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Studies of Stacking in AMP and Its Pd(II) Complexes by Ultrasonic Velocity Measurements

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The self-association of AMP and Pd(II) complexes of AMP was studied by ultrasonic velocity measurements. Using an isodesmic model, equiblibrium constants of the stacking process were obtained.

Two different Pd(II) complexes were used in the reactions with AMP, one in which two of the Pd^{2+} coordination sites were blocked by ethylenediamine (en), and one where three sites were blocked by diethylenetriamine (dien). In the first case two sites are available for coordination with AMP and in the other case one. We find that addition of the metal complexes induce stacking but the enhancement is much greater with $enPd^{2+}$ than with $dienPd^{2+}$.

The stacking constant for AMP^- was found to be, $K = 2.3 \pm 0.5$. Addition of $dienPd^{2+}$ and $enPd^{2+}$ increased the stacking constant with a factor of 3.5 and 14, respectively.

The self-association of nucleic bases, nucleosides and nucleotides has been the subject of intensive discussion in the literature. The field was reviewed, about a decade ago [1,2] and Sigel *et al.*[3] give a good review on later explorations in the field.

Different techniques have been employed to evaluate equilibrium constants for the aggregation of these compounds. Vapour pressure osmometry,[4–11] NMR-studies [2,3,12,13] and sedimentation equilibria [6,14,15] give information on the geometry and extent of the self-association. It is now generally accepted that the aggregation is due to stacking of the planar bases.

The interplay between metal ions and nucleic acid constituents has been subject of extensive research lately.[16–19] Especially since the antitumor action of *cis*-$[PtCl_2(NH_3)_2]$ was discovered in 1969,[20] has the effort in the field been steadily growing.

The strong ion–ion interactions make such methods as vapour pressure osmometry less useful. Only spectroscopic methods have been successfully applied to measure association of charged compounds (nucleotides).[21]

Quite recently, ultrasonic velocity measurements were utilized by Hemmes *et al.*[22] to evaluate equilibrium constants by stacking of nucleic bases. Hemmes *et al.* based their calculations on the isodesmic model of indefinite non-cooperative stacking. We applied their method to evaluate stacking constants of AMP and Pd(II) complexes of AMP.

THEORETICAL SECTION

The models for self-association have been reviewed by Magar.[23] We have applied the sequential equal constant-model (SEK), also referred to as the isodesmic model. The basic idea is that adding a monomer into an aggregate is characterized by the same equilibrium constant independent of the size of the aggregate. The various stages of association can be described as follows:

$$N_1 + N_1 \overset{K_1}{\rightleftharpoons} N_2 \quad K_1 = C_2/C_1$$

$$N_1 + N_2 \overset{K_2}{\rightleftharpoons} N_3 \quad K_2 = C_2/C_2C_1 \qquad (1)$$

$$N_1 + N_n \overset{K_n}{\rightleftharpoons} N_{n+1} \quad K_n = C_{n+1}/C_nC_1$$

where $K_1 = K_2 = K_3 = \ldots K$

The total concentration C can be expressed in terms of the concentrations of the aggregates.

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$$C = C_1 + 2C_2 + 3C_3 + \ldots \quad (2)$$

By introducing the equilibrium constant, the concentration is expressed as a function of the monomer concentration, C_1, and K.

$$C = \sum_{n=1}^{\infty} nK^{n-1}C_1^n \quad (3)$$

$$C = C_1/(1+KC_1)^2 \quad (4)$$

$$\Delta u/u = A_1C_1 + A_2C_2 + \ldots \quad (5)$$

$$A_n = nA_1 - 2n\Delta + 2\Delta \quad (6)$$

$$\Delta u/u = \sum_{i=1}^{\infty} n(A_1 - 2\Delta)K^{n-1}C_1^n + 2\Delta K^{n-1}C_1^n \quad (7)$$

$$\Delta u/uC = A_1 + 2\Delta KC_1 \quad (8)$$

$$\left(A_1 - \frac{\Delta u}{uC}\right) = 2\Delta KC_i \quad (9)$$

$$\left[\left(A_1 - \frac{\Delta u}{uC}\right)/C\right]^{1/2} = \left(\frac{K}{2\Delta}\right)^{1/2}\left[2\Delta - \left(A_1 - \frac{\Delta u}{uC}\right)\right] \quad (10)$$

$$\left[\left(A_1 - \frac{\Delta u}{uC}\right)/C\right]^{1/2} = \left(\frac{K}{2\Delta}\right)^{1/2}\left(A_1 - \frac{\Delta u}{uC}\right) + (2\Delta K)^{1/2} \quad (11)$$

$$A_1 = \lim_{c \to 0} \frac{\Delta u}{uC} \quad (12)$$

This series converges, when $KC_1 < 1$, giving eqn. (4). Rao[24] has shown that the contribution of non-interacting solutes to the ultrasonic velocity are additive. That is eqn. (5). Following the arguments of Hemmes *et al.*,[22] eqn. (6), where $n = 1, 2, 3, \ldots$ and Δ is the reduction in the A_n coefficients resulting from the association of two monomers. Combining eqn. (5) and eqn. (6), gives eqn. (7). This equation is simplified by dividing by C to eqn. (8); rearranging eqn. (8) gives eqn. (9), which still has three unknowns, C_1, Δ and K. Dividing by C, taking the square root and using eqn. (4) eliminates C_1, and gives eqn. (10); rearranging this equation gives us the linear eqn. (11) cited by Hemmes *et al.*, K and Δ are determined from the plot of $[(A_1 - \Delta u/uC)/C]^{1/2}$ *versus* $(A_1 - \Delta u/uC)$. A_1 is measured from the intercept of $\Delta u/uC$ *versus* C, eqn. (12).

MATERIALS AND METHODS

AMP of the best quality was purchased from Sigma Chemical Co., and used without further purification. Nitrates of dienPd$(H_2O)^{2+}$ and enPd$(H_2O)_2^{2+}$ were prepared as described earlier.[25,26] Solutions were prepared in the concentration range 0.16 to 0.01 M. The pH of the solutions corresponded to the nucleotide with a neutral adenine ring and monodeprotonated phosphate, (AMP^-).

The ultrasound velocity was measured at 25 °C ±0.01 by the resonant method, as described by Garnsey *et al.*[27] The method was improved by controlling the return signal by means of an oscilloscope. This made it possible to adjust the amplitude of the signal to a constant level. The resonance frequency of the x-cut crystals was 9 MHz. The ultrasonic cell was made from glass, with piezoceramic transducers in brass mountings. The cell had a volume of 5 ml, with an ultrasonic path of 5 cm. The accuracy of the ultrasonic velocity was ±1 cm/s, this makes the accuracy of the relativ velocity in the order of 10^{-3}%.

RESULTS AND DISCUSSION

Vapour pressure osmometry cannot give information on equilibrium constants of the charged nucleotides, without coarse approximation of the effect of the ionic interactions on the osmotic coefficients. In the ultrasonic method the effects of interacting forces are reflected in the limiting slope, A_1 (Fig. 1). Stacking constants can be obtained, because the solute–solvent interactions are eliminated as K is determined from the concentration dependence of the difference $(A_1 - \Delta u/uC_T)$. This difference is equal to the decrease in the slope of relative velocity *versus* concentration, resulting from the association of the nucleotides.

The application of the isodesmic model to the charged AMP-systems, can be justified from earlier investigations. The osmotic coefficients of 5'-AMP-methylester compared to NaH_2PO_4 verifies a strong association in water solutions.[8] Also ultracentrifuge studies by Rossetti *et al.*[14,15] show

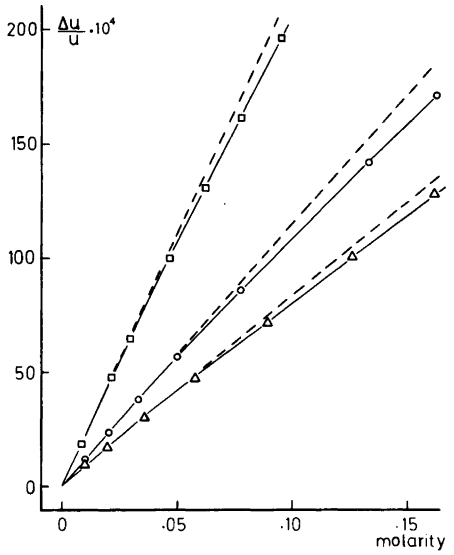

Fig. 1. Relative velocity *versus* concentration at 25 °C for ○ AMP^-, □ $dienPdAMP^+$ and △ $enPdAMP^+$. Dashed lines mark the limiting slopes.

that aggregation occurs beyond the dimer stage.

The results obtained are summarized in Table 1. Our value for AMP^-, $K=2.3\pm0.5$ is in good agreement with values published for AMP^{2-} by Sigel *et al.*[3] $K=2.1\pm0.4$ M^{-1} (27 °C), Neurohr and Mantsch,[12] $K=1.9$ M^{-1} (30 °C) and by Imoto,[28] $K=2.6$ M^{-1} (28 °C). Results for ATP show only a slight decrease in K upon deprotonation of the terminal phosphate.[29]

Comparison of the association constant for $dienPdAMP^+$ with the corresponding value for uncomplexed AMP^- shows an increase in the constant by a factor of 3.5 (*cf.* Table 1). In $Mg(ATP)^{2-}$, Sigel *et al.*[3] found a three-fold increase in going from the uncomplexed ATP^{4-}. In the magnesium complex there is no metal–purine coordination, and hence no intermolecular interaction induced by the metal ion. The behaviour

Table 1. Association parameters and stacking constants.

	$A_1[M^{-1}]$	$\Delta[M^{-1}]$	$K[M^{-1}]$
AMP^-	0.1175	0.0295	2.3 ± 0.5
$dienPdAMP^+$	0.2290	0.0398	8 ± 2
$enPdAMP^+$	0.0865	0.00605	33 ± 6

of dien Pd^{2+}, binding to AMP, has recently been described by Vestues and Martin.[30] The palladium coordinates to the purine ring in AMP, but since it already has three of its four available coordination sites blocked by dien, it is not able to form intermolecular crosslinks between different AMPs. The situation could be comparable to protonation of N(1) in the free nucleotide. Studies of ATP[29] show an almost threefold increase in the association constant upon protonation of N(1).

The ion–ion interactions in solution, and especially the few results reported, make a discussion of A_1, the limiting slope and the Δ quantity very difficult. The $A_1(AMP^-)=0.1175$ M^{-1} is much larger than the corresponding value for adenosine (0.0499 M^{-1}).[22] This is probably a result of the ionic interactions in the AMP solutions. $\Delta(AMP^-)=0.0295$ M^{-1} compared to Δ(adenosine) $=0.0355$ M^{-1}, indicates that the charge of the AMP has only a small effect on the actual association process.

A further treatment of the data for A_1 and Δ, in terms of compressibilities and solvation of the aggregates are of little value, regarding the ionic strength of the solutions.

A very interesting feature arises when comparing the association constant of $enPdAMP^+$, with $dienPdAMP^+$ and AMP^-. An increase in the self-stacking tendency by a factor of 4 and 14, respectively, indicates that there is a very strong additional effect operating, besides the effect of fixing a positive charge onto the adenine ring.

The reaction between $enPd^{2+}$ and AMP has been characterized by means of ^1H NMR.[31] Polymerization occurs within the actual pH-range, with relatively short stacks in the complex of +1 overall charge, and long stacks in the complex of net zero charge. Preliminary results from a single-crystal X-ray study, under progress in this laboratory, seem to confirm this polymerization. The two adjacent coordination sites in $enPd^{2+}$ form bridges between two nucleotides by coordination to N7. Another $enPd^{2+}$ ion bridges from N1 in these two AMPs to N1 in new nucleotides. The polymerization can be described as

$$(-\text{enPd}-\overset{\frown}{\text{N7}}\ \overset{\frown}{\text{N1}}-\text{enPd}-\overset{\frown}{\text{N1}}\ \overset{\frown}{\text{N7}}-\text{enPd}-)$$

It is to be expected that intermolecular interactions of this type would influence the association constant derived from an isodesmic

model. Sigel *et al.*[3] observed similar effects in the reactions between Zn^{2+} or Cd^{2+} and ATP^{4-}. The average association constants derived, with the assumption of the isodesmic model, were 11.1 and ~17, respectively. These values are explained with a high equilibrium constant for the dimerization of two metal $(ATP)^{2-}$ units. The metal ion coordinates to the phosphate of one ATP^{4-} and to N7 of the adenine in the next. The same arguments can be put forward in this case where the $enPd^{2+}$ binds to two adjacent adenine rings. This demonstrates limitations in applying the isodesmic model to get quantitative measurements of the stacking process, but the qualitative arguments still hold.

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