MOLECULAR ORBITAL STUDIES ON THE CONFORMATION OF THE TERMINAL AMINOACYLADENOSINE MOIETIES OF tRNA

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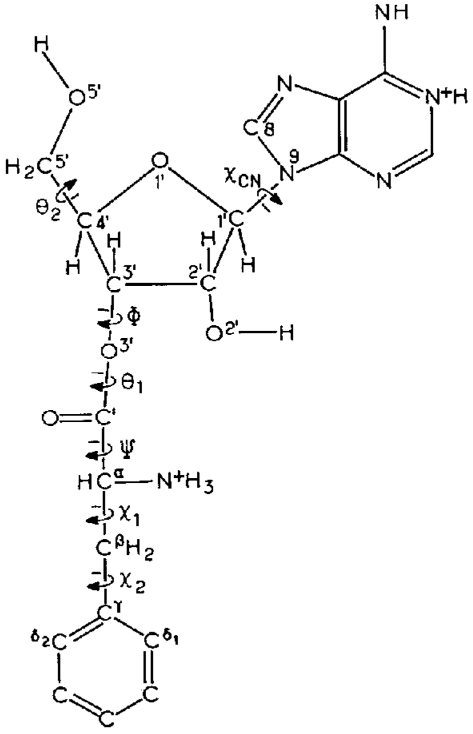
1. Introduction

We have recently carried out a series of computations on the conformational properties of the constituents of proteins [1–3] and nucleic acids [4, 5] employing the molecular orbital method PCILO (Perturbative configuration interaction using localized orbitals). This note describes the results of an extension of this study to a molecular entity representing an association between a nucleic acid and a protein component. A particularly important case of such a combination is given by the terminal aminoacyladenosine moieties (TAAM) of tRNA's. We have investigated the principal conformational properties of such moieties as represented by scheme I, on the example of 3′-*O*-phenylalanyladenosine. Calculations have also been carried out for 3′-*O*-alanyladenosine. Parallel, we have also performed similar computations on puromycin II (which differs from 3′-*O*-phenylalanyladenosine essentially in having an amide instead of an ester linkage to the position C(3′) of the sugar), because of the known X-ray structure of this molecule and the proposal that it may be considered as a model for TAAM [6, 7].

2. Methods

A description of the PCILO method may be found in [1, 4, 8]. A more detailed account and the program are available from the Quantum Chemistry Program Exchange at Indiana University, Bloomington.

The geometry adopted for the adenosine moiety was based on the X-ray study of ATP by Kennard et al. [9, 10]. Standard dimensions and angles were taken for the remaining parts of the molecule.



Scheme I.

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The torsion angles are defined as:

Φ = C2′–C3′–O3′–C′

Ψ = O3′–C′–C^{α}–N^+

χ_{CN} = O1′–C1′–N9–C8

χ^1 = C′–C^{α}–C^{β}–C^{γ}

χ^2 = C^{α}–C^{β}–C^{γ}–$C^{\delta 1}$

θ_1 = C3′–O3′–C′–C^{α}

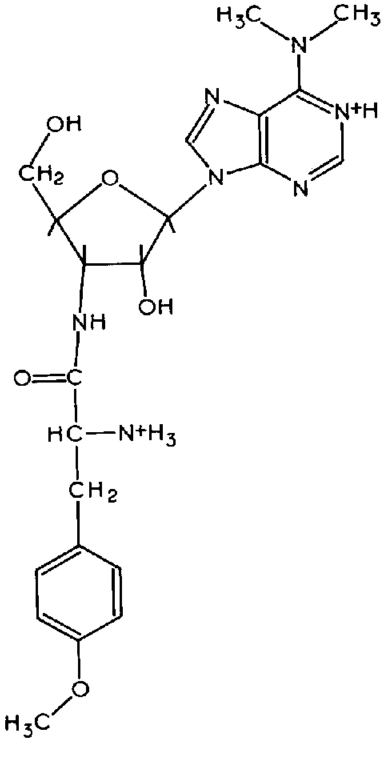
θ_2 = C3′–C4′–C5′–O5′

(We remind that the torsion angle τ of the bonded atoms A–B–C–D is the angle between the planes A B C and B C D; viewed from the direction of A, τ is positive for clockwise and negative for anticlockwise rotations. The value $\tau = 0°$ corresponds to the planar-*cis* arrangement of the bonds AB and CD).

Because of the large number of degrees of freedom available reasonable simplifying assumptions have been adopted with respect to the conformational properties of the two constituent fragments, the main object of the study being the determination of the gross features of their resultant interaction.

For calculations on I and by analogy with the crystallographic data for puromycin, the sugar, considered to have the 3′-endo conformation, was fixed in the *anti* arrangement with respect to the base with $\chi_{CN} = 20°$. The exocyclic CH_2OH group was fixed in the *gg* orientation as it is also in puromycin ($\theta_2 \approx 60°$). The formation of an intramolecular H-bond between this group and N_3 of the adenine ring was forbidden because in the biological medium this group is bound through a phosphate link to the backbone of t-RNA. The ester group was considered planar ($\theta_1 = 180°$) in agreement with X-ray results on the related 3′-*O*-acetyl adenosine [12] and 3′-*O*-acetyl-4-thiothymidine [12]. Preselected values of $\chi^1 = 60°$ and 180° for the C^{α}–C^{β} bond and of $\chi^2 = 90°$ for the C^{β}–C^{γ} bond were adopted. In 3-*O*-alanyladenosine the methyl group of the amino acid residue was considered staggered ($\chi^1 = 60°$ or 180° or 300°). These values correspond to the preferred torsions about these bonds in amino acids and in globular proteins, as indicated both by experimental and theoretical evidence [13].

Similar assumptions were adopted in puromycin, with the amide group considered planar. Moreover, in the calculations for this molecule the CH_3-groups of N_6 of adenine and the OCH_3-group on the phenyl ring were replaced by H atoms.



II

Scheme II.

Finally the α-amino group and N_1 of adenine were considered protonated in both molecules.

The conformational energy maps were then constructed as a function of the torsion angles about: 1) the C(3′)–O bond in scheme I or C(3′)–N bond in scheme II (angle Φ) and 2) the C′–C^{α} bond (angle Ψ). The rotations were carried out in 30° increments.

3. Results and discussion

Fig. 1 presents the conformational map of 3′-*O*-phenylalanyladenosine for the preselected values of $\chi^1 = 180°$, $\chi^2 = 90°$. It indicates the existence of two energetically equivalent global minima centered both on $\Psi = 180°$ but one around $\Phi = 60°$ and the other around $\Phi = 300°$. The first of these two minima is much larger and corresponds thus to a much greater probability. In fact, it is associated with a relatively broad stable conformational zone extending between

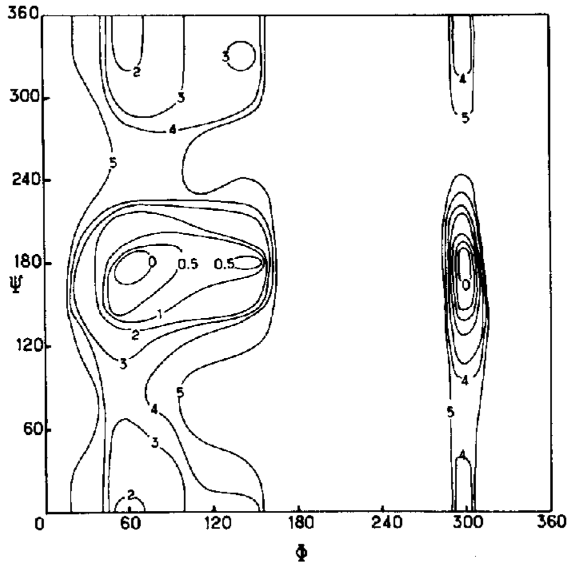


Fig. 1. Conformational energy map of 3′-*O*-phenylalanyladenosine. (χ^1 = 180°, χ^2 = 90°). Isoenergy curves (kcal/mole) with respect to the global minimum taken as energy zero.

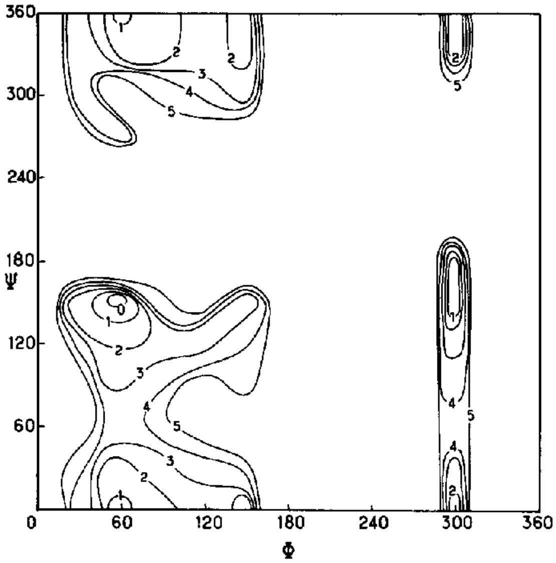


Fig. 2. Conformational energy map of 3′-*O*-phenylalanyladenosine. (χ^1 = 60°, χ^2 = 90°). Isoenergy cyrves (kcal/mole) with respect to the global minimum taken as energy zero.

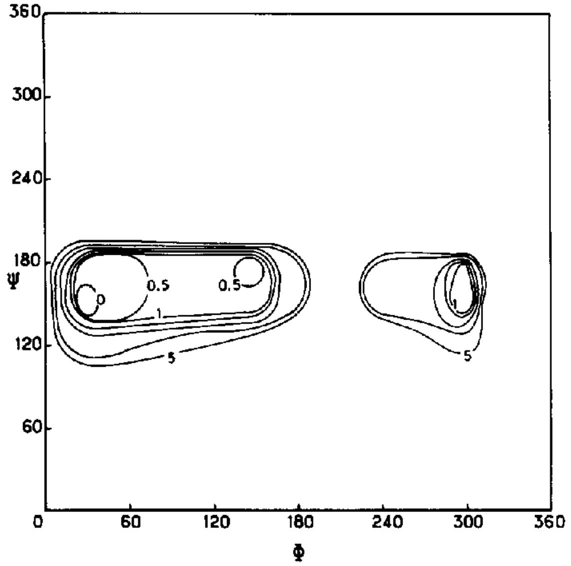


Fig. 3. Conformational energy map of puromycin (χ^1 = 180°, χ^2 = 90°). Isoenergy curves (kcal/mole) with respect to the global minimum taken as energy zero.

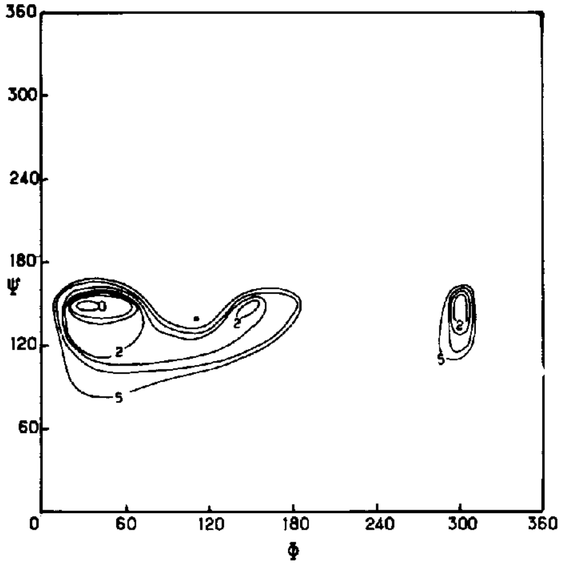


Fig. 4. Conformational energy map of puromycin (χ^1 = 60°, χ^2 = 90°). Isoenergy curves (kcal/mole) with respect to the global minimum taken as energy zero. • X-ray result.

Φ = 60° and Φ = 150° within the 1 kcal/mole isoenergy curve. It corresponds to an elongated structure, in which the phenyl ring points away from the adenine moiety. Fig. 2 presents results for the same compound,

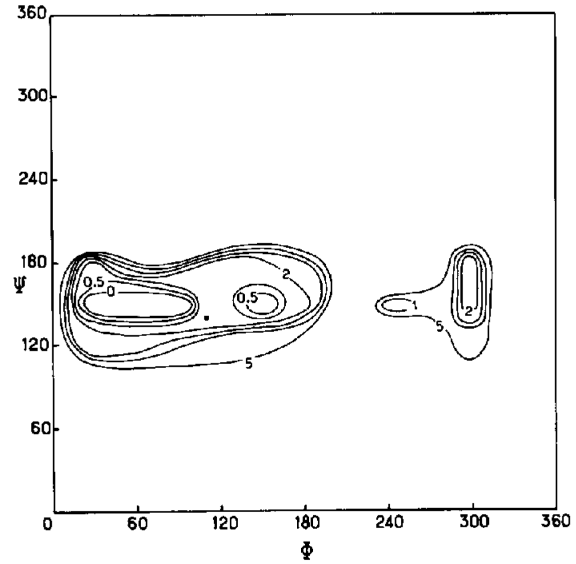


Fig. 5. Conformational energy map of puromycin ($\chi^1 = 74°$, $\chi^2 = 75°$). Isoenergy curves (kcal/mole) with respect to the global minimum taken as energy zero. ● X-ray result.

constructed with the preselected values $\chi^1 = 60°$, $\chi^2 = 90°$. The available conformational space within the same limits of 5 kcal/mole above the global minimum is more restricted than in fig. 1. There is now one global minimum localized around (Φ, Ψ) = (60°, 150°) about 1.4 kcal/mole higher than that of fig. 1. It corresponds to the same type of an elongated conformation.

A conformational energy map was also constructed, as said, for 3'-*O*-alanyladenosine (with the terminal methyl in a staggered position, $\chi^1 = 60°$, 180° or 300°). It will not be reproduced here as it is practically identical to that of fig. 1.

Figs. 3 and 4 present results of similar calculations performed with the precedingly quoted simplifications, for puromycin, fig. 3 with $\chi^1 = 180°$ and fig. 4 with $\chi^1 = 60°$. They indicate that: 1) the allowed conformational space within the same limit of 5 kcal/mole above the global minimum is more restricted in puromycin than in 3'-*O*-phenylalanyladenosine, a situation due probably to the hindering effect of the H atom of the amide group; 2) there is one global minimum on both figures, around $\Phi = 30°$, $\Psi = 150°$. The minimum of fig. 3 is about 2 kcal/mole lower than that of fig. 4 and its low energy contour, say within 1 kcal/mole, is broader. It corresponds to an extended form of the molecule analogous to that of 3'-*O*-phenylalanyladenosine.

The X-ray results for puromycin indicate for the angles Φ, Ψ the values of 110° and 142°, respectively [14]. The values of χ^1 and χ^2 are both approximately of 75° so that the observed conformation should be compared preferentially with the map of fig. 3. (The observed values of the other torsion angles are close to the preselected ones). The agreement is moderate, the experimental conformation being somewhat displaced with respect to the theoretical global minimum and at the borderline of the stability region as concerns the angle Ψ.

Among the possible reasons for this moderate agreement we may consider: 1) the effect of crystal forces, 2) the simplifications in the model molecule, 3) the effective values of the angles χ^1 and $\chi^2 \approx 75°$ as compared with the preselected values of $\chi^1 = 60°$, $\chi^2 = 90°$.

This last possible cause is the easiest to investigate and we have therefore performed calculations on puromycin with the experimental values of $\chi^1 = 74°$ and $\chi^2 = 75°$ (and by putting also the other preselected torsion angles at their experimental value: $\chi_{CN} = 20°$, $\theta_1 = 178°$ and $\theta_2 = 54°$). The results are indicated in fig. 5 and they are certainly very striking. The conformationally stable zone is substantially enlarged with respect to that of maps 3 and 4 and so are also the dimensions of the global minimum. On this map the experimental conformation is close to the global minimum and anyway well within the stable zone. This situation points to the delicate problem with respect to the preselection of the rotational states on the basis of model studies. As such a preselection, in cases as complex as this one, is a practical necessity, it cannot be avoided. Variations around preselected values should, however, obviously be explored whenever possible.

4. Conclusion

Altogether the present results appear highly suggestive as to the probable structure of the aminoacyladenosine moieties of tRNA's for which they predict, in agreement with the suggestion of Sundaralingam [6,7], an elongated structure. The hypothesis of

Raacke [15] that puromycin can assume a U-shaped conformation, with the benzene ring stacked under the dimethyladenine seems improbable, at least within the limits of the values adopted here for the fixed torsion angles and the same is true about the aminoacyladenosines. Without explicit calculations it cannot of course be ascertained that the results of fig. 1 and 2 are representative for all the amino acid residues of proteins although it seems probable that *grosso modo* they should be so. It is also evident that although the structure of puromycin may be considered, as suggested also by Sundaralingam [6, 7], as a model for the structure of the aminoacyladenosines, it must be borne in mind that as indicated by the comparison of figs. 1 and 2 with figs. 3–5 these last molecules may have somewhat more conformational freeedom. It may, however, be remarked that the torsion angle Φ has a very similar value in 3′-*O*-acetyl-4-thiothymidine (120°), 3′-*O*-acetyladenosine (97.8°) and puromycin (110°), in spite of the marked differences in the constitution of these molecules and in some aspects of their conformation (3′-*O*-acetyladenosine in *syn* while the other two molecules are *anti* with respect to χ_{CN}) and that an elongated structure similar to that of I and II have also been found recently in 8-bromo-3′-*O*-triisopropyl-benzenesulfonyl-adenosine (but not in its 2′-isomer) [16] and in uridine-3′-*O*-thiophosphate methyl ester [17].

Acknowledgement

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