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Nucleic acid reactive antibodies—Specificities and applications

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Abstract. The specificities of nucleic acid reactive antibodies and their applications in cell biology and molecular biology are reviewed.

Keywords. Nucleic acid reactive antibodies; specificities; applications.

Introduction

Nucleic acids are not immunogenic by themselves unlike proteins and carbohydrates. In practice antibodies are raised against nucleic acids and their components using them as haptens in combination with proteins. Nucleic acid reactive antibodies are present in the sera of human-beings and animals affected with certain autoimmune disorders. Currently monoclonal antibodies are also being raised against nucleic acids.

Nucleic acid reactive antibodies are of great interest in clinical medicine, cell biology and molecular biology. They are used as markers to detect certain autoimmune diseases. Nucleic acid reactive antibodies of definite specificities find wide applications in the detection, estimation and isolation of specific nucleic acids and nucleic acid components. They are used as reagents to locate modifications or conformational variations in nucleic acid polymers even when they occur in combination with other macromolecules like proteins. They have good potential as inhibitors of nucleic acid functions. Their use as anticancer agents has been proposed (Senapathy and Jacob, 1979).

Nucleic acid immunology has been reviewed previously (Plescia and Braun, 1967; Lacour *et al.,* 1973; Stollar, 1973, 1975, 1980; Munns and Liszewski, 1980; Erlanger, 1980; Lee 1984). The present review attempts to focus on the specificities and the consequent applications of nucleic acid reactive antibodies in cell biology and molecular biology.

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Abbreviations used: SLE, Systemic lupus erythematosus; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; snRNPs, small nuclear ribonucleoproteins; UV, ultra-violet; i^6 A, isopentenyl adenosine; AAF, N-acetoxy-N_2-acetylaminofluorene; MBSA, methylated bovine serum albumin; CD, circular dichroism.

Nucleic acid reactive autoantibodies

Nucleic acid reactive autoantibodies are present in the serum of individuals affected with systemic lupus erythematosus (SLE), a disease of the immune system (Koffler, 1980). They generally belong to IgG or IgM class (Ross *et al.,* 1978). Some of these antibodies react with single-stranded DNA (ssDNA), and/or double-stranded DNA (dsDNA), Z-DNA, tRNA, rRNA and nucleoproteins like snRNP.

The bases are recognised by ssDNA binding antibodies and cover 5–6 bases (Stollar 1981). The antibodies that bind to dsDNA recognises to a large extent the sugar-phosphate backbone and covers only two or three base pairs. The antibodies that bind to both ssDNA and dsDNA may be recognising mainly the backbone of one DNA strand.

Anti DNA antibodies have been purified on hydroxyapatite column (Fishbein *et al.,* 1980). Different populations of antibodies have been separated by passing the serum successively through ss-poly dT-Sepharose and T_5-dsDNA-Sepharose columns (Gillian *et al.,*1980). Some fractions of dsDNA binding antibodies distinguish between ds-poly [d(AT)] and pBR 322 DNA. The importance of DNA structure in antibody recognition has been demonstrated by competition experiments on the binding of antibodies to [^{3}H]-dsDNA from colicin El plasmids (Casperson and Voss, 1983).

The presence of two distinct populations of antinucleic acid antibodies—one recognising polyribonucleotides and the other polydeoxyribonucleotides have been demonstrated (Eilat *et al.,* 1978). Antibodies specific for rRNA (Eilat *et al.,* 1980) and another for tRNA (Eilat *et al.,* 1977) were detected in a strain of autoimmune mice. The tRNA binding antibodies showed a definite preference for native tRNA as compared to tRNA denatured and crosslinked with formaldehyde.

One other notable group of autoantibodies are those against small nuclear ribonucleoproteins (snRNPs). snRNPs consist of a variety of small stable RNAs complexed with proteins. The interaction of these RNPs with antibodies and their application in studying and classifying these RNPs have been discussed (Lerner and Steitz 1979) and reviewed (Lerner and Steitz, 1981). It has been demonstrated that both the RNA and the protein in the RNP complex are equally important in antigenicity (Mattiöli and Reichlin 1971; Dσuvas *et al.,* 1979). Direct evidence for the involvement of snRNAs in the splicing of adenovirus RNA was obtained using snRNA antibodies (Yang *et al.,* 1981)

With a view to identify the specific antigenic determinants which the SLE antibodies recognise, monoclonal antibodies have been raised from both mouse and human systems (reviewed by Eilat, 1982). Antibodies with some sequence preferences were obtained (Lee *et al.,* 1981); for example, one monoclonal antibody bound to $d(TTC)_n$ but not to $d(TCC)_n$, while another interacted strongly with $d(TG)_n$. $d(CA)_n$ but not with $d(TC)_n$. $d(GA)_n$. In some cases the monoclonal antibodies cross reacted with a wide variety of polynucleotides and phospholipids like cardiolipin, phosphatidic acid and phosphatidyl glycerol (Lafer *et al.,* 1981a). Another monoclonal anti-DNA antibody has been observed to bind to cell surface proteins as detected by cell-binding experiments (Jacob *et al.,* 1984).

The idiotypic features of anti-DNA antibodies have been studied using polyclonal (Andrezejewski *et al.,* 1981; Datta *et al.,* 1983) and monoclonal (Solomon *et al.,* 1983)

antiidiotype antibodies. It was found that antiidiotype antibodies cross-reacted with antibodies from different clones, suggesting that although anti-DNA antibodies are produced by different clones, they have similar antigen binding regions. These antiidiotype antibodies in some cases cross-reacted with antibodies from different individuals. As this indicates that autoantibodies are derived from related families of germlines, cross-reactivities are not surprising.

Antibodies elicited against nucleic acid haptens

Nucleic acids or their components are either electrostatically complexed with basic proteins like methylated bovine serum albumin or chemically coupled to proteins like bovine serum albumin to make them immunogenic. The most widely used methods for chemical coupling are the periodate oxidation method (Erlanger and Beiser, 1964) and the carbodiimide method (Halloran and Parker, 1966). The periodate oxidation method involves the oxidation of the ribose moiety and subsequent formation of a Schiff's base linkage with the aminogroups of proteins. Finally the linkage is stabilized by reduction with sodium borohydride. The carbodiimide method involves the condensation of phosphate moiety of nucleotides to the amino groups of proteins.

Antibodies specific to nucleosides and modified nucleosides

In most of the studies on antibodies to normal and modified ribonucleosides, protein-nucleoside conjugates prepared by the periodate method were used as immunogens. In this method the structure of ribose attached to proteins is altered. The antibodies induced show specificity for the ribose ring-opened trialcohol form than the parent nucleoside structure. Some of them react better with respective deoxynucleoside than the ribonucleoside. These antibodies have been used for radioimmunoassays (Schrader *et al.,* 1978; Hughes *et al.,* 1973). They bind to both RNA and ssDNA. In the case of adenosine antibodies, the populations that bind to RNA were found specific to morpholine derivative of adenine (Raju, 1984).

A wide spectrum of modified nucleosides like methylated nucleosides, carcinogen modified nucleosides, ultra-violet (UV)-induced photoproducts, isopentenyl adenosine, wyosine, queosine etc., give highly specific antibodies (reviewed by Munns and Liszewski, 1980) and have found wide applications. For example, $tRNA^{Arg}$ containing inosine were isolated using antibodies against inosine (Inouye *et al.,* 1973).

Isopentenyl adenosine (i^6A) is of great interest among the modified nucleosides as it is a plant hormone, stimulating cell division and differentiation in plant systems. It is also a component of tRNAs of numerous organisms. Antibodies to i^6A (Hacker *et al.,* 1972; Humayun and Jacob 1974a,b; Jayabaskaran and Jacob, 1983) are produced in very high titres in rabbits (Humayun and Jacob, 1974c) and have been used for radioimmunoassay (Shivkumar *et al.,* 1976; Khan *et al.,* 1977a) and isolation of i^6A, $i0^6A$ and cytokinin containing tRNAs (Senapathy and Jacob, 1981; Jayabaskaran and Jacob 1982) and tRNA precursors (Jayabaskaran, 1984).

Antibodies to m^7G have been used to isolate mRNAs which have a m^7G containing 5′ cap (Meredith and Erlanger, 1979) and m^7G antibodies inhibited *in vitro* translation of chorion mRNA in a wheatgerm system (Munns *et al.,* 1979). m_2^6A antibodies were

used as a probe to pinpoint the location of 16S rRNA by immunoelectronmicroscopy in the 30S ribosomal subunit (Politz and Glitz, 1977). Similarly the position of m^6A in bacteriophage fd DNA was located (Munns and Liszewski, 1980).

Modified bases in DNA are of great interest as the methylated bases, especially m^5C play a role in gene regulation. Availability of specific antibodies have made it possible to detect and quantitate even picomole amounts of m^5C. Recently m^6A and m^7G have been detected and estimated in eukaryotic DNAs using respective antibodies (Achwal and Chandra, 1982; Achwal *et al.,* 1983, 1984).

Immunoprecipitatable DNA probes ('immunonucleic probes') are of use in constructing genetic linkage maps of large genomes. Such probes have been prepared by modifying the probe DNA with N-acetoxy-N_2-acetylaminofluorene (AAF) or iodinated AAF both of which modify G in DNA. The probe hybridizes with large genomic fragments and also gets precipitated by antibodies raised against guanosine-AAF or DNA-AAF (Tchen *et al.,* 1984).

Most chemical carcinogens, cancer-therapeutic drugs, ionizing radiation and UV light cause structural modifications in DNA. Antibodies with high affinity to modified deoxyribonucleosides are of great value in detecting and quantitating these modifycations (reviewed by Adamkiewicz *et al.,* 1984a). Examples are O^6 butyl deoxyguanosine, O^4 butyl deoxythymidine (Saffhill *et al.,* 1982), O^6 ethyl 2′-deoxy guanosine and O^4 ethyl thymidine riboside (Rajewsky *et al.,* 1983, Adamkiewicz *et al.,* 1984b). They have been used to study the modifications caused by alkylating carcinogens on DNA and subsequent repair.

Antibodies have been raised against DNA modified by UV light, X-rays and chemicals. As the modified bases are immunodominant, the antibodies elicited are usually specific to modified nucleosides and other features of the polymer. In case of exposure to UV light, the antibodies are specific for the pyrimidine dimers formed, and also the altered conformational structure associated with these lesions (Wakizaka and Okuhara, 1979). The amount of thymidine dimers formed as well as the repair have been quantitated using antibodies raised against UV exposed calf-thymus DNA (Seaman *et al.,* 1972). One monoclonal antibody raised against UV-ssDNA recognises thymidine dimers in a polynucleotide fragment longer than tetranucleotide, but not isolated thymidine dimers suggesting that the adjacent sequences are also important for antibody binding (Strickland and Boyle, 1981). The binding sites of AAF on Col E1 DNA has been located using AAF-DNA antibodies (Murcia *et al.,* 1979).

Fuchs *et al.,* (1974) raised antibodies to gluteraldehyde conjugated $tRNA^{phe}$ and found that the antibodies are primarily directed towards the highly modified nucleoside Y and not to the tRNA itself.

Antibodies to mononucleotides

Antibodies raised against ribonucleotides by the periodate method do not discriminate between DNA and RNA even though they are base specific. Antibodies raised against pG by the carbodiimide method bound to RNA at pG residues but not to DNA (Iswari, 1981). pA antibodies were specific for the whole molecule and had very low cross reactivity with pG, pC and pU (Ali, 1981).

Antibodies raised against dpA (Humayun and Jacob, 1973) dpG (Jayaraman and

Jacob, 1980a) dpC (Reddy and Jacob, 1983a) dpT (Chandrasekharappa, 1981) and dpm^{5}C (Reddy, 1980) show very high specificity to the respective hapten when tested by radioligand binding and competition experiments. For example dpA antibodies showed a high degree of discrimination among the four deoxyribomononucleotides by a minimum factor of 8000. It had a high degree of specificity for the whole structure of the nucleotide. The nucleoside, base, or sugar phosphate were poor inhibitors of dpA binding. Blocking of the 3'OH group of dpA by acetylation resulted in 10 fold decreased affinity for the antibody sites. The immunodominance of 2 and 6 positions of adenine moiety is evident from a comparison of the inhibition by dpA, dpG and dpT. The binding of dpA antibodies to [^{3}H]-dpA could not be inhibited by native DNA. Denatured DNA showed inhibition only at very high concentrations indicating low affinity. Affinity of mononucleotide antibodies to oligonucleotides have been studied by inhibition analysis of mononucleotide binding. They prefer oligonucleotides with the respective hapten residues at the 3′ end. For example dpG antibodies have thousand fold less affinity to dpGpA compared to dpApG or other oligonucleotides with dpG at the 3′ end. 2, 6 and 8 positions of guanine seem to be important in antibody recognition. Some of the nucleotide antibodies can distinguish well between respective deoxy and ribonucleotides. For example dpC antibodies (Reddy and Jacob, 1981a) show 1400 fold more affinity to dpC compared to pC and can distinguish between DNA and RNA (Reddy and Jacob, 198 1b) and bind to denatured DNA at dpC residues. dpC antibodies unexpectedly showed higher affinity to dpm5C than dpC (Reddy, 1980).

Antibodies raised against nucleotides are heterogenous and there are populations of antibodies which bind to dsDNA with base specificity. This has been demonstrated in the case of dpG and dpC antisera (Jacob and Jacob, 1982). dsDNA binding dpG antibodies have been purified and their interaction with dsDNA and human metaphase chromosomes studied (Jacob, 1984). The interactions are base specific. The staining of chromosomes by indirect immunoperoxidase method using dpG antibodies showed differences in staining intensities between chromosomes in a pair and between chromatids in a chromosome. As the staining is dpG specific, it is expected that the differences in staining are due to differences in content or environment of dpG residues in the presumably identical chromatids.

Antibodies to oligonucleotides

With a view to obtain antibodies specific to nucleotide sequences, antibodies have been raised against oligonucleotides chemically coupled to proteins or electrostatistically complexed with methylated borine serum albumin (MBSA).

Antibodies to ApC, CpA, CpU and UpA recognised both component bases and the nucleoside attached to the carrier protein seemed to be immunodominant (Beiser and Erlanger, 1966; Estrada-Parra and Garcia-Ortigoza, 1972). Antibodies against ApApC, ApUpG and ApApU showed reactivity with the respective hapten (D'Alisa and Erlanger, 1974; Bonavida *et al.,* 1972).

Antibodies to MBSA complexes of ApApCp, ApApUp, ApGpCp, ApGpUp, GpUpUp and ApUpGp showed some degree of specificity to the respective hapten sequences, but in some cases the specificity appeared to be to the sugarphosphate backbone (Plescia *et al.,* 1968).

Antibodies raised against deoxyribo di- and tri-nucleotide haptens by the car-

bodiimide method have shown good sequence specificity. In the case of dpApT antibodies, dpApT was the best inhibitor of binding to ^{3}H-dpApT where as the reverse sequence dpTpA was required in more than 2000 fold concentration to bring about equivalent inhibition. The antibodies were formed against the whole molecule, as dApT, dpA and dpT were bad inhibitors. The higher inhibitory capacity of dpT compared to dpA showed that the nucleotide at the 3′ end of the hapten is immunodominant. The antibodies show good affinity to oligonucleotides having dpApT at the 3′ end (Khan *et al.,* 1977b). dpApT antibodies purified on AH-Sepharose-dpApT column bound to denatured colitis phage DNA at dpApT sequences as shown by inhibition studies (Reddy and Jacob, 1983b). Certain populations of dpApT antibodies are bound to dsDNA (Jacob and Jacob, 1982) and they are likely to be specific to a particular conformation of dpApT. Antibodies raised against the dinucleotide dpTpA also showed good sequence specificity (Chandrasekharappa, 1981; Gopalakrishnan, 1976). Antibodies raised against the deoxytrinucleotides dpApTpA and dpApApT showed good specificity to the respective haptens (Khan and Jacob, 1977). From studies on the trinucleotide hapten coupled to protein through 5′ phosphate or 3′ phosphate it seems that the immunodominant part of the oligonucleotide is the one farthest away from the point of coupling to the protein (Jayaraman and Jacob, 1980b).

Antibodies to double stranded and triple stranded polynucleotides

Antibodies have been raised against MBSA complexes of double stranded and triple stranded polynucleotides (reviewed by Lacour *et al.,* 1973; Stollar 1975). The method used to form the ds or ts helices was to mix the complementary homopolymers in optimum proportions. Antibodies against these polynucleotides seem to be specific mainly for the sugar phosphate backbones.

Antibodies raised against poly (A). poly (U) have been found to react well with poly (I). poly(C) or viral dsRNAs (Raso and Schreiber, 1978). Purified poly(I)·poly(brC) antibodies react well with several ds-polyribonucleotides such as poly (A) poly(U), poly(I)·poly(brC), but not poly(G) poly(C), poly(X) ·poly(U) or DNA (Guiges and Leng, 1976). Antibodies to ds ribonucleotides have been used to detect the presence of dsRNA in a variety of cells infected with Sindibis virus (Stollar and Stollar, 1970; Stollar *et al.,* 1972), Reovirus (Silverstein and Schur, 1970) as well as uninfected cells (Stollar *et al.,* 1978).

Antibodies specific for triple stranded polynucleotides clearly distinguish ts and ds structures. Examples are antibodies to poly(U)·poly(A)-poly(U), poly(U)·(dA)·poly(U), poly(U)·poly(A)·poly(I) (Rainen and Stollar, 1978a; Nahon-Merlin *et al.,* 1971; Papallian *et al.,* 1980). Antibodies to poly (A)·poly (U)·poly (I) have been fractionated into three antibody populations, each recognising a different conformational feature of the triple helical immunogen (Rainen and Stollar, 1978b). Antibodies distinguish helices built on poly (A) from that built on poly (dA) (Stollar and Raso, 1974).

Antibodies to RNA-DNA hybrids

Antibodies raised against poly (A)·poly (dT) have been shown to react with poly (I)·poly (dC) and also natural RNA-DNA hybrids (Rudkin and Stollar, 1977).

Antibodies to $\phi \times 174$ DNA-RNA hybrids have been shown to be specific to naturally occurring DNA·RNA hybrids and they did not bind to synthetic hybrid homopolymers like poly·(A) poly (dT) (Nakazato, 1979). It has been shown that poly (A)·poly (dT) is a better immunogen than poly (I) poly (dC) in eliciting antibodies to RNA·DNA hybrids (Kitagawa *et al.,* 1982). This may be because poly (A)·poly (dT) forms a more stable helix than poly (I)·poly (dC).

Antibodies against poly (A)·poly (dT) were used to detect DNA·RNA hybrids in polytene chromosomes of *Drosophila* (Rudkin *et al.,* 1977). Using the antibodies the regions carrying 5S rRNA genes on the chromosomes were located after hybridizing with 5S rRNA. Similarly antibodies to poly (A) poly (dT) were coupled to Sepharose and was used to enrich and isolate rDNA of Dictyostelium (Stumph *et al.,* 1978). The DNA was found to contain sequences coding for 17S and 26S rRNAs and adjacent regions. Regions of active transcription on the polytene chromosomes of *Trichosia* were pinpointed using antibodies (Busen *et al.,* 1982).

Antibodies to Z-DNA

Z-DNA is a left handed double helical form of DNA which was characterized by X-ray crystallographic studies on hexanucleotide CpGpCpGpCpG (Wang *et al.,* 1979). In Z-DNA was found to contain sequences coding for 17S and 26S rRNAs and adjacent syn conformation. Alternating purine-pyrimidine sequences other than AT attain Z-conformation at high salt concentrations. Chemical modifications like bromination, methylation or iodination of poly d(GC) make it attain Z-form under physiological ionic conditions. Z-DNA gives characteristic circular dichorism (CD), Raman and [^{31}P]-NMR spectra distinct from that of B-DNA. Immunological studies on Z-DNA have been reviewed (Rich *et al.,* 1983, 1984; Lafer *et al.,* 1983a; Leng *et al.,* 1983).

Z-DNA antibodies have been found to occur in sera of certain autoimmune mice (Lafer *et al.,* 1981b), SLE patients (Lafer *et al.,* 1983b), patients affected with Crohn's disease, polyradiculoneuritis, and amylotrophic lateral sclerosis (Allinquaint *et al.,* 1984). Antibodies to Z-DNA form a valuable probe for identifying and localizing Z-DNA in different systems. Such identification using antibodies is more reliable than physical techniques that rely on changes in physical properties that are consistent with Z-DNA, but not necessarily restricted to Z-DNA (Tomasz *et al.,* 1983).

Polynucleotides in Z-form are complexed with MBSA to elicit antibodies in rabbits or mice. Brominated poly[d(GC)] (Lafer *et al.,* 1981b), poly[d(GC)] modified on N^7 of guanosine with chlorodiethylene triaminoplatinum (dien-Pt) (Malfoy and Leng 1981) poly[d(G^s-C)] in which phosphorothioate group is present instead of phosphate group, poly[d(G-x/y/zC)] in which the 5 position of cytosine is methylated, brominated or iodinated, were found to be immunogenic (Zarling *et al.,* 1984). DNAs incapable of assuming left-handed conformation under physiological salt conditions were weakly or non-immunogenic. These include unmodified poly[d(GC)] as well as poly[(d(AC)]. poly[d(GT)] family of sequences bearing pyrimidine C-5 substitutions. Z-DNA reactive antibodies were obtained against poly[d(GC)] modified with *cis*-diamine dichloroplatium even though this polymer is not either in B- or Z-forms under physiological ionic conditions as seen from CD and [^{31}P]-NMR studies (Malinge *et al.,* 1984).

The Z-DNA antibodies are specific for the left-handed DNA conformation, with

characteristic binding preferences indicative of structural variations in Z-DNA itself. The structural features recognised include the sugar phosphate backbone, convex surface and minor grove. Antibodies raised against br-poly[d(GC)] bound to Z- [^{3}H]-br poly[d(GC)]. The binding was inhibited by br-poly [d(GC)] but not by poly[d(GC)], br-poly (dG), poly(dG), br poly (dG). poly (dC) or denatured calf thymus DNA, baby hamster kidney RNA or poly (A). poly (dT). Studies on the precipitation of [^{3}H]-poly[d(GC)] by poly[d(GC)]-dien-pt antibodies as a function of NaCl concentration, showed that there was a sudden increase in precipitation at 1.2 M NaCl which correlated well with the formation of Z-DNA (Malfoy and Leng, 1981). Lack of interaction of these antibodies to Z-poly[d(I.br^{5}C)] suggest that the aminogroup of guanine is important for antibody binding. From quantitative precipitin curves it has been estimated that the antibody binding site covers 3-5 nucleotides (Malfoy *et al.,* 1982). Poly[d(A·xC)] poly[d(G·yT)] and poly[d(G·zC)] families of Z-DNA display common as well as distinct antigenic determinants (x, y · z indicate methyl, bromo or iodo substitutions). Antibodies elicited against Z-br poly[d(GC)] bound to both Z-poly[d(GC)] and Z-poly[d(AC)] · poly[d(GT)] in high salt RIA (Zarling *et al.,* 1984). Unmodified Z-poly[d(GC)] was recognised by all Z-DNA antibodies tested. The binding sites recognised by anti poly[d(G· br^{5}C)] are different from those recognised by br-poly[d(GC)] antibodies. The cytidine 5 position appeared to be important not only for stabilization of left handed conformations as already stated but also serves as a major site of antibody recognition.

In the case of monoclonal antibodies raised against Z-br-poly[d(GC)] (Moller *et al.,* 1982), one bound to Z-poly[d(G · m$_5$C)] but another did not though both bound to Z-poly[d(GC)]. Two monoclonal antibodies raised against Z-br poly[d(GC)] were studied by Zarling *et al.* (1984). Both bound Z-poly[d(GC)], Z-br poly [d(GC)] and Z-poly[d(G s C)] but not Z-poly[d(A·m^{5}C)]-poly[d(G·T)]. It appears that a bromine or methyl group at the 5-position of the pyrimidine can block the antibody binding site, which is likely to be on the convex surface of DNA.

Binding of anti Z-DNA antibodies to plasmid and viral DNAs

It has been demonstrated by electrophoresis that Z-DNA structure is induced by negative supercoiling in recombinant plasmids containing poly[d(GC)] inserts, under physiological ionic conditions (Singleton *et al.,* 1982). Nordheim *et al.* (1982) were able to demonstrate that Z-DNA antibodies bound to two recombinant plasmids, one containing 32 and the other 14 GC repeats. Using ethidium bromide and topoisomerase, plasmids with different degrees of supercoiling were generated. It was found that the plasmid with the longer insert bound to the antibodies at a lower degree of supercoiling. Bound antibodies were cross-linked to the plasmids and then cut with restriction enzymes. The antibody bound fragments were trapped on nitrocellulose filter and the filterate was analysed. It was seen that the binding of the antibodies was to the $(GC)_n$ insert. Surprisingly pBR322 also showed binding to the antibodies, the sequence bound being CACGGGTGCGCATG. It should be mentioned that all plasmids formed Z-DNA at physiological degrees of supercoiling itself. Studies on plasmids containing inserts of d $(CA)_{32}$ · d (G T)$_{32}$ also gave similar results (Nordheim and Rich, 1983a). Studies on SV40 genome showed that Z-DNA is present in

transcriptional enhancer regions. Three regions which formed Z-DNA are at map locations 258-265, 199-205 and 128-133, the sequences being ATGTGTGT, GCATGCAT and GCATGCAT respectively (Nordheim and Rich, 1983b).

Zarling *et al.,* (1984) studied the binding of different Z-DNA antibodies to pBR322 and SV40 DNAs by agarose electrophoresis. It was found that these antibodies bound only to supercoiled DNA and occasionally crosslinked the DNA.

Pohl *et al.* (1982) observed that both monoclonal and polyclonal antibodies bound to Form V DNA which is formed by the annealing of complementary circular ssDNA. This DNA has a linking number of zero. The CD spectrum of Form V DNA had indicated the presence of left handed sequences.

Thomae *et al.* (1983) were able to isolate Z-DNA containing plasmids from *Escherichia coli* using purified monoclonal Z-DNA antibodies coupled to Sephacryl S-1000. From the amount of DNA bound, it seems that 1 % of the plasmids contain Z-DNA.

Binding anti-Z-DNA antibodies to eukaryotic chromosomes

The presence of Z-DNA in eukaryotic chromosomes was checked by different groups of workers using Z-DNA antibodies. Nordheim et al., (1981) demonstrated by indirect immunofluorescence that Z-DNA antibodies bound to fixed polytene chromosomes of *Drosophila.* The binding was restricted only to the interband regions and the intensity varied among different interbands in a reproducible manner. The binding could be inhibited by Z-DNA but not B-DNA. Lemeuneier *et al.* (1982) did similar experiments on polytene chromosomes of *Chironomus* but found that the binding was mainly in the band regions. Detailed studies were done by Arndt-Jovin *et al.* (1983) using antibodies against Z-br-poly[d(GC)], Z-poly[d(Gm^5C)] and Z-poly[d(G^5-C)]. They were found to bind to both *Drosophila* and *Chironomus* chromosomes and the binding was mainly in the band regions. It was calculated that approximately 0·02 to 0·1 % of DNA is in Z form. The presence of Z-DNA in the nucleus of the ciliate protozoan *Stylonichia mytilus* during different stages of life cycle was studied by Lipps *et al.* (1983) using anti Z-br poly[d(GC)] antibodies. In the vegetative cells the Z-DNA antibodies bound only to the macronucleus but not to the micronucleus, but an anti-B-DNA antibody bound both equally well. In the life cycle, no binding was seen at the polytene chromosome stage, but there was binding after the DNA elimination stage.

In vetebrates, Morgenegg *et al.* (1983) has shown by immunohistochemical techniques that anti Z-DNA antibodies bind to nuclei of various rat tissues, but differentially. Studies on Z-DNA immunoreactivity of fixed metaphase chromosomes of man and a primate *Cebus albifrons* (Viegas-Pequignot *et al.,* 1983) showed different levels of staning. In *Cebus,* intense staining was located in the R-Band positive heterochromatin segments. In contrast the human karyotype did not show any binding to R and positive heterochromatin segments.

All the chromosome studies discussed above involved the classical method of fixing chromosomes in 45 % acetic acid. Hill and Stollar (1983) were able to show that Z-DNA antibody binding can be induced in chromosomes by fixing in 45 % acetic acid. Polytene chromosomes isolated in native state by microsurgical procedures without acid fixation did not bind Z-DNA antibodies. But the same chromosomes after acid fixation showed intense binding. If the chromosomes were exposed to 45 % acetic acid

for 5 sec, returned to neutral pH and treated with antibody, the binding was found to be in the interbands and puffs. But if the treatment was prolonged for 25 sec, there was a great increase in the binding, and it was predominantly in the band regions. This explains the discrepancy in earlier reports that in some cases the binding being to bands and in others interbands. It appears that acid treatment induces Z-DNA formation in some parts of chromosomal DNA.

Exposure to 45 % acetic acid can extract four core histones of nucleosomes which can induct negative supercoils into chromosomal DNA. This may cause B $\rightarrow$ Z transition. The possibility of this mechanism was established by studies using DNase I and Topoisomerase I.

Z.DNA appears to be of wide spread occurrence, existing as isolated or multiple sites in both prokaryotic and eukaryotic genomes under certain conditions and upon stabilization with specific ligands. It is possible that different Z-DNA structures and sequences have both common and unique functions (Zarling *et al.,* 1984).

Conclusion

Nucleic acid reactive antibodies have proved to be valuable tools for the radioimmunoassays of nucleic acid components, for the detection of modifications on DNA and RNA, and as probes for Z-DNA in genomes, the methods being highly selective and sensitive.

Just as antibodies are available for particular proteins, antibodies against individual mRNAs, tRNAs etc. would be of immense value. As the antigen binding sites of nucleic acid reactive antibodies span a stretch of 4–5 nucleotides it seems feasible to elicit antibodies against oligonucleotides of that size, but enough efforts have not been made in this direction. If the oligonucleotides are chosen with proper planning it may be possible to elicit antibodies specific to individual nucleic acids or to sequence specific conformations of DNA. Thus there are exciting possibilities for the immediate future.

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