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Interaction of L-Tryptophan with Inosine and Guanosine

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L-Tryptophan, guanosine, inosine

The equilibrium constants for the complex between L-tryptophan and inosine are established by solubility measurements at different temperatures, the order of magnitude of these constants being about 10^3 times bigger than the corresponding one for the formation of guanosine-tryptophan or any other of the nucleoside-tryptophan complexes. The mode of association of these molecules is governed mainly by stacking interactions as it has been suggested from previous PMR results. The differences in magnitude obtained for the equilibrium constants of (I+T) with respect to (G+T) are discussed in terms of differences in solute-solvent and solvent-solvent interactions. Thermodynamic parameters inferred from the equilibrium constants at different temperatures support this suggestion. Infrared results on I-T complex suggest that changes at the ribose moiety occurs and that possible hydrogen bridges are involved in the mechanism of association besides the stacking interactions.

Introduction

A molecular study of the interaction between amino acids and nucleic acids as well as related molecules may help to understand the chemical specificity of several protein-nucleic acids interactions ocurring at the cell level. In this respect some information on the origin of the genetic code could also arise from these investigations.

An approach to simplify the study of the complex molecular interaction between polymers is to establish first the nature of chemical bondings between the monomeric constituents. The chemical interaction between aromatic amino acids and mononucleosides or derivatives have been measured by different methods [1–6]. The formation of complexes between aromatic amino acids and polynucleotides (single and double stranded) have been also established by means of fluorescence, proton magnetic resonance and temperature studies [7–9].

In general all these studies suggest that the interaction between aromatic amino acids and the different nucleobases in aqueous solutions are governed mainly by stacking forces responsible for the partial overlapping of the aromatic rings. To the authors' knowledge, there is no data concerning the molecular interaction of L-tryptophan with inosine or its derivatives. In this work a quantitative study of the complex formation between inosine and L-tryptophan is reported. The results obtained are based on solubility measurements at different temperatures. A comparison between the chemical nature of this complex and that of guanosine with tryptophan is also established. The much greater binding affinity of L-tryptophan with inosine as compared to guanosine (which has been shown to have the greatest affinity as compared with the rest of the nucleosides [4, 5]) may be indicative of a possible regulation action that this interaction may play in the synthesis of proteins.

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Experimental

Guanosine (Lot 106B-0910) and inosine (Lot 91C-1810) were obtained from Sigma. We want to draw attention to the fact that very often guanosine reagents are contaminated with ions (as detected by PMR methods from the broad aromatic H 8 resonance). Guanosine samples were purified on a Dowex W-50 column. This method is not completely satisfactory * but in this particular work the effects of remaining impurities on solubility measurements have proved to be negligible.

Crystalline L-tryptophan was obtained from Calbiochem.

Solubility measurements were carried out at different temperatures in a regulated water bath (Gallenkamp). Solubility studies with guanosine were performed with saturated nucleosides solutions, to which different amounts of L-tryptophan were added. The procedure and method used is reported in a previous communication [4].

Inosine solutions 10^{-2} M were prepared, to which different amounts of L-tryptophan were added. The range of concentrations of L-tryptophan used was selected according to the solubility of this amino acid in water at the temperature considered.

Optical density measurements were done with a Beckman DU instrument. The concentration of each nucleoside and L-tryptophan, were calculated from the optical density at 254, 248, and 280 nm.

Infrared spectroscopy was performed with a Perkin Elmer 621 instrument. Solid samples in KBr of the inosine tryptophan complex and a 1 : 1 mechanically mixture of tryptophan and inosine were prepared. The inosine tryptophan complex was obtained by mixing V/V a $2 \cdot 10^{-2}$ M solution of inosine with one of the same concentration solution of tryptophan.

Once the precipitation was completed, the white precipitated complex was isolated by filtering. The solid was washed several times with small amounts of water and then dried in vacuum.

Results and Discussion

Solubility of guanosine and inosine as a function of temperature and concentration of tryptophan (at equilibrium)

As can be seen from Fig. 1 the ratio $[\Delta S]/[S]$ of guanosine, at neutral pH, increases linearly with increasing concentration of tryptophan and decreases with an increse in temperature. This behaviour was observed over the entire range of temperature studied. Within the experimental error all straight lines intersect the origin which indicates a 1 : 1 stecheometry. The equilibrium constants at different temperatures were calculated according to the equations:

$$G + T \rightarrow G - T$$

for which

$$K = \frac{[G\,T]}{[G]\,[T]} \quad \text{or} \quad K = \frac{[\Delta S]}{[S]\,[T]} .$$

$[\Delta S]$ = Increase in solubility of guanosine;
$[S]$ = solubility of pure guanosine;
$[T]$ = tryptophan concentration, at equilibrium.

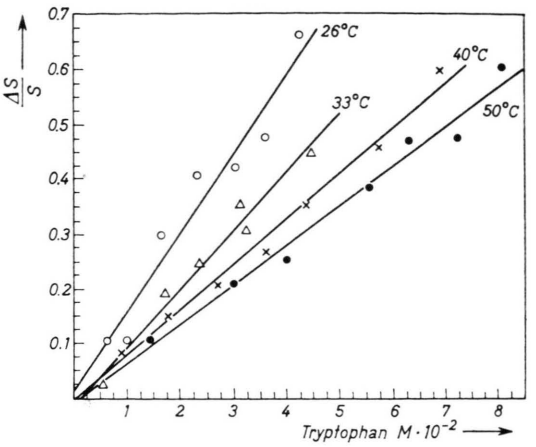

Fig. 1. Solubility of guanosine as a function of L-tryptophan for different temperatures (at neutral pH). Standard error for the slope (K) at 51 °C = ±0.5; 40 °C = ±0.4; 33 °C = ±0.9; 26 °C = ±1.2.

As pointed out by the authors in a previous work [4] and independently by others using PMR techniques [5], the interaction of purine ribonucleosides with L-tryptophan in aqueous solutions at acid pH proceeds *via* stacking interactions of the aromatic rings. The upfield shifts for the aromatic protons of the ribonucleosides as a function of the tryptophan concentration supports this structural interpretation.

A comparison of the different slopes of the curves in Fig. 1 shows that K decreases with increasing temperature. Therefore if stacking interactions are present these will be affected by temperature variations. This is in accordance with the hypothesis of stacking interaction being present in complex formation: As temperature increases thermal agitation

* M. Pieber, P. Kroon, J. H. Prestegard, and S. I. Chan, J. Amer. chem. Soc. **95**, 3408 [1973].

of the molecules will result in a decreasing amount of complex being formed, *i. e.*, in a decreasing K with increasing temperature.

These studies as well as the NMR data may thus provide a more accurate picture of the geometry of stacks in solution.

A rather unexpected feature was found for the case of inosine-tryptophan complex formation: The aggregation of these molecules led to the formation of a insoluble compound even at low concentration of the reactants (10^{-2} M).

In Fig. 2 a plot of 1/I *vs* tryptophan concentration (at equilibrium) for different temperatures shows a linear relationship. In terms of the equilibrium constant the following equation holds:

$$I + T \rightarrow I - T$$

for which

$$K = 1/[I] \cdot [T].$$

The equilibrium constants for the complex I – T and G – T as calculated from the slopes of Figs. 2 and 1 are strikingly different. In general the magnitude of K for the I – T complex at any temperature is of the order of 10^3 times greater than for guanosine tryptophan complex. A graphical representation of log K of inosine and guanosine complex *vs* 1/T (Fig. 3) summarizes these results.

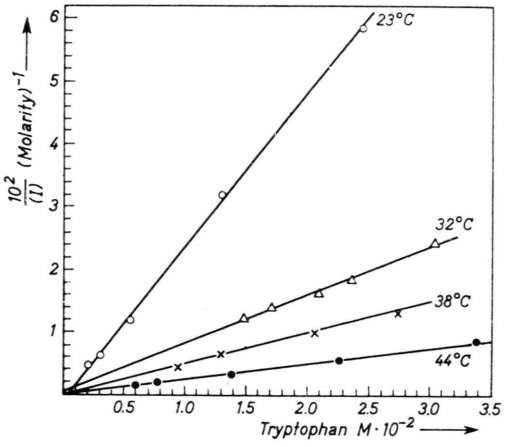

Fig. 2. Inverse concentration of inoside as a function of the concentration L-tryptophan (at equilibrium, for different temperatures at neutral pH). Standard error of the slope (K) at 44 °C = ±0.02; 38 °C = ±0.2; 32 °C = ±0.3; 23 °C = ±0.3.

The ability of inosine and guanosine to self associate *via* vertical stacking at neutral pH has been established by vapor pressure osmometry and NMR methods [10, 11]. However, although NMR methods have yielded information about the geometrical arrangements of the stacks in solution, this information is still incomplete. In this connection we wish to point out that the binding constants calculated from PMR studies on the stacking interaction between L-tryptophan with pyrimidine and purine nucleosides are larger for the latter; the differences in any case are small. (In both cases the calculated binding constants are of the same order of magnitude.) The values of these binding constants, on the other hand, correlate well with the equilibrium constants obtained from solubility data as described in a previous work [4]. All these results could then suggest that the stacking interaction energy between L-tryptophan and the different nucleosides are also of the same order of magnitude. Therefore there is more reason to believe that this should hold for two chemically similar molecules such as guanosine and inosine.

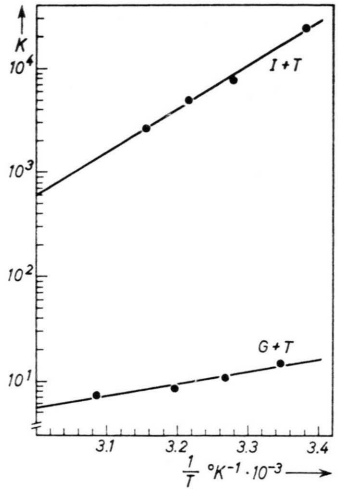

Fig. 3. A semilogarithmic plot of the equilibrium constant values *vs* the inverse of the absolute temperature. Upper: (I+T); lower: (G+T).

Studies on selfassociation of inosine and methylguanosine by NMR methods [12] have shown that the magnitude of the upfield shifts at H8 proton (when the concentrations of the nucleosides are increased) are practically identical. Hence, if these shifts are of the same magnitude and are indeed an indication of the degree of aggregation correlating therefore with the magnitude of the interaction energy, it is then also possible to assume that the magnitudes of the stacking energy of I – T and G – T complexes should be essentially equal to each other. The same conclusion should also hold for the corresponding

Table I. Equilibrium constants and thermodynamic parameters for the association of guanosine and inosine with L-tryptophan in aqueous neutral solutions.

	T [°C]	K	$-\Delta F$ K_{cal}	$-\Delta H$ K_{cal}	$-\Delta S$(ue)
	25	14.3 ± 0.5 *	1.57 ± 0.03		
(G+T)	35	10.7 ± 0.5	1.45 ± 0.03	5.3 ± 0.9	12.6 ± 0.3
	45	8.1 ± 0.3	1.32 ± 0.03		
	25	$(1.8 \pm 0.1) * 10^4$	5.82 ± 0.03		
(I+T)	35	$(6.5 \pm 0.2) 10^3$	5.37 ± 0.03	19.2 ± 1.1	45.0 ± 0.3
	45	$(2.4 \pm 0.1) 10^3$	4.93 ± 0.03		

* Means standard error.

degree of association if stacking forces are mainly responsible for the complex formation.

Our experimental results show that the equilibrium constant for the association of inosine with L-tryptophan is about 10^3 times greater than for the G – T complex. The sole absence of a polar group (NH_2) in inosine as compared to guanosine seems unlikely to affect the stacking interaction energy to such an extent as to account for the differences in the observed association constants at the different temperatures considered. The authors are more inclined to believe that these differences may arise from differences in solute-solvent and solvent-solvent interactions as a result from differences in intermolecular bonding other than stacking, and/or as a consequence from a different geometrical arrangement between the aromatic rings having notwithstanding a similar interaction energy.

In Table I the thermodynamic parameters free energy (ΔG), free enthalpy (ΔH), and free entropy (ΔS) are correlated with the equilibrium constants at different temperatures for the I – T and G – T complex formation.

H was calculated using the well known integrated Van't Hoff equation $\log K = -\Delta H/R\,T + C$, where ΔH is obtained from the slope of the straight line in Fig. 3 ($\Delta F = -R\,T \ln K$ and $\Delta S = (\Delta H - \Delta G)/(T)$. As it can be deduced from the values of these parameters, it is clear that the formation of I – T complex in a water system takes place with a release of energy (ΔF) of about four times higher than that for the G – T complex formation. The differences in entropy indicates that a higher ordering of the molecules is achieved when the I – T complex is formed. As already described, the association of L-tryptophan with inosine leads to the formation of an insoluble compound. This feature is related with the idea that solute-solvent interactions may be important when complexing occurs. The lack of a polar group (NH_2) in inosine and structural changes at the level of the ribose moiety could account for the changes in these forces. Aiming to get some information about possible changes in the ribose moiety infrared studies were performed.

Fig. 4 shows the results on the IR spectra of the I – T complex, as compared with a mixture of ino-

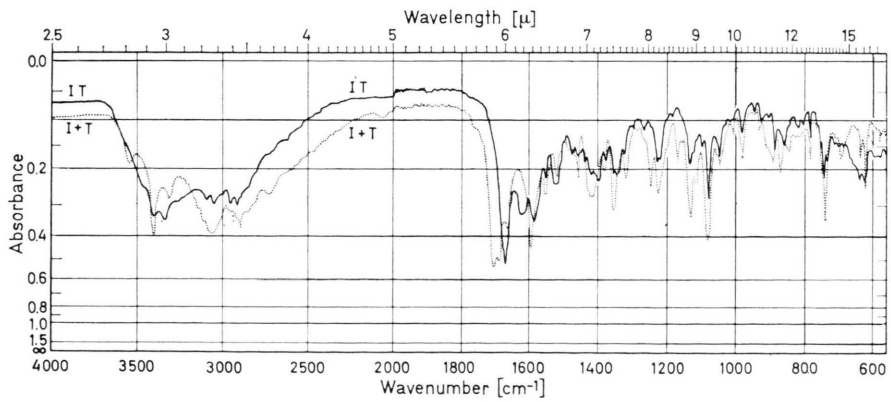

Fig. 4. Infrared spectra for inosine-tryptophan complex (solid). Upper: I-T complex; lower: A 1:1 mechanically mixture of inosine and L-tryptophan.

sine and tryptophan. In spite of the complexity of these spectra the following conclusions may be drawn from the data [14]:

1. 3550 cm^{-1} (where stretching vibrations of OH groups occurs): It is found that the absorption band is considerably modified in the case of the I – T complex. The disappearance of this band may then be indicative of changes at the OH protons of the ribose moiety.

2. 1100 – 1250 cm^{-1}: In this zone stretching vibrations are attributed mainly to C – O – C and C – OH bonds. As it can be observed, these bands are also strongly modified in the I – T complex. These changes may then also arise from changes in the ribose moiety.

3. 1610 – 1630 cm^{-1}: In this region a new band appears when tryptophan is complexed with inosine. This new band can be attributed to an increase in the aromaticity of the inosine and tryptophan ring and as a result of the stacking interaction.

4. 740 cm^{-1}: The modification of this band in the I – T complex can be attributed only to a change in the aromaticity of the aromatic tryptophan ring which will be affected if this ring is stacked to the inosine one.

5. 1650 – 1720 cm^{-1}: In this region, the stretching vibration of the CO and NH group of inosine appears shifted to shorter wavelengths in the I – T complex. The shape of these absorbancy bands are also modified. The shift to shorter wavelengths of these groups could be interpreted as if these groups were involved in hydrogen bridges.

In conclusion the observed changes in the IR spectra suggest that important changes in the ribose moiety occurs, and that the aromatic ring of inosine may be involved in hydrogen bridges besides the occurence of stacking.

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