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DNA在土壤活性颗粒表面的结合机制及其稳定性和生物活性*

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采集我国地带性土壤山东泰山天外村棕壤，分离提取粗胶体（0.2～2.0 μm）和细胶体（＜0.2 μm），对两部分胶体进行去有机质和不去有机质处理，得到0.2～2.0 μm含有机质粘粒、0.2～2.0 μm去有机质粘粒、＜0.2 μm含有机质粘粒和＜0.2 μm去有机质粘粒。以上述不同类型的棕壤胶体、蒙脱石、羟基铝-蒙脱石复合物，高岭石和针铁矿为试验材料，研究DNA在土壤胶体和矿物表面的吸附解吸行为和结合机制、固定态DNA的稳定性和细胞转化、固定态质粒DNA的PCR扩增以及土壤胶体和矿物对微生物代谢活性的影响，获得如下主要结果：

1. 运用吸附和解吸等化学方法和现代仪器分析手段如衰减全反射傅立叶变换红外光谱（ATR/FT-IR）、圆二色光谱（CD）、荧光光谱、微量热分析技术，系统探讨了恒电荷土壤活性颗粒表面DNA吸附、解吸及固定的特点，基本揭示了DNA分子在不同类型土壤活性颗粒表面的结合机制。DNA在土壤胶体和矿物表面的最大吸附量顺序为：蒙脱石≫去有机质细粘粒＞含有机质细粘粒＞高岭石＞去有机质粗粘粒＞含有机质粗粘粒。体系pH从2.0增加到5.0，DNA在含有机质粘粒和蒙脱石表面的吸附量显著降低，pH大于5.0时，DNA在含有机质粘粒表面的吸附量几乎忽略不计。对于去有机质粘粒和高岭石，pH从2.0增加到9.0，DNA的吸附量逐渐降低。Mg^{2+}比Na^{+}更能促进DNA在土壤胶体和矿物表面的吸附。土壤胶体和矿物表面吸附态DNA依次用10 mmol/L Tris-HCl、100 mmol/L NaCl和100 mmol/L Na_2HPO_4-NaH_2PO_4溶液解吸。Tris-HCl和NaCl溶液对含有机质粘粒和蒙脱石表面DNA的解吸率为53.7%～64.4%，对去有机质粘粒和高岭石表面DNA的，29.7%解吸率仅为10.7%～15.2%；Na_2HPO_4-NaH_2PO_4溶液对去有机质粘粒、含有机质粘粒、蒙脱石和高岭石表面DNA的解吸率分别为39.7%～42.2%，23.6%～28.8%和11.4%～29.7%。DNA在含有机质粘粒和矿物表面的吸附为吸热反应（1.1 kJ/g ＜$\triangle H_{ads}$＜3.5 kJ/g），而在去有机质粘粒表面的吸附为放热反应（－0.3 kJ/g ＜$\triangle H_{ads}$＜－0.1 kJ/g）。上述结果表明，DNA可能主要通过脱水作用和静电力作用吸附在含有机质粘粒和蒙脱石表面，而在去有机质粘粒和高岭石表面，氢键和配位交换可能是DNA吸附的主要作用力。ATR/FTIR结果显示固定在去有机质粘粒和高岭石表面的DNA构型由原来的B型变为Z型；而蒙脱石和含有机质粘粒表面DNA的构型仍为B型。通过荧光光谱和CD谱观察到从高岭石表面解吸的DNA为C型，而蒙脱石和土壤胶体表面解吸的DNA仍为B型。

2. 查明了不同类型的有机和无机配体对土壤活性颗粒表面DNA吸附的影响。体系中柠檬酸、酒石酸和磷酸浓度从0 mmol/L增加到5 mmol/L，DNA在蒙脱石和高岭石表面的吸附量显著降低；随着配体浓度继续增加，DNA在蒙脱石和高岭石表面的吸附量逐渐增加。在土壤胶体体系中，随着配体浓度的增加，DNA的吸附量降低，各配体抑制效率的大小顺序为：磷酸＞柠檬酸＞酒石酸。和去有机质粘粒相比，配体对含有机质粘粒表面DNA吸附的抑制效果更强。这些结果表明，配体对DNA吸附的影响和配体的类型、浓度以及土壤组分的表面性质密切相关。在配体加入体系之前先加入DNA

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的处理使 DNA 在土壤胶体表面的吸附量最大。对于蒙脱石和高岭石而言，在 DNA 加入之前先加入配体的处理使 DNA 吸附量最大。

3. 选取土壤中 2 种常见的细菌如革兰氏阳性菌苏云金芽胞杆菌（*Bacillus thuringiensis*）和革兰氏阴性菌恶臭假单胞菌（*Pseudomonas putida*），研究了细菌对土壤胶体和矿物体系吸附 DNA 的影响。发现 2 种细菌细胞对 DNA 的吸附量没有显著差异，DNA 主要通过范德华力和静电力作用吸附在细菌表面，恶臭假单胞菌表面的 DNA 更易被解吸。除了恶臭假单胞菌对高岭石体系中 DNA 的吸附影响较小之外，在土壤胶体或矿物体系中加入苏云金芽孢杆菌和恶臭假单胞菌均可显著促进 DNA 的吸附。与恶臭假单胞菌相比，苏云金芽孢杆菌更能促进 DNA 在土壤胶体表面的吸附。

4. 合成了含有不同浓度羟基铝的蒙脱石复合物（含铝量为 2.5、10.0、20.0 mmol/g，用 $AM_{2.5}$、AM_{10} 和 AM_{20} 表示），探讨了 DNA 在其表面的吸附解吸行为和结合机制。发现羟基铝在蒙脱石表面的包被显著降低了蒙脱石对 DNA 的吸附量，但增强了对 DNA 的结合强度，使吸附态 DNA 分子很难被解吸。在 Na_2HPO_4-NaH_2PO_4 体系中，DNA 在蒙脱石或羟基铝蒙脱石复合物表面的吸附量显著高于其在 Tris-HCl 体系中的吸附量，但 DNA 的吸附亲和力较低；随着蒙脱石表面羟基铝含量的增加，DNA 的吸附量显著降低，这表明在羟基铝-蒙脱石复合物表面，磷酸根阴离子与 DNA 分子形成了强烈的竞争作用。体系中 $CaCl_2$ 浓度的增加或 pH 的降低均可促进 DNA 在羟基铝-蒙脱石复合物表面的吸附。蒙脱石，$AM_{2.5}$，AM_{10} 和 AM_{20} 表面的吸附态 DNA 用 Tris-HCl 充分洗涤，解吸率分别为 65.01%，30.00%，8.04% 和 5.18%，这表明蒙脱石表面羟基铝的存在可显著增强 DNA 的吸附强度。应用等温滴定微量热技术测定了 DNA 在蒙脱石和羟基铝-蒙脱石复合物表面吸附的焓变，结果显示 DNA 在蒙脱石表面的吸附为吸热反应（$\triangle H_{ads}=1.15$ J/g），而在羟基铝-蒙脱石复合物表面的吸附为放热反应（-9.50 J/g $<\triangle H_{ads}<-6.64$ J/g）。DNA 分子的碱基和磷酸基团参与了吸附过程，固定在 AM_{10} 和 AM_{20} 表面的 DNA 构型由原来的 B 型变为 Z 型，且和 DNA 分子之间形成了氢键。上述结果表明，DNA 可能主要通过静电引力、氢键和配位交换等多种作用力吸附在羟基铝-蒙脱石复合物表面。扫描电镜结果显示，DNA 可在 AM_{10} 表面形成一层薄膜，而在蒙脱石表面没有观察到。

5. 联合应用凝胶电泳技术和等温滴定微量热技术，揭示了土壤胶体和矿物体系（表面）固定态染色体 DNA 的稳定性特点，基本阐明了固定态 DNA 抗核酸酶降解的机制。凝胶电泳结果显示，核酸酶质量浓度为 2 μg/mL 时，DNA 的条带消失，DNA 完全被降解。体系中核酸酶质量浓度为 20 μg/mL 时，高岭石、去有机质粗粘粒和细粘粒体系中的 DNA 在 23 kb 处的条带全部消失，仅剩一些大约 2 kb和 4 kb 的碎片段。对于蒙脱石、含有机质粗粘粒和细粘粒体系中的 DNA 而言，即使核酸酶质量浓度增加到 40 μg/mL，DNA 的条带均无明显变化。等温滴定微量热结果显示，游离态 DNA、固定在去有机质粗粘粒、含有机质粗粘粒、高岭石和蒙脱石表面 DNA 的水解热分别为 -4.76、-4.06、-2.38、-2.36 和 -0.22 mJ。上述结果表明，土壤胶体和矿物可保护 DNA，使之抗核酸酶的降解；在供试的土壤胶体和矿物中，高岭石表面的 DNA 最易被降解，而蒙脱石表面的 DNA 较难降解；与去有机质粘粒相比，含有机质粘粒表面的 DNA 更难被核酸酶降解。DNA 在土壤胶体或矿物表面的降解与 DNA 的固定强度及 DNA 构型的变化无关，土壤中有机质和 2∶1 型矿物（如蒙脱石）的存在以及土壤胶体和矿物对核酸酶的吸附程度可能是固定态染色体 DNA 抗核酸酶降解的主要原因。

6. 使用 $CaCl_2$ 处理的大肠杆菌 TG1 感受态细胞，系统区分了土壤胶体和矿物对固定态质粒 p34S DNA 转化活性和抗核酸酶降解的影响，基本明确了固定态 DNA 的转化机制。不同 Ca^{2+} 浓度下制备了土壤胶体或矿物-质粒 p34S DNA 的复合物，复合物的转化效率随 Ca^{2+} 浓度的增大而增大。高岭石表面固定态质粒 DNA 的转化效率最低，尤其是在 Ca^{2+} 浓度为 5～100 mmol/L 时，没有观察到转化子。与含有有机质粘粒和细粘粒相比，去有机质粘粒和粗粘粒表面固定态质粒 DNA 的转化效率较低。加入 10 ng DNase Ⅰ，10 μg 游离质粒 DNA 的转化子数下降了 99.8%，而土壤胶体和蒙脱石表面固定态质粒 DNA 的转化子数仅下降了 2.0%～57.8%。加入 100 ng DNase Ⅰ，去有机质粘粒表面固定态质粒 DNA 的转化子数下降了 92.3%～

93.8%，DNase Ⅰ的量达到1 000 ng时，固定态质粒DNA完全被降解，检测不到转化子。对于含有机质粘粒和蒙脱石表面质粒DNA，即使加入2 000 ng DNase Ⅰ，转化子数仅下降了64.0%～98.0%。固定在粗粘粒表面质粒DNA的转化子数下降比率高于细粘粒。蒙脱石、含有机质粘粒和细粘粒对质粒DNA有较强的保护作用。质粒DNA在土壤胶体和矿物表面的吸附亲和力以及构型的变化可能是影响固定态质粒DNA转化效率高低的主要因素。

7.首次利用PCR技术扩增了土壤胶体和矿物表面固定态质粒pGEX-2T DNA中600 bp碱基对序列。土壤胶体、高岭石或蒙脱石-质粒DNA复合物直接用于PCR扩增，无扩增产物出现；土壤胶体或高岭石-DNA复合物稀释10倍或20倍后，可观察到扩增产物，蒙脱石-质粒DNA复合物即使稀释到100倍，仍不能进行扩增；对于固定在针铁矿表面的质粒DNA而言，复合物在不稀释、稀释10倍和20倍条件下，均可观察到扩增产物。因此，PCR体系中粘土矿物类型和浓度显著影响固定态质粒DNA的PCR扩增结果。

8.应用等温微量热技术，首次获得了土壤胶体或矿物体系下大肠杆菌的代谢热曲线。通过拟合指数方程，发现大肠杆菌在LB液体培养基中的生长速率常数(k)为0.074/min，而LB中加入去有机质粗粘粒、高岭石、含有机质粗粘粒、蒙脱石和针铁矿后，k分别为0.073、0.058、0.054、0.045和0.020 min。供试的土壤胶体和矿物对大肠杆菌生长代谢有显著的抑制作用。3种矿物中，对大肠杆菌代谢抑制能力最强的是针铁矿，最弱的是高岭石，蒙脱石居中。土壤中有机质对大肠杆菌的生长代谢也有较强的抑制作用。

关键词 DNA；土壤胶体；矿物；吸附；解吸；降解；转化；扩增；代谢活性

Binding mechanisms, stability and biological activity of DNA on soil active particles

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Typical zonal soil such as Brown soil was sampled from Tianwai village, Taishan, Shandong Province in China. Two soil colloidal components i. e, fine clay (<0.2 μm) and coarse clay (0.2-2.0 μm) were separated by centrifugation. Two treatments applied to fine and coarse clays were organic matter left on the samples (organic clays) and organic matter removed from the samples by H_2O_2 (inorganic clays). Brown soil was divided into four types of clays: coarse organic clay (0.2-2.0 μm, organo-mineral complexes), coarse inorganic clay (0.2-2.0 μm, H_2O_2-treated clay), fine organic clay (<0.2 μm, organo-mineral complexes) and fine inorganic clay (<0.2 μm, H_2O_2-treated clay). The adsorption, desorption and binding mechanism of DNA on Brown soil colloid or mineral such as montmorillonite, hydroxyaluminum-montmorillonite, kaolinite and goethite, the ability of transforming competent cells of bound DNA and the resistance to DNase Ⅰ degradation, the PCR amplification of bound plasmid DNA and the effects of soil active particles on microbial metabolic activities were investigated. The main results were as following:

1. Adsorption-desorption of DNA, ATR/FTIR, circular dichroism (CD), fluorescence spectroscopy, microcalorimetry were used to clarify the adsorption mechanism of DNA on permanent-charge soil active particles. The maximum amount of DNA adsorbed followed the order: montmorillonite≫fine inorganic clay>fine organic clay>kaolinite>coarse inorganic clay>coarse organic clay. A marked decrease in the

adsorption of DNA on organic clays and montmorillonite was observed with the increase of pH from 2.0 to 5.0. Little DNA was adsorbed by organic clays above pH 5.0. As for inorganic clays and kaolinite, a slow decrease in DNA adsorption was found with increasing pH from 2.0 to 9.0. Magnesium ion was more efficient than sodium ion in promoting DNA adsorption on soil colloids and minerals. DNA molecules adsorbed on soil colloids and minerals were desorbed by sequential washing with 10 mmol/L Tris-HCl, 100 mmol/L NaCl and 100 mmol/L sodium phosphate buffer at pH 7.0. A percentage of 53.7%-64.4% of DNA adsorbed on organic clays and montmorillonite was released, while only 10.7%-15.2% of DNA on inorganic clays and kaolinite was desorbed by Tris-HCl and NaCl. The percent desorption of DNA from inorganic clays, organic clays, montmorillonite and kaolinite by sodium phosphate buffer was 39.7%-42.2%, 23.6%-28.8%, 29.7% and 11.4%-29.7%, respectively. DNA adsorption on organic clays was endothermic ($1.1 < \triangle H_{ads} < 3.5$ kJ/g), whereas that on inorganic clays was exothermic (-0.3 kJ/g$< \triangle H_{ads} < -0.1$ kJ/g). Dehydration effects and electrostatic interactions dominated DNA adsorption on organic clays and montmorillonite, and DNA was adsorbed predominantly by ligand exchange and possibly hydrogen bonding on inorganic clays and kaolinite. ATR/FTIR spectra showed that the binding of DNA on kaolinite and inorganic clays changed its conformation from the B-form to the Z-form, whereas montmorillonite and organic clays retained the original B-form of DNA. A structural change from B- to C-form in DNA molecules desorbed from kaolinite was observed by CD spectroscopy and confirmed by fluorescence spectroscopy and DNA molecules desorbed from soil colloid or montmorillonite were still B-form.

2. The effects of various organic and inorganic ligands on DNA adsorption on active soil particles were studied. An obvious decrease in DNA adsorption was observed on montmorillonite and kaolinite with increasing anion concentrations from 0 to 5 mmol/L. However, the amount of DNA adsorbed by montmorillonite and kaolinite was enhanced when ligand concentration was higher than 5 mmol/L. In the system of soil colloids, with the increase of anion concentrations, a steady decrease was found and the ability of ligands to depress DNA adsorption followed the order of phosphate$>$citrate$>$tartrate. Compared to inorganic clays, a sharp decrease in DNA adsorption was observed on organic clays with the increase of ligand concentration. The results indicated that the influence of anions on DNA adsorption varies with the type and concentration of anion as well as the surface properties of soil components. Introducing DNA into the system before the addition of ligands had the maximum amount of DNA adsorption on soil colloids. Organic and inorganic ligands promoted DNA adsorption on montmorillonite and kaolinite when ligands were introduced into the system before the addition of DNA.

3. Adsorption and desorption of DNA on *Bacillus thuringiensis* and *Pseudomonas putida* and their composites with soil colloids or minerals were investigated. *B. thuringiensis* and *P. putida* did not show significant difference in the amount of DNA adsorption although the two bacterial cells had different surface properties. DNA was adsorbed on bacteria mainly through van der Waals force and electrostatic force. Compared with *B. thuringiensis*, DNA adsorbed by *P. putida* was desorbed more easily. There was no significant difference in the amount of DNA adsorption on kaolinite between the absence and the presence of *P. putida*. Except for the effect of *P. putida* on DNA adsorption on kaolinite, the presence of *B. thuringiensis* and *P. putida* significantly promoted DNA adsorption on soil colloids and minerals, and the promotion of *B. thuringiensis* was stronger than that of *P. putida* in the system of soil colloids.

4. Adsorption of DNA on different hydroxyaluminum-montmorillonite complexes ($Al(OH)_x$-M) containing 2.5, 10.0 and 20.0 mmol coated Al/g clay ($AM_{2.5}$, AM_{10} and AM_{20}) was studied in Tris-HCl and sodium phosphate buffers at pH 7.0. The coatings of montmorillonite by hydroxyaluminum species

decreased the amount of DNA adsorption, but increased the affinity of DNA adsorption. At the same pH, the amount of DNA adsorption on montmorillonite or $Al(OH)_x$-M complexes in sodium phosphate was greater than that in Tris-HCl, suggesting that the nature of a buffer solution strongly affected DNA adsorption on clays. As for $Al(OH)_x$-M complexes, the higher the level of $Al(OH)_x$ coatings, the lesser the amount of DNA was adsorbed in sodium phosphate buffer. The reduction of DNA adsorption in sodium phosphate buffer with the increase of the level of $Al(OH)_x$ coatings may be ascribed to the strong competition of phosphate anions with DNA molecules on surface sites of $Al(OH)_x$-M complexes. An increase of the concentration of Ca^{2+} and/or a decrease of the values of pH helped DNA adsorption on montmorillonite and $Al(OH)_x$-M complexes. The desorption percent of DNA from montmorillonite, $AM_{2.5}$, AM_{10} and AM_{20} was 65.01%, 30.00%, 8.04% and 5.18% by Tris-HCl buffer. It suggests that the larger the OH-Al loading on M surface, the greater the binding energy of DNA. DNA adsorption on montmorillonite was endothermic ($\triangle H_{ads} = 1.15$ J/g), whereas that on $Al(OH)_x$-M complexes was exothermic (-9.50 J/g $< \triangle H_{ads} < -6.64$ J/g). The bases and phosphate groups of DNA are involved in DNA adsorption on clays and DNA changes its conformation from the B-form to the Z-form as the result of its binding on AM_{10} and AM_{20}. Electrostatic forces, hydrogen bonding and ligand exchange dominated DNA adsorption on $Al(OH)_x$-M complexes. SEM showed that a thin layer was formed on the surface of AM_{10} after the binding of DNA, while that was not observed on montmorillonite surface.

5. Electrophoresis and thermometric TAM III were used to investigate the degradation of chromosomal DNA in the system of active soil particles or bound on soil colloid or mineral by DNase Ⅰ. When nuclease concentration was 2.0 μg/mL, DNA was completely degraded. In systems of kaolinite and coarse and fine inorganic clays, DNA was degraded to 2-4 kb segments at 20 μg/mL of nuclease. For DNA in systems of montmorillonite and coarse and fine organic clays, no evident change was observed in the patterns with 40 μg/mL nuclease in comparison with no nuclease. The heat released from the hydrolysis of DNA, free or bound on coarse inorganic clay, coarse organic clay, kaolinite and montmorillonite by the nuclease was -4.76, -4.06, -2.38, -2.36, -0.22 mJ, respectively. These results indicated that soil colloids and minerals could exert an effective protection for DNA to resist degradation by the nuclease. Among the soil colloids and minerals studied, montmorillonite and organic clays provide more protection for DNA against degradation by DNase Ⅰ than kaolinite and inorganic clays. The protection of DNA was not a result of the adsorption affinity of DNA for soil colloid or mineral and the changes in DNA structure. The presence of organic matter and an efficient adsorption of nucleases on soil colloids and minerals appeared to be responsible for the lower degradation of DNA in soil ecosystems.

6. The ability of bound plasmid p34S DNA on soil colloids and minerals to transform competent cells of $CaCl_2$-treated *Escherichia coli*, and the resistance of bound plasmid DNA to degradation by DNase Ⅰ were investigated. The transformation efficiency of bound plasmid DNA increased with increasing concentrations of Ca^{2+} at which soil colloid or clay mineral-plasmid DNA complexes were formed. Plasmid DNA bound by kaolinite showed the lowest transformation efficiency, and especially no transformants were observed with kaolinite-plasmid DNA complex prepared at 5-100 mmol/L Ca^{2+}. Compared with organic clays and fine clays, plasmid DNA bound on inorganic clays and coarse clays showed a lower capacity to transform *E. coli* at different Ca^{2+} concentrations. Transformation by 10 μg of free plasmid DNA was inhibited 99.8% by 10 ng of DNase Ⅰ. As for the same amount of plasmid DNA bound by soil colloids and montmorillonite, 100 ng of DNase Ⅰ resulted in 92.3%-93.8% inhibition of transformation by plasmid DNA bound on inorganic clays, whereas 2000 ng of DNase Ⅰ caused only 64.0%-98.0% inhibition of transformation by that on organic clays and montmorillonite. The percentage of reduction of

transformants by plasmid DNA bound on coarse clays was higher than that on fine clays. Montmorillonite, organic clays and fine clays showed stronger protective effects for plasmid DNA than that of inorganic clays and coarse clays. The adsorption affinity of plasmid DNA for soil colloid or mineral and a conformational change in the plasmid DNA molecule bound on clays may determine the efficiencies of transformation.

7. The polymerase chain reaction (PCR) was used to amplify a 600-base pair (bp) sequence of plasmid pGEX-2T DNA bound on soil colloidal particles and three different minerals (goethite, kaolinite, montmorillonite). DNA bound on soil colloids, kaolinite, and montmorillonite was not amplified when the complexes were used directly but amplification occurred when the soil colloid or kaolinite-DNA complex was diluted 10- and 20-fold. The montmorillonite-DNA complex required at least 100-fold dilution before amplification was detectable. DNA bound on goethite was amplified whether the complex was used directly, or diluted 10- and 20-fold. The PCR amplification of mineral-bound plasmid DNA was markedly influenced by the types and concentrations of minerals used.

8. The thermodynamic data of the metabolic activity of *E. coli* as influenced by soil colloids and minerals were analyzed. The growth rate constant (k) of *E. coli* in LB was 0.074/min, and the k values of *E. coli* in the system of coarse inorganic clay, kaolinite, coarse organic clay, montmorillonite and goethite were 0.073, 0.058, 0.054, 0.045 and 0.020/min, respectively. It suggested that the selected soil colloids and minerals inhibited significantly the exponential growth of *E. coli*. The inhibitory ability of the three minerals on metabolic activity of *E. coli* followed the sequence of goethite>montmorillonite>kaolinite. Compared with inorganic clay, organic clay showed a higher inhibitory effect on metabolic activity of *E. coli*.

Key words　DNA; soil colloid; mineral; adsorption; desorption; degradation; transformation; amplification; metabolic activity

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