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The self-organization of adenosine 5′-triphosphate and adenosine 5′-diphosphate in aqueous solution as determined from ultraviolet hypochromic effects

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Abstract

The self-association of adenosine 5′-triphosphate (ATP) and of adenosine 5′-diphosphate (ADP) was studied in aqueous solution at different pH values, over the concentration range from 5×10^{-6} to 5×10^{-2} M, by ultraviolet spectroscopy. Measures of the molar absorptivity of the ultraviolet bands of these compounds with increasing concentration have shown two hypochromic effects, at concentrations below 10^{-3} and above 10^{-3} M, respectively. These results can be interpreted in terms of self-association processes involving the formation of dimers and of polymers. From the fitting of the experimental curves of hypochromic effects, self-association constants for dimerization and polymerization were calculated. The results obtained are discussed in relation to the values reported in the literature and indicate the influence of the concentration range not only on the numerical value but also on the order of magnitude of the association constants. Comparison of ATP with ADP shows that the length of the phosphate chain may be a relevant feature in the nature of the self-organization processes in these adenine nucleotides in aqueous solution.

Keywords: Self-organization; Self-association; Adenosine 5′-triphosphate; Adenosine 5′-diphosphate; Hypochromism; Ultraviolet spectroscopy

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1. Introduction

The increasing interest for understanding non-covalent intermolecular interactions, which are of particular importance in the structure and function of biopolymers, has inspired many studies on the self-association of model compounds in aqueous solution. The self-association represents an advanced stage of intermolecular interaction and it can also be regarded as a first step of self-organization, the basis for the ulterior reactivity of these kinds of molecules.

Our studies on self-association of nucleobases, nucleosides and nucleotides [1], and of their corresponding building blocks [2–4], have shown a behaviour of self-association in two steps, which may be interpreted in terms of the formation of dimers and polymers. We consider that it may be worthwhile to extend these studies to the self-association of adenosine 5′-triphosphate (ATP), a singular compound from different aspects. ATP is recognized as a major source of energy for biological activity, maintaining all cellular biochemical reactions and used by higher organisms to power their muscles [5]; therefore, it plays a central role in many biological processes. It is well known that the energy-rich forms of the monomeric units of nucleic acids, such as ATP, can unite to give macromolecular chains, even without the catalytic assistance of enzymes [5], which indicates the interest to study the ability of ATP to self-associate itself. Moreover, the concentration of ATP has been shown to regulate the activity of certain allosteric enzymes [6,7], a fact which could be related to the self-association of ATP. Several studies on self-association of ATP in aqueous solutions have been carried out by different authors [8–19]. In the present paper, the self-association of ATP has been studied by ultraviolet spectroscopy, with the aim of covering a wider concentration range in which the formation of dimers and polymers can be expected to occur, giving hypochromic effects similar to those of the monomeric units of nucleic acids and of the building blocks previously studied by us.

The study of the self-association of ATP is complemented by that of adenosine 5′-diphosphate (ADP), with the objective to test the influence of the length of the phosphate chain in the ability of self-association of these nucleotides, which have adenine as nucleobase. Both compounds were studied at different pH values (acidic, neutral and basic), to test the sensitivity of the self-association to different states of protonation and ionization available to these molecules in aqueous solution. Other complementary experiments were carried out for ATP in pure water, to test the occurrence of self-association without added ions and correlate it with the eventual variation of pH in a non-buffered medium. Self-association of ATP was also studied in water in the presence of Mg^{2+} ions to obtain the $MgATP^{2-}$ complex, which is the active form of ATP in many biological processes, taking also into account that Mg^{2+} has been shown to promote the self-association of this compound [9–11,14,18,19].

2. Experimental

The compounds studied in this work were ATP and ADP, disodium salts, of the best purity available from Boehringer-Mannheim and Aldrich. The experimental method applied to the study of the self-association of these compounds is based on the detection of deviations from the Beer–Lambert law with increasing concentration and has been reported by us previously in a critical study of the application of ultraviolet spectroscopy to the self-association of adenine, adenosine and adenosine 5′-monophosphate (AMP) in aqueous solution [1]. The self-association of each compound was studied over the widest concentration range available, taking into account the limits of absorption. Thus, the range from 5×10^{-6} M to 5×10^{-2} M was covered. The absorbance as a function of concentration has been measured at the wavelength which showed maximum absorption to obtain the greatest accuracy of detection. These measurements were performed usually in the range $0.1 < A < 1.2$, according to the general rule for photometric measurements [20]. Observing this precaution requires the use of cuvettes with various path lengths (4.0 cm, 1.0 cm, 1.0 mm and 0.025 mm)

for the optimization of the values of absorbance for each concentration. It is important to point out that the study of solutions with concentrations higher than 1×10^{-2} M requires the use of cuvettes with a path length as low as 0.025 mm. In contrast, the study of more dilute solutions may be easily carried out with standard cells with more common path lengths, such as 4.0 cm, 1.0 cm and 1.0 mm. The ultraviolet absorption spectra were recorded on a Perkin-Elmer Lambda 6 spectrophotometer with double monochromator, which offers the advantage of a low level of stray light that is significant for the measure of high values of absorbance ($A > 2$). Spectrophotometer functional checks were performed as usual and the absorbance scale was shown to be linear up to 4.0 A with solutions of potassium dichromate. The spectra were recorded with a spectral bandwidth of 1 nm, a scan speed of 20 nm min^{-1} and a response of 1 s. Data acquisition was performed with PECSS software, using a suitable computer on line with the spectrophotometer. The spectra were digitized at 0.5-nm intervals. The values of molar absorptivity given in the figures have been calculated from values of absorbance measured with these experimental criteria and corrected for background absorption, with a reproducibility within 1%. The positions of maxima and shoulders were checked with the second-derivative spectra.

The pK_a values at 25°C of the compounds studied are: for ATP, 1.0; 1.0; 1.7 (phosphate chain); 4.06 (adenine residue); 6.53 (terminal phosphate) and > 12.5 (ribose, estimated from adenosine); for ADP, 1.0; 1.7 (phosphate chain); 3.93 (adenine residue); 6.44 (terminal phosphate) and > 12.5 (ribose, estimated from adenosine); for MgATP, 3.80 (estimated for the adenine residue) and 4.60 (terminal phosphate) [15,21–28]. Thus, ATP and ADP may exist in aqueous solution in different forms depending on pH (Figs. 1 and 2). The self-association of the different species of these compounds, protonated or ionized, may be conveniently studied in solutions with the suitable pH value. Accordingly, solutions were prepared at pH 2.8 with HCl and at pH 11.0 with NaOH, both from Merck-Titrisol, and at pH 6.9 with a standard 0.05 M phosphate buffer, using Milli-Q water. The pH values of all solutions were checked with an accuracy of 0.01 pH, using Ingold electrodes calibrated with buffer solutions at the pH values of 4.00, 7.02 and 9.26, and were

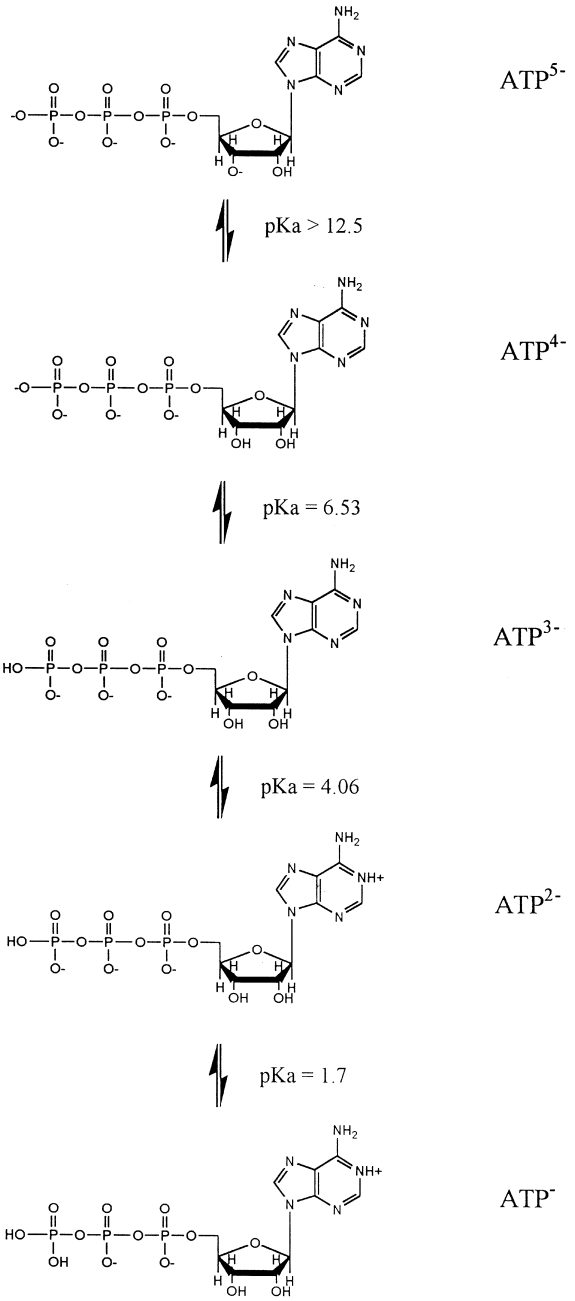

Fig. 1. Equilibria of ionization and protonation of ATP in aqueous solution.

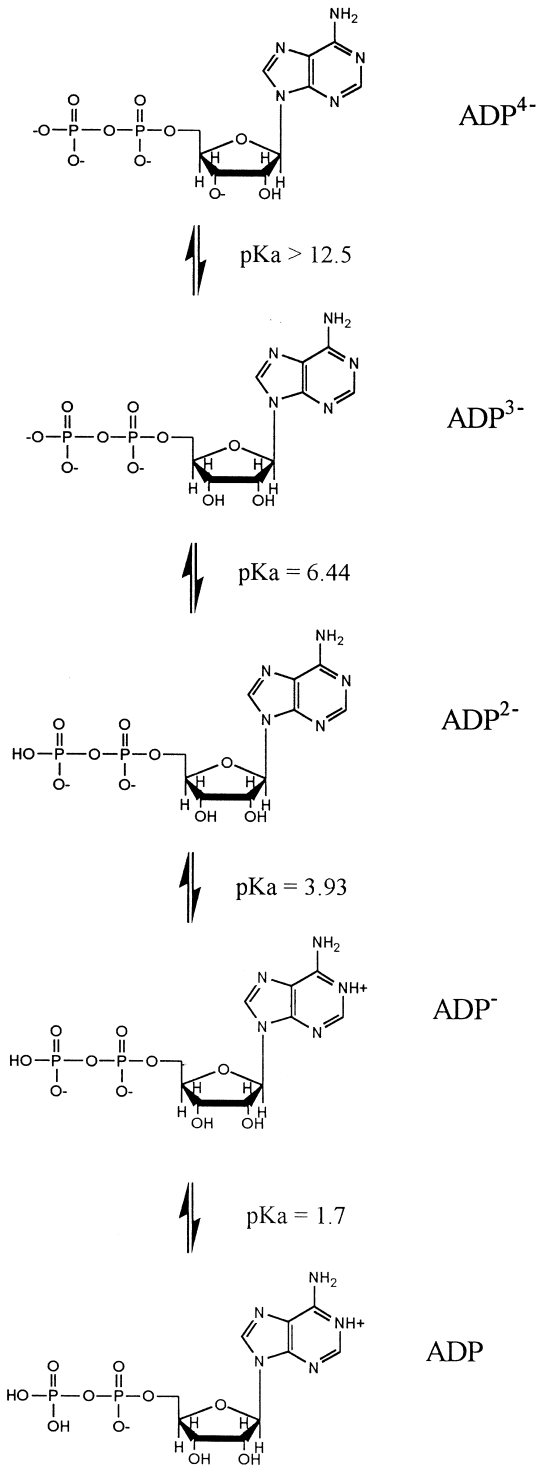

Fig. 2. Equilibria of ionization and protonation of ADP in aqueous solution.

re-adjusted when necessary. For the experiment of self-association of ATP in the presence of Mg^{2+} ions, solutions of this compound were prepared in 0.1 M $MgCl_2$ from Merck.

3. Results

For the compounds studied in this work, hypochromic deviations from the Beer–Lambert law with increasing concentration are observed under all the analysed conditions. There is a first hypochromic effect in dilute solutions ($c < 10^{-3}$ M) and a second hypochromic effect for more concentrated solutions ($c > 10^{-3}$ M). This double hypochromic effect is similar to that observed in other nucleic acid constituents (nucleobases, nucleosides and nucleotides), and several heteroaromatic compounds (derivatives of pyrimidine and imidazole) in aqueous solution, and may be explained in terms of a multiequilibrium of intermolecular self-association of these compounds, as discussed in previous papers [1–4]. Since self-association is still evident in dilute solutions, the first hypochromic effect may be interpreted in terms of the formation of the simplest possible complexes, the dimers

$$M + M \rightleftarrows M_2 \tag{1}$$

where M is the monomer with an equilibrium concentration c_1 and M_2 is the dimer with a concentration c_2. The association model for analysing spectroscopic data has been described in detail previously [1]. The basic relations may be summarized as follows.

Mass balance $$c = c_1 + 2c_2 \tag{2}$$

Association constant $$K_2 = c_2/c_1^2 \tag{3}$$

Beer–Lambert law $$\varepsilon c = \varepsilon_1 c_1 + \varepsilon_2 c_2 \tag{4}$$

Dimer absorptivity $$\varepsilon_2 = 2\varepsilon_1 - 2\delta_2 \tag{5}$$

Interaction parameter

$$\delta_2 = \varepsilon_1 - (\varepsilon_2/2) = \varepsilon_1 - \varepsilon_\infty \quad (6)$$

Combination of these expressions gives the following equation for dimer formation

$$\varepsilon = \varepsilon_\infty + \frac{2(\varepsilon_1 - \varepsilon_\infty)}{1 + (1 + 8K_2c)^{1/2}} \quad (7)$$

where ε, c are the experimental data; ε_1, ε_∞ are the values of molar absorptivity per residue corresponding to monomer and dimer, respectively, and K_2 is the dimerization constant. These parameters may be determined by fitting the experimental curves of the first hypochromic effect.

However, the second hypochromic effect is consistent with the formation of associated species higher than dimers, i.e. 'polymers', assuming an indefinite, sequential scheme [1]. The simplest assumption which can be made is that the association constants for the consecutive steps of aggregation are approximately equal, regardless of the size of the polymer. Consequently, the formation of these polymers may be described by a equation similar to that of the formation of dimers

$$\varepsilon = \varepsilon_\infty + \frac{2(\varepsilon_1 - \varepsilon_\infty)}{1 + (1 + 4K_nc)^{1/2}} \quad (8)$$

where ε_1, ε_∞ are the values of molar absorptivity per residue corresponding to monomer and polymer, respectively, and K_n is the polymerization constant.

Both equations may be used to obtain the values of the parameters characteristic of the processes of self-association, such as association constants. To this purpose, the experimental data

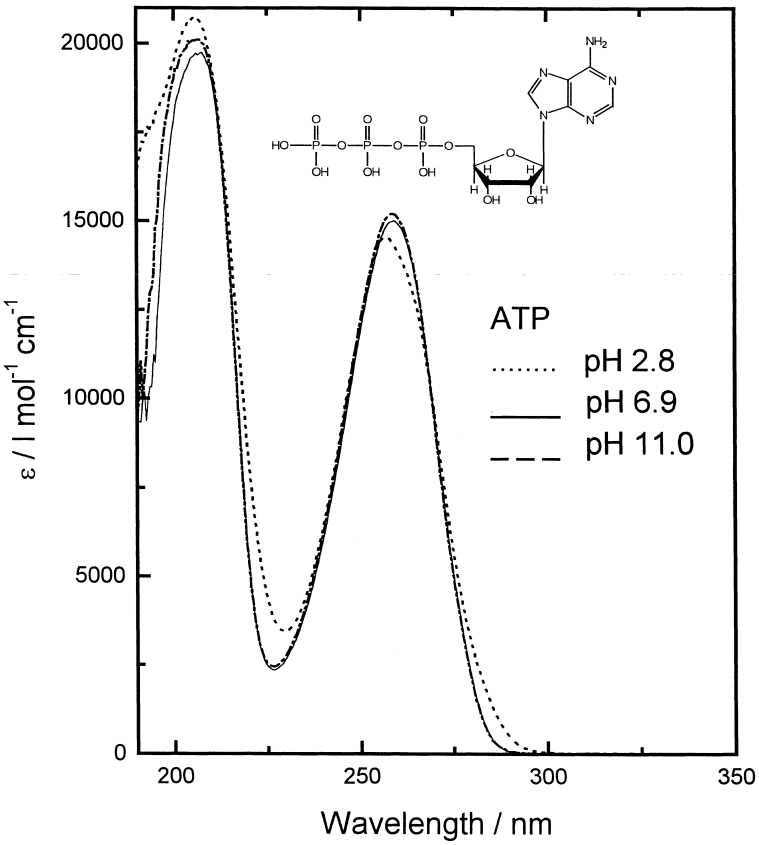

Fig. 3. Ultraviolet absorption spectra of ATP (1.00×10^{-4} M) in aqueous solution, at pH values of 2.8, 6.9 and 11.0.

of apparent molar absorptivity as a function of concentration have been fitted by non-linear least squares regression using the programs 'EnzFitter' from Biosoft and 'Origin 4.1' from Microcal Software. Both programs are based on the Levenberg–Marquardt algorithm and have been adapted for the data analysis with the equations of self-association, yielding identical results. The plots shown in the figures have been obtained directly from 'Origin 4.1'; the curves plotted are those calculated from the fittings.

3.1. Self-association of ATP at different pH values

Ultraviolet absorption spectra of ATP recorded at pH values of 2.8, 6.9 and 11.0, are plotted in Fig. 3. In general, these spectra are quite similar, showing two bands with maxima near 205–206 and 257–259 nm, respectively. At pH 2.8, the adenine ring becomes protonated and a shoulder is observed in the long wavelength side of the band at 257 nm, which can be related to the possible contribution of $n \rightarrow \pi^*$ transitions to this band [29].

The values of molar absorptivity at the maxima of both bands decrease as concentration increases, for all the pH values studied. These hypochromic deviations from the Beer–Lambert law, dependent on concentration, suggest the existence of processes of self-association similar to those reported for other derivatives of purine and pyrimidine [1–4]. The curves of these hypochromic effects are plotted in Fig. 4. The results obtained at each pH value will be discussed separately.

3.1.1. Results at pH 6.9

The study of self-association of ATP at neutral pH is specially relevant to the behaviour of this compound in living systems. The experimental results at pH 6.9 show a double hypochromic effect at 259 nm (Fig. 4b). The value of $\varepsilon_m = 15\,400$ reported in the literature [30] is compatible with our results at a molar concentration near 1×10^{-5} M, but it does not take into account the dependence on concentration observed by us. Calculations of the association constants for the formation of dimers, K_2, and for the formation of 'polymers', K_n, using the method reported above, give the results listed in Table 1. The most striking result is the different order of magnitude of both constants: $K_2 = 8 \times 10^3$ and $K_n = 50$, which is the consequence of the different concentration ranges in which dimerization and polymerization occur. These values are higher than those reported by other authors [9–12,19], but the discrepancy may be explained by the wider concentration range available to ultraviolet spectroscopy with regard to other instrumental techniques. In general, ultraviolet spectroscopy is applicable to solutions more dilute than those accessible to other techniques such as nuclear magnetic resonance, and therefore yields higher values of the constants. To check this point further, our data of concentrated solutions were fitted over truncated intervals, more similar to those studied in other works, and the values of constants so obtained were similar to those in the literature. As an example, the fitting of data points at concentrations higher than 4×10^{-3} M yields the result $K_n = 1.2 \pm 0.7$ M^{-1}, in agreement with the value of 1.3 ± 0.2 obtained for the range 1×10^{-2}–4×10^{-1} M by Scheller and Sigel, using nuclear magnetic resonance (NMR) spectroscopy [11,19]. It may be concluded that the same process of association has been analysed by different techniques. However, the value of the constant of self-association is dependent on the concentration range covered in the experiments.

Measurements of the band with maximum at 206 nm confirm the hypochromic effect occurring at high concentration, assigned to polymerization, but the dispersion of the experimental points at lower concentrations, due to the strong background absorption at low wavelength in the cells used for the dilute solutions, hinders the accurate determination of the first hypochromic effect, assigned to dimerization.

3.1.2. Results at pH 2.8

The study of self-association at acidic pH is interesting since it has been shown that the association is favoured with the protonation of the adenine ring [10,12,15,16]. However, the rele-

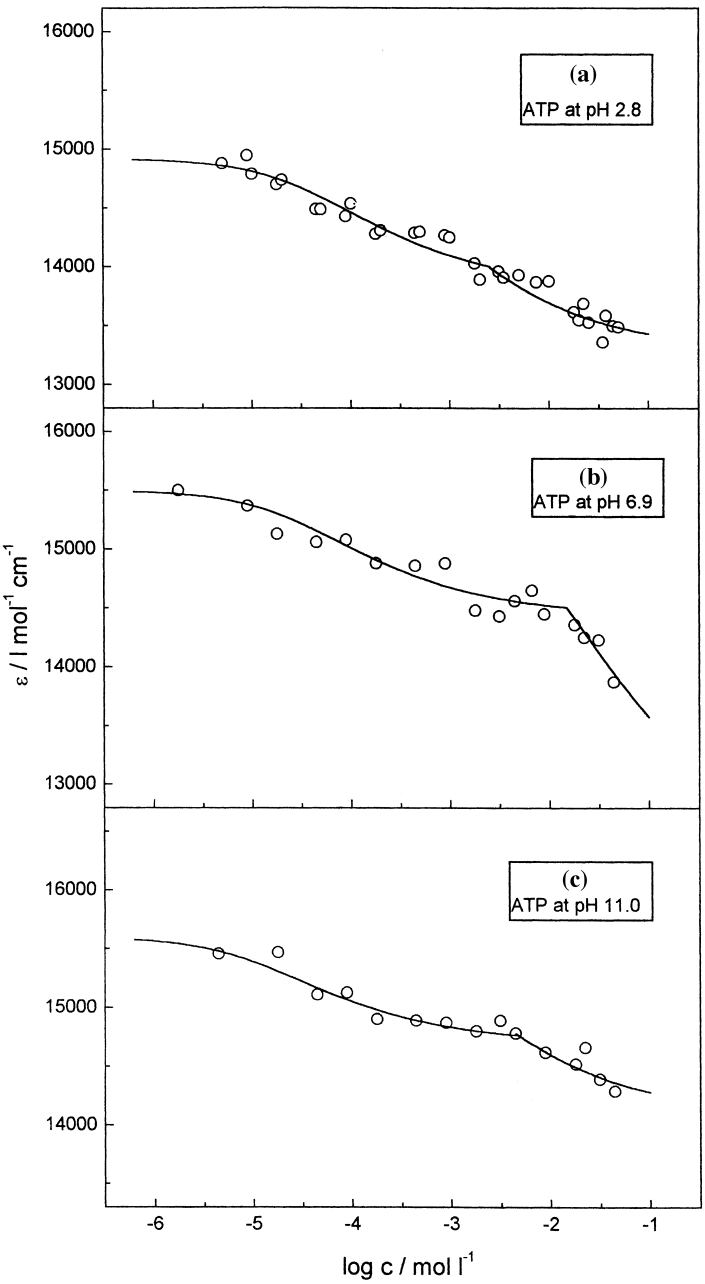

Fig. 4. Curves of hypochromic effects of ATP: (a) pH 2.8, 257.0 nm; (b) pH 6.9, 259.0 nm; (c) pH 11.0, 259.0 nm. The circles are experimental points. The curves have been calculated from the association model involving dimerization and polymerization.

vance of this study to the biological activity of ATP is not so clear as that of neutral pH; in particular, mitochondria, which are the cell's power sources and the places where ATP is synthesized, become inactivated at pH values lower than 4.4 [31].

To test the reproducibility, experiments of self-association were carried out using samples of

Table 1
Wavelengths (nm) and calculated self-association constants (l mol^{-1}) of ATP and ADP in aqueous solution, at different pH values, at 25°C

Compound	pH	λ_{max}	K_2	K_n
ATP	2.8	257.0	$(6 \pm 3) \times 10^3$	$(1.2 \pm 0.8) \times 10^3$
	6.9	259.0	$(8 \pm 6) \times 10^3$	$(5 \pm 4) \times 10^1$
	11.0	259.0	$(2 \pm 1) \times 10^4$	$(6 \pm 2) \times 10^2$
ATP in H_2O	Variable	259.0–257.0	$(5 \pm 2) \times 10^3$	$(4.2 \pm 0.6) \times 10^2$
ATP in $MgCl_2$ 0.1 M	Variable	258.5–256.5	$(8 \pm 4) \times 10^3$	$(7 \pm 2) \times 10^2$
ADP	2.8	256.5	$(7 \pm 6) \times 10^3$	$(10 \pm 4) \times 10^1$
	6.9	259.0	$(5 \pm 3) \times 10^3$	$(5.1 \pm 0.3) \times 10^1$
	11.0	259.0	$(1.0 \pm 0.6) \times 10^4$	$(5 \pm 2) \times 10^1$

ATP from two sources: disodium salt hydrate from 'Aldrich' and crystallized disodium salt from 'Boehringer Mannheim', obtaining excellent agreement between both series. Measurements of molar absorptivity at the maximum at 257 nm indicate a double hypochromic effect similar to that of neutral pH (Fig. 4a,b, respectively). The value of $\varepsilon_m = 14\,790$ reported in the literature [30] is compatible with our results at a molar concentration near 1×10^{-5} M. Calculation of values of K_2 and K_n (Table 1) shows that $K_2 = 6 \times 10^3$ is similar to the value obtained at neutral pH, but $K_n = 1.2 \times 10^3$ is significantly higher, confirming that self-association of ATP is stronger at acidic pH [10]. In this case, the double hypochromic effect may also be observed in the band with maximum at 206 nm.

The fit of the data points corresponding to concentrated solutions, performed over different intervals of concentration, yields good agreement with the results obtained by other authors. Thus, for the range 1×10^{-4} to 5×10^{-2} M, the value of $K_n = 160$ M^{-1} is similar to the value of 158 obtained for the range 1×10^{-4} to 2.2×10^{-2} M using circular dichroism spectroscopy [10]; for the range 1×10^{-3} to 5×10^{-2} M, the value of $K_n = 30$ M^{-1} is similar to the value of 23.0 obtained by spin labelling at pH 4.0 [12]. These results indicate that, independently of pH changes, the value of the association constant may be conditioned by the concentration range available to each technique; when more dilute solutions are included in the fitting, higher values of the constant are obtained. In general, techniques of ultraviolet spectroscopy allow the study of more dilute solutions than those accessible to other techniques such as NMR spectroscopy, vapour pressure osmometry, etc., and therefore tend to give higher constants.

3.1.3. Results at pH 11.0

At pH 11.0, the adenine ring of ATP is neutral, as at neutral pH, but the phosphate chain is fully ionized and the ribose ring is close to becoming ionized (Fig. 1). Therefore, the study of ATP at pH 11.0 should give information about the influence of negative charges on the self-association behaviour of this compound. The curve of molar absorptivity as a function of concentration shows a double hypochromic effect at the maximum at 259 nm, very similar to that observed at neutral pH (Fig. 4c). The value of $\varepsilon_m = 15\,400$ [30] corresponds to a molar concentration near 2×10^{-5} M. The calculated values of the constants (Table 1) indicate that K_2 is similar at all the pH values studied, but $K_n = 6 \times 10^2$ at basic pH is intermediate between the values corresponding to neutral pH and acidic pH. Considering that the self-association should increase in the order $ATP^{4-} < ATP^{3-} < ATP^{2-}$ [15,16], this result suggests that the species $Na(ATP)^{3-}$ is probably involved in the self-association at pH 11.0 instead of ATP^{4-}, due to relatively high concentrations of Na^+ ions. The second hypochromic effect is confirmed by measurements in the band at 205 nm. Comparison with results of other authors is not possible

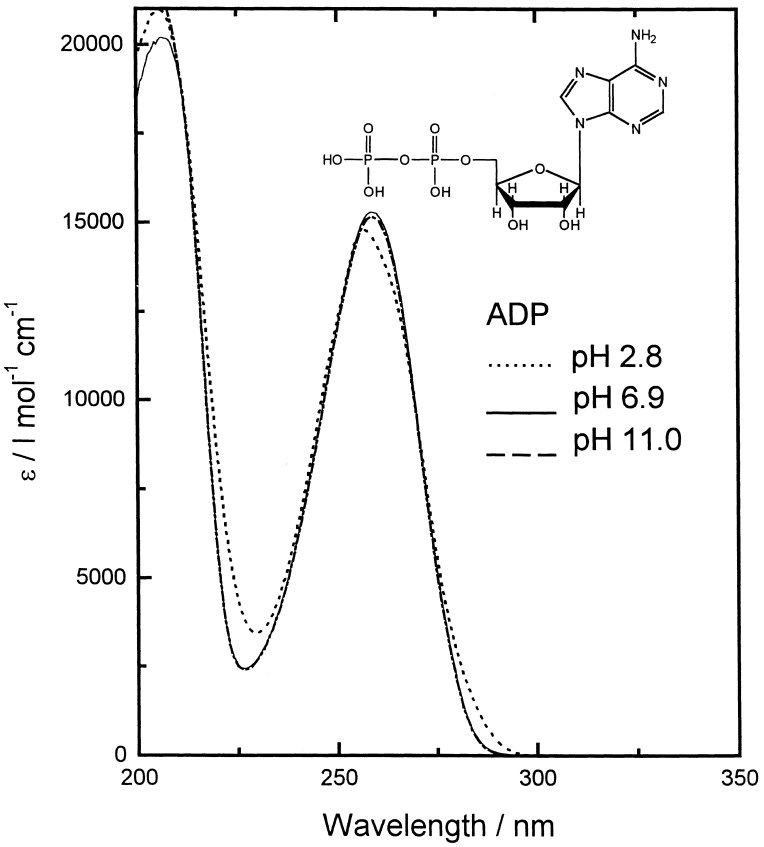

Fig. 5. Ultraviolet absorption spectra of ADP (1.00×10^{-4} M) in aqueous solution, at pH values of 2.8, 6.9 and 11.0.

since values of constants at basic pH have not been found in the literature.

3.2. Self-association of ADP at different pH values

Ultraviolet spectra of ADP at different pH are similar to those of ATP (Fig. 5). The shoulder detected at pH 2.8 could be interpreted in a similar way. Curves of molar absorptivity as a function of concentration are plotted in Fig. 6. The values of $\varepsilon_m = 15\,100$ at pH 2.0 and $\varepsilon_m = 15\,400$ at pH 7.0 and 11.0 [30] are compatible with our results at a molar concentration near 2×10^{-5} M for each of the pH values studied.

3.2.1. Results at pH 6.9

Measurements at the maximum at 259 nm show a double hypochromic effect (Fig. 6b) similar to those discussed for ATP. The calculated constants for dimerization and polymerization are listed in Table 1. The value of $K_2 = 5 \times 10^3$ is similar to that of ATP at this pH, within the experimental uncertainty, and the value of $K_n = 50$ is identical in both compounds. Therefore, at neutral pH the length of the phosphate chain does not exert a significant influence on the polymerization tendency of ADP and ATP.

When the data points at 259 nm corresponding to the concentrated solutions are fitted to polymerization, excluding the data points for dilute solutions, the value of the constant so obtained is $K_n = 10$, very similar to that obtained by spin labelling [12], i.e. $K = 9.6$ M^{-1}. This result confirms that when similar concentration ranges are considered in the fitting, similar values of the association constant are obtained, independently

of the technique used, since the value of the constant is mainly conditioned by the shape of the curve over the interval subjected to the fitting.

The second hypochromic effect is confirmed by measurements in the band at 205 nm. The calculated constant for these data points, over the

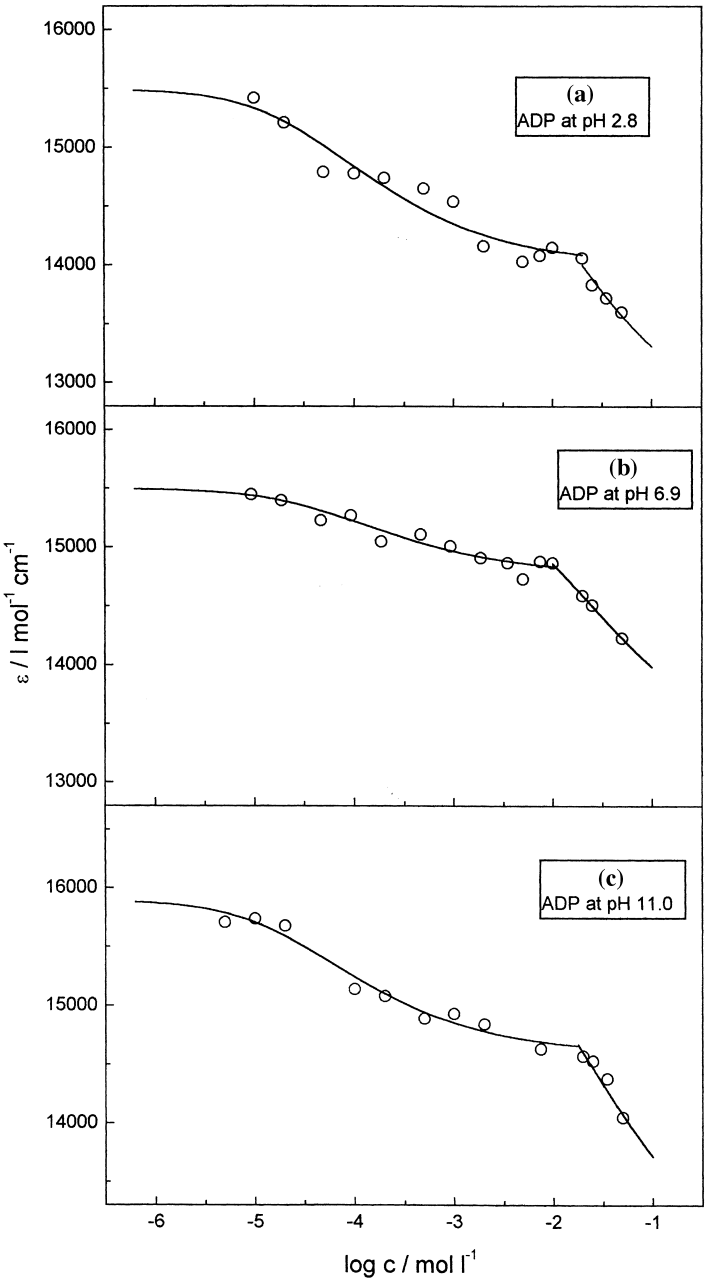

Fig. 6. Curves of hypochromic effects of ADP: (a) pH 2.8, 257.0 nm; (b) pH 6.9, 259.0 nm; (c) pH 11.0, 259.0 nm. The circles are experimental points. The curves have been calculated from the association model involving dimerization and polymerization.

concentration range from 1.8×10^{-3} to 5.0×10^{-2} M, is $K_n = 2 \pm 1$, in good agreement with that obtained by NMR spectroscopy over the concentration range 3×10^{-3}–4×10^{-1} M by Scheller and Sigel [14], that is 1.8 ± 0.5 M^{-1}.

3.2.2. Results at pH 2.8

The curve of molar absorptivity at the maximum at 257 nm as a function of concentration is plotted in Fig. 6a and shows a double hypochromic effect similar to that found in the other cases. The values obtained for the constants are $K_2 = 7 \times 10^3$ and $K_n = 100$ (Table 1), and are higher than those at pH 6.9, confirming that self-association is stronger at acidic pH; however, the comparison with results of ATP discussed above indicates that K_n is higher in ATP. The double hypochromic effect is confirmed by the measurements performed in the band at 206 nm. The fitting of data points at 257 nm corresponding to concentrated solutions yields the value $K_n = 20$, similar to that obtained by spin labelling, which is 22.7 [12].

3.2.3. Results at pH 11.0

The measurements of molar absorptivity at 259 nm also indicate a double hypochromic effect (Fig. 6c). The corresponding values of the constants are $K_2 = 1 \times 10^4$ and $K_n = 50$ (Table 1). Therefore, with relation to neutral pH, K_2 increases at basic pH but K_n is identical in both cases. With relation to ATP at basic pH, K_2 and K_n are higher in ATP. The second hypochromic effect is confirmed by the data obtained at 205 nm. As in the case of ATP, comparison with results of other authors is not possible since values of constants for ADP at basic pH have not been found in the literature.

3.3. Self-association of ATP in pure water

A complementary experience was carried out for ATP in pure water, to remove the possible influence of the ions added to regulate the pH of the medium on self-association of this compound. In this case, a significant variation of pH was detected with increasing concentration of ATP; the pH values decreased from 6.50 at 5×10^{-6} M to 2.92 at 5×10^{-2} M. In this regard, the dependence of the acid–base properties of ATP on its concentration, and therefore on the effect of the self-association, has been discussed by Corfú and Sigel [17].

The curve of molar absorptivity at the maximum at 259–257 nm as a function of concentration is shown in Fig. 7 (open circles). A double hypochromic effect similar to those discussed above is evident. In this case, the first effect occurs with the adenine ring in neutral form and the second effect occurs with the adenine ring in protonated form. When the concentration of ATP in water is increased, the variation of pH is such that the polymerization of ATP can be developed under conditions of optimum pH, which are those of acidic pH.

The values obtained for the association constants in pure water are $K_2 = 5 \times 10^3$ and $K_n = 4.2 \times 10^2$ (Table 1). The last value is lower than that obtained at acidic pH. It may be remarked that in pure water the influence of added ions does not exist, as in the corresponding experiences with buffered solutions at constant pH.

The fitting of the data points of concentrated solutions yields the value $K_n = 70$, which is casually identical to the value $K_2 = 70.2$, obtained by sedimentation equilibrium in a very similar concentration range [8]. The second hypochromic effect is confirmed by the measurements in the band at 206–205 nm.

3.4. Self-association of ATP in water in presence of Mg^{2+} ions

As discussed above, the study of the influence of Mg^{2+} ions is interesting, not only by the physiological significance of the complex with ATP, which is the active form in many biological processes, but also by the promotion of the self-association of ATP, emphasized in several papers [9–11,14,18,19]. In this work, an experiment of self-association of ATP was performed in pure water with added $MgCl_2$ 0.1 M. This salt concentration was chosen to be intermediate between those used by other authors [9,10].

In this experiment, the variation of pH with increasing ATP concentration was significant, as

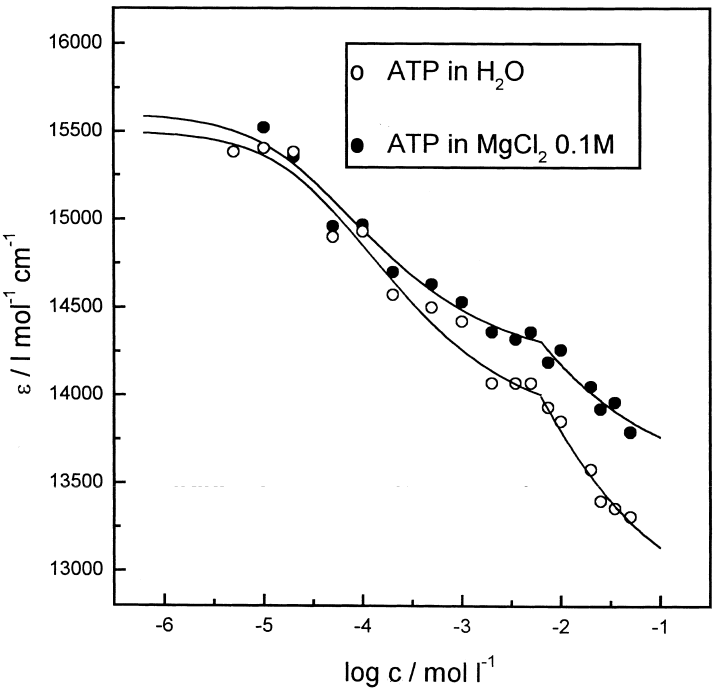

Fig. 7. Curves of hypochromic effects of ATP: (○) in pure water; (●) in water in presence of Mg^{2+} ions.

observed in the experience with pure water above described: from 5.43 at 5×10^{-6} M to 2.43 at 5×10^{-2} M. A double hypochromic effect was also observed at the maximum at 258.5–256.5 nm (Fig. 7, solid circles), in such way that the first effect occurs with the adenine ring in the neutral form and the second effect with the adenine ring in protonated form, as in pure water. As can be observed in Fig. 7, both curves of ATP in water, with added Mg^{2+} ions and without these ions, tend to coincide at infinite dilution. The values of the constants $K_2 = 8 \times 10^3$ and $K_n = 700$, obtained in the presence of Mg^{2+} ions (Table 1), are increased by a factor close to 1.7 in relation to those in pure water, showing the favourable influence of Mg^{2+} in the self-association. With regard to the experiences at constant pH, K_2 is identical to the value at neutral pH, but K_n is lower than the value at acidic pH. It may be concluded from these results that the self-association of ATP is favoured by Mg^{2+} with respect to pure water, although the addition of this ion does not improve the self-association substantially with relation to the experiences in buffered solutions at constant pH. Indeed, the ability for polymerization has been found to be optimum at acidic pH.

The fitting of data points for concentrated solutions, corresponding to acidic pH values, over different concentration ranges, yields values of the constant K_n which are higher than those reported in the literature, obtained in the presence of Mg^{2+}, but using solutions buffered at neutral pH [9,13,14,18]. This result confirms that the pH value is a factor that governs the self-association tendency of ATP in aqueous solution.

4. Discussion

The experimental results discussed above have shown that the occurrence of a double hypochromic effect dependent on concentration is a general phenomenon for ATP and ADP in aqueous solution under all the conditions examined in this work. Indeed, the double

hypochromic effect, indicative of the formation of dimers and polymers, is detected in buffered solutions over all the pH range, and also in non-buffered water, pure or with added Mg^{2+} ions. This behaviour of self-association is not due entirely to the presence of the purine ring as a building block of these nucleotides; in the molecule of purine, a double hypochromic effect is evident at neutral pH, but only the first hypochromic effect is observed at acidic or basic pH. Therefore, the ability of purine to form dimers and polymers is found in the neutral species but not in the charged species [4]. In contrast, the ability to form dimers and polymers under different conditions emerges in the series of adenine, adenosine and 5′-AMP [1], and seems to be initiated by the insertion of an exocyclic amine group at the purine ring and completed by the presence of the phosphate group. For the self-association of ADP and ATP, quantitative variations are observed in relation to these simplest adenine derivatives, especially in the behaviour of polymerization. The influence of the phosphate chain is shown in the increase of the polymerization constant K_n as the chain length increases. Indeed, the ability to polymerization becomes maximum in ATP under optimum conditions, which are those corresponding to acidic pH.

However, the behaviour of self-association of ATP and of ADP in two steps, leading to the formation of dimers in dilute solutions and of polymers in more concentrated solutions, can be considered as indicative of the ability of these compounds for self-organization in aqueous solution, and could be related to the regulation of the activity of certain enzymes by means of the variations of concentration of ATP and ADP which have been shown in several cases [6,7]. In particular, the oscillations in the concentration range from 10^{-2} to 10^{-3} M could be related to the 'plateau' occurring between the two hypochromic effects detected in this paper, i.e. in the transition from the dimers to the polymers, which correspond to two different steps of self-organization. Other oscillations of this kind have been reported for the coenzymes NAD and NADH [7], which also contain the adenine ring in their molecules and undergo self-association processes in two steps [32], similar to those described above for ATP and ADP.

In summary, studies on self-association phenomena in aqueous solution such as those reported in the present paper for ATP and ADP, as well as those of monomeric units of nucleic acids, may provide a conceptual framework to elucidate the rules that govern the self-organization of these compounds towards subsequent processes of structural and functional significance.

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