Notes

Efficient Coupling of DNA on a Silica Support with Psoralen Derivatives as Anchoring Agents for High-Performance Liquid Affinity Chromatography

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The weak-affinity chromatography or high-performance liquid affinity chromatography (HPLAC) is a promising tool for the identification, isolation and systematic investigation of the components of weak (readily reversible) bioaffinity reactions.^{1,2} A high concentration of a ligand coupled to the solid phase is a prerequisite in this case.

In addition to some enzyme-substrate³ and IgG-antigen⁴ combinations to which the method has been successfully applied, weak bindings of biological importance involve a DNA-drug interaction. This interaction is usually evaluated by spectral titration and equilibrium dialysis.⁵ Since these methods require time-consuming procedures, they do not appear to be suitable for a systematic investigation of a series of drug analogues. In addition, the methods are only applicable to systems with relatively strong affinity. In this context, an HPLAC using a DNA-immobilized support appears to be promising to study DNA-drug interactions.⁶ So far, an efficient coupling method is not available for DNA, since polynucleotides lack highly reactive groups.

We report here on a general and convenient method for covalent coupling of double-stranded DNA on a solid support. This method relies on a psoralen derivative (**1**) which can form a photoadduct with the duplex DNA. Since coupling chemistry permits a large amount of DNA to be immobilized on a macroporous silica support (10 mg g-gel⁻¹), the concept of weak-affinity chromatography can be applied. Preliminary results are presented regarding DNA-drug interactions by using a DNA-loaded column.

1
(*n*=2, **1a** ; *n*=6, **1b**)

Experimental

Synthesis of anchoring reagents (**1**)

4'-[[*N*-(2-Aminoethyl)amino]methyl]-4,5',8-trimethylpsoralen (**1a**; *n*=2) was synthesized according to a method described by Lee *et al.*⁷ **1b** (*n*=6) was prepared similarly, though the product was isolated as a dihydrochloride and then recrystallized from methanol-diethyl ether. Yield, 16.2%. Elemental analysis of the salt: Found: C, 56.89; H, 6.85; N, 6.43%. Calcd for C₂₁H₃₀O₃N₂Cl₂·0.75H₂O: C, 56.95; H, 7.16; N, 6.32%.

Coupling of DNA to silica gel

A silica gel (Nagel, Nucleosil 1000-5; diameter, 5 μm; pore, 1000 Å; surface area, 25 m² g⁻¹) was modified with epoxy groups according to a method described in the literature.^{1,3} **1** was then reacted with the epoxy-silica (the epoxy group content, 46 μmol g⁻¹) in 0.1 M sodium pyrophosphate (pH 8.5) for 40 h at 25°C (1 g-gel with 3 μmol of **1** in 4 ml of the buffer). The reaction and the subsequent procedures were all dark adapted. The psoralen-modified silica was treated with an aqueous solution (1 M) of 2-mercaptoethanol for converting the residual epoxy groups into hydrophilic diols. The psoralen content was determined for gel suspension in 67 wt-% aqueous sucrose (6 mg-gel ml⁻¹) by UV spectrophotometry (λ_{max}, 300 and 340 nm), and was found to be 2.07 and 1.12 μmol g-gel⁻¹ for **1a** and **1b**, respectively.

The psoralen-bearing silica gel (1 g) was suspended in a 3 ml solution (20 mg ml⁻¹) of sonicated calf thymus DNA (10² – 10³ bp) in a buffer (10 mM phosphate, 1 mM EDTA, 0.2 M NaCl, pH 6.6). The suspension in a Pyrex vessel was rotated on an ice bath with a rotary evaporator, and then was irradiated (*ca.* 60 mW cm⁻²) with a high-pressure Hg lamp for 2 h. The silica gel was washed successively with water, 10 mM Tris-HCl–2 M NaCl (pH 7.2), 10 mM Tris-HCl–1 mM EDTA–0.1 M NaCl (pH 7.2), 0.4 M MES (pH 6.0), and 1 M phosphate

(pH 7.6). The silica gel was preserved in 10 mM Tris-HCl-1 mM EDTA-0.1 M NaCl (pH 7.2) at 5°C. For determining the amount of DNA bound to the silica, the gel dried *in vacuo* (50 mg) was digested with 1.2 ml of 0.4 M perchloric acid for 30 min at 70°C; the nucleotides in the supernatant were determined by the diphenylamine reaction.⁸

High-performance liquid affinity chromatography

The DNA-loaded silica support was packed into a 4.6 mm i.d.×7.5 cm stainless-steel column by standard slurry procedures. A Shimadzu Model LC-5A liquid chromatograph with a Rheodyne Model 7125 sampling valve and a 50 µl injection loop was operated at 23°C with a Union-Giken Model MCPD-350PC detector system. The mobile phase consisted of a mixture of Tris-HCl (10 mM, pH 7.4)-EDTA (1 mM)-NaCl (a prescribed concentration) and acetonitrile (9:1 by volume). The flow rate was 0.5 ml min⁻¹.

Results and Discussion

The amount of DNA immobilized was determined to be 8.4 and 10.2 mg g-gel⁻¹ for **1a**- and **1b**-bearing gel, respectively, by using the diphenylamine method for perchloric acid digests. The values were in fair agreement with those estimated from UV absorption at 260 nm of the gel suspension in 67 wt-% aqueous sucrose. The UV spectra also showed that the absorbance at 340 nm was reduced by a photochemical reaction to 30% of the original, indicating that at least 70% of the psoralen derivative gave a photoadduct with DNA.⁹ Thus, an efficient coupling of DNA covalently to the silica gel support (up to 10 mg g-gel⁻¹) was attained with the aid of **1** residues.

Cyanogen bromide has been widely used to couple DNA with biopolymer-based supports including agarose gel, though the amount of DNA immobilized was rather limited (typically, 3–30 µg ml-gel⁻¹).^{10,11} Sonicated

DNA has been attached to dextran gel in high concentrations (10–38 mg ml-gel⁻¹) by a carbodiimide method.¹² Supports made of polysaccharides are, however, improper for use in HPLC. Modifications of DNA with reactive group(s) made it possible to conjugate DNA with silica and organic gels.^{6,13,14} These methods, however, require expert synthetic procedures for pre-modifying each DNA of interest.

In our method presented here, the chemistry is based on the intercalation of furocoumarins into DNA and photosensitized conjugation between them. Since this reaction is general, a series of double-stranded DNA can be covalently immobilized on any 1-bearing solid support.

A preliminary experiment was carried out to evaluate the interaction between DNA and polycyclic aromatic compounds (PAC's), some of which are known to be DNA-intercalators; PAC's are mutagenic and/or carcinogenic due to some interaction with DNA. The results are summarized in Table 1 along with those for DNA-free **1b**-bearing packings used as a reference. The capacity factor (*k'*) is found to be dependent on the structure of the compounds, as well as the ionic strength of the eluent. The values of *k'* for methyl 9-acridine-carboxylate and 4,5',8-trimethylpsoralen appear to be correlated with the magnitudes of the association constant (*K*_{ass}) determined for their intercalative binding with DNA.^{15,16} This suggests that the retention on a DNA-loaded column of the compounds is governed by their affinity toward DNA. It is reported that the affinity with DNA of a certain intercalator decreases with increasing ionic strength.¹⁷ This was the case in our data for nonionic compounds; the capacity factors decreased with increasing concentration of NaCl in the eluent. Tricyclic compounds, such as phenanthrene are, therefore, considered to be intercalative to DNA with a *K*_{ass} of the order of 10³ M⁻¹.

As for anionic compounds, on the other hand, there was almost no retention for 9-anthracenecarboxylic acid, probably due to an electrostatic repulsion with poly-

Table 1 Retention of polycyclic aromatic compounds on the DNA-loaded column^a

	Capacity factor (<i>k'</i>) ^b				<i>K</i> _{ass} ^c /M ⁻¹
	NaCl 0 M	0.01 M	0.05 M	0.1 M	
9-Anthracenecarboxylic acid	0 (0.51)	0	0	0.27 (0.21)	1.54×10 ³ ¹⁵ [0.1 M]
Methyl 9-acridinecarboxylate	2.08 (2.01)	1.74	1.62	1.54 (2.17)	
Fluorene	2.05 (1.62)	2.22	1.87	1.89 (1.42)	
1-Pyrenebutyric acid	3.85 (4.50)	4.06	5.20	5.39 (16.98)	
Phenanthrene	5.36 (5.16)	5.62	5.28	5.11 (4.17)	4.0×10 ³ ¹⁶ [0 M]
4,5',8-Trimethylpsoralen	5.56 (5.12)	5.00	3.99	3.98 (3.14)	
Anthraquinone	5.62 (4.02)	5.18	5.02	4.83 (4.40)	
Quinacrine	not detected				1.13×10 ⁵ ¹⁷ [0.1 M]

a. The column packing used was the **1b**-derivatized silica coupled with DNA. b. Results for the DNA-uncoupled, **1b**-derivatized silica packing are shown in parentheses. The amount of **1b** on silica was 2.6 µmol g-gel⁻¹. c. Association constant determined for the compound with DNA.^{15–17} Concentration of NaCl in the measurement is shown in brackets.

anionic DNA; 1-pyrenebutyric acid, however, showed a significant retention, which increased when the ionic strength was raised. The latter can be explained by the relatively long distance between the carboxylate group and the intercalating pyrene ring, leading to a reduced repulsive interaction between 1-pyrenebutyrate and DNA. Quinacrine, a positively charged compound, was not eluted due to the strong affinity ($K_{\text{ass}} > 10^5 \text{ M}^{-1}$).¹⁷

The retention behaviors obtained for the DNA-carrying column can be consistently explained in terms of some familiar interactions with DNA. In contrast, the capacity factor on a DNA-free, psoralen-loaded column varies for each compound with no straightforward relationship; the underlying interactions seem to be too complicated to delineate in a simple manner. The non-specific interactions observed here could pose a problem when applying DNA-immobilized packings to affinity chromatography when the surface transformation of the packings with DNA happens to be incomplete. The coupling chemistry presented, however, should be useful

for a wide range of DNA-dependent bioaffinity reactions, since the method endorses a very high binding capacity.

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