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(54) Title: DNA HYBRIDS AND ENVIRONMENT CLEANING SYSTEM EMPLOYING DNA HYBRIDS

(57) Abstract: A DNA hybrid which comprises a porous oxide matrix and DNA immobilized thereon, and is useful for environmental clean-up, where the hybrid is prepared by removing a dispersion medium from a dispersion of colloidal oxide and DNA.

DESCRIPTION

DNA HYBRIDS AND ENVIRONMENT CLEANING SYSTEM
EMPLOYING DNA HYBRIDS

5

TECHNICAL FIELD

The present invention relates to a DNA hybrid. More particularly, the invention relates to a DNA hybrid where DNA is immobilized firmly in an oxide matrix, having water resistance; retaining selective recognition function of DNA and allowing intercalation into the double helix of DNA. It also relates to an environmental clean-up system using this DNA hybrid.

15 BACKGROUND ART

DNA (deoxyribonucleic acid) carrying genetic information in living organisms is one of the most important materials for life process. DNA has the capability of extremely precise molecular-recognition, because DNA forms a double-stranded structure between two complementary strands via base pairs between them. Based on this principle, genetic diagnosis that uses the DNA chip has been developed. Application to biosensors and molecular devices is also expected.

25 Also since the DNA double helix allows selective intercalation of an aromatic compound having a planar chemical structure, it is useful in removing a

carcinogenic compound and the like, and is expected to be useful as an environmental cleaning material for removing a harmful substance in air or water (see FUNCTION & MATERIALS, Vol.19, 1999).

5 For example, DNA chips carrying a lot of DNA fragments (DNA probes) on the surface of a substrate like a slide glass have been developed. Since DNA is naturally water-soluble, DNA must be immobilized onto the substrate for such applications. In order to
10 immobilize a biological substance on a substrate, organic crosslinking reaction is conventionally utilized. For instance, there is a method of supporting a biological substance such as nucleic acid, protein and peptide on the roughened surface of
15 a slide glass having a surface layer of a carbon-based material (see Japanese Patent Application Laid-open No. 2002-211954). In this method, first the carbon surface is carboxylized and subjected to dehydration-polycondensation with carbodiimide and N-
20 hydroxysuccinimide to form active ester groups such as N-hydroxysuccinimide ester group on the end of the hydrocarbons through an amide bond, and then the biological substance to be supported is bonded to the activated surface. Also a method and a kit for
25 quantitatively detecting nucleic acid are disclosed in Japanese Patent Application Laid-open No. H11-148935.

Furthermore, a method for immobilizing nucleic acid on a substrate is disclosed where the substrate is activated by using atomic-oxygen plasma to immobilize nucleic acid thereon (see Japanese Patent Application Laid-open No. 2002-218976). Any one of these technologies has such problems that the supporting region is limited to the surface of the substrate and the process is complicated.

In addition to the organic crosslinking reaction, a crosslinking reaction with a metal ion was also proposed. Japanese Patent Application Laid-open No. H7-41494 discloses a method for immobilizing a deoxyribonucleic acid by coagulating an alkaline metal salt of the deoxyribonucleic acid and an alkaline metal salt of alginic acid using a divalent metal-containing compound.

Meanwhile, concerning the use of DNA as an environmental cleaning material, Japanese Patent Application Laid-open No. 2001-81098 proposes a material where DNA is solidified and immobilized on a support by irradiating UV light of 250 to 270 nm to a liquid film or thin layer of water-soluble DNA on the support. Further, Japanese Patent Application Laid-open No. H10-175994 discloses a DNA complex where DNA is immobilized on an inorganic solid. In these methods DNA is crosslinked to develop water resistance, but there are such problems that the

exposed area of DNA is small and functions of DNA are not developed efficiently.

US Patent 6303290 discloses a method for immobilizing a biological material on a matrix of silica colloid. In this method, although the matrix has a large number of micropores, the matrix strength is not enough.

DISCLOSURE OF THE INVENTION

10 The DNA carriers obtained by the above methods are not adequate as a DNA composite material applicable to selective absorption process or environmental clean-up with high efficiency, in view of the DNA-supporting strength and function
15 expression. The present invention has been made to solve such problems in the prior arts, and an object of the present invention is to provide a DNA hybrid where DNA is effectively immobilized and exhibits sufficient function, a preparing method therefor, and
20 further an environmental clean-up system using the DNA hybrid.

The invention includes the following aspects.

(1) A DNA hybrid which comprises a porous oxide matrix and a DNA immobilized on the matrix, where the
25 DNA hybrid is characterized by that it is prepared by removing a dispersion medium from a dispersion of a colloidal oxide and DNA.

(2) The DNA hybrid described in the above (1) characterized in that the dispersion medium is removed by heating the dispersion at 25°C to 200°C.

(3) The DNA hybrid described in the above (1),
5 characterized in that the DNA content in the hybrid is 0.01 to 15% by mass.

(4) The DNA hybrid described in the above (1) or (3), characterized in that the colloidal oxide is a mixture of a colloidal silica and at least one
10 colloidal oxide of a metal whose valence is 3 or 4.

(5) The DNA hybrid described in the above (1), characterized in that the oxide comprises at least one oxide selected from the group consisting of aluminum oxide, iron oxide, titanium oxide and
15 zirconium oxide.

(6) A method for producing a DNA hybrid which comprises the steps of preparing a dispersion comprising a colloidal oxide, a DNA and a dispersion medium; and removing the dispersion medium from the
20 dispersion to immobilize the DNA in a porous matrix made of the oxide.

(7) The method described in the above (6), characterized in that the dispersion medium is removed by heating the dispersion at 25°C to 200°C.

(8) The method described in the above (6),
25 characterized in that the DNA content of the DNA hybrid is 0.01 to 15% by mass.

(9) The method described in the above (6) or (8), characterized in that the colloidal oxide is a mixture of colloidal silica and at least one colloidal oxide of a metal whose valence is 3 or 4.

5 (10) The method described in the above (9), characterized in that the oxide comprises at least one oxide selected from the group consisting of aluminum oxide, iron oxide, titanium oxide and zirconium oxide.

10 (11) An environmental cleaning system for removing a harmful substance in water, which comprises a DNA hybrid described in the above (1) and means for bringing the DNA hybrid into contact with a water containing a harmful substance.

15 (12) An environmental cleaning method by removing a harmful substance in a water, characterized in that a DNA hybrid described in the above (1) is brought into contact with water containing a harmful substance to adsorb the harmful
20 substance by the DNA hybrid.

BEST MODE FOR CARRYING OUT THE INVENTION

In the invention, colloid aggregation or gelling occurs in the DNA containing oxide colloid
25 dispersion as of the dispersion medium is evaporated or because of the presence of a third component, and finally a porous oxide gel is formed after the

dispersion medium is removed. DNA is taken in the oxide colloid during stages from dispersion to gellation to be immobilized in the porous matrix.

DNA employed in the invention is not limited particularly for its size or type, provided that it achieves the purpose of use when being immobilized in the matrix. Such DNA may be single-strand or double-stranded DNA obtained from, for instance, soft roe or animal thymus; preferably obtained from soft roe (testis) of salmon, herring or cod; and also preferably obtained from the thymus of mammals or birds such as cow, pig and chicken.

Other example of water-soluble DNA may be a synthetic DNA, for instance, poly (dA)-poly (dT) having base pairs of (dA)-(dT). The molecular weight of DNA is preferably 100,000 or higher, more preferably 500,000 or higher.

The content (by mass) of DNA in the DNA hybrid according to the present invention is in a range of 0.01 to 15% and preferably of 0.1 to 10%. DNA content of 0.01% or more enables sufficient expression efficacy of DNA-originated properties, and DNA content of 15% or less can obviate the cost efficiency problem and allow formation of pores in the DNA hybrid. The pores allow rapid movement of gas and liquid into the DNA hybrid, so that DNA can achieve its function not only on the surface but also

in the pores.

The colloidal oxide employed in the invention may be, for example, colloidal silica, colloidal aluminum oxide, colloidal iron oxide, colloidal gallium oxide, colloidal lanthanum oxide, colloidal titanium oxide, colloidal cerium oxide, colloidal zirconium oxide, colloidal tin oxide, colloidal hafnium oxide and the like. Any of these can be used alone or in combination of two or more. In view of the matrix stability and the cost, it is preferred to use readily available silica colloid as the main matrix component. More preferably, colloidal silica is used in combination with at least one colloidal oxide of trivalent or tetravalent metal. Addition of colloidal oxide of tri- or tetravalent metal causes binding between the phosphate group of DNA and the metal ion, which enhances the DNA-holding ability of the matrix, preventing elution of DNA in the ordinary aqueous environment. As a solid in the colloid, the 3- or 4-valent metal oxide content is preferably 0.1 to 50% by mass. Among those listed above, iron oxide, titanium oxide and zirconium oxide are especially preferred. Any of these colloids can be synthesized by the hydrothermal reaction. Alternatively, an aqueous colloidal dispersion of oxide is commercially available.

Examples of colloidal silica include aqueous

sols such as SNOWTEX 20, SNOWTEX 30, SNOWTEX N, SNOWTEX O, SNOWTEX C (trade names, provided by NISSAN CHEMICAL INDUSTRIES, LTD.) and the like, methanolic sols, and solvent-based sols such as IPA-ST, EG-ST, 5 MEK-ST (trade name, provided by NISSAN CHEMICAL INDUSTRIES, LTD.), OSCAL-1132, OSCAL-1432, OSCAL-1232 (trade name, provided by SHOKUBAI KASEI KOGYO). Examples of colloidal aluminum oxide are those marketed by NISSAN CHEMICAL INDUSTRIES, LTD. under 10 the trade name of ALUMINA SOL 100, ALUMINA SOL 520 and the like.

To immobilize DNA in the oxide matrix, first a mixture of a DNA solution and a colloidal oxide dispersion is prepared and the dispersion medium is 15 removed from the mixture. The surface of colloidal oxide particles may be modified in advance to prevent agglomeration of colloid particles in the dispersion stage. The modification can be accomplished by adding a chelating agent such as a surfactant, 20 titanium compound, silane coupling agent, β -diketone and the like and an organic acid such as acetic acid as appropriate to a colloidal dispersion to partially modify the surface of the colloid particles. The colloidal dispersion thus obtained was combined with 25 an aqueous solution of DNA to obtain a DNA hybrid. In the step of immobilization, the dispersion medium is removed by means of heating, spray drying, vacuum

drying and the like to form an oxide matrix. A drying process with heating is especially preferred because it enhances the matrix strength. The temperature of heat treatment for DNA hybrid is preferably 25°C or higher and 200°C or lower, more preferably not lower than 30°C and not higher than 150°C. If necessary, a third component may be added to strengthen the bond between the oxide colloid particles. The third component is not limited particularly, including acid or base, water-soluble metal compound and metal alkoxide that can promote the aggregation of colloid particles.

The DNA hybrid may be used in a form of powder or bulk. In addition, it may be used as a coating film on a substrate such as plate, tube, fiber, woven fabric and non-woven fabric, as needed. Accordingly, the DNA hybrid according to the present invention can be used in these forms. For instance, DNA hybrid powder can be obtained by spray drying of a dispersion containing colloidal oxide and DNA. Alternatively, first DNA hybrid is formed in the bulk and then the bulk is pulverized.

Furthermore, it is possible to make a module using the above-described DNA hybrid powder, or plate, tube, fiber, woven or non-woven fabric to which DNA hybrid is immobilized. For instance, a column filled with the DNA hybrid powder can be used for extracting

a specific substance from a gas or a liquid. Or a filter module may be contemplated using a fiber or fabric supporting the DNA hybrid to filter cow's milk or mother's milk.

5 Such a module can be applied to an environmental clean-up system for removing hazardous materials from various effluents, waste water, and river or lake water. For instance, as described above, a column containing the DNA hybrid powder, or
10 DNA hybrid held on a plate, a tube, a fiber, a woven or non-woven fabric can be used to purify water containing a harmful substance by passing it through the column.

EXAMPLES

15 (Example 1)

Five parts by mass of double-stranded DNA obtained from salmon testis (molecular weight: 6×10^6) was dissolved in 1000 parts by mass of ion exchange water over a day to obtain an aqueous
20 solution of DNA.

To 70 parts by mass of 30% silica sol (NISSAN CHEMICAL INDUSTRIES, LTD., SNOWTEX CM), 20 parts by mass of 20% by mass alumina sol (NISSAN CHEMICAL INDUSTRIES, LTD., ALUMINA SOL 520) was added with
25 stirring. The resultant sol mixture was combined with 100 parts by mass of the DNA solution, and stirred slowly for 10 minutes. Subsequently, the

resultant DNA dispersion was dried for 24 hours at 50°C to obtain DNA Hybrid 1 whose DNA content was about 2% by mass.

This DNA hybrid was subjected to an elution
5 test. To 20 parts by mass of ion-exchanged water, 0.05 parts by mass of the DNA hybrid powder was added and settled in a sealed condition at room temperature for 48 hours. The absorbance (260 nm) of DNA in the supernatant liquid measured by a spectrophotometer
10 (U-3310, Hitachi) was 0.05 or less. The DNA hybrid was proved to be holding 95 mass% or more of the DNA. When 0.5 parts by mass of the DNA hybrid was put into an aqueous solution of 60 ppm ethidium bromide, the DNA hybrid turned red after three hours, while the
15 red color due to ethidium bromide in the supernatant decreased. When irradiated with ultraviolet light of 366 nm wavelength, the DNA hybrid emitted an orange fluorescence, which ensured that the intercalating function for a hazardous compound having a planar
20 structure was maintained.

The DNA hybrid showed a specific surface area of 135 m²/g, when measured with a nitrogen adsorption method.

(Example 2)

25 To 70 parts by mass of 30% silica sol (NISSAN CHEMICAL INDUSTRIES, LTD., SNOWTEX CM), 40 parts by mass of 20% by mass alumina sol (NISSAN CHEMICAL

INDUSTRIES, LTD., ALUMINA SOL 520) was added with stirring. The resultant sol mixture was combined with 100 parts by mass of the DNA solution prepared in Example 1, and stirred slowly for 10 minutes.

- 5 Subsequently, the resultant DNA dispersion was dried for 24 hours at 50°C to obtain DNA Hybrid 2 whose DNA content was about 1.7% by mass.

This DNA hybrid was subjected to an elution test. To 20 parts by mass of ion-exchanged water,
10 0.05 parts by mass of the DNA hybrid powder was added and settled in a sealed condition at room temperature for 48 hours. The absorbance (260 nm) of DNA in the supernatant liquid measured by a spectrophotometer (U-3310, Hitachi) was about 0.02. The DNA hybrid was
15 proved to be holding 98 mass% or more of the DNA.

(Example 3)

To 70 parts by mass of 30% silica sol (NISSAN CHEMICAL INDUSTRIES, LTD., SNOWTEX CM), 60 parts by mass of a titanium oxide sol (TAKI CHEMICAL CO., LTD.,
20 M-6, 6% by mass) was added with stirring. The resultant sol mixture was combined with 100 parts by mass of the DNA solution prepared in Example 1, and stirred slowly for 10 minutes. Subsequently, the resultant DNA dispersion was dried for 24 hours at
25 50°C to obtain DNA Hybrid 3 whose DNA content was about 1% by mass.

This DNA hybrid was subjected to an elution

test. To 20 parts by mass of ion-exchanged water,
0.1 parts by mass of the DNA hybrid powder was added
and settled in a sealed condition at room temperature
for 48 hours. The absorbance (260 nm) of DNA in the
5 supernatant liquid measured by a spectrophotometer
(U-3310, Hitachi) was about 0.05. The DNA hybrid was
proved to be holding 95 mass% of the DNA.

(Example 4)

To 70 parts by mass of 30% silica sol (NISSAN
10 CHEMICAL INDUSTRIES, LTD., SNOWTEX CM), 100 parts by
mass of a titanium oxide sol (TAKI CHEMICAL CO., LTD.,
M-6, 6% by mass) was added with stirring. The
resultant sol mixture was combined with 100 parts by
mass of the DNA solution prepared in Example 1, and
15 stirred slowly for 10 minutes. Subsequently, the
resultant DNA dispersion was dried for 24 hours at
50°C to obtain DNA Hybrid 4 whose DNA content was
about 1.8% by mass.

This DNA hybrid was subjected to an elution
20 test. To 20 parts by mass of ion-exchanged water,
0.1 parts by mass of the DNA hybrid powder was added
and settled in a sealed condition at room temperature
for 48 hours. The absorbance (260 nm) of DNA in the
supernatant liquid measured by a spectrophotometer
25 (U-3310, Hitachi) was about 0.07. The DNA hybrid was
proved to be holding 96 mass% of the DNA.

(Example 5)

To 100 parts by mass of 30% silica sol (NISSAN CHEMICAL INDUSTRIES, LTD., SNOWTEX CM), 50 parts by mass of a titanium oxide sol (TAKI CHEMICAL CO., LTD., M-6, 6% by mass) was added with stirring. The
5 resultant sol mixture was combined with 20 parts by mass of the DNA solution prepared in Example 1, and stirred slowly for 10 minutes. Subsequently, the resultant DNA dispersion was dried for 24 hours at 50°C to obtain DNA Hybrid 5 whose DNA content was
10 about 0.3% by mass.

This DNA hybrid was subjected to an elution test. To 20 parts by mass of ion-exchanged water, 0.1 parts by mass of the DNA hybrid powder was added and settled in a sealed condition at room temperature for
15 48 hours. The absorbance (260 nm) of DNA in the supernatant liquid measured by a spectrophotometer (U-3310, Hitachi) was not detected. The DNA hybrid was proved to be holding almost all DNA.

(Comparative 1)

20 5 Parts by mass of a silica powder whose particle size was 0.063 to 0.2 mm was combined with 5.6 parts by mass of the DNA solution of Example 1 and mixed uniformly. The resultant paste was dried for 24 hours at 50°C to obtain a silica powder whose
25 DNA supporting level was 0.56% by mass. 0.040 Parts by mass of the resultant silica powder was combined with 5 parts by mass of ion exchange water to conduct

the elution test. The absorbance of the supernatant at 260 nm was 0.56. Thus, it was revealed that about 61% by mass of the DNA was eluted.

The present invention is not limited to the
5 above embodiments and various changes and
modifications can be made within the spirit and scope
of the present invention. Therefore to apprise the
public of the scope of the present invention, the
following claims are made.

CLAIMS

1. A DNA hybrid comprising a porous oxide matrix and a DNA immobilized by the matrix, the DNA
5 hybrid prepared by removing a dispersion medium from a dispersion of a colloidal oxide and DNA.

2. The DNA hybrid according to Claim 1, wherein the dispersion medium is removed by heating the dispersion at 25°C to 200°C.

10 3. The DNA hybrid according to Claim 1, wherein the DNA content in the hybrid is 0.01 to 15% by mass.

4. The DNA hybrid according to Claim 1 or 3, wherein the colloidal oxide is a mixture of a colloidal silica and at least one colloidal oxide of
15 a metal whose valence is 3 or 4.

5. The DNA hybrid according to Claim 1, wherein the oxide comprises at least one oxide selected from the group consisting of aluminum oxide, iron oxide, titanium oxide and zirconium oxide.

20 6. A method for producing a DNA hybrid comprising the steps of:

preparing a dispersion comprising a colloidal oxide, a DNA and a dispersion medium; and,

removing the dispersion medium from the
25 dispersion to immobilize the DNA in a porous matrix made of the oxide.

7. The method according to Claim 6, wherein the

dispersion medium is removed by heating the dispersion at 25°C to 200°C.

8. The method according to Claim 6, wherein the DNA content of the DNA hybrid is 0.01 to 15% by mass.

5 9. The method for producing a DNA hybrid according to Claim 6 or 8, wherein the colloidal oxide is a mixture of colloidal silica and at least one colloidal oxide of a metal whose valence is 3 or 4.

10 10. The method according to Claim 9, wherein the oxide comprises at least one oxide selected from the group consisting of aluminum oxide, iron oxide, titanium oxide and zirconium oxide.

15 11. An environmental cleaning system for removing a harmful substance in water comprising a DNA hybrid according to Claim 1 and means for bringing the DNA hybrid into contact with a water containing a harmful substance.

20 12. An environmental cleaning method by removing a harmful substance in a water comprising a step for bringing a DNA hybrid according to Claim 1 into contact with a water containing a harmful substance to adsorb the harmful substance by the DNA hybrid.

INTERNATIONAL SEARCH REPORT

International Application No
T/JP2004/007472

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C07H21/00 C02F1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q C07H C02F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; YAMADA, MASANORI ET AL: "UV-irradiated DNA matrix selectively accumulates heavy metal ions" XP002298608 retrieved from STN Database accession no. 137:290487 abstract & BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN , 75(7), 1627-1632 CODEN: BCSJA8; ISSN: 0009-2673, 2002, ----- -/--	1-12

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	MEYER-ALMES, FRANZ JOSEF ET AL: "Mechanism of intercalation into the DNA double helix by ethidium" BIOCHEMISTRY , 32(16), 4246-53 CODEN: BICHAW; ISSN: 0006-2960, 1993, XP002298606 abstract -----	1-12
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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