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Electrochemical Detection of Oligo-DNA only with the Difference of Single Nucleotide Polymorphisms using the Polyaniline Intercalator

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The human gene all are not identical, and there is slight difference. Especially, difference between a base in the gene, it is indicated that various diseases are caused by Single Nucleotide Polymorphisms(SNPs). SNPs exists about one for every 1000 bases in all genomes. Lifestyle habit illness and side effect of drug, etc. is caused by SNPs. Therefore, SNPs becomes a marker which shows human constitution.
In the meantime, the chip for DNA diagnosis is divided roughly into result of utilizing the fluorescene and thing that has electrochemically active molecules (intercalator). The former, gene segment or oligo-DNA are fixed on the substrate, and the hybridization (it combines complementarily) of target DNA is done, and the detection is carried out by the fluorochrome, and there are DNA chips of Affymetrix Co., etc.. Using the intercalator the latter electrochemical measurement has done. The gold electrodes implanted DNA related with cancers etc. are evaluated by potentiodynamics. The part practical application stage of Toshiba Co., etc. may have been entered. It is advantageously expected that the electrochemical measuring method can be comparatively accurately judged fluorescene method measuring device as DNA chip in the next generation. However, the high precise electrical measurement technology is required in the electrical diagnosis using SNPs in order to electrically evaluate. Therefore, the real application to which diagnoses the constitutions which are changeable to the lifestyle habit illness, etc. has not been developed still.

The target of this study is the constitution which is changeable to the lifestyle habit illness (hypertension, diabetes mellitus, obesity, hyperlipidemia, arteriosclerosis, etc.). SNPs with these disease pathopoiesis is fixed on the gold electrode as probe DNA. The complementary DNA is dropped in this. The existence of the hybridization is detected by the intercalator. Finally, by cooperating with the medical institution, DNA chip and measuring device which evaluate the constitution which is changeable to the lifestyle habit illness will be developed.

Single strand oligo-DNA: The 4 kinds of DNA in which one base which is related to obesity and diabetes mellitus within the lifestyle habit illness is polymorphic DNA as an object : They are trp64, arg64, pro12, ala12. From the specific gene sequence, specific amino acid chain. This amino acid becomes the protein with space structure, and in addition, it functions in the internal. There is a function which is related to the energy consumption on the β3 adrenergic receptor (protein). Amino acid of 64 is whether it becomes the tryptophan (Trp ; whole 80 % almost) and whether it becomes arginine (Arg;20%), and slimness, offal fat decrease type or obesity and offal fat accumulated respectively appear in the phenotype (constitution). This tryptophan or arginine, decide the phenotype, slimness, offal fat decrease type or obesity and offal fat accumulated are respectively shown. The difference of the one base of DNA on the gene does the predetermination of whether it becomes this Trp and whether it becomes Arg. 25mer oligo-DNA given to both sides of part (thymine (T) or Cytosine (C)) of this SNPs in the each 12 bases. It is respectively expressed with Trp64 and Arg64, and it is used as a probe DNA for complementing target DNA in the condition that floats submerged. And, it is expressed the result to of add sulfhydryl group (mercapto

group) at the 5' end with trp64-SH,arg64-SH, and fixed on gold electrode, it is used as probe DNA. And the following are expressed with trp64-com,arg64-com : Probe DNA and target DNA with the complementary base sequence.

Trp64,(Trp64-SH) : (SH-) 5'-GTGGCCATCGCC T GGACTCCGAGAC-3'
Trp64com : 3'-CACCGGTAGCGG A CCTGAGGCTCTG-5'
Arg64, (Arg64-SH) : (SH-) 5'-GTGGCCATCGCC C GGACTCCGAGAC-3'
Arp64com : 3'-CACCGGTAGCGG G CCTGAGGCTCTG-5'

In the meantime, PPARγ2 shows adiposity type, insulin resistance, and the 12th amino acid is whether it becomes proline (Pro ; whole 96 % almost) and whether it becomes alanine (Ala;4%), and or, they are hard to consist phenotype (constitution) each diabetes mellitus be easy to, they appear. 25mer oligo-DNA that SNPs did this predetermination and that it gave it to both sides of part (C or Guanine (G)) of this SNPs in each 12 bases It was respectively made to be Pro12 and Ala12. And, it was made the result to of add the mercapto group at the 5' end to be Pro12-SH,Ala12-SH, and target DNA was expressed with Pro12-com,Ala12-com.

Pro12,(Pro12-SH) : (SH-) 5'-TCTCCTATTGAC C CAGAAAGCGATT-3'
Pro12com : 3'-AGAGGATAACTG G GTCTTTCGCTAA-5'
Ala12,(Ala12-SH) : (SH-) 5'-TCTCCTATTGAC G CAGAAAGCGATT-3'
Ala12com : 3'-AGAGGATAACTG C GTCTTTCGCTAA-5'

It was adjusted by probe DNA and standart sodium citrate(SSC;pH7) buffer solution at 10pmol/μl.

Intercalator: In the intercalator, there are methylene blue (MB), bis benz imide (bBI). And, there is ferrocenyl-naphthalene-diimide (FND ; Takenaka et.al., Kyushu Univ.) which shows the oxdation-reduction reaction which was reversibly stabilized with the high selectivity for double strand DNA[1]..

Using polyaniline (PAn) in this study as an intercalator. The polyaniline stably reversibly does the oxdation-reduction reaction[2]. And, because it is a electroconductive polymer, also affected conductivity by pH atmosphere. It can have the material of the resemblance more and more for the dyeing of indigo plant fabric and neurone as a dye, and the acquaintanceship with the biomaterial is good. Used reduction un-doping condition.Mean degree of polymerization is almost 1550 (Nitto Denko Co.,) [3]. As a conductivity in the oxidation state by the electronic drawing for the compensation of the charge this Pan the anion it dopes After the hybridization processing, by following the stage, the introduction of the intercalator carried out it. The stacking interaction between nucleic acid base and aromatic ring of the intercalator is because adverse effect be would not caused the hybridization on this.

Manufacture of the DNA chip : Gold electrode was produced by on pyrex glass, film of Cr and Au is formed by the sputtering, and is respectively forming film of 1200Å and 4000Å. The film formation condition are substrate heating temperature 110℃, RF power, and time is Cr(120W,15min), Au(100W,20min) respectively. The electrode is ϕ6mm circular (0.28cm^2). It was used as a working electrode in the time of the electrochemical measurement. Using gold electrode and mercapto group, the 5' end side of DNA probe was fixed by the Au-S combination on Au. Drop quantity of the DNA-SSC solution is 20μl. After 25℃, 1h neglect, it was washed in the Tris(hydroxymethyl)aminomethane/EDTA ;deoxyribonuclease inhibitor (TE;pH7) solution. In this after, the intercalator was dropped (dark place, 25℃, 10 minutes), and the electrochemiscal measurement was carried out.

The experimental result: First, the base pair size of DNA was measured in microchip type electrophoresis apparatus (2100 bioanalyzer;Agilent technologies) in order to confirm whether it has done actually DNA hybridization. After the hybridization of probe DNA(Trp64 ,Arg64, Pro12, Ala12) and target DNA(Trp64com ,Arg64com ,Pro12com, Ala12com) is done, electrophoresis. The result is shown in Figure 1.

The quadrature axis shows the base pair number, vertical line shows concentration. Laddre is a marker. Trp64-Trp64com, Arg64-Arg64com, Pro12-Pro12com Ala12-Ala12com seemed to cause the hybridization, and it was proven that the concentration was easy to do the hybridization high with 4000nmol/l the above. Though pre- 3 persons respectively agree with 25bp(25+25) in 24,25,24 almost, Ala12-Ala12com becomes 19bp and small value. Next, pairs of the one base difference,Trp64-Arg64com, Arg64-Trp64com, Ala12-Pro12com, Ala12-Pro12com was about 1500～4000nmol/l. It is shown that this is a difference between the one base and that it does completely do not do the hybridization, and that it has gently combined.

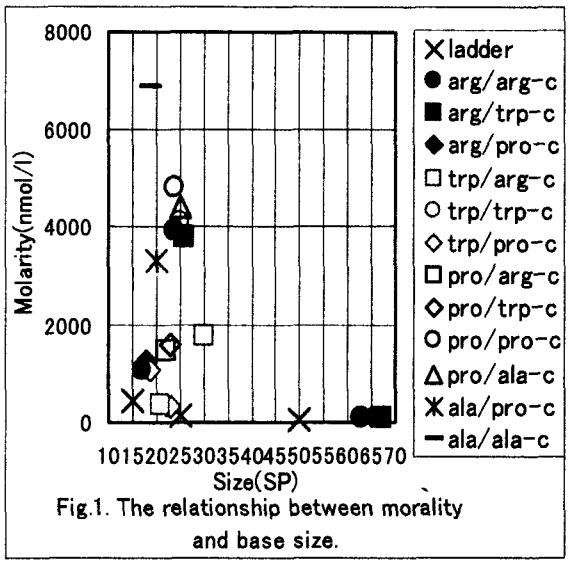

Fig.1. The relationship between morality and base size.

Arg64-Trp64com, Ala12-Pro12com, Ala12-Pro12com concentration was as a half of the result of perfectly doing the hybridization. It was proven that the concentration was almost difficult to do the hybridization of the result (for example, Arg64- Pro64 com) which there is not the other relevance low with 1500nmol/l or less. This result shows the situation of the hybridization of oligo-DNA of about 25mer clearly and is important.

Figure 2 shows Cyclic voltamograms of Trp64 and 4 target DNA(Arg64com 、 Trp64com 、 Pro12com 、 Ala12com). At 0.5Vvs.AgAgCl,Trp64-Trp64com,Trp64-Arg64com, 25 μ A/cm2,21 μ A/cm2 current respectively flowed. Trp64-Trp64com seems to be whether it is not because the exchange of the charge smoothly progresses between the electrode that the density of DNA on the gold electrode greatly comes and that it works, when that 2 chains were chosen by the hybridization, as it was shown in Figure 1,are considered. In Figures 3, the difference between Trp64-Trp64com and Trp64-Arg64com by the difference between the intercalators is shown. At 0.4Vvs.AgAgCl in Pan, it was respectively 16 μ A/cm^2,12 μ A/cm^2 on Trp64-Trp64com,Trp64-Arg64com. And, Figures 4 shows the difference between Arg64-Arg64com and Arg64-Trp64com by the difference between the intercalators. On Pan, at 0.4Vvs.AgAgCl, Arg64-Arg64com,Arg64-Trp64com was respectively 29 μA/cm^2,19 μA/cm^2. The fact is that the current flows without also relating to covering the gold electrode surface than the thing that there is the selectivity of the hybridization which can say from Figure 3,4 and that it

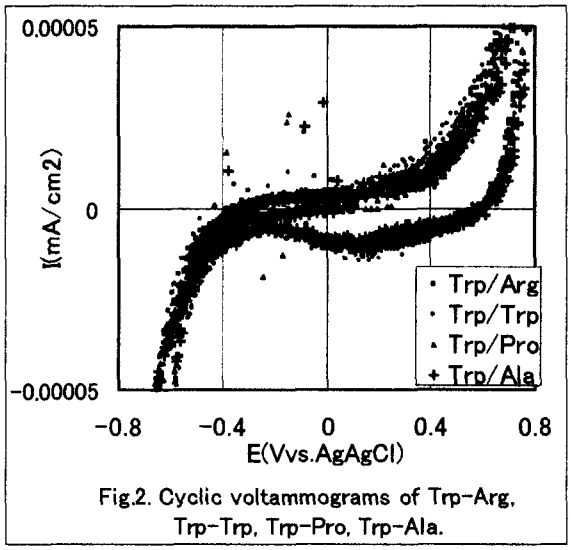

Fig.2. Cyclic voltammograms of Trp-Arg, Trp-Trp, Trp-Pro, Trp-Ala.

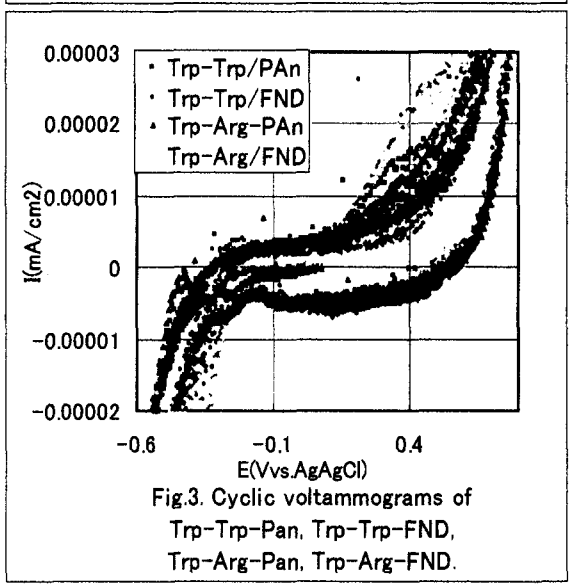

Fig.3. Cyclic voltammograms of Trp-Trp-Pan, Trp-Trp-FND, Trp-Arg-Pan, Trp-Arg-FND.

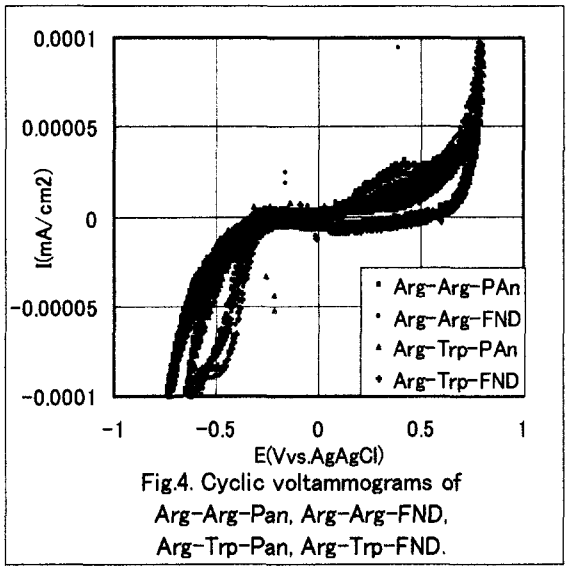

Fig.4. Cyclic voltammograms of Arg-Arg-Pan, Arg-Arg-FND, Arg-Trp-Pan, Arg-Trp-FND.

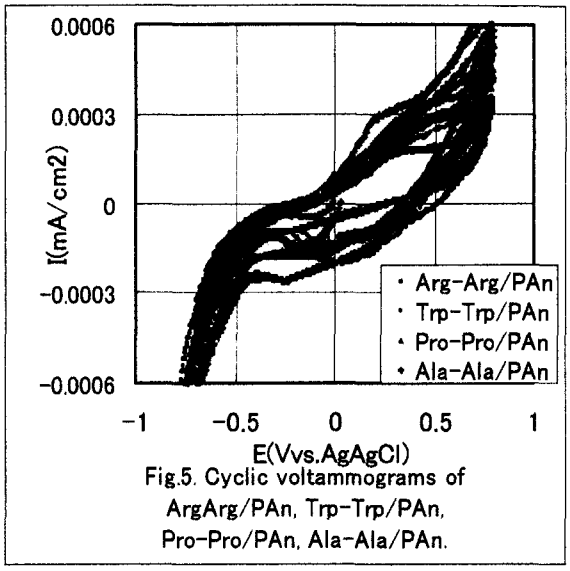

Fig.5. Cyclic voltammograms of ArgArg/PAn, Trp-Trp/PAn, Pro-Pro/PAn, Ala-Ala/PAn.

does not do the hybridization clearly. It is possible to regard this as Pan exchanging charge and electrode through DNA, and, the possibly important suggestion that it is regarded as a doping to Pan, because DNA seems to be the anion in the solution, is contained. Figures 5 observed the hybridization selectivity of Pan. Pan was let in 4 kinds of hybridization (Arg64-Arg64com、Trp64-Trp64com、Pro12-Pro12com、Ala12-Ala12com). The quantity of current in 0.3Vvs.AgAgCl is following order ,Ala12- Ala12com、Pro12-Pro12com、Trp64- Trp64com、Arg64-Arg64com.

Hydrogen bond between adenine A- thymine T are the 2 places here, and it between cytosine C- guanine G is the 3 places. The AT,CG combination number between Arg64-Arg64com is 8 and 17.

In Trp64-Trp64com,Pro12-Pro12com,Ala12-Ala12com, it is respectively 7-18,14-11,14-11. It is more frequent, as the AT combination is more abounding, stacking (that the aromatic ring of the intercalator enters between combination), and it is guessed that the current value may increase, since intercalator and DNA can close.

Conclusion: Lifestyle habit illness and one base polymorphism oligo-DNA of which the relation is deep were used as probe DNA, target DNA, and the DNA chip was produced. Using electroconductive polymer (PAn) as an intercalator for the detection. The result showed that the existence of the hybridization could be electrochemically measured.

Reference

[1] M. Takagi, s. Takenaka, Technical Digest of the 18th Sensor Symposium,(2001) pp.387～393
[2] K. Yoshino, H, Takahashi, S. Morita, T. Kawai and R. Sugimoto, Jpn.J.Appl.Phys.,33(1994)
[3] A. Ohotani, M. Abe,M. Ezoe,T. Doi,T. Miyata,A. Miyake,Synth.Met,55-57,3696-3701(1993)