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The Electrostatic Molecular Potential of Yeast tRNA^{Phe}. III. The Molecular Potential and the Steric Acessibility Associated with the Phosphate Groups

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The molecular electrostatic potential of yeast tRNA^{Phe} is calculated at sites bridging the anionic oxygens of each of the 76 phosphate groups of the molecule. A quantitative measure of the steric accessibility of the anionic oxygens of the phosphates toward a spherical cation is presented. Both the resulting potentials and accessibilities are discussed in terms of the molecular and electronic structure of tRNA.

Key words: Electrostatic potential – Steric accessibility – Phosphate groups – tRNA.

1. Introduction

The importance and complexity of the roles of tRNA's in cellular function have encouraged our laboratory to begin a theoretical study of this family of nucleic acids in an attempt to understand better their molecular and electronic structure and, further, the nature of their interactions with other molecules or macromolecules.

Two previous publications [1, 2] have dealt with the electrostatic molecular potentials due to the combined influence of the phosphate groups of tRNA^{Phe} and with the potentials for individual phosphates and sugars possessing the variety of molecular conformational states observed in this nucleic acid. Both these studies were based on the X-ray crystal structure of yeast tRNA^{Phe} [3].

We now extend our treatment in three ways.

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Firstly, whereas in article I, of this series [1], we considered the combined influence of solely the 76 phosphates of $tRNA^{Phe}$, we now consider in addition the effect of all the sugars and bases of the molecule (which is to say in all 228 molecular subunits containing approximately 2500 atoms). The electrostatic molecular potentials which will presently be discussed consequently derive from a complete $tRNA^{Phe}$.

Secondly, whereas in our former study, planes of potential cutting through the tRNA molecule at various levels were presented, we now consider a selected, particularly significant site at each phosphate unit and follow the variations of the potential of $tRNA^{Phe}$ at this site as we move along the phosphate-sugar backbone of the molecule (through the various stems and loops of its complex folded form).

Thirdly, we attempt a measure of the steric accessibility of the phosphate groups under study. This is of particular importance for a macromolecule with such a convoluted form as tRNA. It translates into quantitative terms the steric hindrance (including that due to energetically favourable tertiary interactions) at any selected site inside the molecule and thus promises to be of considerable importance in interpreting the reactivity of sites within tRNA, for which the role of steric accessibility has been acknowledged and discussed qualitatively by several authors [4–6].

2. Method

A detailed description of our approach to the calculation of the electrostatic molecular potential of a macromolecule has been given in article I of this series [1] and in our publications on the electrostatic potential of B-DNA (7 and references contained therein). In essence the method consists of subdividing the macromolecule into molecular subunits (bases, sugars and phosphates in the case of tRNA), calculating an *ab initio* wavefunction for each of these units and from the latter developing a multipolar expansion of the associated electron distribution which has been shown to lead to a satisfactory reproduction of the electrostatic potential of the units [8]. These individual potentials are then appropriately superposed to yield the total potential of the macromolecule. It is to be noted that the accuracy of the multipolar representation of the electrostatic potential is limited to points at more than 2 Å from constituent atoms of the molecule. Below this distance exact electrostatic potentials must be calculated.

For yeast tRNA, 33 distinct subunits were considered: 10 different conformations of the monoanionic phosphate group, the doubly charged phosphate 1, 8 different conformations of the sugars, the $O_{2'}$ methylated sugar (see article II of this series [2]), 4 "standard" bases, adenine, uracil, guanine and cytosine and the 9 "unusual" or modified bases. The detailed properties of the electronic distributions of these latter bases will be presented in a forthcoming publication. The geometry of all subunits and that of the complete macromolecule were based (as in Ref. [1, 2]) on the crystallographic study of the orthorhombic crystal of yeast $tRNA^{Phe}$ by S. H. Holbrook et al. [3].

The phosphate site studied in this article is located in the plane containing the phosphorus atom and the two anionic oxygens of the phosphate group and is equidistant from these latter by 2.15 Å. This site corresponds to the optimal binding position of a sodium ion to the phosphate group [9] for the atomic orbital basis set that we have employed. Its study is used here for the illustration of the variation of the potential of the phosphates along the sugar phosphate backbone of tRNA. It is of interest also for the study of the effect of screening the charged phosphate by Na^+, which we are currently carrying out.

Because of the complex folded form of tRNA the potentials at the phosphates are very variable, as will be shown. Their values may consequently be expected to be of considerable importance if molecular interactions at these units are to be explained. There is, however, an additional factor which must be taken into account in such a convoluted molecule, namely, the steric accessibility of the sites. For if a reactive species cannot approach a site, reaction clearly cannot take place even if the potential at the site is very favourable for the approaching species.

We have therefore attempted the determination of the accessibility of the reactive atoms within the macromolecule with a view to quantify these steric effects. In this we follow, with some modifications, the earlier attempts at the quantification of the accessibility of a macromolecule towards a solvent molecule as proposed by Lee and Richards [10] and applied to $tRNA^{Phe}$ by Thiyagarajan and Ponnuswamy [11]. Both these studies represented the solvent molecule by a sphere of appropriate radius. We have adapted this approach to our problem in the following way. Firstly, the van der Waals envelope of $tRNA^{Phe}$ was generated by placing spheres of appropriate radii around each atom of the molecule, employing the atomic radii:

H 1.2 Å
C 1.6 Å
N 1.5 Å
O 1.4 Å
P 1.9 Å.

These values being due to Pauling [12] with the exception of that for the carbon atom, for which this author does not give the corresponding value, we consequently employed the radius used by Webb [13]. Unlike the previous representation of Lee and Richards, we did not employ the concept of a single sphere for groups of atoms and we have individually represented all the hydrogen atoms. Next, because one of the main interests in the accessibility towards the phosphate groups is related to their cation binding abilities, we calculated the accessibility of their anionic oxygens toward a simple spherical cation. In the present study the radius of the sphere was taken to be 0.95 Å representing that of an Na^+ ion [12].

For each anionic oxygen of all the phosphates the accessibility was calculated by bringing the cationic sphere into van der Waals contact with the oxygen sphere and then moving the cation over the surface of this latter sphere. For each position

of the cation it was calculated whether or not the cationic sphere intersected with any of the atomic spheres forming the van der Waals envelope of tRNA, that is, whether or not such a configuration was (for a classical "hard sphere" model) accessible. By studying a large number of such configurations ($\simeq$700) over the surface of each anionic oxygen, dividing the number of "accessible conformations" by the total number of conformations tried, and multiplying by the surface area of the anionic oxygen we arrive at the accessible area (henceforth abbreviated to AA) of an atom toward a given interacting species, in this case, Na^+ (we thus extend out notation to AA (Na^+) to recall that the values calculated depend on the interacting species). This parameter can have values between 0.0 corresponding to the minimal accessibility and A_n (the surface area of the atom n concerned, 24.63 $Å^2$ for oxygen) representing maximal accessibility. To calculate the total accessibility of a phosphate group we simply add the accessible areas found for its two anionic oxygens (or of the three anionic oxygens in the case of the doubly ionized phosphate 1).

It should be noted that even for a free phosphate group the accessible area of its anionic oxygen will be less than A_{oxygen} due to steric hindrance caused by other atoms of the group; we term this value the intrinsic accessible area, IAA. The corresponding AA's subsequently calculated within the $tRNA^{Phe}$ macromolecule must then be either, equal to the IAA, if no atoms from other groups hinder the phosphate anionic oxygens, or smaller than the IAA, if such hindrance exists. The IAA is thus useful for comparing the intrinsic accessibilities of various atoms or groups of atoms before the "environmental" steric effects of the macromolecule are taken into account.

This method of calculating the accessibility of an atom or group of atoms can, of course, be equally well applied to other atoms of tRNA and moreover extension is possible to calculate the accessibility towards poly-atomic species of different compositions and shapes. Such a study is currently in progress.

In this paper we shall be considering the electrostatic molecular potentials of the phosphate groups of $tRNA^{Phe}$ in conjunction with their accessibilities. It must be underlined that strictly speaking these two quantities do not refer rigorously to the same space element, being for technical reasons computed the first one for the "bridge" position and the second one as a summation over the surface of the two anionic oxygens. There is, however, no possible doubt that globally speaking they translate to a significant degree the two studied characteristics of the *phosphate groups* and that their evaluation and their examination, one versus the other, should give useful information on their overall interelation.

3. Results and Discussion

3.1. The Electrostatic Molecular Potential at the Phosphates (Bridge Site)

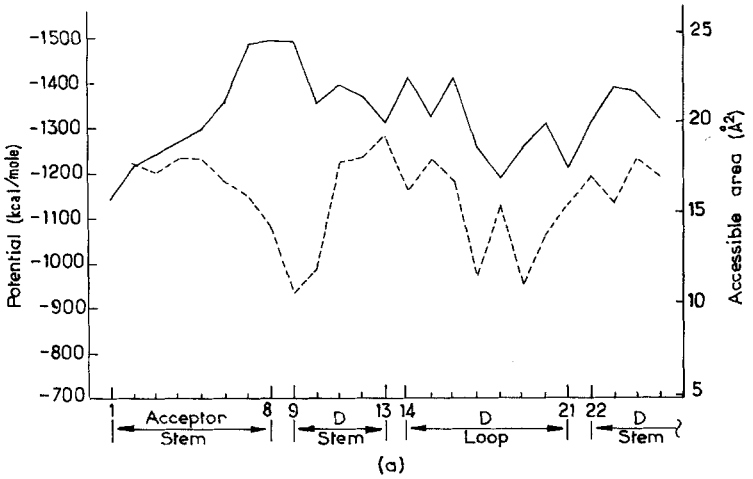
The electrostatic potentials calculated at the bridge sites of the 76 phosphates within $tRNA^{Phe}$ are given in Table 1 and represented graphically by the continuous line (——) in Fig. 1. Fig. 1 is divided into three sections for convenience: (a)

Table 1. Potentials at the phosphate bridge sites and accessibilities of the phosphate anionic oxygens

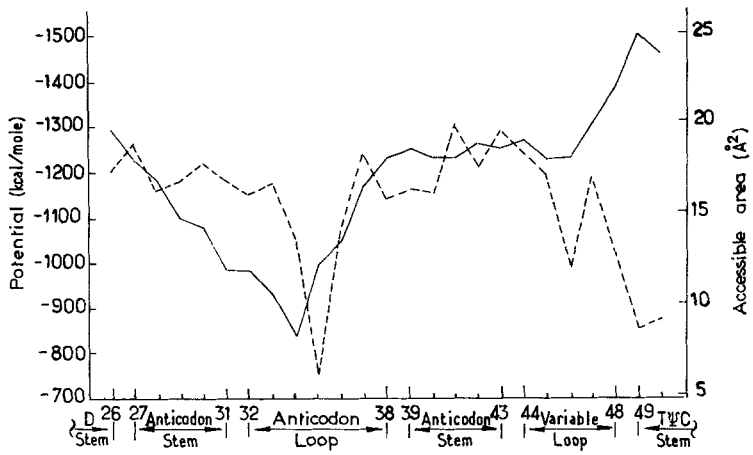
Phosphate	Potential at bridge site (kcal/mole)	Accessible area of anionic oxygens toward Na^+: $AA(Na^+)$ ($Å^2$)	Phosphate	Potential at bridge site (kcal/mole)	Accessible area of anionic oxygens toward Na^+: $AA(Na^+)$ ($Å^2$)
1	−1139.6	21.61	39	−1248.0	16.23
2	−1214.1	18.37	40	−1229.8	15.99
3	−1238.8	17.28	41	−1229.5	19.66
4	−1226.8	18.01	42	−1260.9	17.40
5	−1294.7	17.89	43	−1247.7	19.30
6	−1357.1	16.84	44	−1266.3	18.29
7	−1482.7	16.03	45	−1224.7	17.04
8	−1491.1	14.09	46	−1228.9	12.12
9	−1488.0	10.54	47	−1303.3	16.92
10	−1351.6	12.16	48	−1381.8	12.51
11	−1390.6	17.68	49	−1502.1	8.56
12	−1364.1	17.97	50	−1459.8	9.12
13	−1307.9	19.18	51	−1335.7	16.80
14	−1409.4	16.27	52	−1183.1	17.97
15	−1322.2	17.80	53	−1115.6	18.21
16	−1409.1	16.75	54	−1084.7	17.37
17	−1255.5	11.51	55	−1043.4	16.76
18	−1187.1	15.47	56	−994.5	17.33
19	−1251.4	10.98	57	−1138.0	12.28
20	−1308.6	13.85	58	−1244.6	12.03
21	−1206.0	15.46	59	−1340.7	13.53
22	−1308.6	16.87	60	−1219.0	7.59
23	−1384.5	15.47	61	−1402.3	16.76
24	−1374.3	18.05	62	−1324.3	17.12
25	−1265.8	17.04	63	−1242.3	17.81
26	−1292.2	17.16	64	−1233.4	19.10
27	−1225.4	18.77	65	−1248.5	18.09
28	−1179.2	16.23	66	−1271.4	18.49
29	−1098.0	16.76	67	−1253.9	17.12
30	−1077.3	17.76	68	−1212.1	17.68
31	−982.5	16.71	69	−1156.2	19.54
32	−978.8	16.07	70	−1115.4	19.38
33	−929.0	16.43	71	−1069.4	19.18
34	−835.2	13.45	72	−1017.0	18.29
35	−999.2	6.26	73	−946.0	18.81
36	−1045.2	14.41	74	−913.8	14.74
37	−1166.8	18.09	75	−869.5	13.93
38	−1230.5	15.67	76	−802.1	14.50

residues 1 → 25; (b) residues 26 → 50; (c) residues 51 → 76. Beneath the abcissa the conventional notations of the various loop and stem regions of tRNA are indicated. The potentials are given in kcal/mole by the left-hand ordinate, more negative values being higher on this axis.

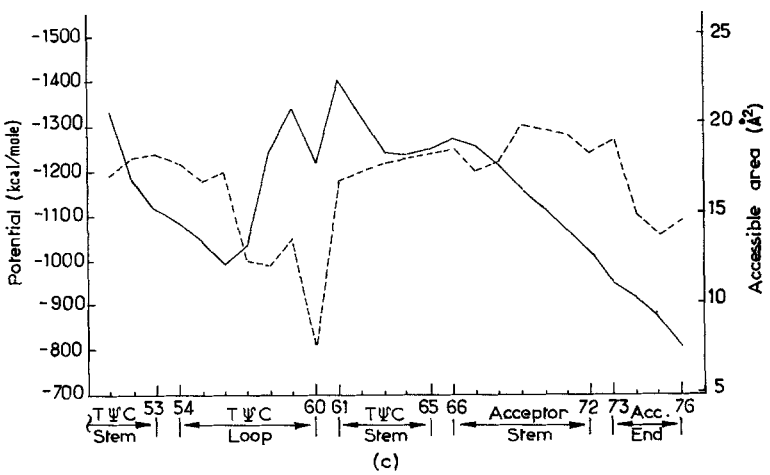
A first point to note is the variability of the potential of the phosphates as one passes along the backbone of tRNA. The most negative value of


Potential (kcal/mole)
-1500
-1400
-1300
-1200
-1100
-1000
-900
-800
-700
Accessible area (Å²)
25
20
15
10
5
1 Acceptor 8 9 D 13 14 D 21 22 D
Stem Stem Loop Stem

(a)


Potential (kcal/mole)
-1500
-1400
-1300
-1200
-1100
-1000
-900
-800
-700
Accessible area (Å²)
25
20
15
10
5
D 26 27 Anticodon 31 32 Anticodon 38 39 Anticodon 43 44 Variable 48 49 TΨC
Stem Stem Loop Stem Loop Stem

(b)


Potential (kcal/mole)
-1500
-1400
-1300
-1200
-1100
-1000
-900
-800
-700
Accessible area (Å²)
25
20
15
10
5
TΨC 53 54 TΨC 60 61 TΨC 65 66 Acceptor 72 73 Acc. 76
Stem Loop Stem Stem End

(c)

−1502.1 kcal/mole at phosphate 49 is separated by 700 kcal/mole from the least negative value of −802.1 kcal/mole at phosphate 76. This is in contrast to the situation in a nucleic acid of regular structure such as B-DNA, where the phosphate bridge potentials are almost constant along the backbone of the double helix (the small variations being due to the influence of the sequence of the neighbouring bases).

The presently calculated variability is a direct result of the folding of tRNA which produces a "clustering" of many phosphate groups near the center of the molecule and consequently much more negative potentials than those seen in the loops or stems at the extremities of the structure.

We may consider these potentials in more detail moving along the backbone from phosphate 1 to phosphate 76 (Phosphate X is abbreviated to PX in the following discussion for convenience). As an aid to this discussion a schematic representation of the bridge site potentials is given in Fig. 2, where circles of radii proportional to the values of the potentials have been centered on each phosphate group of the molecule. This graphic may be used in conjunction with Fig. 1 and Table 1.

Beginning then with the acceptor stem for residues P1 to P8 the potentials change almost monotonically from −1139.6 kcal/mole to −1491.1 kcal/mole. This

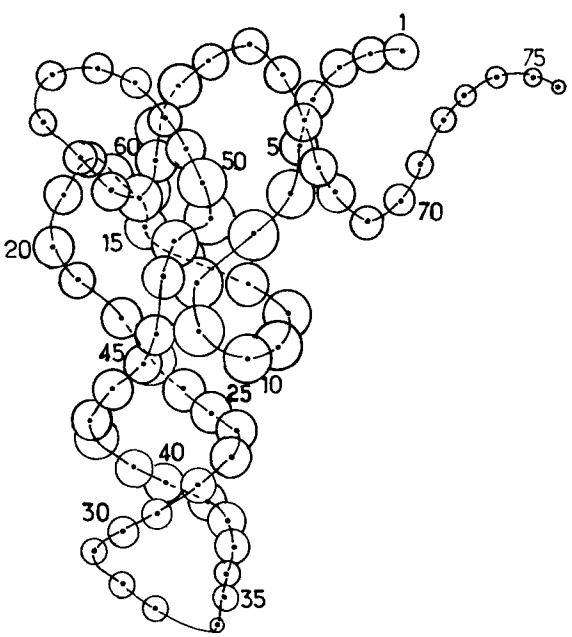


Fig. 2. Schematic representation of the potentials at the phosphate bridge sites. (The radii of the spheres about each phosphate being proportional to the corresponding bridge site potentials.)

Fig. 1. Potentials at the phosphate bridge sites (——) and accessibilities of the phosphate anionic oxygens (– – – –); (*a*) phosphates 1–25; (*b*) phosphates 26–50; (*c*) phosphates 51–76

reflects the approach toward the "core" of the tRNA which may be loosely defined by the residues P7 to P9, P49 and P50.

Along the D stem and D loop, from P10 to P26 the potentials vary considerably from one phosphate to the next. P14, P16 and P23–P24 have strong potentials, both groups being relatively close to the core of the molecule, while the most distant residues P18 and P22 show potentials more than 100 kcal/mole less negative.

Moving into the anticodon stem and then into the anticodon loop, the potentials fall rapidly in magnitude to the second weakest potential observed for the bridge sites of −835.2 kcal/mole at P34. The anticodon loop, being one of the regions most distant from the core of the molecule, thus represents a section of the backbone associated with relatively weak potentials. This may be clearly seen in Fig. 2.

From P38 to P46 in the second branch of the anticodon stem and the variable loop, the potentials are relatively stable around a value of −1250 kcal/mole which represents, approximately, the mean value for the bridge site potentials calculated. Moving into the TΨC stem we return once more to the core of the molecule and another strong negative potential of −1502.1 kcal/mole is observed at P49. This is the most negative potential calculated for the sites studied.

Further along the backbone we move to another extremity of the molecule at the TΨC loop (see again Fig. 2), where a least negative potential of −994.5 kcal/mole is calculated for P56. Leaving the TΨC loop leads us once more through the core of the molecule where strong negative potentials are observed for P59 and P61, after which the potentials diminish steadily along the second branch of the acceptor stem and into the acceptor end. This latter region represents the third extremity of the molecule where the least negative potential of all the sites studied is calculated for P76 (−802.1 kcal/mole).

It may be noted (by reference to article I of this series [1]) that the phosphate bridge site potentials currently presented are similar in magnitude to the potentials in corresponding regions, calculated by the superposition of the potentials of the phosphate groups only, without the influence of the bases or sugars. The global electrostatic potential of $tRNA^{Phe}$ is thus seen to be, understandably, dominated by the influence of the anionic phosphate groups. (The only difference to be noted is in the region of P1 where a correctly doubly ionized phosphate group has, in the present study, replaced the singly ionized phosphate used in our previous work. As a result, the potentials in this region are approximately 100 kcal/mole more negative.

3.2. Accessibility of the Phosphates

We began our studies of accessibility by calculating the IAA (Na^+) of individual anionic oxygens of the free phosphate groups having the conformations observed in $tRNA^{Phe}$. The values obtained show little variation, being between 10.62 $Å^2$ and 11.75 $Å^2$ for all the anionic oxygens studied. Similarly, the IAA(Na^+) of the

free phosphate groups themselves are rather stable, lying between 22.9 $Å^2$ (P76) and 23.1 $Å^2$ (P39) (summed areas of the two anionic oxygens of the group).

The corresponding results for the phosphate groups contained within the macromolecule, $AA(Na^+)$, are given in Table 1 and are represented graphically by the broken line (– – –) in Fig. 1, where the values are given by the right-hand ordinate ($Å^2$), larger accessible areas being higher on this axis. Note that the accessibility of P1 is not shown on this graphic. It is much larger than that for the remaining 75 phosphates, being a summation of the accessible areas for the three anionic oxygens available for this phosphate in contrast to only two such atoms for the remaining groups.

As for the potentials, considerable variation in the accessible areas is calculated for the phosphates along the tRNA backbone. Although many phosphate groups have $AA(Na^+)$ values >17 $Å^2$, indicating only slight steric hindrance of the anionic oxygens in comparison to the free phosphate, there are several regions of much greater hindrance which may be correlated with known structural features of $tRNA^{Phe}$. We choose, somewhat arbitrarily, to consider phosphates with $AA(Na^+)$ values of less than 15 $Å^2$ as specially hindered. The location of these phosphates may be seen in Fig. 3 where they are indicated by the dark bars along the backbone.

It may be useful to consider in some details these hindered phosphates. The first hindered region occurs between the acceptor stem and the D stem for P8 to P10 for which the calculated $AA(Na^+)$ are 14.09 $Å^2$, 10.54 $Å^2$ and 12.16 $Å^2$, respectively. Reference to the discussions of the structure of $tRNA^{Phe}$ [6, 13] yields an explanation for these low accessibilities. Residues 7 to 10 form a relatively tight bend which is maintained by base-backbone and backbone-backbone hydrogen

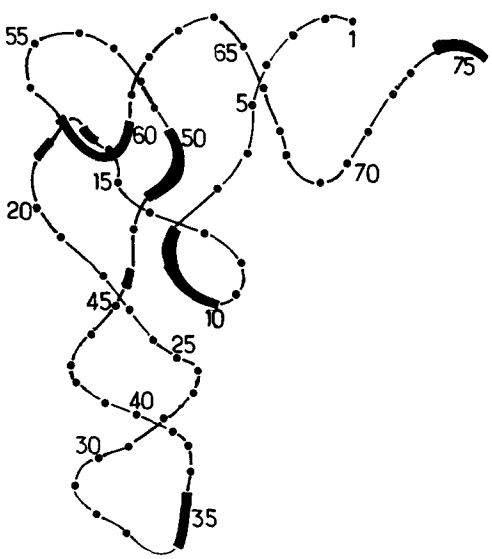


Fig. 3. Representation of the phosphate groups having anionic oxygens with low accessible areas (——)

bonding, notably the binding of P9 to cytosine 13 (N4) and 7-methylguanine 46(N2) (see Ref. [12]), which explains the particularly low accessibility at P9.

The next hindered region in Fig. 1 is seen to comprise phosphates 17 and 19 in the D loop. The two phosphates fall within the "arch" conformation of the D loop variable residues. At residue 17 the base (dihydrouracil) points outwards from the D loop consequently hindering the region around the anionic oxygens of the opposed phosphate. While at P19 the hindrance is caused by the proximity of sugars 17 and 18.

The next hindered phosphates occur in the anticodon loop; namely, phosphates 34 to 36, especially P35, for which the lowest $AA(Na^+)$ of 6.26 $Å^2$ is calculated. This hindrance is due to the U-turn of the anticodon loop which brings uracil 33 unusually close to the P35 anionic oxygens. In addition P36 appears to be hydrogen bonded by one of its anionic oxygens to uracil 33 (N3).

Continuing along the backbone from the anticodon loop to the anticodon stem and then to the variable loop we see a low accessibility for P46 and P48 which appears to be due to the expulsion of uracil 47 from the interior of the molecule which brings residues 46 and 48 close together. Passing to P49 and P50 the accessibility falls still further due to the large number of tertiary interactions in this region. In particular, hydrogen bonds are thought to exist between one of the anionic oxygens of P49 and ribose 7 ($O_{2'}$) and between one of the anionic oxygens of P50 and ribose 47 [13].

Further along the backbone low accessibilities are calculated in the TΨC loop for phosphates 57 to 60. At P59 there is a U-turn similar to that in the anticodon loop which brings pseudouracil 55 close to the anionic oxygens of P57, reducing their accessibility. At residues 59 and 60 there is again an "arch" conformation and tertiary interactions are thought to exist for phosphates 58 and 60 with pseudouracil 55(N3) and, with ribose 58($O_{2'}$) and cytosine 61(N4), respectively.

Finally, in the acceptor end the accessibilities of the phosphates 74 to 76 are seen to be slightly below 15 $Å^2$. However, in this region there is considerable disagreement between the published crystal structures of $tRNA^{Phe}$ and the present $AA(Na^+)$ results may have limited significance.

4. Conclusion

The variations of the potentials for the phosphate bridge sites may be summarized by the observation that phosphates at the two extremities and at the corner of the L-shaped molecule, namely, in the acceptor end, the anticodon loop and the TΨC loop, have potentials smaller in magnitude than those of the phosphates situated in the core of the molecule, notably, at the intersection between the acceptor stem and the D stem around phosphate 8 in the TΨC stem around phosphate 49.

The accessibilities of the phosphate anionic oxygens toward a Na^+ cation show some phosphates to be particularly hindered. This hindrance sometimes, but not always, correlates with low potential regions. Conflicts between these two factors,

which both play an important role in determining the biochemical reactivity of the nucleic acid thus exist for certain phosphates. No experimental data seem to be available, at present, that would enable us to estimate the respective roles of these two factors in such cases.

Extension of these computations, of potentials and of accessibilities, for other sites of the macromolecule and to interactions with different polyatomic species, presently being carried out, should be susceptible to provide more precise answers to this question.

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