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Precise ultraviolet absorbance study of the interactions of amino acids and mononucleosides in aqueous solution

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The association constants of various amino acids or their derivatives (methyl esters, amides, etc.) with mononucleosides in aqueous solutions have been measured by using precise ultraviolet difference absorbance photometry. Some of the results are in agreement with those of the previous solubility experiments. The superiority of this ultraviolet absorbance method over the solubility experiments is that it can discriminate between stacking and hydrogen-bonding interactions. New types of specific interactions of some amino acids with nucleic acid bases by using a peptidyl carboxylate ion and another donor or acceptor in their side chains have been found using this technique.

1. Introduction

Heredity (or replication of DNA) and metabolism (or protein synthesis) are the two fundamental functions of the biosystem. Replication of DNA is known to be based on the specific base-base hydrogen-bonding interaction, which is very weak and difficult to measure in aqueous solutions [1]. The association constants beween the bases have been accurately determined only in nonaqueous hydrophobic solutions such as dimethyl sulfoxide (DMSO), chloroform, cyclohexane, etc.

In contrast to the replication process of DNA, protein synthesis is a rather complicated system. Furthermore, even the most elementary amino acid-base interactions have not yet been well studied in aqueous solution. The fundamental form of interaction may be hydrogen-bonding, electrostatic, hydrophobic, and stacking interactions, similarly to the case of base-base interaction [2]. The main obstacles to such a study are again the weakness of the interactions in aqueous solution and, in addition, insolubility of amino acids in hydrophobic solvents.

Attempts were made to measure the association constants of the nucleosides and amino acids by determining the solubility enhancement of the nucleosides in concentrated amino acid solutions due to complex formation [3,4]. The magnitudes of the apparent association constants obtained were quite scattered and clear discrimination between specificity and nonspecificity was difficult, although some cases were attributed to the specific interaction by using two hydrogen bonds, such as that between arginine and cytidine or between glutamate and guanosine, on the basis of molecular structural discussions.

NMR experiments to measure the downfield shift of hydrogen-bonded protons were carried out in nonaqueous solutions for nucleic acid bases (or nucleosides) and amino acid analogues. Various specific interactions were observed, the best

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studied being the strong association of carboxylate ions to guanine with an association constant of 110 M^{-1} in DMSO [5]. This value decreased to 18 M^{-1} in a 3:1 DMSO/water solution, and the value in pure water was too small to be measured accurately using this method. A competition association measurement in aqueous solution [2] showed that this interaction is stronger than that between guanine and cytosine, whose association constant was estimated to be about 0.6 M^{-1} in water [1].

In this paper, the results obtained from a precise ultraviolet absorbance photometry experiment to determine the association constants of various amino acids and their derivatives such as methyl esters, amides, etc., with mononucleosides in aqueous solution are reported. The characteristics of this technique is that it can discriminate between hydrogen-bonding and stacking interactions by using the hyper- and hypochromicity of the absorbance, respectively. The results of another biologically important measurement for the detection of the specific interaction of the anticodonic dinucleoside monophosphate and the cognate amino acid, correlated with the origin of the genetic code, have already been reported [6] and the results described in this paper would certify the accuracy of the photometric measurement in the abovementioned paper.

2. Materials and methods

All chemicals were purchased from Sigma and used without purification. It was anticipated that the signal resulting from the binding of amino acids to nucleosides would be very small in water. A Beckman DU-8B spectrophotometer, which can register an absorbance of 0.0003 A units under optimum conditions, was used to detect such a small signal (about 0.5–2% of the total absorbance). The ultraviolet and visible sources were on continuously throughout the experiment and the ultraviolet lamp was exchanged every 20 days. The cleanness of the ultraviolet cell with a lid (1 cm light path) was carefully checked and the temperature of the solution was maintained at 25.0 ± 0.1° C.

Buffers used were 50 mM sodium cacodylate at pH 6.00 ± 0.01. Absorbance was measured over the range 1.2–1.7 A units at 270–250 nm (near maximum absorbance), the nucleoside concentration being of the order of 100 μM. The association constant was obtained from Scatchard plots: the equilibrium equation for an amino acid a and a nucleoside b is defined by

$$K = \frac{[c]}{[a][b]}$$

where c is the complex between these two compounds and K the association constant (in M^{-1}). Since $[a]_0 = [a] + [c]$ and $[b]_0 = [b] + [c]$, where the subscript 0 denotes the initial concentration, and the difference in absorbance $\Delta = k[c]$, where k is a constant, this equation can be transformed to

$$K(k[b]_0 - \Delta) = \frac{\Delta}{[a]_0}$$

when $[a]_0 \gg [b]_0$. The gradient of the Δ vs. $\Delta/[a]_0$ plot gives the association constant K. 2 M solutions of amino acids (this high concentration restricts the variety of the amino acids for this experiment) and their derivatives were used and absorbance was measured after successive addition of 60 μl solutions to 1000 μl nucleoside solutions. The difference absorbance Δ was calculated from $\Delta = b^{(n)} - (b^{(0)} + 0.060na^{(0)})/(1.000 + 0.060n)$, where n is the number of addition of the amino acid solution, $b^{(n)}$ the observed absorbance of the n-th mixture, and $a^{(0)}$ the absorbance of the 2 M amino acid solution (0.05 – 0.43 A units). Then, Δ can be determined with a better accuracy by more than one order of magnitude.

3. Results and discussion

3.1. Comparison with the results of solubility experiments

All measured association constants are listed in table 1. The signal for the interaction studied is sometimes hyperchromic (indicated by +) or hypochromic (indicated by −). The observed constants from the solubility experiments performed

Table 1

Association constants of nucleosides and amino acids or their derivatives

Values determined by Thomas and Poddar [3] and by Bruskov [4] are denoted by round and square brackets, respectively.

	Gly	Gly methyl ester	Gly amide	Gly-Gly	Ser	Glu	Lys	Arg	Guanidine
Guo	0.5 (0.45) [0.51]	0	0	0	1.0 (0.35?)	1.5 [0.71]	1.2 (1.27) [0.85]	special (see text) [1.91]	−1.4
Ado	0 (0.04) [0]	0	0	0	0	0 [0]	0.7 (0.55) [0.06]	−1.3 [1.14]	−1.4
Cyd	0 [0.27]	0	0	0	0	0 [0]	1.0 [0.63]	−2.1 [1.5]	−1.6
Urd	0 [0]	0	0	0	0	0 [0]	0 [0]	−2.0 [0.6]	−1.6

by Thomas and Poddar [3] and Bruskov [4] are also compared in table 1.

The agreement of our measured association constants with those obtained from the solubility experiments is generally good. Rather large differences can be seen in only four cases. (1) In the case of guanosine-serine, our value is about 3-times larger than that of Thomas and Poddar. It is known that the OH group in the side chain of serine interacts with bases. Furthermore, the uniform attachment of all amino acids with guanosine suggests that the carboxylate ion in the peptidyl moiety of the amino acid can form two hydrogen bonds with $-NH_2$ and $>NH$ of guanosine with an association constant of about 0.5 M^{-1}, as suggested by the guanosine-glycine case (it is remarkable that all measured K values for these three experiments agree with each other in this case, possibly due to the fact that the high solubility of glycine decreases the inaccuracies in the solubility measurements). Consequently, it appears unlikely that K for this case is as low as 0.35 M^{-1}. (2) For adenosine-lysine, the result given by Bruskov is smaller than ours and that of Thomas and Poddar and may be rejected. (3) In the case of guanosine-arginine, we found a special cooperative interaction which could not be detected in the solubility experiment. This will be described later. (4) For uridine-arginine, Bruskov used thymidine instead of uridine to avoid the effect of the high solubility of uridine whereas we used uridine. The difference in K values between the two may be due to the different selection of nucleosides.

The above comparison appears to confirm the accuracy of our measurements. In the case of cytosine-glycine, the ultraviolet measurement did not enable accurate determination of the association constant despite the repetition of experiments. This appears to limit the detectional ability of our experiment for the association constant to 0.2 M^{-1}. (The variation ΔK of the association constant $K = K_0 \exp(-h/kT)$ on the change in sample temperature ΔT is expected to be $\Delta K = -K \cdot h/kT \cdot \Delta T/T \approx -1 \times 10/0.5 \cdot \Delta T/300 \approx -0.1\Delta T$ M^{-1}). Since $\Delta T = 0.1°C$ in this experiment, the inaccuracy of K with respect to ΔT is less than the above detectional ability.)

3.2 Hyperchromicity

Hyperchromicity appears to occur in the case of hydrogen bonding: some amino acids are concluded to form two hydrogen bonds with bases as inferred from various NMR studies in hydrophobic solvents [2]. In the case of the carboxylate ion $-COO^-$, it has two acceptors to bind with two donors of guanosine ($-NH_2$ and $>NH$). In the present ultraviolet experiments, these interactions have led to hyperchromic signals being detected. For instance, the association constant of acetate

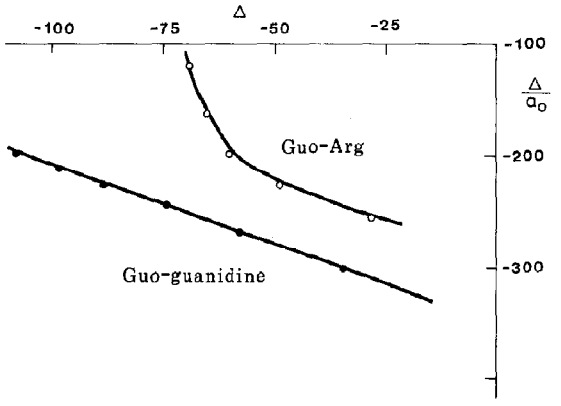

Fig. 1. Scatchard plots for association of guanosine (Guo) to arginine and guanidine. Δ is the signal (in 10^{-4} A units) and a_0 the initial concentration of arginine or guanidine (in M).

and guanosine in aqueous solution was measured to be 0.8 M^{-1} (hyperchromic) in this experiment. This value is consistent with that expected (18 $M^{-1} \gg K > 0.6$ M^{-1}) from NMR experiments [2] described in section 1 and confirms that the hyperchromic signal arises from the hydrogen bonding.

Guanosine interacts hyperchromically with the peptidyl COO^- of all amino acids; in particular, its affinity to aspartate and glutamate is large, since both aspartate and glutamate have another carboxyl group in their side chains. Interestingly, hyperchromicity appears in the cases of adenosine-lysine and cytidine-lysine. A hydrogen bond between the amino group in the side chain of lysine and these nucleosides is too weak to result in the measured association constants in table 1 [2]. Model building shows that the COO^- of lysine can also bind to the NH_2 group of the nucleosides to form another hydrogen bond by changing the conformation of the lysine side chain. This is a new type of specific interaction between the base and the whole amino acid. (It should be noted that arginine cannot undergo this type of interaction, since it contains a rigid large planar guanidine group.) In the cases of serine, aspartate, and glutamate, the same type of hydrogen-bonding interaction on changing of the conformation of the side chain of the amino acids may also occur. On the other hand, the methyl ester or amide does not interact with guanosine, since one of the acceptors in the peptidyl carboxyl group is modified by CH_3 or NH_2.

In the above discussions, the possibility that the peptidyl amino group can hydrogen bond to any of the acceptors on the bases has been ignored. This may be concluded from the fact that glycine hardly interacts with adenosine (the association constant may be less than 0.2 M^{-1}), since, if the peptidyl amino residue acts simultaneously with the peptidyl carboxyl residue, they should form a complex with adenine via two hydrogen bonds. It can also been shown from NMR that the lines of the peptidyl amino group always disappear completely in aqueous solution, since the pK value of this group is low and proton exchange with water occurs very rapidly. Such protons cannot form a stable hydrogen bond. Although not explicitly shown in table 1, we have observed that 9-ethylguanine and 9-ethyladenine interact with the amino acid with almost the same association constants to guanosine and adenosine, respectively. This suggests that the ribose moiety of the nucleoside does not interact directly with the amino acid.

The positive charge of a hydrogen-bonded proton appears to neutralize partially the excess negative charges on the acceptor, the side chain of a nucleic acid base. Then, the base ring current would increase. This may cause the hyperchromicity detected in this experiment. (This effect may also occur in the cases of the specific interaction between bases such as G ≡ C and A = U as a result of hydrogen-bond formation. The mutual influence of the large ring currents of the paired bases is, however, overwhelmingly greater than the above neutralization effect and the ultraviolet absorbance in the case of paired bases is always hypochromic.)

3.3. Hypochromicity

It has been found from ultraviolet absorbance (and NMR) spectroscopy that tryptophan and the nucleosides stack with association constants of several M^{-1} [7]. The observed hypochromicity was ascribed to electron transfer from the π-bonded indole ring to the purine or pyrimidine ring. In our experiments, the arginine alone, which has a π-bonded guanidine group in its side chain, shows

hypochromicity. It is generally confirmed in our experiment that the guanidine group shows hypochromicity in the case of the nucleosides (table 1). This suggests that hypochromicity arises from the stacking interaction. (Although not shown in table 1, we observed a hypochromic signal for the case of the cytidine-histidine methyl ester. However, the rapid decomposition of the methyl ester in water prevents precise measurement of the association constant.) The behaviors of guanidine and arginine are, however, not necessarily similar. The association constant for guanidine to guanosine by hydrogen bonding in DMSO as measured by NMR amounts to about 1.2 M^{-1} [2]. In aqueous solution this may be less than 0.2 M^{-1}. (Similarly, that of Cl^- contained in the arginine reagent to guanosine may also be neglected in this experiment, since the value in DMSO is about 1 M^{-1} [8].) Consequently, the measured association constant for guanidine may be totally of stacking type. Scatchard plots in the cases of nucleoside-guanidine are always linear. The association constant for arginine to adenosine, cytidine, and uridine are almost the same as those for guanidine, as expected. However, that of arginine to guanosine is unusual. The Scatchard plot for this case shows a similar behavior to the case of guanidine for small signals, but then increases in slope for large signals to give large affinity (fig. 1). We changed the cacodylate buffer to a Tris buffer (50 mM Tris, 50 mM $NaNO_3$, pH 7.70) but obtained a similar curve, although the magnitudes of the signals were different. In the case of guanosine, the peptidyl $-COO^-$ of arginine becomes attached to $-NH_2$ and $>NH$ of the base. Cooperativity of this binding to the guanosine-guanidine moiety interaction appears to cause the above unusual behavior.

4. Conclusions

It may be argued that the difference absorbance detected by precise ultraviolet photometry in an amino acid-nucleoside interaction is hyperchromic for cases of hydrogen bonding and hypochromic for those of stacking. The difference absorbance in the base-base interaction is, in contrast, always hypochromic. Consequently, the ultraviolet absorbance method may be used to discriminate between both kinds of interaction and in discussing the molecular structure of the amino acid-nucleoside complex, similarly to the case of the downfield and upfield chemical shifts, to discriminate between hydrogen-bonding and stacking interactions, respectively, in nonaqueous solution by NMR. A general agreement of our results with those from solubility experiments may guarantee the accuracy of our ultraviolet photometric method.

The amino acid-nucleoside interaction in aqueous solution may be classified as follows, by summarizing all the information obtained so far:

(1) Hydrogen-bonding interaction

(a) Side chain-base interaction

A moiety in the side chains of an amino acid interacts with the side chains of a base with two hydrogen bonds. This case is well studied by using the amino acid analogues in nonaqueous solution in NMR experiments.

(b) Whole amino acid-base interaction

The carboxyl group in the peptidyl moiety of an amino acid forms a hydrogen bond with a donor of the base and an amino or carboxyl group in the side chain of the amino acid makes another hydrogen bond with the side chain of the base. This is a new type of specific interaction found in this experiment.

(2) Stacking interaction

Aromatic amino acids can stack with the (side) plane of a base as already found in previous experiments. The guanidine group can also stack with the side plane of the nucleoside. In the case of guanosine and arginine, a cooperative effect of hydrogen bonding and stacking interaction was detected.

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