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A THEORETICAL STUDY OF THE EFFECT OF STRUCTURAL VARIATIONS ON THE BIOCHEMICAL REACTIVITY OF YEAST $tRNA^{Phe}$ AND YEAST $tRNA^{Asp}$

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The ASIF index which combines both steric and electronic factors is applied to the comparative study of the reactivity of yeast $tRNA^{Asp}$ and yeast $tRNA^{Phe}$ using the coordinates deduced from their crystal structures. The results compared with the known experimental reactivities in solution are somewhat less perfect for $tRNA^{Asp}$ than for $tRNA^{Phe}$. The reasons for this situation are probably related to the differences existing between the structures of $tRNA^{Asp}$ in the crystal and in solution.

1. Introduction

Transfer ribonucleic acids (tRNAs) form a family of nucleic acids of great interest because of the role played by these molecules in many biological processes and, particularly, in the protein-synthesizing machinery. Although their main functions are common to all tRNAs, they involve elements of specificity proper to each of them (e.g., attachment to specific aminoacyl-tRNA synthetases).

In the search for a better understanding of the interactions of different tRNAs with chemical and biochemical reagents, our laboratory has undertaken a theoretical study of this family of nucleic acids, based on the exploration of their steric and electronic characteristics. A series of calculations have been conducted on the first structure resolved by X-ray determination [1], that of yeast phenylalanine tRNA, $tRNA^{Phe}$ [2–9].

With the recently resolved crystal structure of yeast asparagine tRNA, $tRNA^{Asp}$ [10], it has now become possible to make a similar investigation of the electronic and steric properties of this second tRNA and a comparison of the two molecules.

The respective nucleotide sequences of yeast $tRNA^{Asp}$ and yeast $tRNA^{Phe}$ are recalled in fig. 1 (fig. 1a from ref. 11 and fig. 1b from ref. 12). A structural comparison between the two molecules has been presented [10]. For the sake of the forthcoming discussion let us stress that, although the variable loop comprises four residues in $tRNA^{Asp}$ and five in $tRNA^{Phe}$, we have followed the procedure of refs. 13 and 14 so as to preserve the same numbering of residues in the two molecules in order to facilitate their comparison. This was obtained by giving the number 48 in $tRNA^{Asp}$ to the nucleotide following the one numbered 46, with a lack of nucleotide 47.

2. Method

In view of comparing the reactivities of the two tRNAs, we have appealed to a recently introduced index of reactivity, called ASIF [9], which has the particular advantage of combining in a unique expression electronic and steric factors relevant to this reactivity, and which has been used very successfully for the study of the reactivity of yeast $tRNA^{Phe}$ for a series of different chemical reagents

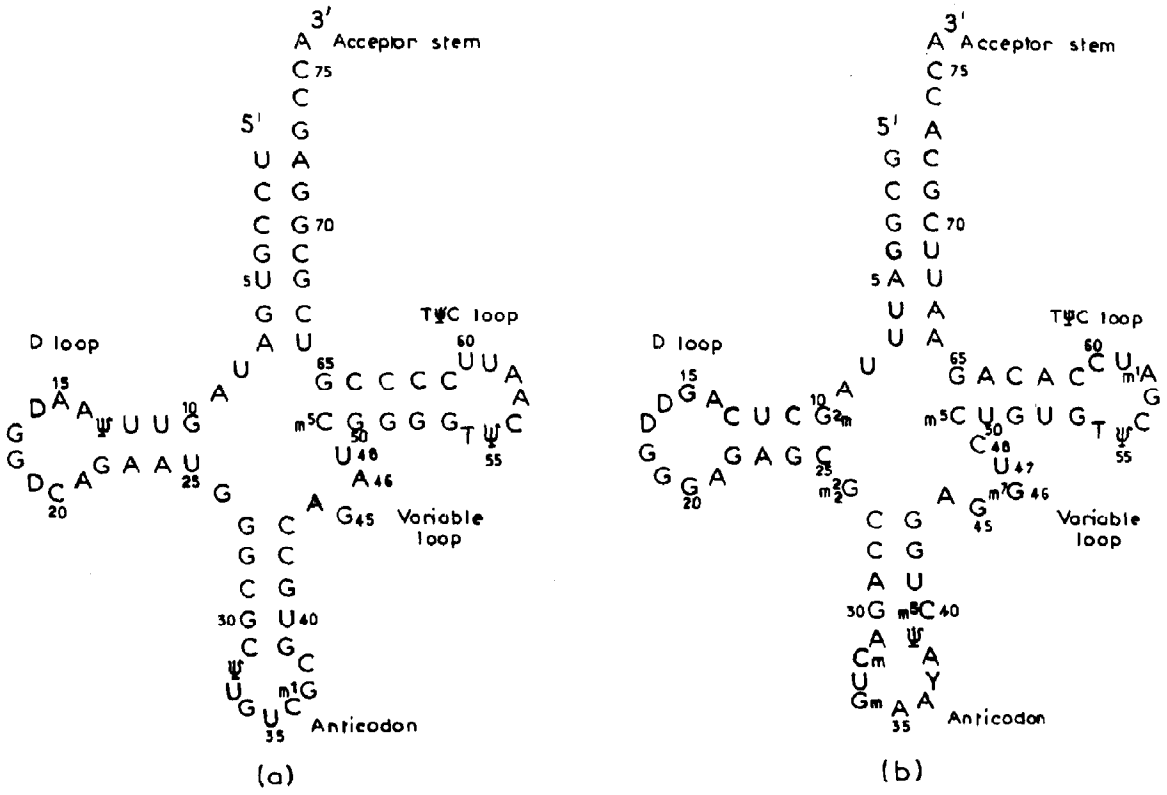

Fig. 1. Cloverleaf diagram of: (a) $tRNA^{Asp}$ (from ref. 11), (b) $tRNA^{Phe}$ (from ref. 12).

[9]. This index relies on the simultaneous consideration of the electrostatic field and the accessibility at any target within the macromolecule. The method for its evaluation is described in detail in the reference quoted above and we shall thus recall here only its general features.

The target atom of the macromolecule is treated as a sphere of a pertinent van der Waals radius. On the surface of this sphere a grid of uniformly distributed points is generated by the Korobov technique [15]. The chosen attacking probe is put into contact with the macromolecular target atom. A grid point of the target atom is considered as being accessible when the attacking species touching this point does not intersect any of the van der Waals spheres of the other atoms of the macromolecule. The fraction of the total number of grid points which correspond to accessible positions of the probe may be used as a measure of the accessibility of the target atom tested [16]. Considering polyatomic attacking species it has been shown that it is frequently possible to represent them by a spherical model, provided, however, that their radii are appropriately chosen. A search has been conducted successfully in that way in our laboratory for several types of polyatomic cations representing species important in reactions with tRNAs [9].

The electronic factors are taken into account via the macromolecular electrostatic field. This is calculated at the center of the attacking sphere, for each of the accessible grid points on the attacked sphere, and the numerical integration of the radial component of the field over the corresponding accessible area generated by the center of the probe is carried out. In order to distinguish the

attraction or repulsion of the target towards the attacking electrophilic species, the net field is replaced, at each point, by its projection on the radius vector $\vec{r}$ joining the center of the attacked atom to the center of the attacking sphere. The ASIF (accessible surface integrated field) index of reactivity is then defined as:

$$\int_S \vec{F} \cdot \vec{r}_u \mathrm{d}S$$

where $\vec{r}_u$ is a unit vector along the direction $\vec{r}$ and S the accessible surface area.

The integral will be positive or negative for a target repulsive or attractive, respectively, towards the positively charged electrophilic probe. It was noted that ASIF represents, in fact, the flux of the electrostatic field through the accessible surface [9]. Its values are given in units of V Å.

We will present here the results of the ASIF values computed for the bases and phosphates of yeast $tRNA^{Asp}$ for different alkylating reagents. In order to compare the resulting values with those previously calculated for the residues of yeast $tRNA^{Phe}$ [9] and compare all these data with the experimental reactivities observed for both tRNAs [13,14], the investigated positions are the following ones:

guanines for reaction with dimethyl sulfate (DMS) which alkylates the N7 position of these bases;

adenines for reaction with diethyl picrocarbonate (DEPC) which alkylates the N7 position of these bases;

cytosines for reaction with DMS which alkylates the N3 positions of these bases;

phosphates for reaction with ethylnitrosourea (ENU) which alkylates one of the two anionic oxygens.

For each of these positions – A(N7), G(N7), C(N3) and anionic oxygens of phosphates – ASIF is calculated using the radius probe pertinent for the involved alkylation. The radii employed are those previously determined [9]: for DMS, which involves an attack by a methyl cation, 1.8 Å; for ENU which involves an attack by an ethyl cation, 2.5 Å; for DEPC, because of the complexity of this reagent and the lack of information about the exact attacking species, the calculations were repeated for three different values of the radius of the attacking sphere: 2, 3 and 4 Å.

3. Results

3.1. ASIF values of bases for alkylation with DMS and DEPC

The experimental data on the reactivity of $tRNA^{Asp}$ are much more restricted than those for $tRNA^{Phe}$. For the sake of space economy and simplification we present here the ASIF values for sites which have been tested in both tRNAs, especially as those are distributed throughout the macromolecules. The values of ASIF computed for the alkylation of all the sites are, however, available upon request.

The comparison of the experimental reactivity of these chosen residues towards DMS and DEPC has been established at the Institut de Biologie Moléculaire et Cellulaire in Strasbourg [13,14] and the results obtained there are recalled in table 1. In

Table 1

Positions investigated and their chemical reactivity (●, reactive; ○, unreactive) for alkylation of N7 of purines (guanines with DMS and adenines with DEPC) and N3 of cytosines (with DMS) (from ref. 13)

	$tRNA^{Asp}$	$tRNA^{Phe}$
N7 of purines	A9 ○	A9 ○
	G4 ●	G4 ○
	G10 ○	G10 ○
	A14 ○	A14 ○
	A15 ○	G15 ○
	G17 ●	G18 ●
	G18 ●	G19 ●
	A21 ●	A21 ○
	G22 ○	G22 ○
	A23 ○	A23 ○
	A24 ○	G24 ○
	G30 ●	G30 ●
	G34 ●	G34 ●
	A44 ○	A44 ○
	G45 ○	G45 ●
	G51 ○	G51 ○
	G53 ○	G53 ○
	A57 ○	G57 ○
	G65 ○	G65 ●
	G71 ○	G71 ●
	G73 ●	A73 ○
N3 of cytosines	C56 ○	C56 ○
	C61 ○	C61 ○
	C63 ○	C63 ○

this table the symbols ● and ○ represent, respectively, the presence or absence of experimental reactivity of the N7 sites of purines towards DMS (for alkylation of guanines) or DEPC (for alkylation of adenines) and of the N3 sites of cytosines towards DMS. For facility of discussion we will term ASIF-G(N7) the ASIF computed for the N7 atom of guanines for alkylation with DMS, ASIF-A(N7) that computed for the N7 atom of adenines for alkylation with DEPC, and ASIF-C(N3) that computed for the N3 atom of cytosines for alkylation with DMS.

Fig. 2 presents ASIF values calculated for the alkylation of N7 of the guanines enumerated in table 1 in the two tRNAs. They are listed along vertical axes, which indicate on their left-hand side the scale of ASIF and on their right-hand side the numbers of the residues considered (see fig. 1) and the symbols of their reactivity or of the lack of it. We recall that a positive or zero value of ASIF means that the tested target is unlikely to be reactive towards the involved probe, and that, on the contrary, the more negative the ASIF is, the more likely is the reaction to occur. In the same way fig. 3 gives the corresponding results for the tested adenines, and fig. 4 those for the tested cytosines. In the case of alkylation by DEPC, where three values of radius were considered for the attacking sphere, the values of ASIF-A(N7) relative to the smallest radius are given on the left-hand side of the scale and those relative to the greatest one on its right-hand side.

Concerning the N7 sites of guanines (fig. 2), we observe that the values of ASIF-G(N7) of the common guanines G10, G22, G51 and G53 correlate well with the experimental lack of reactivity observed: the values of G10 and G22 are zero in both tRNAs, that of G51 is positive in both tRNAs and the value for G53, though negative, is very small in both tRNAs. G15, G24 and G57 exist only in $tRNA^{Phe}$ (adenines are found at these

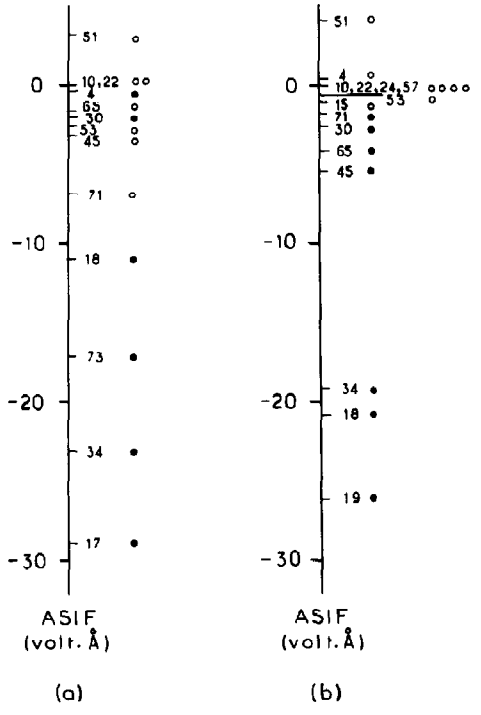

Fig. 2. ASIF (on the left-hand side of the axis) of N7 sites of guanines: (a) in $tRNA^{Asp}$; (b) in $tRNA^{Phe}$. Symbols represent reactivity towards DMS. The numbers (on the right-hand side of the axis) refer to the nucleotide positions in the tRNAs and the symbols indicate the experimental reactivities. (● Reactive, (○) unreactive.

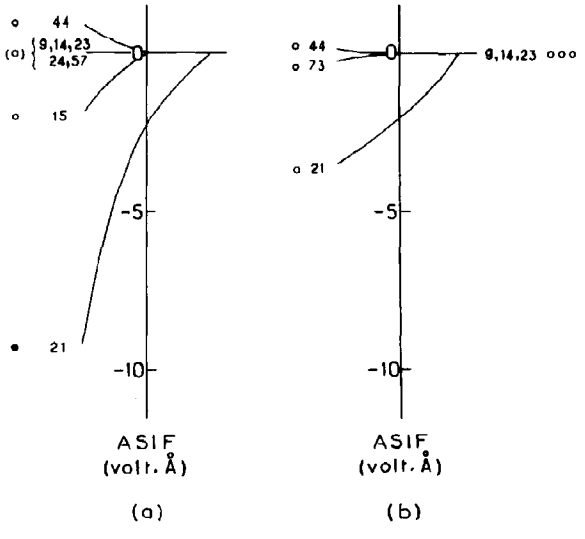

Fig. 3. ASIF (on the axis) of N7 sites of adenines (numbered at the left extremities of the curves). (a) In $tRNA^{Asp}$, (b) in $tRNA^{Phe}$. Symbols (as in fig. 2) represent reactivity towards DEPC.

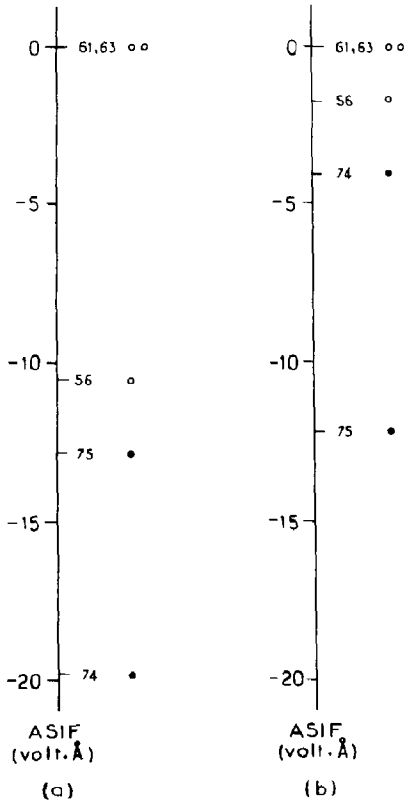

Fig. 4. ASIF (on the left-hand side of the axis) of N3 sites of cytosines. (a) In $tRNA^{Asp}$, (b) in $tRNA^{Phe}$. Symbols (as in figs. 2 and 3) represent reactivity towards DMS.

positions in $tRNA^{Asp}$) and the corresponding ASIFs-G(N7) correlate again well with their lack of reactivity: the values calculated for G24 and G57 are both equal to zero and the negative value for G15 is very small. The values of ASIF-G(N7) of G30 and G34, lying in the anticodon stem and the anticodon loop, respectively, are negative with a stronger value for G34, in both tRNAs. In $tRNA^{Phe}$ the two constant Gs of the D-loop, G18 and G19, show both strong negative values of ASIF-G(N7), well correlated with experimental reactivity. In $tRNA^{Asp}$ the two constant Gs of the D-loop are G17 and G18 instead of G18 and G19 in $tRNA^{Phe}$. The value of ASIF-G(N7) of G17 is strongly negative while that of G18 is much smaller (but remains in the range of the strong absolute values of ASIF). Both residues are said to be reactive, the experimental results not being precise enough to indicate a quantitative difference among them. Should their reactivity be similar, the difference between the calculation and the experiment could possibly be due to a conformational flexibility of the D-loop of $tRNA^{Asp}$ advocated by the experimentalists [10,17], which could result in a modification of the steric and electronic surroundings of the involved targets in solution. We may recall that the conformation of $tRNA^{Phe}$, for which our computations gave very satisfactory results, has been shown to be similar in the crystal and in solution [18,19].

Particularly interesting is the case of the common guanines G4, G45, G65 and G71, the behaviour of which is different in the two tRNAs. G4 is reactive in $tRNA^{Asp}$ but unreactive in $tRNA^{Phe}$. This result is accounted for by the computations which indicate the ASIF to be negative for the former but positive for the latter. The remaining three guanines are reactive in $tRNA^{Phe}$ but unreactive in $tRNA^{Asp}$. The computed ASIF values account satisfactorily for this situation as concerns G45 and G65 whose ASIF values are more negative in $tRNA^{Asp}$ than in $tRNA^{Phe}$. They fail, however, to account for the behaviour of G71, whose ASIF is substantially more negative in $tRNA^{Asp}$ than in $tRNA^{Phe}$ while this residue is only reactive in the latter. Altogether, considering the individual tRNAs the agreement between theory and experiment is better for $tRNA^{Phe}$, in which all the reactive guanines are grouped together at the lower part of the scale of fig. 2. Nevertheless, for $tRNA^{Asp}$ only two reactive bases (G4 and G30) are interspersed between the unreactive ones.

Concerning the N7 sites of adenines (fig. 3) the situation is rather satisfactory. It may be noted from table 1 that out of all these bases, the only reactive one with DEPC is A21 of $tRNA^{Asp}$. This situation is remarkably accounted for in fig. 3 provided that the radius to be considered as representing the attacking species does not exceed 2.8 Å.

The situation is similarly altogether satisfactory as concerns the reactivity of the N3 sites of cytosines towards DMS (fig. 4). The absence of reactivity of C61 and C63 in both tRNAs and that

of C56 in $tRNA^{Phe}$ is easily accounted for by the zero or small negative values of their ASIF indices. C74 and C75 are both situated in the lower part of the ASIF scale related to each tRNA, in agreement with their reactive behaviour. The non-reactivity of C56 of $tRNA^{Asp}$ may be somewhat surprising, its ASIF falling within the range of the ASIF of the reactive C74 and C75 (although above these values for reactive bases in $tRNA^{Asp}$). A possible reason for this last discrepancy can perhaps again reside in a conformational modification of yeast $tRNA^{Asp}$ in solution with respect to its structure in the crystal, which could concern the possible hydrogen-bonded interaction of C56 with G18. Such an interaction exists in the crystal of $tRNA^{Phe}$ between its C56 and G19, the consequence being the inaccessibility of the N3 atom of C56. No similar interaction with G18 exists in the crystal of $tRNA^{Asp}$ leaving N3 of its C56 largely accessible. It is not impossible, however, for such an interaction to occur in solution following a rotation of G18 around its glycosidic linkage and this would make the situation in the two tRNAs similar.

Thus, dealing with the reactivity or absence of reactivity of representative bases of both $tRNA^{Asp}$ and $tRNA^{Phe}$ towards DMS and DEPC, we observe that the correlation between our computed ASIF values of the involved sites and their experimental reactivities, though somewhat less complete for $tRNA^{Asp}$ than for $tRNA^{Phe}$, is nevertheless satisfactory for the former.

3.2. ASIF values of phosphates for alkylation with ENU

We have computed the ASIF indices of the anionic oxygens of the phosphate groups of $tRNA^{Asp}$ for alkylation with ENU, using an attacking sphere of radius 2.5 Å for this reaction which involves an ethyl cation. Since the ethylation may occur at either of the two anionic oxygens, we have performed the calculations for these two atoms and made an average of the two values obtained. Similar calculations were previously conducted on $tRNA^{Phe}$ [9] and we recall these results in fig. 5 together with those obtained now for $tRNA^{Asp}$ (fig. 5a, $tRNA^{Phe}$; fig. 5b, $tRNA^{Asp}$). Note that in these two figures the numbers of the different phosphate residues are indicated on the horizontal axis and that the absolute values of ASIF increase from bottom to top on the vertical scale. A parallel presentation of the experimental reactivities is reported in fig. 6 (fig. 6a, $tRNA^{Phe}$; fig. 6b, $tRNA^{Asp}$) [13,14]. In fig. 5c we give the differences in ASIF, termed Δ(ASIF), between each phosphate in $tRNA^{Asp}$ and its homologue in $tRNA^{Phe}$, calculated as:

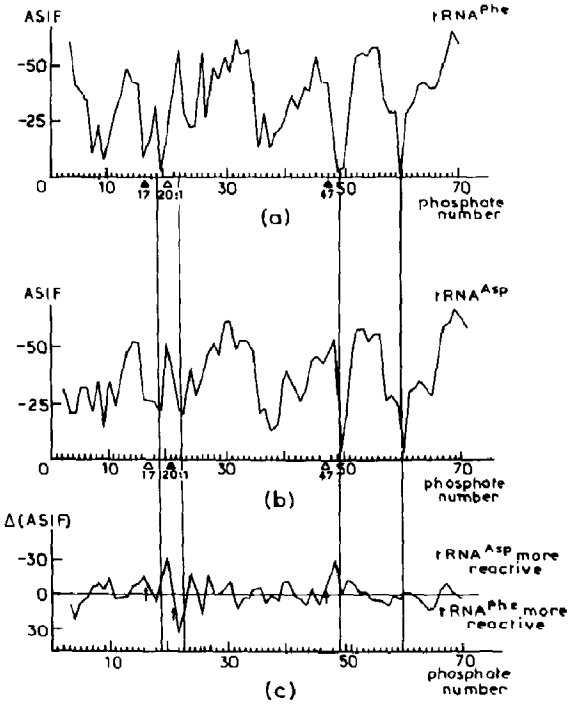

Fig. 5. Average ASIF of the two anionic oxygens of the phosphates of: (a) $tRNA^{Phe}$, (b) $tRNA^{Asp}$, (c) $tRNA^{Asp}$–$tRNA^{Phe}$ (Δ(ASIF) defined in text) for alkylation with ENU. (▲ and △) Phosphates which are present or absent, respectively, in the corresponding position according to the numbering of fig. 7.

$$\Delta(\mathrm{ASIF}) = \left[\frac{\mathrm{ASIF(O1)} + \mathrm{ASIF(O2)}}{2}\right]^{tRNA^{Asp}}_{\text{at position } n} - \left[\frac{\mathrm{ASIF(O1)} + \mathrm{ASIF(O2)}}{2}\right]^{tRNA^{Phe}}_{\text{at position } n}$$

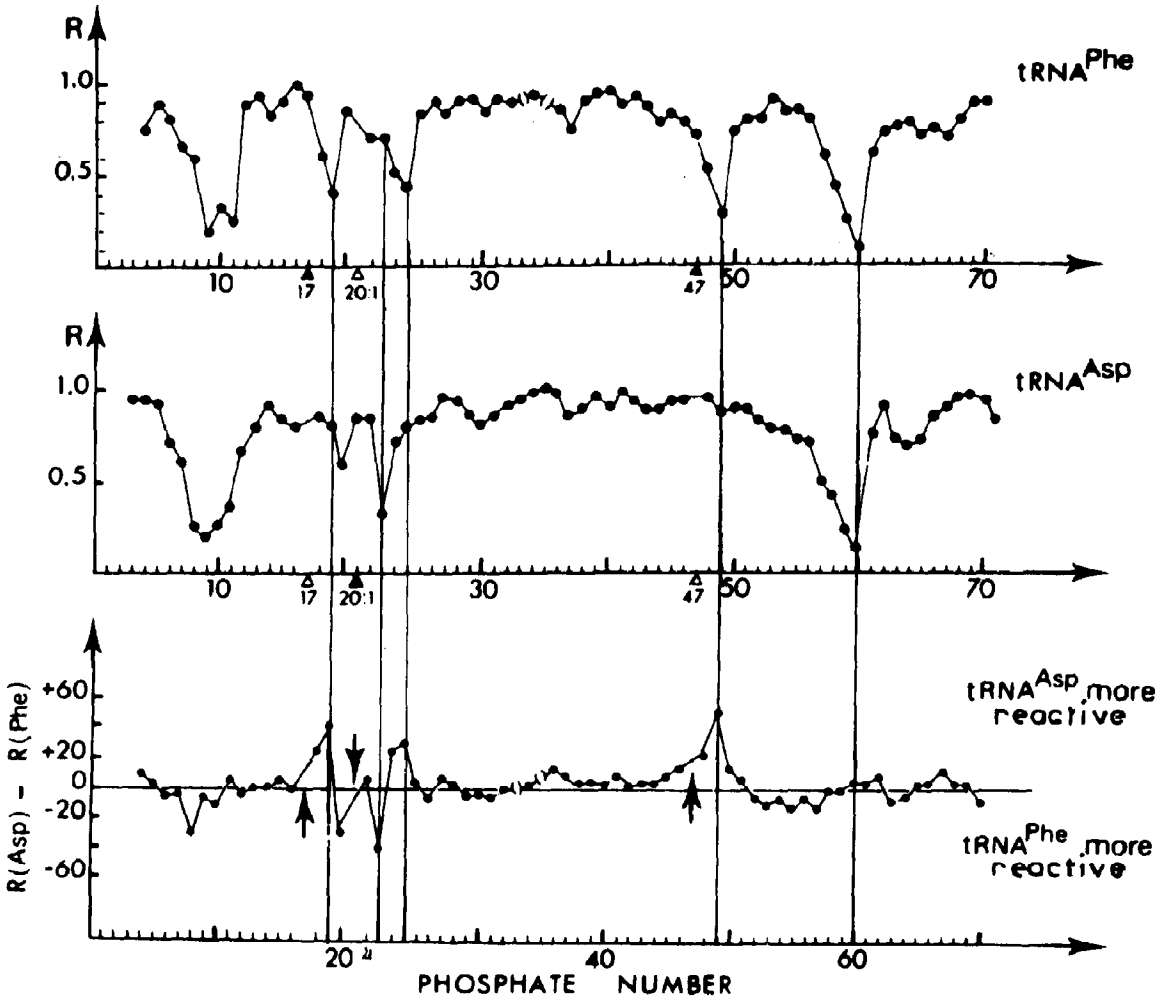

Fig. 6. Experimental reactivities of the phosphate groups of: (a) $tRNA^{Phe}$, (b) $tRNA^{Asp}$, (c) $tRNA^{Asp}-tRNA^{Phe}$ towards ENU. (▲ and △) As in fig. 5.

Thus, when Δ(ASIF) is negative the phosphate at the investigated position of $tRNA^{Asp}$ is more reactive towards the chosen attacking sphere than that of $tRNA^{Phe}$ and the situation is reversed when Δ(ASIF) is positive. The corresponding curve of differences deduced from the experimental data is shown in fig. 6c. Note that the numbering used by the experimentalists for the phosphate residues is not the same as that used for the bases. To make possible the comparison between our theoretical results and the available experimental observations [13,14] we have adopted the same numbering (shown in fig. 7) for both tRNAs (from the same reference).

The comparison of figs. 5a and 6a shows a good agreement between the calculations and the experimental reactivities of the phosphates of $tRNA^{Phe}$ (as already shown in ref. 9). The correlation between ASIF and reactivity for the phosphate residues of $tRNA^{Asp}$ (figs. 5b and 6b) is

perhaps somewhat less satisfactory. In fact, two main disagreements are observed: they concern the couple P20-P21 and P49. The absolute value of ASIF computed for the anionic oxygens of P20 of $tRNA^{Asp}$ would suggest a greater reactivity than that experimentally observed with ENU, while the situation is reversed for P21 whose ASIF would suggest a smaller reactivity than that experimentally observed. It may be observed that these two phosphates lie in the D-loop of $tRNA^{Asp}$, i.e., in the most flexible region of the molecule [17]. Thus, it is likely that the conformation of all this part of $tRNA^{Asp}$ is not the same in the crystal and in solution, a feature which could change the situation concerning the computed values.

P49 is the site of the most striking disagreement: this residue is situated at a very unfavourable position on the ASIF scale but shows a strong reactivity towards ENU. In $tRNA^{Phe}$ the anionic oxygens of P49 are protected from ENU because of the overcrowding found in this part of the molecule. Moreover, one of them is hydrogen bonded to the hydrogen atom of the hydroxyl group of the ribose 7. In $tRNA^{Asp}$ the distance between the nuclei of these two atoms is too large (exceeding 3.5 Å) to allow the formation of such a bond. Nevertheless, it is too small to permit the attacking sphere to approach the involved anionic oxygen. Moreover, we have calculated, from the coordinates derived from the crystal analysis, the distances between the anionic oxygens of P49 and their nearest neighbours and have found that the smallest distances are of the same order of magnitude in both tRNAs. Thus, the distances between either one of the anionic oxygens of P49 and some atoms of A7, U8 and U48 in $tRNA^{Asp}$ are indicative of significant steric hindrance in this part of the molecule, as in $tRNA^{Phe}$. This leads, in both

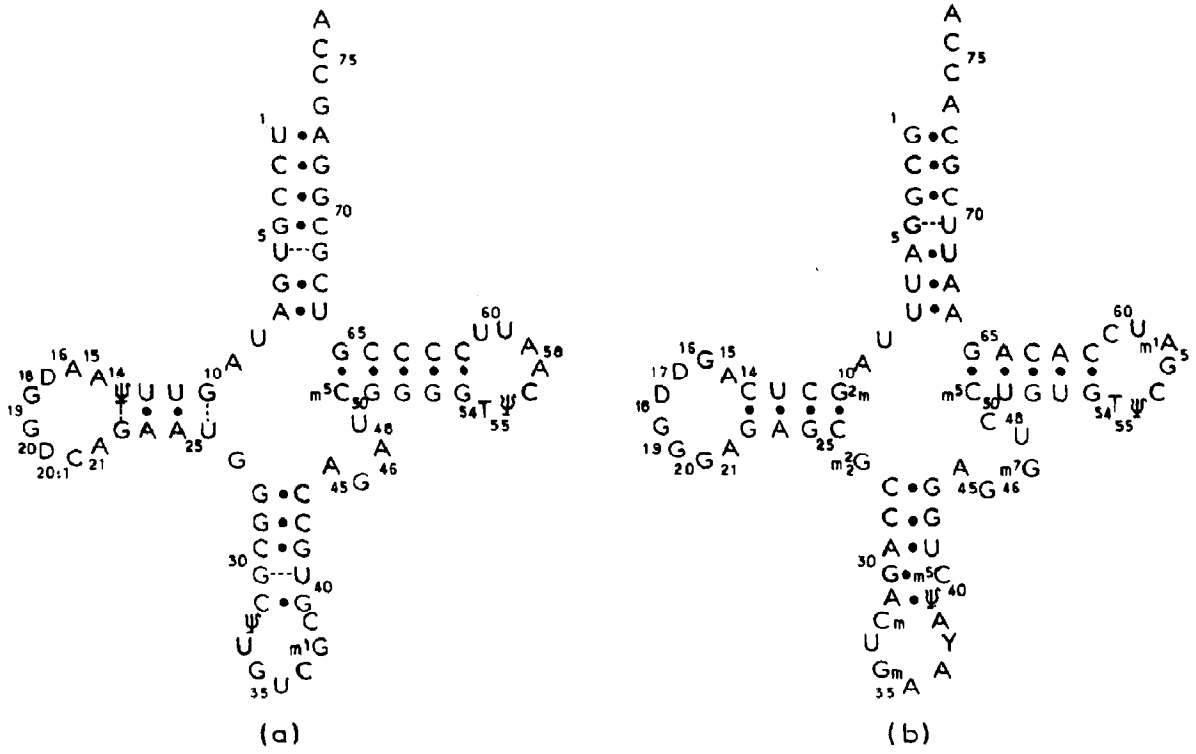

Fig. 7. Numbering of the phosphates of: (a) $tRNA^{Asp}$, (b) $tRNA^{Phe}$ adopted for comparison of reactivities towards ENU (from refs. 13 and 14).

tRNAs, to a low value of the accessibility of P49, which determines a low ASIF value. It is thus difficult to understand the significant experimental reactivity of P49 of $tRNA^{Asp}$. A modification of the conformation of this part of the molecule in solution could, of course, be responsible for this discrepancy [14].

A straightforward depiction of the differences of the reactivities of similar phosphates in the two tRNAs is given in figs. 5c (theoretical) and 6c (experimental). We observe that in a general manner the differences of reactivity suggested by our calculations agree with those experimentally observed with the exception of some departures due to the disagreements mentioned between theoretical calculations and experimental observations on $tRNA^{Asp}$. Thus, P8 and P20 are more susceptible to alkylation by ENU in $tRNA^{Phe}$ than in $tRNA^{Asp}$ and P21 in $tRNA^{Asp}$ than in $tRNA^{Phe}$, while the ASIF values suggest the reverse situations: P49 is observed to be more reactive towards ENU in $tRNA^{Asp}$ than in $tRNA^{Phe}$, while the computations suggest no difference of behaviour for this residue. This lack of correlation of some residues could be due to a change in conformation of the corresponding part of $tRNA^{Asp}$ when passing from the crystal to solution.

4. Conclusion

Results of computations of the index of reactivity ASIF for different target sites of $tRNA^{Asp}$ towards appropriate chemical reagents (ASIF of guanines and cytosines for alkylation with DMS, ASIF of adenines for alkylation with DEPC and ASIF of phosphates for alkylation with ENU) have been compared with those previously obtained for $tRNA^{Phe}$, and the values for all the tested residues of both tRNAs have been compared with the experimental reactivities observed in solution. The agreement between theory and experiment, altogether quite satisfactory, is somewhat less complete in $tRNA^{Asp}$ than in $tRNA^{Phe}$. This situation could arise from two possible causes. The first may be due to the necessity of a further refinement of the atomic coordinates. We have demonstrated in a recent work [20] how significantly a slight modification of the steric environment of a target site can influence the value of the index of reactivity of this site. The second reason, the most probable one, is the possible different conformations of some regions of this macromolecule in the crystal and in solution. This hypothesis is supported by the work of the Strasbourg group which indicates [17] a much greater lability of $tRNA^{Asp}$ as compared to $tRNA^{Phe}$, particularly in the D-loop and in its relation to the T-loop.

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