

Unconventional Method Based on Circular Dichroism to Detect Peanut DNA in Food by Means of a PNA Probe and a Cyanine Dye

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Dedicated to Professor Piero Salvadori, Pisa, on the occasion of his 70th birthday.

ABSTRACT In this paper we report an innovative and unconventional method based on circular dichroism for the identification of peanut DNA in food, which can be detected after PCR amplification at the nanomolar level by using an achiral PNA probe complementary to a tract of the peanut Ara h 2 gene and an achiral 3,3'-diethylthiadicarbocyanine dye [DiSC₂(5)]. Peanuts are one of the most common causes of severe allergic reactions to foods and are particularly dangerous when they are "hidden" (undeclared) in food. For better protection of consumers, detection methods are required to specifically detect the presence of hidden allergens in a wide variety of food items. Alternative to the detection of the proteins is the determination of species-specific DNA, which is more resistant to technological treatments. PNAs are very specific probes able to recognize DNA sequences with high affinity and evidence for the binding can be obtained by using the DiSC₂(5) dye, which aggregates onto the PNA–DNA duplex giving rise to a characteristic visible band at 540 nm. Because the PNA–DNA duplex is in a right-handed helical conformation, the aggregation of the dye to the duplex gives also rise to a strong CD signal in the 500–600 nm region with a strong exciton coupling due to the formation of multimeric species, since the handedness of the helix is transferred to the dye aggregate. The dye does not interact with the free single-stranded DNA and although aggregating on the achiral PNA, this interaction is obviously not detectable by circular dichroism. Thus, only the formation of the PNA–DNA duplex, which takes place only upon specific Watson–Crick hydrogen binding between the PNA and the DNA bases, is detected, ensuring a very high specificity and sensitivity. The method has been optimized in a model system by using a synthetic oligonucleotide complementary to the PNA probe, showing that the intensity of the signal is linearly related to the amount of the DNA. The optimized method has been applied to the identification and quantitation of DNA extracted and amplified by PCR from peanuts and from peanut-containing foods, allowing for a very sensitive detection at a very low level (few pmol). © 2005 Wiley-Liss, Inc. *Chirality*

KEY WORDS: PNA; DNA recognition; circular dichroism; cyanine dye; induced chirality; allergens; peanuts

The incidence of allergic reactions to foods and food intolerance has increased significantly in the last few decades and represents an important public health problem. In some cases, severe food-induced anaphylaxis can result in death. It is estimated that approximately 2.5% of children and about 1% of the adult population suffer from food allergy.¹ The most common allergens are cow's milk, eggs, fish, seafood, tree nuts, peanuts, soy, sesame, and wheat. Usually food allergens are proteins or glycoproteins with a molecular mass ranging from 10 to 70 kDa. The allergenicity of these molecules can be due to linear or conformational epitopes or to post-translational modifications (e.g., glycosylation). Peanuts are one of the most common causes of severe allergic reactions to foods

even at minimal doses (100 µg of peanut proteins),^{2,3} leading to life-threatening anaphylaxis and even death.^{4,5} Avoidance of peanut-containing foods can be sometimes difficult for allergic patients because of mislabeling of the

Contract grant sponsor: MIUR; Contract grant numbers: PRIN2002, PRIN2004.

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Received for publication 25 February 2005; Accepted 2 May 2005

DOI: 10.1002/chir.20194

Published online in Wiley InterScience (www.interscience.wiley.com).

products, recycling of peanut-containing foods, or cross-contamination during processing. In order to protect the consumer, detection methods are required which specifically detect the presence of hidden allergens in a wide variety of food items.

Enzyme-linked immunosorbent assays (ELISA)⁶ are currently the most common methods to determine allergenic proteins in food products; however, industrial processes often involve heat treatments, which may denature food proteins,⁷ thus modifying the protein structure and so interfering with their detection. In this case, an alternative is represented by the detection of the specific DNA of a determined species. DNA is less modified under heat and pressure processing, and therefore it can provide a food index for the presence of the hidden allergens in foods.⁸ The DNA extracted from food must be amplified by using PCR (polymerase chain reaction). The presence of the amplified DNA product, which can be easily verified by gel electrophoresis, is taken as a proof of the identity of the specific DNA, although this is not always sufficient for a definitive confirmation, because aspecific amplifications can often generate false positives, thus further confirmatory tests are required.^{9–11}

One of the most promising approaches to confirm the DNA sequences after PCR amplification makes use of peptide nucleic acids (PNAs), oligonucleotide analogues with a pseudopeptide backbone (Fig. 1),¹² which bind to complementary DNA sequences through standard Watson–Crick hydrogen bonds and show a higher affinity for DNA than DNA itself, due to the absence of electrostatic repulsion between negative charges present in DNA–DNA duplexes. Moreover, PNA–DNA duplexes are also characterized by a very high sequence specificity.¹³ An HPLC method has been recently developed by our group for identifying specific DNA sequences by means of PNA probes,¹⁴ in particular for the confirmation of the presence of hazelnut DNA previously amplified by PCR.¹⁵

In 1999, Armitage et al. reported that hybridization of PNA to DNA sequences can be readily visualized by using

3,3'-diethylthiadicarbocyanine dye [DiSC₂(5), Fig. 1], which strongly aggregates to PNA–DNA duplexes, changing its absorption properties: the solution containing the dye alone in its monomeric state has a typical blue color, whereas upon aggregation to the PNA–DNA duplex, the solution becomes purple.¹⁶

However, although outstanding results have been obtained for the detection of point mutations related to genetic diseases in humans,^{17,18} several problems hamper its widespread use. First, the DiSC₂(5) dye has a strong tendency to aggregate also on free PNAs, often generating confusing results. Moreover, colorimetric detection has low sensitivity, requiring a DNA concentration in the low micromolar range, which is not easily obtained.

In this paper we report for the first time the use of the DiSC₂(5) dye and of a PNA probe in order to confirm DNA sequences after PCR amplification by means of CD spectroscopy. The rationale derives from the well-known fact that, upon aggregation on PNA–DNA duplexes, not only does a diagnostic absorbance band at 540 nm wavelength appear but a strong CD signal is also generated in the same region, due to the asymmetric conformation of the DiSC₂(5) dye aggregate induced by the PNA–DNA double helix. Moreover the right-handedness of the PNA–DNA helix also induces a right-handed helical arrangement of the dye aggregate, thus generating a strong exciton coupling effect that defines the shape of the CD band.¹⁶ The use of CD can overcome the limitations of the absorbance measurements, since it gives a positive signal only in the presence of chiral compounds: the DiSC₂(5) dye, which is achiral, aggregates on the PNA–DNA duplex and on single-strand PNA, but only the first one is a chiral complex, because DNA is chiral but PNA is not. Moreover, the strong intensity of the generated CD band may allow for sensitive detection of DNA, even at very low concentrations.

The DNA target of this work is an internal sequence of the peanut Ara h 2 gene (GenBank accession number L77197), which codifies for one of the major allergens in peanuts. DNA detection, after amplification by polymerase chain reaction (PCR), is based on the use of a complementary 15-mer PNA probe and the DiSC₂(5) dye. The appearance of the CD signal in the 500–600 nm region with a right-handed exciton coupling confirms the presence and the identity of the amplified DNA. This method will be applied both to model systems and foods.

MATERIALS AND METHODS

PNA Design

The PNA sequence was designed in order to minimize any secondary structure which would result in a loss of hybridization efficiency, using the program Mfold (version 3.1),¹⁹ available online. The sequence specificity of the probe was first evaluated using the BLAST homology search system from DDBJ (<http://www.ddbj.nig.ac.jp/E-mail/homology.html>) and then it was checked in order to avoid hybridization with any other nontarget region among other amplified products. The sequence chosen for the analyses is H-CGATCAACACATTCA-NH₂.

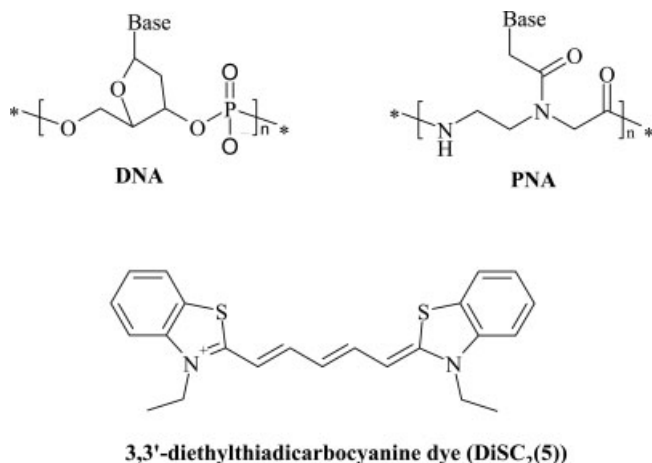


Fig. 1. Structure of DNA, PNA, and DiSC₂(5) dye.

PNA Synthesis

The PNA H-CGATCAACACATTCA-NH₂ was synthesized by solid-phase synthesis on a ABI 433A Peptide Synthesizer (Applied Biosystems, Foster City, CA) with the Boc strategy and HBTU/DIEA coupling, as described previously.^{14,15} Swelling, downloading, and cleavage of the PNA from the resin were done manually. Two 2-(2-aminoethoxy)ethoxyacetic acid linkers were added at the N-terminus of the PNA for its eventual linking to solid surfaces (i.e., microarrays). The crude product was cleaved from the resin by using a 2:6:1:1 TFMSA/TFA/thioanisole/*m*-cresol mixture, precipitated by Et₂O, and purified by reversed-phase HPLC using a Phenomenex C18 peptide column (3 μ m, 250 mm $\times$ 10 mm) with a binary gradient (flow rate: 4 ml/min); eluent A, water/TFA = 100:0.2; eluent B, water/acetonitrile/TFA = 60:40:0.2; detector, UV (260 nm). The purified product was characterized by ESI mass spectrometry (Micromass ZMD, positive-ion mode): calcd MW, 4305 Da; calcd *m/z*, 862.0 (MH₃⁺), 718.5 (MH₆⁺), 616.0 (MH₇⁺); found *m/z* 861.9, 718.5, 615.9.

Oligonucleotide Sequences

The oligonucleotide fully complementary to the PNA synthesized (5'-TGAATGTGTTGATCG-3'), to be used in the model experiments, and a noncomplementary oligonucleotide (5'-GAGCATTGAAATCAT-3') to be used as control in the same experiments, were purchased from Thermo Electron (Waltham, MA).

Food Samples

Unroasted peanuts without shells were provided by an Italian food factory.

Muesli snacks with cereals and milk chocolate were commercially available. Declared content: milk chocolate (21%), sugar syrup, extruded cereal (wheat flour, rice, maize malt, sugar, and salt) toasted oat flakes, toasted peanut bits (7%), brown sugar, hydrogenated vegetable oil, corn flakes (maize, sugar, salt, malt), whole condensed milk, toasted wheat flakes, toasted almond bits (1%), honey (1%), malt, emulsifying agent: mono- and diglycerides of fatty acids; salt, flavors. Average cereal content: 36%.

Chocolate wafers were commercially available. Declared content: cream filling 76% (filling with cocoa 38%: sweetener maltitol, vegetable fats, cocoa 11%, whole-milk powder, rice starch, flavors; filling with vanilla flavor 38%: vegetable fats, sweetener maltitol, whole-milk powder, rice starch, lecithin emulsifier agents, flavors), wheat flour, potato starch, vegetable oils, raising agents (sodium hydrogen carbonate), lecithin emulsifier agent, salt.

DNA Extraction

DNA extraction was performed using the commercial kit Wizard® Plus Miniprep DNA purification system (Promega, Madison, WI), modified as described in a previous work.¹⁹ The modifications can be summarized as follows: once the DNA was extracted and purified following the instructions provided by the commercial

kit, it underwent a further purification step by addition of 0.1 vol of 3 M sodium acetate (pH 5.2) and 2 vol of cold 95% ethanol, followed by incubation at -70°C for 30 min. After precipitation, the DNA was pelleted by centrifugation at 12,000 rpm for 15 min at 4°C. The DNA pellet was washed with 80% ethanol and incubated at room temperature for 10 min, and was then centrifuged for 5 min at 12,000 rpm; the DNA pellet was finally dried and then dissolved in 100 μ l of water.

Polymerase Chain Reaction (PCR)

In order to develop a valid PCR method for the detection of specific DNA sequences coding for peanut allergens, several primer pairs were designed on known sequences obtained from on line available databases. The target sequence was the Ara h 2, one of the major allergens in peanut (GenBank accession number L77197); the specificity of the possible PCR was first evaluated by aligning the designed amplicon with the available sequences using the database DDBJ Homology search system and then tested by PCR with other non-target DNA extracted from 16 vegetables. The target sequence was a 201-bp region identified by the primers Ara h 2_455 (5'-GGCGGCAGAGACAGATACTA-3') and Ara h 2_655 (5'-GACAACGCCATAAAAGCACTC-3').

Polymerase chain reactions were performed in a final volume of 100 μ l. All the reagents were supplied by Euroclone (Whettherby, U.K.), and the primers were purchased from Thermo Electron).

The DNA extracted from peanuts underwent a double amplification: the first step was used to amplify the target sequences, while the second step was an asymmetric PCR used to selectively amplify the target DNA single strand to be hybridized with PNA, by using the double-stranded DNA obtained from the first PCR as the template. The reagent concentrations in the first PCR were as follows: reaction buffer (Blue Taq Euroclone) 3 mM MgCl₂, 0.2 mM dNTPs, 0.4 μ M of each primer, 0.1 U/ μ l of hot-start Blue Taq, and 150 ng of extracted peanut DNA. For the second step (the asymmetric PCR), the reagent concentrations were the same, except the primer concentrations were 0.2 μ M for the primer copying the nontarget DNA strand and 2.0 μ M for the primer copying the target DNA strand. Both PCR procedures were carried out in a PCR-Sprint thermal cycler (Thermo Hybaid, Thermo Electron) using the following conditions: one cycle of DNA denaturation and Blue Taq activation at 95°C for 5 min; 40 cycles consisting of DNA denaturation at 95°C for 50 s, primer annealing at 60°C for 50 s, and elongation at 72°C for 50 s; and one final step of elongation at 72°C for 5 min.

The DNA extracted from commercially available cereal snacks and chocolate wafers underwent asymmetric PCR directly. In order to avoid false-negative results generated by the residual presence of possible PCR inhibitors, the extracted genomic DNA was diluted 20 times and only 4 μ l of the diluted solution was added to the PCR mixture. All other reagent concentrations and conditions for the asymmetric PCR were the same as described above.

DNA–PNA Hybridization

DNA–PNA hybridization tests were performed in a final volume of 2 ml: phosphate buffer (NaCl 100 mM, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 10 mM, EDTA 0.1 mM; pH 7), PNA (300 nM), DNA, and water to 2 ml. The DNA used in the model experiments is a synthetic 15-mer oligonucleotide complementary to the PNA used at different concentrations (from 1 to 300 nM). The DNA in the experiments making use of real food samples was the product of asymmetric PCR of unknown concentration (100 μl used). All samples were previously hybridized at 95°C for 5 min and then cooled to –20°C.

Addition of the DiSC₂(5) Dye

DiSC₂(5) dye was purchased from Sigma-Aldrich (St. Louis, MO). A stock solution of DiSC₂(5) in methanol (500 μM) was added to each sample up to a final concentration of 10 μM in a total volume of 2 ml at room temperature; CD spectra were then immediately recorded.

Spectral Measurements

CD spectra were recorded on a JASCO J-715 spectropolarimeter equipped with a Peltier thermostat (measurements done at 20°C): acquisition range 400–700 nm, resolution 1 nm, accumulation 1, band width 1.0 nm, response 1 s, scan speed 100 nm/min. All spectra were treated with the noise reduction software included in the program J-700 for Windows Standard Analysis, version 1.33.00.

RESULTS AND DISCUSSION

The experimental design of the approach here proposed is shown in Figure 2.

As a model system to test our hypothesis, we used a 15-mer PNA designed to be complementary to a sequence contained in the Ara h 2 gene (the sequences are given in Materials and Methods), a gene that codes for the major peanut allergen.

In order to assess if a viable CD signal could be obtained by the dye aggregation onto the PNA–DNA duplex present at low concentration and in order to define the best working concentration of the dye, we mixed the PNA strand (300 nM) with its complementary synthetic oligonucleotide (30 nM) in a phosphate buffer (pH = 7) and we added the DiSC₂(5) dye to the solution in concentrations ranging from 1 to 30 μM . The concentration of the complementary oligonucleotide was kept very low in order to simulate a likely DNA concentration of a PCR product. A well-defined CD spectrum was recorded starting from a 5 μM dye concentration, and the CD band appeared to level out at 10 μM , since addition of 20 or 30 μM DiSC₂(5) dye did not increase the band intensity (data not shown). This simple experiment demonstrated that a very small amount of DNA can be detected by circular dichroism using PNA probes in connection with the DiSC₂(5) dye, and, according to the data obtained, a 10 μM concentration of the DiSC₂(5) dye was shown to be the optimal working amount.

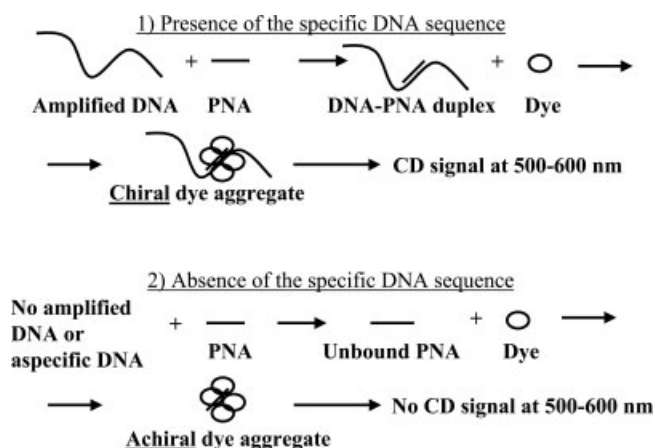


Fig. 2. Rationale of the proposed method. (1) If the correct DNA sequence is amplified, the PNA will form a specific PNA–DNA duplex. Aggregation of the dye on this chiral duplex will generate a CD signal at 500–600 nm due to the induced chirality in the dye aggregate. (2) If no amplification occurs or if a wrong DNA sequence is specifically amplified, the PNA will not bind the DNA. The dye will nevertheless aggregate on the free PNA, but because both the PNA and the dye are achiral, no CD signal will be generated at 500–600 nm.

To test the specificity of the sequence recognition, the 15-mer PNA was also mixed with a noncomplementary oligonucleotide in a phosphate buffer (pH = 7), and DiSC₂(5) dye (10 μM) was added to the solution. The CD spectra of the PNA (300 nM) mixed with the fully complementary oligonucleotide (30 nM) and with a noncomplementary oligonucleotide (30 nM), both with DiSC₂(5) dye (10 μM) added, are reported in Figure 3. A CD spectrum of the solution containing only the dye and the complementary oligonucleotide, without any PNA, is also reported.

The appearance of the CD band was clearly related to the presence of the specific PNA–DNA duplex, because in all cases when the duplex was not present (PNA with noncomplementary oligonucleotide or oligonucleotide alone), the signal was not significantly different from the baseline noise. It should be emphasized that, although no CD signal was detected when PNA was mixed with the noncomplementary oligonucleotide, as is consistent with the likely absence of any PNA–DNA duplex, the solution nevertheless absorbed strongly at 540 nm and therefore became purple, because the dye strongly aggregated on the free PNA. This aspecific aggregation, although very disturbing in the case of absorbance measurements, is totally “invisible” in CD spectrophotometry, as no chiral species are involved. Actually, because a fraction of the dye does aggregate on the free PNA, this could result in a decrease of the dye available to aggregate on the PNA–DNA duplex and could ultimately lead to a decrease of the potential CD signal. However, the fact that the CD signal did not increase with dye concentrations from 10 to 30 μM confirmed that, even at a 10 μM concentration, the dye was still present in large excess when compared to the PNA and DNA strands (and also when compared to the PNA–DNA duplex), and the fraction aggregated on the free PNA did not affect the intensity of the CD signal.

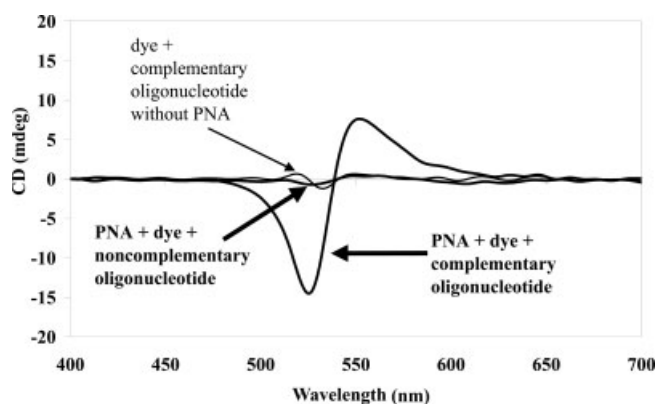


Fig. 3. CD spectra obtained by adding the DiSC₂(5) dye (10 μ M) to (i) a solution containing the 15-mer PNA complementary to a tract of the Ara h 2 gene (300 nM) and its complementary oligonucleotide (5'-TGAATGTGTTGATCG-3', 30 nM); (ii) a solution containing the same PNA and a non complementary oligonucleotide (5'-GAGCATTGAAATCAT-3', 30 nM); (iii) a solution containing the complementary oligonucleotide alone (5'-TGAATGTGTTGATCG-3', 30 nM).

The absence of significant CD signals in the presence of the oligonucleotide and the dye is also of particular importance; because DNA is a chiral molecule, if the dye interacts with the single-stranded DNA it may result in potentially interfering CD bands. In order to definitively rule out interaction of the single-stranded DNA with the dye, we have also added an oligonucleotide that is non-complementary to the PNA to the solution containing the PNA, the complementary DNA, and the dye. We observed that the intensity and the shape of the CD spectrum were not influenced by this addition (data not shown). It should be noted that ruling out interaction of the dye with the single-stranded DNA is of a particular importance in real applications with DNA coming from a PCR amplification, where a short target DNA sequence is embedded in a very long DNA tract.

Additional experiments were then performed in order to test the correlation between the oligonucleotide concentration and the CD signal, by mixing different concentrations of the oligonucleotide (1, 5, 10, 50, 100, 300, and 500 nM) with the complementary PNA (300 nM) and with the DiSC₂(5) dye (10 μ M). Spectra are shown in Figures 4 (1–100 nM oligonucleotide concentration) and 5 (50–500 nM oligonucleotide concentration).

A plot of the CD signal versus the oligonucleotide concentration could be obtained by using the total CD signal (CD_{Tot}), defined as $CD_{Tot} = CD_{555nm} - CD_{525nm}$, where CD_{555nm} is the intensity of the CD signal at 555 nm (positive) and CD_{525nm} is the intensity of the CD signal at 525 nm (negative). This formula gives the sum of the absolute intensities of the two CD bands generated by the exciton effect. Every experiment at every different concentration was repeated three times, and the average results were used in the plots (inserts in Figs. 4 and 5).

The value recorded at an oligonucleotide concentration of 1 nM was not significantly different from the value recorded with the noncomplementary oligonucleotide; thus the limit of detection of this method can be estimated

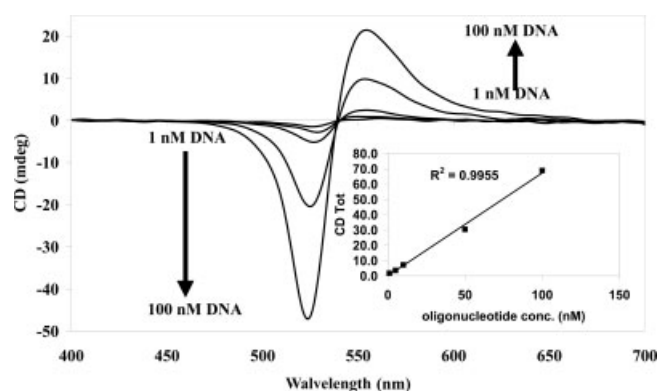


Fig. 4. Increase of the CD signal according to the oligonucleotide concentration in solutions containing the 15-mer PNA complementary to a tract of the Ara h 2 gene (300 nM), the complementary oligonucleotide (5'-TGAATGTGTTGATCG-3', concentrations: 1, 5, 10, 50, 100 nM) and the DiSC₂(5) dye (10 μ M). The plot of the total CD signal against the oligonucleotide concentration is shown in the insert with the corresponding linear regression.

to be 5 nM DNA concentration. It is immediately evident that the response is related to the oligonucleotide concentration: it is also linear up to 100 nM (Fig. 4), but the signal tends to level off at higher concentrations (Fig. 5). Therefore, in order to obtain a linear response, it appears that the PNA must be present in excess with respect to the DNA strand. The linearity of response has important consequences as far as DNA quantitation is concerned. If we assume that the intensity of the CD signal depends only on the concentration of the PNA–DNA duplex, then it should not be strongly influenced if the target DNA sequence is part of a much longer DNA sequence, as occurs in a PCR product. Therefore, we can use the linear regression obtained with the complementary oligonucleotide to “calibrate” the method, thus making an accurate quantitation of the amount of DNA after the PCR amplification feasible.

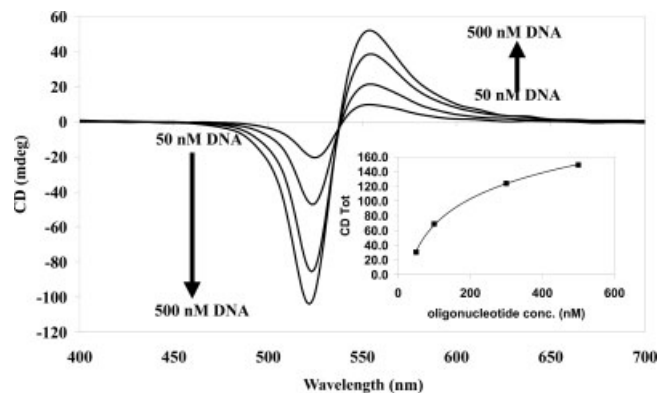


Fig. 5. Increase of the CD signal according to the oligonucleotide concentration in solutions containing the 15-mer PNA complementary to a tract of the Ara h 2 gene (300 nM), the complementary oligonucleotide (5'-TGAATGTGTTGATCG-3', concentrations: 50, 100, 300, and 500 nM) and the DiSC₂(5) dye (10 μ M). The plot of the total CD signal against the oligonucleotide concentration is shown in the insert with the corresponding regression.

On the basis of the promising results obtained in the model system with the complementary oligonucleotide, the method was tested in real samples, where PCR products instead of synthetic oligonucleotides were used.

Unroasted peanuts made up the first sample: the DNA was extracted, and a 201-bp region of the Ara h 2 gene was amplified by standard PCR procedures. Since PCR yields double-stranded DNA, and given that PNA can hybridize only to single-stranded DNA, a further PCR was then performed by using different concentrations of the two primers (asymmetric PCR), in order to obtain a discrete amount of single-stranded DNA that could be recognized and bound by the PNA. Details of DNA amplification starting from peanuts are reported in Materials and Methods. After the asymmetric amplification, 100 μ l of the mixture containing the amplified DNA was mixed with the PNA (300 nM) and the DiSC₂(5) dye (10 μ M). A strong signal was obtained, as reported in Figure 6, allowing us to confirm the presence of DNA containing the target sequence, i.e., a part of the Ara h 2 gene, as was expected.

We then assessed the presence of peanut DNA in cereal snacks containing peanuts (declared peanut content: 7%). The DNA was extracted, and the region of the Ara h 2 gene was directly amplified by using only asymmetric PCR, with no preamplification of the DNA by standard PCR. To test a negative sample, the DNA from a chocolate wafer that did not contain peanuts was also extracted and amplified by asymmetric PCR. After the PNA probe and the DiSC₂(5) dye were added, the CD spectra were recorded (Fig. 6). These experiments consistently gave positive signals only in the presence of the peanut-containing sample.

The linear regression obtained as reported above was used to quantitate the single-stranded DNA present in the two positive cases, by assuming that the aggregation of the dye is influenced only by the PNA–DNA duplex. We

obtained a value of 41 nM in the case of DNA from 100% peanuts (corresponding to an absolute amount of 82 pmol of single-strand DNA) and a value of 9 nM for the DNA from the cereal snacks (corresponding to an absolute amount of 18 pmol of single-strand DNA).

It should also be noted that the extreme sensitivity of the method requires the use of a very specific PNA probe. Whenever amplified DNA is present in the post-PCR mixture which does not correspond to the target sequence, in order to avoid false positives, it is absolutely necessary that the PNA probe bear fewer than 4–5 bases complementary to any tract along the overall DNA sequence. In fact, preliminary experiments showed that a complementarity of 5 bases or more will give rise to PNA–DNA duplexes stable enough to elicit DiSC₂(5) dye aggregation and therefore the appearance of CD signals which, in that case, are false positives.

CONCLUSIONS

In this paper we report for the first time a CD-based method for the confirmation, after PCR amplification, of a specific DNA sequence in foods by using a PNA probe and diethylthiadicarbocyanine dye. The method is based on the appearance of a diagnostic absorption band at 540 nm, due to aggregation of the dye onto the PNA–DNA duplex, accompanied by the selective elicitation of a CD signal at the same wavelength, due to the chirality of the dye aggregate induced by the right-handedness of the PNA–DNA duplex. The method appears to be very selective, because the free PNA–dye aggregate, which exists in solution and is achiral, does not give rise to any CD signal and the dye does not aggregate on free DNA. The method was demonstrated to be very sensitive, allowing the confirmation of DNA down to a concentration of 5 nM, and the response was shown to be linear in the 5–100 nM range.

Application to real food samples demonstrated that the method can be used to confirm the presence of peanut DNA after PCR amplification in complex food matrices, making this approach a specific and efficient way to confirm the presence of hidden allergens in food.

ACKNOWLEDGMENTS

The MIUR (Ministry of Education, University and Research) is gratefully acknowledged for its financial support given to Projects of Relevant National Interest (PRIN). This work has been supported by PRIN 2002 ("Peptide Nucleic Acids (PNAs) as Probes for Food Diagnostics") and PRIN 2004 ("Biotechnologies and Nanotechnologies Applied to the Identification of Plant Allergens in Foods").

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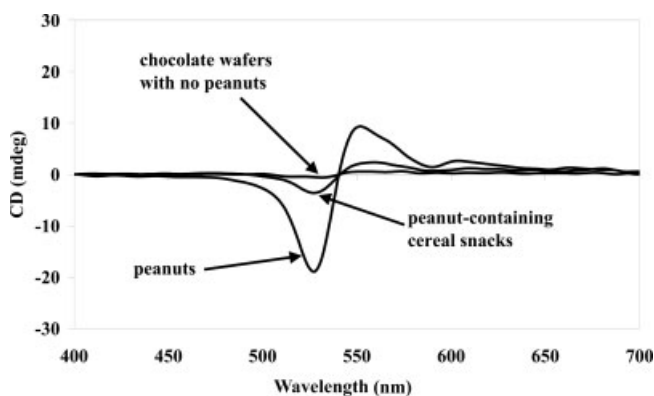


Fig. 6. CD spectra obtained by adding the DiSC₂(5) dye (10 μ M) to (i) a solution containing the 15-mer PNA complementary to a tract of the Ara h 2 gene (300 nM) and the DNA extracted and amplified from unroasted peanuts; (ii) a solution containing the 15-mer PNA complementary to a tract of the Ara h 2 gene (300 nM) and the DNA extracted and amplified from peanut-containing (7%) cereal snacks; (iii) a solution containing the 15-mer PNA complementary to a tract of the Ara h 2 gene (300 nM) and the DNA extracted and amplified from chocolate wafers with no peanuts.

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