Neurosci.* **1996**, *19*, 79−107.
(2) Campuzano, V.; Montermini, L.; Molto, M. D.; Pianese, L.; Cossee, M.; Cavalcanti, F.; Monros, E.; Rodius, F.; Duclos, F.; Monticelli, A.; Zara, F.; Canizares, J.; Koutnikova, H.; Bidichandani, S. I.; Gellera, C.; Brice, A.; Trouillas, P.; De Michele, G.; Filla, A.; De Frutos, R.; Palau, F.; Patel, P. I.; Di Donato, S.; Mandel, J.-L.; Cocozza, S.; Koenig, M.; Pandolfo, M. *Science* **1996**, *271*, 1423−1427.
(3) Poirier, J.; Ohshima, K.; Pandolfo, M. *Hum. Mutat.* **1999**, *13*, 328−330.
(4) De Michele, G.; Cavalcanti, F.; Criscuolo, C.; Pianese, L.; Monticelli, A.; Filla, A.; Cocozza, S. *Hum. Mol. Genet.* **1998**, *7*, 1901−1906.
(5) Heller, M. *Annu. Rev. Biomed. Eng.* **2002**, *4*, 129−153.
(6) Palecek, E.; Fojta, M. *Anal. Chem.* **2001**, *73*, 74A−83A.
(7) Popovich, N.; Thorp, H. *Interface* **2002**, *11*, 30−34.
(8) Wang, J. *Anal. Chim. Acta* **2003**, *500*, 247−257.
(9) Palecek, E. *Trends Biotechnol.* **2004**, *22*, 55−58.
(10) Yang, I. V.; Thorp, H. H. *Anal. Chem.* **2001**, *73*, 5316−5322.
(11) Palecek, E.; Fojta, M.; Jelen, F. *Bioelectrochemistry* **2002**, *56*, 85−90.
(12) Palecek, E.; Kizek, R.; Havran, L.; Billova, S.; Fojta, M. *Anal. Chim. Acta* **2002**, *469*, 73−83.
(13) Jelen, F.; Yosypchuk, B.; Kourilova, A.; Novotny, L.; Palecek, E. *Anal. Chem.* **2002**, *74*, 4788−4793.
(14) Fojta, M.; Havran, L.; Billova, S.; Kostecka, P.; Masarik, M.; Kizek, R. *Electroanalysis* **2003**, *15*, 431−440.
(15) Wang, J.; Xu, D.; Erdem, A.; Polsky, R.; Salazar, M. A. *Talanta* **2002**, *56*, 931−938.
(16) Wang, J.; Polsky, R.; Merkoci, A.; Turner, K. *Langmuir* **2003**, *19*, 989−991.
(17) Fan, Z. H.; Mangru, S.; Granzow, R.; Heaney, P.; Ho, W.; Dong, Q.; Kumar, R. *Anal. Chem.* **1999**, *71*, 4851−4859.
(18) Palecek, E. *Methods Enzymol.* **1992**, *212*, 139−155.
(19) Kizek, R.; Havran, L.; Fojta, M.; Palecek, E. *Bioelectrochemistry* **2002**, *55*, 119−121.
(20) Ohshima, K.; Montermini, L.; Wells, R. D.; Pandolfo, M. *J. Biol. Chem.* **1998**, *273*, 14588−14595.

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