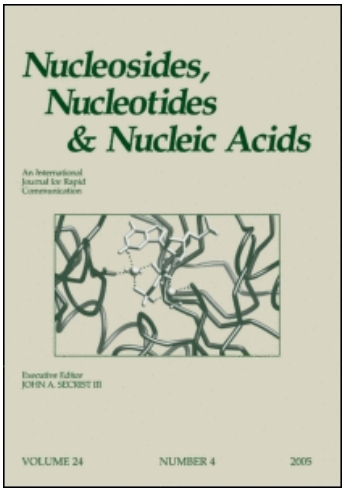

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Affinty Electrophoresis for the Separation of Oligo- and Polynucleotides

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AFFINTY ELECTROPHORESIS FOR THE SEPARATION OF OLIGO- AND POLYNUCLEOTIDES

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ABSTRACT. Affinty electrophoretic procedures for the fractionation of free ribose- or sulphur-containing nucleic acids are presented.

INTRODUCTION

The chemical attraction between two molecules is the basic principle encompassing the field of so-called affinity techniques. It is the chemical interaction between the partners which, in contrast to physical forces of, for example, counter ions, brings about the specificity which has been exploited in all areas of "bio-specific" separations. Whereas the principle may be universal, the methods which have been devised in order to bring the interacting partners into mutual proximity reflect both the nature of the molecules and the nature of the subsequent analytical steps. For example, in the Southern hybridization procedure, a usually high M_r DNA fragment, bound physically to a membrane, is allowed to interact, by diffusion, with a DNA fragment of complementary sequence. One would, in this case, not consider linking one of the two interacting partners to a column and bringing about an affinity recognition by using hydrodynamic pressure as the driving force. For such experiments, the use of membranes and diffusion offer a much faster and more convenient route to specific interactions. Yet, for other purposes the passage of nucleic acids through a column containing a covalently bound complementary nucleic acid has, until recently, been the method of choice. In this case, it is has been the selection of poly(A)-containing mRNAs by passage over a poly(U) or poly(dT) column.

Although columns (hydrodynamic pressure) and membranes (diffusion) form the carriers of the bulk of affinity methods, a third matrix, polyacrylamide gel (electric field), has also been demonstrated as being a

useful "matchmaker" in the technique of affinity electrophoresis. This procedure has been an established tool in immunoelectrophoresis for many years and the nature of a number of enzyme-substrate or lectin-sugar complexes has been examined[1]. However, as was the use of membranes till recently a preserve of nucleic acids, so was affinity electrophoresis firmly in the domain of proteins.

Recently[2-4], we have demonstrated that the covalent incorporation of small molecules, known to interact specifically with certain structural elements in nucleic acids, can lead to the fractionation of nucleic acid species not separable by other methods. Our initial investigations involved the interaction of a boronic acid with diol functions present as such, or added subsequently, in a variety of nucleic acids. Clearly, this is not the only possible binding phenomenon which can be exploited. Experience in the field of affinity chromatography of proteins and solution studies of nucleic acids has shown that an even stronger affinity exists between an organomercurial and sulphur-containing macromolecules.

The results summarized in this communication are intended to demonstrate the application of affinity electrophoresis to the field of nucleic acids, in general, and, in particular, of the use of the boronate and mercurial matrices for the analysis of specific structural motifs.

RESULTS & DISCUSSION

Copolymerization of underivatized acrylamide, bisacrylamide and acryloylaminophenylboronic acid (APB) or acryloylaminophenylmercuric chloride (APM) leads to gel matrices suitable for affinity electrophoretic retardation of nucleic acids bearing intact cis-diol (e.g. ribose) or sulphur-containing species (e.g. phosphorothioates, modified bases).

Analysis of terminal ribose residues. The presence, absence or blockage of a free ribose moiety lends itself to analysis by affinity electrophoresis on boronate gels, as summarized in TABLE I for RNA.

Analysis of sulphur-containing nucleic acids. The strong affinity of mercurials for thiol compounds and the wide range of chemical environments in which the sulphur atom may occur, suggested to us the possibility of fractionating sulphur-containing nucleic acids by affinity electrophoresis not only on the criterion of presence versus absence of sulphur,

Table I. Application of APB-Gels to the Termini of RNA

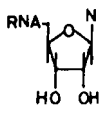
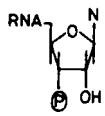
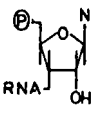
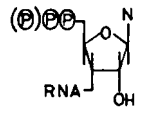
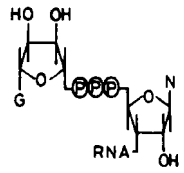
	3'		5'		
Terminal structure	Free ribose	Phosphate	Monophosphate	Di-/Triphosphate	Cap-like
					
Occurrence	Mature RNAs; tRNAs	RNase degradation products; Processing intermediates	RNase degradation products; Processing intermediates; Mature RNAs	Primary transcripts	Eukaryotic mRNAs
Specific test in conjunction with affinity electrophoresis	Sensitive to IO_4^-	Sensitive to P-ase	Adenylation with RNA ligase; shift eliminated by IO_4^-	Guanylation with guanylyltransferase; shift eliminated by IO_4^-	Sensitive to IO_4^-
Mobility shift	▼	▲	▲	▲	▼

Table II.

Interaction between phosphorothioate substituted nucleic acids and Hg(II).

Interaction constants (μM), where measured, are given in parentheses. All gels were run in the presence of 7 M urea. nd, not determined

	ssDNA	dsDNA	RNA
5'Phosphorothioate monoester			
monophosphorothioate	strong (0.8)	strong	strong (0.3)
triphospho-γ-thioate	nd	nd	strong (<0.1)
3'Phosphorothioate monoester			
monophosphorothioate	nd	nd	strong
Phosphorothioate diester	no detectable interaction		

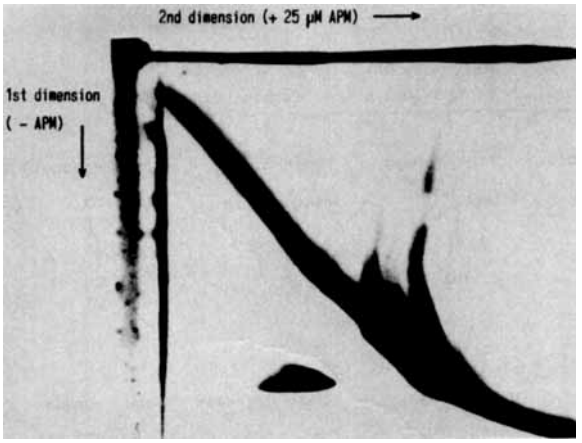


FIG. 1
A component of bulk chloroplast tRNA from maize is retarded by affinity electrophoresis in an organomercurial gel (APM).

Total chloroplast tRNA was 3'-terminally labelled with α^{32}P ATP by the exchange reaction catalyzed by a nucleotidyl transferase activity in a homologous extract. The material was applied to a 10% polyacrylamide, 7 M urea two-dimensional gel, containing APM in the second dimension.

but also as a function of the chemical reactivity of these sulphur substitutions. The effect of the environment of the interacting functional group on the fractionation on an organomercurial affinity gel is exemplified in TABLE II for a series of phosphorothioate modified nucleic acids. Quantification of the strength of the interaction as well as visual inspection of the retardation, clearly indicates that while phosphorothioate monoesters are strongly bound, phosphorothioate diesters show no affinity for the gel.

As far as naturally occurring sulphur-containing nucleic acids are concerned, 4- or 2-thiouridine and their derivatives, for example, are widespread in prokaryotic tRNAs. They occur less frequently in other organisms, but may be detected in, for example, chloroplast tRNAs by the retardation of a tRNA component in a two-dimensional electrophoresis system, containing an immobilized mercurial in the second dimension (FIG. 1).

CONCLUSIONS

Affinity electrophoresis has been demonstrated to be applicable to the analysis of specific structural features of nucleic acids. As a corollary, enzymes involved in the formation of these structures may also be assayed by this method.

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