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A stereochemical model for cytokinin activity

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The three-dimensional structures of several cytokinins were compared and a stereochemical model for cytokinin activity is proposed. This model is used to explain the difference in activity of *cis*- and *trans*-zeatin.

Cytokinin; Zeatin; Stereochemistry

1. INTRODUCTION

Cytokinins are a group of plant hormones which regulate plant growth and development [1–4]. In mammalian systems, some of the cytokinin nucleosides exhibit immuno-suppressive activity [5] and inhibit the growth of leukemic cells [6,7]. Because of the key role that cytokinins play in plant growth and development, a full understanding of the molecular detail of cytokinin action is essential. A careful examination of the molecular structures of the hormones can provide information on the stereochemical requirements that influence the extent and nature of the hormonal response.

The X-ray crystal structures of several cytokinins are currently known. These include N^6-(Δ^2-isopentenyl)adenine (i^{6}Ade) [8], N^6-benzyladenine (b^{6}Ade) [9], kinetin (f^{6}Ade) [10] and N^6-(Δ^2-isopentenyl)-2-methylthioadenine (2MeSi^{6}Ade) [11]. In addition, the structures of *N*-(purin-6-ylcarbamoyl)-L-threonine (PCT) [12] and *N*-(purin-6-ylcarbamoyl)glycine (PCG) [13] are also available. These latter two N^6-substituted adenines do not exhibit cytokinin activity and provide the possibility of distinguishing stereochemical features of the cytokinins which are important for activity. The crystal structures of two cytokinin ribosides, namely kinetin riboside (f^{6}Ado) [14] and benzyl adenosine (b^{6}Ado) [15], as well as *N*-(purin-6-ylcarbamoyl)-L-threonine riboside [16] (which is not a cytokinin) are also available. Based on tobacco pith culture bioassays [17], these N^6-substituted free adenine bases are shown to be extremely potent plant growth hormones and the corresponding ribosides, although not as potent as the free bases, are also known to stimulate plant growth. However, it is still not known whether activity is due to the cytokinin riboside per se or to its metabolites [18].

In an attempt to determine the stereochemical aspects of cytokinin activity, we have compared the structures of several N^6-substituted adenines and adenine ribosides. Based upon this study, we propose a hypothesis to explain the 50-fold greater activity of *trans*-zeatin as compared to *cis*-zeatin [17].

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2. EXPERIMENTAL

Three-dimensional X-ray crystallographic coordinates of several N^6-substituted adenines and adenine ribosides were initially examined graphically using an Evans and Sutherland Picture System 330 computer employing the FRODO software package developed by A. Jones. FRODO is a general purpose molecular modeling program. Subsequently, values for the torsional angles of the side-chains of the molecules were calculated using the following definitions:

$$\phi_1 = \cos^{-1}(\vec{A} \times \vec{B}) \cdot (\vec{B} \times \vec{C}) / |\vec{A} \times \vec{B}| \, |\vec{B} \times \vec{C}|$$

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$$\phi_2 = \cos^{-1}(-\vec{B}\times\vec{C})\cdot(-\vec{C}\times\vec{D})/|\vec{B}\times\vec{C}||\vec{C}\times\vec{D}|$$

$$\phi_3 = \cos^{-1}(-\vec{C}\times\vec{D})\cdot(-\vec{D}\times\vec{E})/|\vec{C}\times\vec{D}||\vec{D}\times\vec{E}|$$

In these expressions, $\vec{A}$ is the vector drawn from atom C62 to atom C63, $\vec{B}$ is the vector drawn from atom C62 to atom C61, $\vec{C}$ is the vector drawn from atom C61 to N6, $\vec{D}$ is the vector drawn from atom N6 to atom C6 and $\vec{E}$ is the vector drawn from atom C6 to atom N1. In these expressions, the cross-products yield the vector normal to the plane defined by the atoms involved and the dot-products yield the angles between the planes determined by the atoms, i.e. the torsional angles.

3. RESULTS AND DISCUSSION

All of the molecules studied share a minimum of three torsional degrees of freedom which must be specified in order to describe the conformation of the N^6-substituent with respect to the adenine ring. Fig.1 is a two-dimensional molecular drawing of i^6Ade which illustrates these torsional degrees of freedom. In table 1, we present the values of these torsional angles for the compounds studied. ϕ_1 is the angle between C61 and C62 of the side chain, ϕ_2 is the angle between N6 and C61 and ϕ_3 is the angle between N6 and C6. Defining equations for these angles are given in the legend to fig.1. In the case of f^6Ade, i^6Ade, b^6Ade and 2MeSi^{6}Ade, the molecules are observed as tautomers in the crystal and the negatives of the values quoted for these compounds are also observed.

As indicated in table 1, ϕ_3 is restricted to a small range of angles for both the N^6-substituted adenines as well as the corresponding ribosides. These values of ϕ_3 place the N^6-substituent in an orientation which is essentially distal to the imidazole moiety of the adenine ring. Presumably this conformation is stabilized by π-π overlap between the lone pair of electrons on N6 and the delocalized π-orbital of the adenine ring. ϕ_2 appears to be clustered about a value of 90° with a larger spread in allowed orientations. The observed values for ϕ_3 and ϕ_2 have the effect of positioning the N^6-substituent into an orientation where the bulk of the side-chain points away from the adenine ring. ϕ_1 shows the largest spread of values in the molecules studied and reflects the high degree of torsional freedom available for the remainder of the N^6-substituent.

Cytokinin bases, f^6Ade, i^6Ade and b^6Ade are all observed to crystallize isomorphously. Not surprisingly, they all have very similar values for all of their torsional angles and, therefore, have extremely similar three-dimensional conformations. Because of their similarity in structure, it has been suggested [19] that these molecules exhibit the active conformation. This cannot be proven unequivocally from an inspection of the structures of f^6Ade, i^6Ade and b^6Ade, because it is not clear whether or not crystal lattice forces have imposed this particular conformation on these molecules. However, 2MeSi^{6}Ade, b^6Ado and f^6Ado crystallize in very different crystal forms and nevertheless have values of ϕ_2 and ϕ_3 which are similar to those observed for f^6Ade, i^6Ade and b^6Ade. It is therefore likely that the conformation about ϕ_2 and ϕ_3 is energetically stable and physiologically relevant.

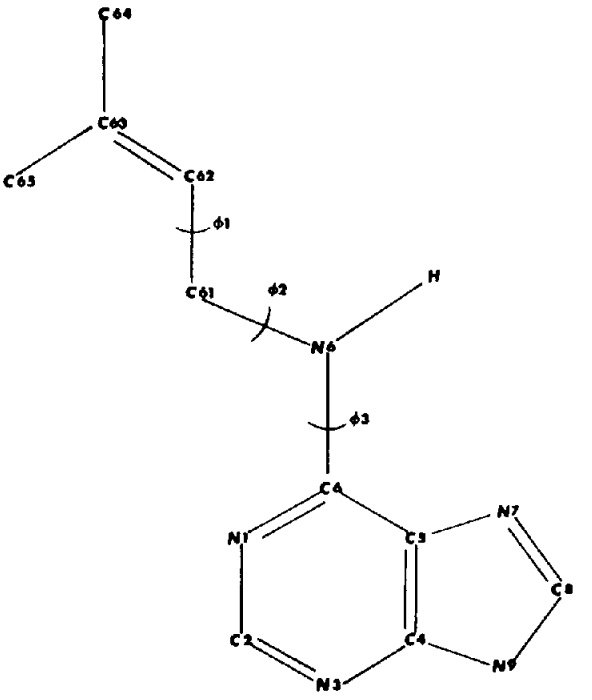

Fig.1. Molecular drawing of N^6-(Δ^2-isopentenyl)adenine. This drawing illustrates the torsional degrees of freedom in the molecule.

Although ϕ_1 is observed to be highly variable, there is indirect evidence that values of ϕ_1 near $-70°$ define the physiologically relevant conformation. Studies on the effect of aromatic substituents on the activity of b^6Ade [20] provide support for this hypothesis. The activity of *ortho*-substituted derivatives of b^6Ade depends on the nature of the substituent. *ortho*-OMe or CF_3

Table 1

Torsion angles in degrees for the side chains of several cytokinins

Molecule	ϕ_1	ϕ_2	ϕ_3
f^{6}Ade	73	110	7
i^{6}Ade	63	87	2
b^{6}Ade	72	102	6
2MeSi^{6}Ade	216	103	1
b^{6}Ado	145	78	−6
f^{6}Ado	57	106	12

substitution greatly diminishes cytokinin activity, while *ortho*-Cl substitution has only a small effect. From model building studies, it is evident that the *ortho*-substituted moiety must be in the proximity of N1 of the adenine ring. Otherwise, even for the smallest substituent, an extremely short van der Waals' contact will occur between the substituent and the proton on N6 if the benzene-moiety is flipped by 180° into a conformation where the *ortho*-substituent is proximal to N7 of the adenine ring. On the other hand, only the *ortho*-Cl substituted b^{6}Ade can assume a conformation similar to that observed for b^{6}Ade, since in both the *ortho*-OF_3 and *ortho*-OMe substituted compounds, steric hindrance between the *ortho*-substituent and N1 would preclude an orientation similar to that observed for b^{6}Ade. For these reasons it is not unlikely that the molecular conformation observed in the crystal structures of the N^6-substituted adenines is close to the physiologically active conformation. Although 2MeSi^{6}Ade and b^{6}Ado do not exhibit the preferred orientation about ϕ_1, model-building studies suggest that these molecules have unrestricted freedom of rotation about ϕ_1 and can achieve the conformation observed for the N^6-substituted adenines.

Based on the close conformational similarity of the sidechains of the molecules discussed, it is reasonable to assume that both *cis*- and *trans*-zeatin also exhibit similar side chain conformations. Fig.2A and B illustrates this conformation for *cis*- and *trans*-zeatin, respectively. As can be seen in this figure, *cis*-zeatin forms a perfect hydrogen bond to N1 of the adenine ring, while it is conformationally impossible to form a similar hydrogen bond for the *trans*-isomer. Since N1 of the adenine ring is known to be essential for activity [17], the existence of a strong hydrogen bond between the OH group of *cis*-zeatin and N1 of the adenine ring most likely blocks N1 thereby precluding the interaction of N1 with the appropriate receptor molecule. A similar argument has been proposed by Parthasarathy, Ohrt and Chheda to explain the lack of cytokinin activity of

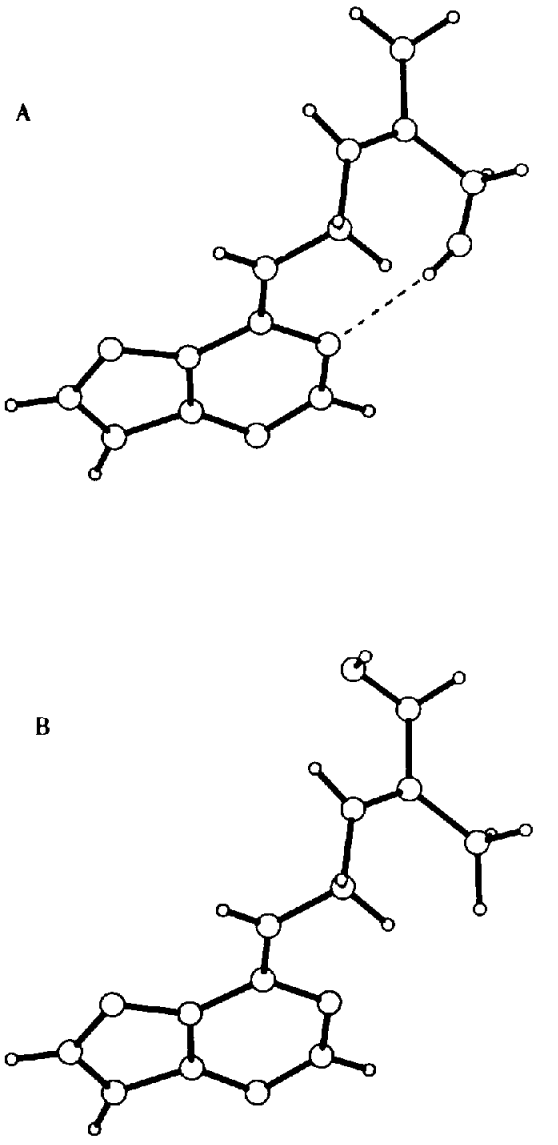

Fig.2. Hypothetical stereochemical conformations of *cis*-zeatin (panel A) and *trans*-zeatin (panel B). The hydrogen bond between the hydroxyl hydrogen and N1 of the adenine ring is shown as a dotted line for *cis*-zeatin. The N1–O distance is approx. 2.80 Å.

N-(purin-6-ylcarbamoyl)-L-threonine and *N*-(purin-6-ylcarbamoyl)glycine. In these molecules, the NH group of the amino acid moiety forms a hydrogen bond to N1 of the adenine ring, and these authors suggest that this hydrogen bond is responsible for the lack of cytokinin activity of these molecules.

Iwamura et al. [21] used an empirical relationship to correlate cytokinin activity to the size of the N^6-substituent after accounting for differences in the electronic structure and hydrophobicity of the N^6-substituent. They concluded that for the N^6-substituted adenines, a maximum in cytokinin activity occurred for those molecules which have an N^6-substituent width of 5.2 Å. While the size and chemical nature of the N^6-substituent are undoubtedly important in the binding of the cytokinins to their appropriate receptors, the hypothesis of Iwamura et al. [21] cannot explain the difference in activity of the *cis*- and *trans*-isomers of zeatin because the side-chains of both isomers are similar in size and composition.

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