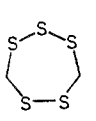
unhalogenated sesquiterpenoids.[55] In a study of Hawaiian *L. nidifica*, Erickson and her collaborators have described the isolation and structure elucidation of an interesting sesquiterpenoid alcohol, **40**,[56] a possible link to dactyloxene B (**41**), isolated from the sea hare *Aplysia dactylomela*.[57] Several species of benthic algae are currently being investigated in my laboratory. In one case we have found that *p*-(methoxymethyl)phenol (**42**) is responsible for the foul odor of the red alga *Martensia fragilis*.[58]



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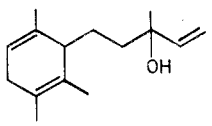

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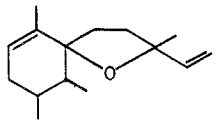

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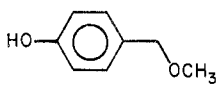

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It is a great pleasure to acknowledge the efforts of my collaborators, especially B. J. Burreson, J. A. Pettus, Jr., P. P. Roller, and F. X. Woolard, whose work is discussed here. I am very grateful to the National Science Foundation and to the Donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

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Spin-Labeled Nucleic Acids

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Received May 7, 1976

Numerous spectroscopic techniques have been used to study the relationships which exist between the structure and the function of nucleic acid molecules. Some of the significant structure–function problems in the macromolecular nucleic acid field are: (a) helix–coil transition, (b) unfolding of the tertiary structure by temperature or chemical agents, (c) binding of metal ions such as magnesium, (d) interaction and intercalation of drugs, causing perturbations of the native structure, (e) recognition site on the transfer RNA (tRNA) by the corresponding aminoacyl-synthetase, and (f) interaction of specific proteins with RNA in the ribosomes.

Certain aspects of these problems have been illuminated by spin-labeling studies.

Nitroxide spin-labels are stable synthetic organic free radicals that can interact either covalently or noncovalently with biological macromolecules of interest. Much of the organic chemistry of nitroxides is presented elsewhere.[1-7] Spin-labels can provide information concerning structure and conformational changes because they are particularly sensitive to molecular motion and orientation, as well as to electric and magnetic environments. All these features can be found in biological systems, and they can be detected and monitored by using an electron paramagnetic resonance (EPR) spectrometer.

The spin-label technique or method has been applied to a variety of biological systems, but polynucleotides have received little attention despite publication of an initial study in 1967.[8] Compared to other biosystems, nucleic acids present a particular challenge because they contain essentially only four types of monomers or bases (G, C, A, U, and/or T), which most of the time are either stacked or hydrogen-bonded to one another. Thus, specific spin-labeling of a particular site or base becomes almost impossible!

Because of this difficulty, most specific labeling studies of nucleic acids have concerned transfer RNAs, in which some rare bases are selectively reactive toward certain spin-label reagents. This Account treats this topic in depth and gives less exhaustive attention to investigations of DNA and RNA molecules. Spin-labeled mononucleotides were reviewed recently by Gaffney[9] and are not covered here. Numerous articles or books treat the general topic of biological applications of spin-labeling.[10-19]

Hermann Dugas received his B.Sc. (1964) from the Université de Montréal and his Ph.D. (1967) in organic synthesis from the University of New Brunswick. After 1 year of postdoctoral work at New Brunswick and 2 years at the National Research Council of Canada in Ottawa, he joined the staff of the Département de Chimie of the Université de Montréal in 1970. Professor Dugas's research interests relate to the study of structure and function of biological systems using NMR and EPR techniques.

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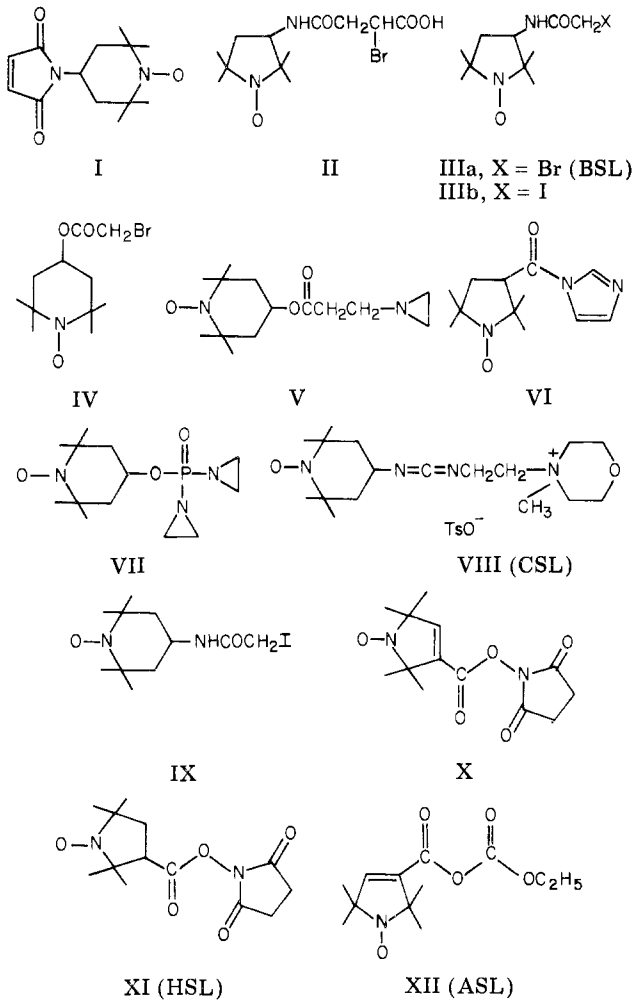

Figure 1. Chemical structures of spin-label reagents that have been used for probing the structure and function of nucleic acids. The abbreviations ASL, BSL, CSL, and HSL are used in the text and in Figure 5.

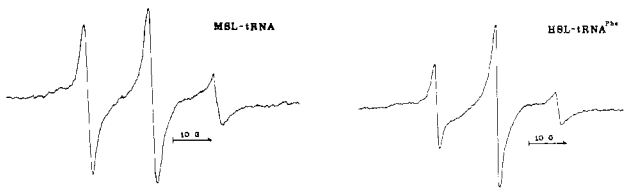

Figure 2. EPR spectra of unfractionated tRNA labeled at the 3′ end using the transformation shown in Figure 3 and spin-labeled tRNA^{Phe} labeled at position X_{47} with the *N*-hydroxysuccinimide spin label XI (HSL). The spectra were obtained at 22 °C in 0.02 M Tris-HCl (pH 7.5), 10 mM $MgCl_2$ (taken from ref 44 with permission).

Structures for several covalent-binding nitroxide free radicals that have been used to study nucleic acids are given in Figure 1.

Analysis of EPR Spectra in Solution

Let us start by considering the typical EPR spectra of a nitroxide spin-label and the way information is extracted from them, with attention especially to the spectra of MSL-tRNA and HSL-tRNA^{Phe} as reproduced in Figure 2. MSL is used to label selectively the 3′ end of unfractionated tRNA according to the transformation shown in Figure 3, whereas HSL is a label that acylates exclusively the rare base X at position 47 in the variable loop of tRNA^{Phe}.

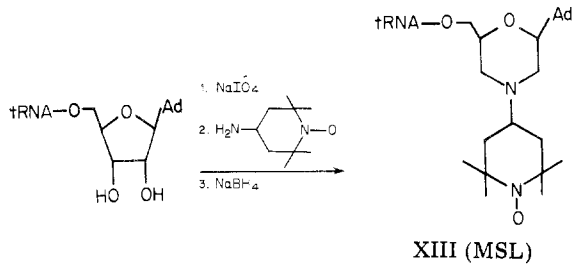

Figure 3. The chemical modifications of the vicinal hydroxyl groups of the 3′-end adenosine ribose ring of tRNA into a morpholino spin-label, XIII (MSL).

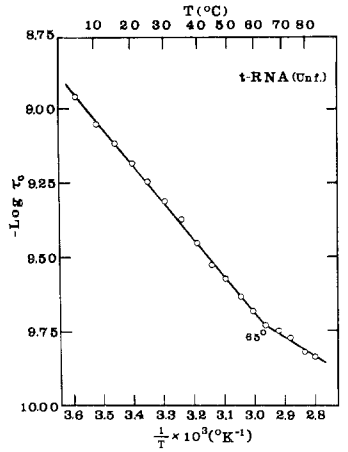

Figure 4. Dependence of the spin-label correlation time upon absolute temperature for MSL-tRNA (unfractionated) in 0.02 M Tris-HCl buffer (pH 7.5), 10 mM $MgCl_2$. It is clear that there exists a single break point at 65 °C corresponding to a spin transition temperature (taken from ref 45 with permission).

From the relative heights of the EPR lines in the spectra, it is possible to estimate the correlation times (τ_c) for rotational reorientation of the label. Naively, τ_c can be defined as simply the average time required for a nitroxide group to undergo a rotation of one radian by Brownian motion. In the limit of rapid motion (<5 ns), τ_c is calculated using the empirical expression

$$\tau_c = C\Delta H_0[\sqrt{h_0/h_{-1}} + \sqrt{h_0/h_{+1}} - 2] \quad (1)$$

where h_{+1}, h_0, and h_{-1} are the amplitudes of the low-field, center, and high-field lines, respectively, and ΔH_0 is the width of the center line in gauss. The constant C is characteristic of the nitroxide group used and varies from 5 to 7 × 10^{-10} s/G. From this expression, the τ_c values for MSL-tRNA and HSL-tRNA^{Phe} are respectively 1.28 and 1.68 ns. In both cases the EPR line shapes show that the labels are not buried inside the macromolecule. They are said to be weakly immobilized by their immediate environment; they reorient themselves rapidly. In fact, the τ_c values tell us that the spin label at the 3′ terminal of tRNA has more motional freedom than the one in the variable loop region.

This Account will show that in nucleic acids τ_c has been used extensively to monitor localized conformation events. For instance, in the case of a thermally induced

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unfolding of tRNA structure, τ_c values are obtained from a series of spectra taken between the temperatures of 5 and 80 °C and are often presented graphically in the form of Arrhenius plots of log τ_c vs. the reciprocal of absolute temperature. An example is presented as Figure 4.

A "spin-transition" temperature is observed, as manifested by a discontinuity in the slope and different slope values for the two segments. Each segment is adequately described by eq 2. Therefore, each segment

$$\tau_i = \tau_i^0 e^{-\text{Hi spin}/RT} \quad (2)$$

corresponds to a particular mode of motion of the spin label; thus a particular conformation of the tRNA allows the label to move with a specific spin enthalpy.

Caspary et al.[20] have recently counseled caution in respect to the effect of change in viscosity of the medium in measuring τ_c of polynucleotides, especially if the spin label is rigidly attached to the biopolymer.

Spin-Labeled DNA

Most of the work on deoxyribonucleic acids has been done by Russian workers,[21-25] although spin-labeling of calf thymus DNA with *N*-nitroxylmaleimide (I), α-bromosuccinic nitroxylamide (II), and nitroxylbromoacetamide (IIIa) is mentioned by Smith and Yamane.[8,21] To my knowledge these studies were limited mainly to the labeling of the DNA, and no detailed studies were carried out with these spin-labeled DNA preparations. Chemical modification of T2 phage and calf thymus DNA samples has been accomplished with the bromoacetoxy spin-label IV.[22] Only weakly immobilized EPR spectra were obtained. Spin-label V has been synthesized[23] for studying conformational changes in DNA during acid denaturation. T2 phage DNA was also spin-labeled with the imidazole spin label VI.[25]

Spin-label VII, an analog of an antitumor agent, was synthesized[26] in the hope that it might be possible to develop the EPR technique into a useful diagnostic tool for detection of cancer. Recently, Hong and Piette[27] developed a spin-labeled technique for the study of carcinogen–DNA intercalation mechanisms utilizing a modified ethidium bromide as a model ligand and specifically synthesized spin labels of carcinogenic aromatic amines such as 2-aminofulvene, 2-aminoanthracene, and 6-aminochrysene. Temperature studies in which the heat-induced release of the spin label in DNA is monitored by EPR give an accurate measure of complex dissociation characteristics and appear to parallel the helix–coil cooperative melting transition monitored by absorbance measurements. This study suggests that the spin-label method may be a useful tool in studying the sequence of molecular events related to carcinogenesis.

Spin-Labeled RNA

In early studies, alkylating spin labels such as I, II, and IIIa were found to react with yeast RNA.[8,21] The estimated per cent labeling was low (at best 5% labeling with IIIa), and the spectra correspond to mobile spin labels attached to a large molecule. Isolation of the individual bases after digestion showed that all monomers could be labeled, although there was a greater reactivity toward the purine bases. Position N-7 appeared to be the side of labeling. The reaction of IIIa with poly(G) at pH 5.5 (acetate buffer) yielded an EPR spectrum corresponding to an immobilized spin-label; this was indicative of a high degree of secondary structure in poly(G) at low pH. Studies of the order–disorder transitions for poly(A) and poly(G) were made; the resultant p*K* value of 11.5 agreed with optical spectroscopic methods. Isoclinic points (by analogy with isosbestic points in absorption spectra) observed in superposition of EPR spectra indicated that each of these large polynucleotides existed in only two forms—one ordered and one disordered—and therefore no intermediate forms were present.

A study of the conformational change in ribosomal 16S RNA isolated from cells of *B. subtilis* was made possible with the aziridine spin-label V.[28] This was accomplished by relating the EPR line shapes to the rotational correlation time τ_c. With this parameter in hand, a temperature dependence of the EPR spectra of the spin-labeled RNA was used to estimate the temperature for the beginning of melting of the polynucleotide structure. This spin transition melting temperature increased from 29 to 43 °C when the ionic strength of the medium changed from 0.01 M to 1.0 M in NaCl.

The spin-label water-soluble carbodiimide VIII was first synthesized in 1970.[29] This compound reacted in high yield under mild conditions with poly(U) and tRNA at position N-3 of uridine residues.[30] In a further study,[31] the dinucleotide UpU and poly(U) were extensively modified by reaction with VIII, and spin-exchange interaction between spin-labels residing on adjacent nucleotide residues was observed. Valuable information on location and mobility of spin-labels can be obtained by means of this spin-exchange technique. This approach has promise for future studies of spin-labeled natural nucleic acids.

Bobst[32] performed a more elaborate study on spin-labeled samples of poly(A), poly(U), and poly(G) using the spin-label iodoacetamide IX. The nitroxide radical was incorporated to a greater extent in poly(A) and poly(U) than in poly(G). The degree of incorporation was about one label for every 600 nucleotides. No incorporation was observed in the case of poly(C); this

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showed that the sugar and phosphate backbone were not tagged with the spin-label. The τ_c values were smaller in spin-labeled single-strand poly(U) (0.43 ns) than in poly(A) (0.71 ns), suggesting less freedom of motion of the label in the adenylic acid building blocks. The conformational properties of labeled poly(A) were followed as a function of pH. The EPR data suggested that the single-strand–double-strand transition takes place in three steps. Two of these steps appear to be related to forms A (partially protonated state) and B (fully protonated state) of the polynucleotide while the third phase may consist of a large aggregate involved in the transition of the less protonated double strands to those of complete protonation.[32]

The temperature-dependent transitions of spin-labeled poly(A)–poly(U) complexes have also been studied.[33] At 26 °C, the τ_c values increased by about 40% in the duplex as compared to the corresponding single-stranded polynucleotides, indicating that the label participates in the ordering characteristics of the duplex. It was shown that the strand separation of the double helix to single strands was accompanied by a well-defined abrupt change in the mobility on the spin-label. From a plot of $-\log \tau_c$ vs. inverse absolute temperature, the spin denaturation temperature for the duplex was estimated to be 47.5 °C. These results are in agreement with optical melting techniques. It was also verified that changes in rotational freedom of the label with temperature were reversible in the temperature range 26 to 66 °C. Therefore, the spin-label method can indeed be used to monitor conformational transitions from double helices to single-stranded systems in nucleic acids.

More recently it has been shown that the 3′ end of an RNA polymer provides a unique site for spin-labeling.[34] This was accomplished by carrying the modifications of the terminal adenosine ribose ring shown in Figure 3. As mentioned earlier, such a chemical transformation of vicinal hydroxyl groups into a morpholino spin label (MSL) was used advantageously to label selectively the 3′ end of tRNA molecules.

Spin-Labeled tRNA

The first effort to spin-label tRNA selectively must be credited to Hoffman, Schofield, and Rich.[35,36] They first aminoacylated *E. coli* $tRNA^{Val}$ and $tRNA^{Phe}$. Then they synthesized the spin-labeled *N*-hydroxysuccinimide analog X and allowed it to react with the α-amino group of the corresponding aminoacyl residue. The amino acids used were radioactive (^{14}C); thus quantitative evaluation of the extent of labeling could be made. Some nonspecific labeling of *E. coli* Phe-$tRNA^{Phe}$ occurred, but this product could be separated by chromatography. It was not characterized, but it could be the product resulting from an acylation of the rare base 3-(3-amino-3-carboxy-*n*-propyl)uridine, X_{47}. The rate of tumbling of the spin-label was measured as a function of temperature and was found to be strongly dependent on the ionic strength of the medium. The results were presented in the form of Arrhenius plots of $\log \tau_c$ vs. the reciprocal of absolute temperature. For example, in 0.01 M Tris-HCl with 0.01 M $MgCl_2$ at pH 7.4, the temperature at which the discontinuity in the slope occurs is 70 °C. This value is somewhat lower that the melting temperature obtained by thermal–optical profiles. The process is reversible and was interpreted in terms of two molecular states of the tRNA in each of which the molecular constraint on the spin-label differed. There was not enough information to allow interpretation of the data in terms of a detailed molecular model, but the variations in the spin mobility observed showed that the 3′ end of the molecule can be freed while the bulk of the molecule is still unperturbed. Also, it appears that the 3′ end, although involved in the folding, is not buried in the interior of the macromolecule but is rather located near the periphery. A similar study[37] was performed on yeast Cys-$tRNA^{Cys}$ labeled with the nitroxyliodoacetamide IIIb on the free sulfhydryl group.

Soon thereafter Nishimura and co-workers[38] reported that the 4-thiouridine (s^4U) residue, occurring occasionally in *E. coli* tRNAs, could be labeled selectively on the sulfur atom with nitroxylbromoacetamide IIIa. Interestingly, the amino acid acceptance activities of spin-labeled $tRNA^{Tyr}$, $tRNA^{fMet}$, $tRNA^{Val}$, and $tRNA^{Phe}$ were unaffected, whereas those of $tRNA^{Glu}$ and $tRNA^{Lys}$ were reduced considerably. The EPR spectra, taken in the absence of Mg^{2+} ions, indicated weakly immobilized spin-labels; the degree of constraint was slightly different among the various tRNA studied. Unfortunately, no further study was reported by this group on these spin-labeled tRNA samples.

In an effort to label selectively other regions of the tRNA molecule, McIntosh et al.[39] succeeded in acylating the rare base 2-thio-5-(*N*-methylaminomethyl)uridine (s^2U^*) in the wobble position of the anticodon of *E. coli* $tRNA^{Glu}$ with the mixed-anhydride spin-label XII. The shape of the EPR spectrum suggested that the spin-label is not buried inside the macromolecule and agreed with a model whereby the orientation of the anticodon loop is at one end of the "L"-shaped tRNA molecule.[40] A temperature-induced conformational study in the presence of Mg^{2+} ions showed a spin transition at 50 °C. This value is well below the expected value of 70 to 75 °C associated with the process of complete denaturation. Hence, it was concluded that the transition might be associated with a localized unfolding of the anticodon region leading to an intermediate state between the native and the random coil structure.

In 1975, Sprinzl et al.[41] introduced the iodoacetamide spin-label IX into a C-75 modified residue to $tRNA^{Phe}$ from yeast. In the presence of Mg^{2+} ions a single melting transition at 47.5 °C was observed. This transition was claimed to be a cooperative process, not associated with a local perturbation of the 3′ end but related to a global conformational change of the ex-

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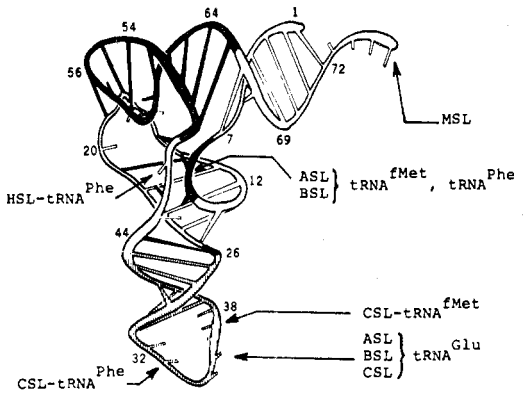

Figure 5. The different sites of spin-labeling on the three tRNAs using the static model deduced by Rich and co-workers[40] from their x-ray work.

tended "L"-shaped tRNA molecule. Their conclusion is at variance with the two previous works presented.[37,39]

EPR spectroscopy has also been used to study the interaction of Mn^{2+} ions with yeast $tRNA^{Val}$ modified with either of the spin-labels I, IX, or X.[42] Label I modified the four pseudouridine (Ψ) residues, label X was attached to the α-amino group of the aminoacyl-tRNA, and label IX reacted with the s^4U residue. The location of the sites of ion coordination was determined by studying the dipole–dipole couplings of the coordinated Mn^{2+} ions with the spin-label selectively attached to the tRNA. The analysis showed that there is a coordination site for Mn^{2+} ions near the 3′ end of the tRNA and also a site close to one of the Ψ residues. Unfractionated tRNA from *E. coli* aminoacylated with phenylalanine and modified at the α-amino group of the amino acid residue with the spin-label X was also examined. The results showed that addition of Mn^{2+} or Mg^{2+} ions aid in the formation of a more rigid and compact tRNA structure. Furthermore, the data implied that a coordination of Mn^{2+} ions also occurs near the s^4U residue. Similar results were obtained in our laboratory, suggesting that Mn^{2+} and Mg^{2+} ions must interact with a site at the apex of the "L"-shaped tRNA molecule for the maintenance of its tertiary structure.[43]

Recently, the preparation and characterization of nine new spin-labeled tRNAs using the spin-labels ASL, BSL, CSL, HSL (Figure 1) as well as MSL was reported.[44,45] The work was carried out on $tRNA^{Glu}$, $tRNA^{fMet}$, and $tRNA^{Phe}$ from *E. coli*. The different sites of spin-labeling are summarized in Figure 5. Arrhenius plots for each spin-labeled tRNA showed that only tRNAs having a spin-label at the s^4U_8 position were able to see two thermal transitions, one at 32 °C and the other at 53 °C. This suggests that the region around s^4U_8 is quite rigid and the label is able to monitor the subsequent dissolution of the remaining ordered regions of the partially melted tRNA molecules. In contrast, labels in the anticodon and variable regions detected only one transition around 52 to 54 °C. Although some labeled tRNAs were not very active toward aminoacylation (Table I), our results enabled us to propose a dynamic model of tRNA that could have possible importance for tRNA function in protein biosynthesis.

Table I
Summary of the Activities of tRNAs Bearing a Spin-Label[67]

Spin-labeled tRNA	Location of the label	Extent of amino-acylation, %
$tRNA^{Glu}$		
ASL	s^2U*-35 (anticodon)	23
BSL	s^2U*-35 (anticodon)	25
CSL	s^2U*-35 (anticodon)	23
$tRNA^{fMet}$		
ASL	s^4U-8	99
BSL	s^4U-8	98
CSL	U-37 (anticodon)	57
$tRNA^{Phe}$		
ASL	s^4U-8	98
BSL	s^4U-8	99
CSL	U-33 (anticodon)	62
HSL	X-47 (variable loop)	99

Table II
Comparison of Melting Transitions for the Thermal Unfolding of tRNA Molecules at Neutral pH Obtained from Various Spectroscopic Techniques[a]

Differential UV Absorption[50] without Mg^{2+}			
25 ± 5 °C (Acceptor stem)	40 ± 4 °C (anticodon arm)	50 ± 5 °C (tertiary structure and cloverleaf structure)	>65 °C (TψC and D helices)
NMR without Mg^{2+}, Combined with Relaxation Kinetics[48]			
30 and 46 °C (D helix and tertiary interactions)	61 ± 2 °C (TψC arm)	70 ± 10 °C (anticodon arm)	77 ± 2 °C (acceptor stem)
NMR[47] without Mg^{2+}			
45 °C (D helix and tertiary interactions)	56 °C (anticodon region)	70 °C (TψC and acceptor stems)	
NMR[47] in the Presence of Mg^{2+}			
60 °C (anticodon region)	68 °C (D helix and tertiary structure)	85 °C (TψC and acceptor stems)	
EPR using Selective Spin-Labeling,[45] with and without Mg^{2+}			
31 ± 2 °C (tertiary interactions and part of D helix)	52 ± 2 °C (anticodon and miniloop regions)	65 ± 2 °C (TψC and acceptor regions)	

[a] The regions affected by the unfolding are given in parentheses under each temperature. D refers to dihydrouridine.

This model is presented in the following section.

Thermal Unfolding of tRNA Structure

To put the reader in perspective, it would be useful to start this section by giving a comprehensive view of some of the researches done in recent years on the denaturation of tRNA by temperature. However, only results obtained from differential UV absorption, EPR (spin-labeling), NMR, and temperature-jump relaxation kinetics are presented. In the spin-labeling study[44,45] three species of *E. coli* tRNA (Glu, fMet, Phe) were investigated, whereas in the NMR and kinetics studies[46-49] only *E. coli* $tRNA^{fMet}$ was examined in

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detail. For the UV absorption studies,[50,51] yeast $tRNA^{Phe}$ was used.

A comparison of the melting transitions obtained with the aforementioned techniques is presented in Table II. Some studies reported up to five distinct melting transitions, while others claimed that only three transitions were involved. One striking observation, based on UV absorption,[50] was the finding that the tertiary structure melts much later than the acceptor stem, but later it was realized that the first transition is indeed related to the opening of the tertiary structure.[51] Crothers et al.[48] observed a first transition at 30 °C, in the absence of Mg^{2+} ions, and suggested that the D loop can open transiently while the tertiary structure is still intact. This low-temperature transition was also observed with the spin-labeling technique.[45]

The NMR results of Wong et al.[47] in the presence of Mg^{2+} ions indicated that, within the accuracy of their measurements, the position of some resonances was subjected to a Mg^{2+} effect and tertiary structure and D loop melted at higher temperatures relative to Mg^{2+}-free samples. On the other hand, it is interesting to observe that localized perturbations of spin-labeled tRNA by temperature were the same with and without Mg^{2+} ions.[45] Although Mg^{2+} ions could very well affect the ribose phosphate backbone of tRNAs as well as some G base stacking,[52] it does not seem to affect to any appreciable extent the tertiary structure of tRNA molecules.[53] It is natural to expect that the structure disrupted in the first thermal transition involves first non-cloverleaf binding, or tertiary structure. Until recently, these extra tertiary hydrogen bonds escaped detection by NMR.[53,54] This local perturbation could then be immediately followed by a loss in the secondary structure of the closeby D helix.

The NMR work of Wong et al.[47] and our own spin-labeling work[45] both agree that the unfolding of the anticodon region follows the D helix but precedes the TΨC helix. This is at variance with the work of Crothers et al.[48] where the TΨC arm unfolds before the anticodon helix. It is difficult to imagine, with the present knowledge of the parameters responsible for helix stability, how the TΨC region could be so prone to unfolding since it is partly shielded from the solvent and forms a continuous double helix with the CCA stem,[40] which often contains a high proportion of GC base pairs. It should be mentioned that this NMR study[48] was carried out with $tRNA^{fMet}$ molecules that were partially cross-linked between s^4U_8 and C_{13}; that could consequently explain the differences in behavior. Despite this irregularity it can be said that although optical, NMR, and EPR melting phenomena may not coincide exactly, the transitions are in general in the same range of temperature.

In fact, the spin-labeling technique has the beneficial potentiality of monitoring localized structural perturbations because of the presence of a spin probe at

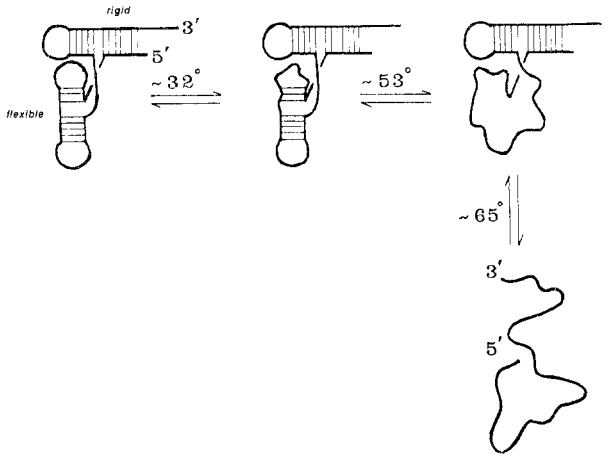

Figure 6. A schematic representation of the sequential unfolding of tRNA structure by temperature, based on the spin-label method.

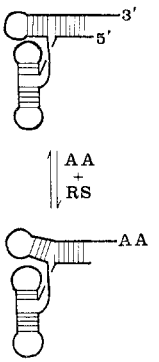

Figure 7. A schematic representation of the conformational change on tRNA upon aminoacylation (AA represents the amino acid and RS the aminoacyl-synthetase). The three tRNA derivatives used to show this change in structure were: $BSL\text{-}tRNA^{Phe}$ (position s^4U_8), $HSL\text{-}tRNA^{Phe}$ (position X_{47}), and $CSL\text{-}tRNA^{Phe}$ (position U_{33}). The EPR spectra of the first two derivatives showed more mobility upon aminoacylation, whereas the last one, labeled in the anticodon, was unchanged.[59]

a specific region. The introduction of a spin-label does not seem to modify appreciably the native structure of tRNA molecules,[45] and the results obtained support the idea that the molecular mechanism of thermal unfolding of tRNA molecules is sequential. This is represented in Figure 6. The spin-labeling results[45] went beyond invoking a sequential mechanism for the thermal unfolding of tRNA. A dynamic role for tRNA molecules in protein biosynthesis was also suggested. Briefly, thermodynamic considerations support the idea of a *semiflexible* structure whereby the 3′ end plus the anticodon and miniloop regions form the flexible portion of the tRNA. These flexible regions would permit a better contact of the tRNA with the mRNA on the ribosome[55] during the reading of the genetic code and would also facilitate the transfer of the growing polypeptide chain.[56] On the other hand, the more rigid portion of the tRNA molecule is composed of the contacts between the D and the TΨC loops plus the TΨC and CCA stems. These regions would have a very important role of fixation to a common recognition site in the ribosomes.[57] Consequently, this rigid portion

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is almost certainly a common architectural feature of all tRNAs.[58]

Conformational Change in tRNA upon Aminoacylation

Recently the spin-label method proved unequivocally that the aminoacylation of tRNA induces a conformational change of structure.[59] *E. coli* $tRNA^{Phe}$ was used, and the labels were located respectively on the s^4U_8 residue, the rare base X_{47} in the variable loop, and residue U_{33} in the anticodon loop. A comparison of the EPR spectra of these three differently located spin labels showed that upon aminoacylation of $tRNA^{Phe}$ the region around the s^4U_8 residue and the variable loop region became more flexible, while the environment of the anticodon loop was not affected.[59] This result strongly suggested that the main difference in structure between charged and uncharged tRNA resides is the release and exposure of the TΨCG loop (Figure 7) for eventual binding to the complementary sequence CGAA on the 5S RNA in the ribosomes.[57,60] This is tantamount to saying that during the normal course of protein biosynthesis some of the tertiary structure of tRNA must be disrupted.[58] Therefore, this interesting observation represents an important contribution toward a better understanding of the dynamic role that tRNA plays in Nature. Such a subtle perturbation of tRNA structure upon aminoacylation was not detected by NMR spectroscopy.[61]

Schwarz et al.[62] have proposed a different model whereby codon–anticodon recognition can cause a conformational change on aminoacyl-tRNA, which results in the binding of the TΨCG sequence to the 50S ribosomal subunit. Binding of the codon triplet AUG to BSL-$tRNA^{fMet}$ resulted in a loosening of the macromolecular structure around the s^4U_8 residue[43] and could correspond to the structural change inferred by Schwarz et al.[62]

Possible Structural Perturbations of Nucleic Acids by the Presence of the Spin-Labels

The possibility of a perturbation of the biosystem by the presence of a spin-label is a constant criticism that the technique suffers from. For instance, one of the first problems examined by the spin-label method was the uptake of oxygen by deoxyhemoglobin.[63] The spin-label I was used to label position Cys-93 of the β chain, and the information obtained at that time was very useful. However, in the case of carboxyhemoglobin an x-ray study of the spin-labeled derivative showed its structure to be different from that of the native protein, despite the retention of cooperative oxygen uptake and various other characteristic properties.[64]

In the field of nucleic acids no detailed work has been carried out in this direction. However, in the case of tRNA derivatives an indirect evaluation of the degree of structural perturbation of the biomolecules can be obtained by measuring the extent of aminoacylation of the spin-labeled tRNAs. Table I shows that in many instances the perturbation of tRNA structure by the spin-label must be minimal. In fact, the lowering in aminoacyl activity encountered in some cases could result from ineffective binding of the enzyme with its cognate tRNA, caused by the presence of the spin-label nearby, rather than by a conformational change in tRNA structure as a result of the presence of the spin-label.

Recent NMR data from Daniel and Cohn[65,66] on BSL-$tRNA^{fMet}$ seem to suggest that the spin-labeled s^4U_8 residue has lost the capacity to make the tertiary hydrogen bond with residue A_{14}. Since all the tRNAs labeled at position s^4U_8 can be fully aminoacylated, it must be assumed that they are still in a conformation very close to the "native active" form, unless the tertiary hydrogen bond involving s^4U_8 is not essential for the activity.

In the study of the thermal unfolding of BSL-$tRNA^{Glu}$, labeled in the anticodon region, two thermal transitions were observed instead of one.[45] Providing that the native form of the spin-labeled tRNA was still intact, one possible explanation for this exception was the capacity for that particular label to be more sensitive to perturbations away from its site of attachment. Also the possibility of an unfolding mechanism different from the general one cannot be excluded. In either case, these results emphasize the importance of using more than one type of spin label, if possible of slightly different size, to monitor localized structural changes in macromolecules before drawing conclusions about the unmodified system.

A last observation deserves consideration. In a study designed to examine whether the tRNA conformation observed in the solid state[40] is a representation of that existing in solution, a series of double spin-labeled *E. coli* $tRNA^{Phe}$ was prepared.[67] Nitroxide–nitroxide interactions were observed only in the case of BSL (on residue s^4U_8) and HSL (on residue X_{47}) doubly labeled $tRNA^{Phe}$. A distance of 18.5 Å was calculated between the two spin-labels. On the yeast $tRNA^{Phe}$ model[68] the distance from O-4 of U_8 to N-3 of U_{47} is 15 Å. The two measurements are very close, especially in view of the fact that addition of a spin-label adds an additional distance between the two residues in question. The tertiary structure of $tRNA^{Phe}$ (and of spin-labeled $tRNA^{Phe}$) in solution is thus comparable to that in the crystalline state. This study is a good illustration of the type of information that can be obtained using the double-labeling technique.

Conclusion

As a reporter group the spin-label can monitor its environment in biopolymers. It is a very effective tool that provides important information pertaining to the local organization of specific regions of polynucleotides, particularly the secondary and tertiary structures of tRNA molecules. Further application of the spin-label method should prove to be exceedingly useful to study

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conformational characteristics of local regions of tRNA and ribosomes during the various step of protein biosynthesis. The principal limitation of the method is the ingenuity of the researcher to synthesize and utilize the appropriate spin-label. The greater the specificity of the labeling and the smaller the perturbance it can create on the system, the richer will be the information from such a study.

Unraveling Reactions with Rotating Electrodes

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Received May 4, 1976

This Account describes how the rotating disk and allied techniques can be used to unravel complicated heterogeneous and homogeneous reactions. The rotating disk geometry permits precise hydrodynamic control of the flux of material to and from a reacting surface, and thereby provides the chemist with a unique experimental variable.[1,2] The application of this concept of flux control via hydrodynamics has been concerned, to date, principally with processes associated with electron transfer.

The rotating ring–disk electrode (RRDE) as conceived by Levich, Frumkin, and co-workers[3-5] provides the chemist with a powerful methodology for studying complicated chemical as well as electrochemical reactions. Prior work relied on single electrode techniques to elucidate the details of electrochemical reactions. However, such approaches can only give inferential evidence about the nature of the electrochemical reaction sequence involved. Supplementing such data with ex post facto information, for example, chemical analysis of the electrolyte, is often necessary, but is inadequate if an unstable or reactive species occurs during electrolysis.

The capability of a RRDE's ring electrode to detect, virtually simultaneously, species generated at the disk electrode provides an elegant and convenient solution to this analytical problem. In addition, the rigorously defined mathematics of the RRDE geometry makes it possible to distinguish between faradaic (electron transfer) and nonfaradaic processes.

In order to place the more recent work in perspective, we review classical rotating disk electrode (RDE) and RRDE theory and practice. Then the recent developments of hydrodynamically programmed RDE and RRDE are presented because of their power to discriminate further among subtly different reaction possibilities. Many of our illustrations involve metal deposition reactions and corrosion phenomena, but it should be kept in mind that the applications of rotating electrodes are much more general. Our object is to present a critical overview of the present state of rotating electrode methodology and to clarify the complementary nature of the various techniques.

Classical Rotating Electrodes

Figure 1 illustrates the geometry of all the rotating electrodes under discussion. P–P′ represents the plane of an insulating disk rotating about an axis O–O′ and containing a disk electrode of radius r_1. The typical streamlines for all electrodes obtained under laminar flow conditions are shown by the dotted lines with the arrowheads.

Rotating Disk Electrode. If the solution contains an electroactive species A, reaction 1 occurs at the disk

$$A + n_D e = B \qquad (1)$$

electrode if a suitable potential, E_D, is applied. It follows from Levich[1,2] that the resultant current for a disk electrode of radius r_1 is given by eq 2, where F is

$$i_D = 0.6025 n_D F \pi r_1^2 D^{2/3} \nu^{-1/6} \omega^{1/2} (C^b - C^s) \qquad (2)$$

the Faraday constant, D is the diffusion coefficient of species A (or B), ν is the kinematic viscosity, ω is the radian velocity of rotation, and C^b and C^s are the bulk and surface concentrations, respectively, of A (or B).

Curve I of Figure 2 represents the disk current–disk potential ($i_D - E_D$) curve that would be obtained for the reversible process in eq 1 in a supporting electrolyte containing only A in solution. The maximum disk current, $(i_D)_L$, occurring when C^s becomes vanishingly small, is remarkably reproducible and a valuable source of diffusion coefficients. An improved form of the

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