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Acidity Constants of Adenosine-5′-mono- and diphosphate in Various Water-Organic Solvent Mixtures

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Summary. The acidity constants of adenosine-5′-mono- and diphosphate (*AMP* and *ADP*) were determined at 25.00 ± 0.1 °C by potentiometric titration in pure water and different solvent mixtures (methanol, ethanol, N,N-dimethylformamide, dimethylsulfoxide, acetone, and dioxane). The ionization of *AMP* and *ADP* depends on both the proportion and the nature of the organic solvent used. The pK_{a1} values for both *AMP* and *ADP* are slightly influenced as the solvent is enriched in ethanol and methanol and remains practically constant in presence of different amounts of *DMF* and *DMSO*. A pronounced change in the pK_{a1} values is observed as the solvent is enriched in acetone or dioxane. It is concluded that the electrostatic effect has only a relatively small influence on the dissociation equilibrium, whereas other solvent effects such as solvent basicity, hydrogen bonding and proton-solvent interactions play an important role.

Keywords. AMP; ADP; Water-organic solvents; Acidity constants; Potentiometry.

Aciditätskonstanten von Adenosin-5′-mono- und -diphosphat in verschiedenen organisch-wäßrigen Lösungsmittelgemischen

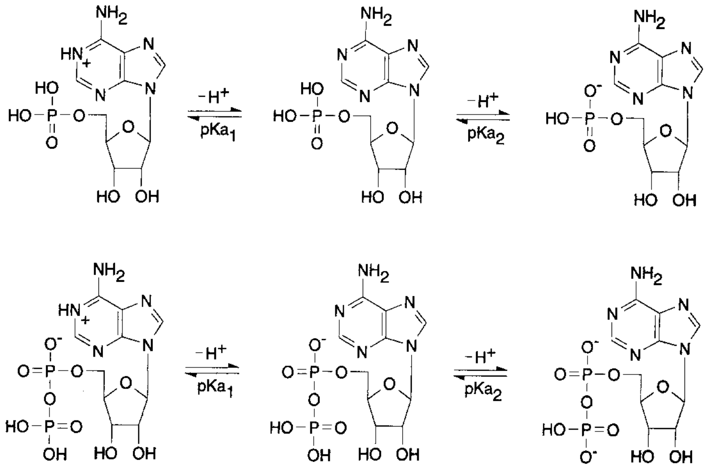
Zusammenfassung. Die Aciditätskonstanten von Adenosin-5′-mono- und -diphosphat wurden bei 25.0 ± 0.1 °C in reinem Wasser und in verschiedenen Lösungsmittelgemischen (Methanol, Ethanol, N,N-Dimethylformamid, Dimethylsulfoxid, Aceton und Dioxan) potentiometrisch bestimmt. Der Ionisierungsgrad von *AMP* und *ADP* hängt sowohl von der Menge als auch von der Art des organischen Lösungsmittels ab. Die pK_{a1}-Werte von *AMP* und *ADP* werden durch Zugabe von Methanol und Ethanol nur wenig, durch verschiedene Mengen von *DMF* und *DMSO* gar nicht, durch Aceton und Dioxan jedoch deutlich beeinflußt. Offensichtlich haben elektrostatische Effekte nur geringe Auswirkungen auf das Dissoziationsgleichgewicht, wogegen andere Faktoren wie Basizität des Lösungsmittels, Wasserstoffbrückenbindungen und Lösungsmittel-Proton-Wechselwirkungen eine bedeutende Rolle spielen.

Introduction

The ribonucleotides adenosine-5′-mono- and -diphosphate (*AMP* and *ADP*) have been considered as biologically important active compounds as they may act as substrates for many enzymic reactions [1–4]. The first and second acid dissociation constants of the disodium salt of *AMP* and *ADP* have been determined from potentiometric data [5]. In previous investigations it has been found that pK_a values of 3.5–4.2 are associated with ionization of the protonated forms of adenine, adenosine, adenosine-5′-monophosphate, adenosine-5′-diphosphate, and adenosine-5′-triphosphate [6–9]. By analogy with aniline (protonated form, $pK_a = 4.78$ [10]) it was stated that ionization stems from the $\overset{+}{N}H_3$ group of the adenine moiety [10–12]. Calorimetric studies [13] provide evidence that ionization of protonated adenine and adenosine involves the aromatic NH group. The second proton ionization was attributed to the phosphate groups. However, the literature is yet scarce of studies concerning the acid-base properties of *AMP* and *ADP* in partially aqueous media. This contribution reports the potentiometric determination of acid dissociation constants of *AMP* and *ADP* in various water-organic solvent mixtures in continuation of the author's work in the field of acidity constant determination of biologically important compounds [14–21]. The organic media used are amphiprotic (methanol), dipolar aprotic (*DMF* and *DMSO*), low basic aprotic (acetone), and non-polar solvents (dioxane).

Results and Discussion

The equilibria to which pK_{a1} and pK_{a2} of *AMP* and *ADP* refer may be illustrated by the following scheme:



The refined pK^*_{a1} and pK^*_{a2} values of *AMP* and *ADP* in different aquo-organic solvent mixtures are given in Tables 1 and 2. The values obtained for pK_{a1} and pK_{a2} in pure water agrees with literature data [5]. Careful examination of the results reveals three important features:

(*i*) With an increase in methanol and ethanol content in the aqueous medium, the change in both pK^*_{a1} and pK^*_{a2} values is rather small. In most cases the values are slightly decreased when the medium is enriched in methanol or ethanol.

(*ii*) With an increase in *DMF* or *DMSO* content, pK^*_{a1} and pK^*_{a2} of *AMP* remain practically constant whereas pK^*_{a2} of *ADP* is slightly increased. The pK^*_{a1} value of *ADP* also remains unchanged.

Table 1. Refined pK^*_{a1} and pK^*_{a2} values of *AMP* (5×10^{-4} *M*) for different mass fractions *w* of organic solvents at 25.00 ± 0.1 °C; S_1, S_2: standard deviations of pK^*_{a1} and pK^*_{a2}

Organic solvent	w	pK^*_{a1}	pK^*_{a2}	S_1	S_2
Methanol	0.00	3.798	6.214	0.012	0.011
	0.10	3.772	6.164	0.021	0.012
	0.20	3.798	6.154	0.022	0.023
	0.30	3.785	6.074	0.011	0.021
	0.40	3.707	6.104	0.014	0.022
	0.50	3.715	6.094	0.015	0.024
Ethanol	0.00	3.798	6.214	0.012	0.011
	0.10	3.731	6.099	0.014	0.015
	0.20	3.687	6.134	0.022	0.014
	0.30	3.698	6.116	0.023	0.014
	0.40	3.733	6.124	0.021	0.016
	0.50	3.701	6.137	0.014	0.013
DMF	0.00	3.798	6.214	0.012	0.011
	0.10	3.790	6.306	0.013	0.012
	0.20	3.770	6.354	0.014	0.011
	0.30	3.788	6.384	0.011	0.014
	0.40	3.785	6.424	0.012	0.014
	0.50	3.779	6.314	0.016	0.011
	0.55	3.792	–	0.018	–
DMSO	0.00	3.798	6.214	0.012	0.011
	0.10	3.774	6.204	0.022	0.014
	0.20	3.706	6.364	0.014	0.022
	0.30	3.786	6.534	0.023	0.023
	0.40	3.792	6.464	0.022	0.022
	0.50	3.788	6.344	0.024	0.021
	0.55	3.790	–	0.020	–
Acetone	0.00	3.798	6.214	0.012	0.011
	0.10	3.870	6.463	0.027	0.012
	0.20	4.053	6.817	0.022	0.027
	0.30	4.088	6.982	0.028	0.022
	0.40	4.158	6.837	0.026	0.024
	0.50	4.319	6.912	0.021	0.025
Dioxane	0.00	3.798	6.214	0.012	0.011
	0.10	3.801	6.372	0.027	0.023
	0.20	3.838	6.613	0.022	0.021
	0.30	4.155	6.814	0.026	0.022
	0.40	4.295	6.863	0.027	0.024
	0.50	4.658	6.925	0.026	0.022
	0.55	4.783	–	0.020	–

Table 2. Refined pK^*_{a1} and pK^*_{a2} values of *ADP* (5×10^{-4} *M*) for different mass fractions *w* of organic solvents at 25.00 ± 0.1 °C; S_1, S_2: standard deviations of pK^*_{a1} and pK^*_{a2}

Organic solvent	*w*	pK^*_{a1}	pK^*_{a2}	S_1	S_2
Methanol	0.00	3.952	6.363	0.021	0.024
	0.10	3.926	6.313	0.024	0.021
	0.20	3.953	6.303	0.022	0.022
	0.30	3.939	6.223	0.023	0.032
	0.40	3.917	6.253	0.021	0.026
	0.50	3.896	6.243	0.011	0.021
Ethanol	0.00	3.952	6.363	0.021	0.024
	0.10	3.942	6.248	0.024	0.022
	0.20	3.924	6.283	0.023	0.024
	0.30	3.913	6.265	0.026	0.020
	0.40	3.830	6.273	0.021	0.021
	0.50	3.703	6.243	0.032	0.030
DMF	0.00	3.952	6.363	0.021	0.024
	0.10	3.942	6.453	0.022	0.022
	0.20	3.932	6.483	0.023	0.017
	0.30	3.950	6.513	0.024	0.018
	0.40	3.951	6.553	0.021	0.016
	0.50	3.944	6.413	0.023	0.018
	0.55	3.930	6.463	0.024	0.011
DMSO	0.00	3.952	6.363	0.021	0.024
	0.10	3.942	6.569	0.030	0.022
	0.20	3.938	6.803	0.031	0.021
	0.30	3.937	7.061	0.027	0.017
	0.40	3.940	6.897	0.021	0.018
	0.50	3.875	6.842	0.020	0.012
	0.55	3.920	6.872	0.022	0.018
Acetone	0.00	3.952	6.363	0.021	0.024
	0.10	3.024	6.612	0.022	0.021
	0.20	4.207	6.964	0.023	0.018
	0.30	4.242	7.146	0.024	0.017
	0.40	4.312	6.993	0.022	0.016
	0.50	4.317	7.053	0.025	0.017
Dioxane	0.00	3.952	6.363	0.021	0.024
	0.10	3.999	6.529	0.025	0.017
	0.20	4.094	6.760	0.024	0.018
	0.30	4.309	6.960	0.022	0.027
	0.40	4.504	7.010	0.021	0.017
	0.50	4.317	7.070	0.028	0.018
	0.55	4.937	–	0.029	–

(*iii*) By increasing the acetone or dioxane content in the aqueous medium, the pK^*_{a1} and pK^*_{a2} values of *AMP* and *ADP* increase.

Effect of amphiprotic solvents. Solvent effects on acid-base equilibria in amphiprotic media are often interpreted in terms of changes in the permittivity (electrostatic effects) and in basicity (non-electrostatic effects). Consider an acid $\mathrm{H}A$ of molarity M in a mixed amphiprotic solvent (SH). It is assumed that the permittivity of the mixed solvent is sufficiently large to permit a certain amount of ionization. The proton activity $a_{\mathrm{H}}{}^{+}$ as a measure of acidity can be expressed in terms of $K_{\mathrm{H}A}$ and $K_{S\mathrm{H}}$, which are the dissociation constants of $\mathrm{H}A$ and $S\mathrm{H}_2^+$, respectively [33].

$$a_{\mathrm{H}} = \sqrt{M \cdot K_{\mathrm{H}A} \cdot K_{S\mathrm{H}} \cdot \frac{{}^{v}S\mathrm{H}_2^+ \cdot {}^{v}\mathrm{H}A}{{}^{v}S\mathrm{H} \cdot {}^{v}A^-}} \qquad 1$$

Here, $v_{S\mathrm{H}^+}$, $v_{\mathrm{H}A}$, $v_{S\mathrm{H}}$, and v_{A^-} are the activity coefficients of the species $S\mathrm{H}_2^+$, $\mathrm{H}A$, $S\mathrm{H}$, and A^-, respectively. A decrease in the permittivity usually causes $v_{S\mathrm{H}_2^+}/v_{S\mathrm{H}}$ and $v_{A^-}/v_{\mathrm{H}A}$ to increase, and it is possible that both of these ratios will increase by about the same amount [35]. Hence, according to Eq. 4, a lowering of the permittivity due to the addition of methanol or ethanol may only have little effect on the acidity of an unbuffered solution of *AMP* or *ADP*.

With respect to solvent basicity effects, the autoprotolysis constant of water is slightly influenced by the addition of methanol or ethanol (pK of pure water is changed from 14.0 to 14.90 $\mathrm{mol \cdot dm^{-3}}$ for a 1:1 ethanol-water mixture). Accordingly, it is deduced that changing the medium basicity by addition of methanol or ethanol to the aqueous medium has little influence on the first and second dissociation stage of *AMP* and *ADP*. Comparing the values of pK_{a1} and pK_{a2} of *AMP* and *ADP* in 0.1 M $\mathrm{KNO_3}$ in water (Tables 1 and 2) with those at the same salt concentration but in mixed water-ethanol or water-methanol solution, it can be seen that in ethanolic or methanolic solution the values of pK_{a1} and pK_{a2} are only slightly changed compared with those for aqueous medium. Such behaviour is common for dipolar ions [35].

The results presented in Tables 1 and 2 with respect to ethanolic solutions can conveniently be discussed in terms of $\Delta G_{(\mathrm{ion})}$ defined as $2.303 \cdot RT \cdot (\log K^{(\mathrm{w})} - \log K^{(\mathrm{s})})$, *i.e.* the difference between the standard free energies of ionization in the mixed solvent and in water [35]. The ionization process can be represented by the general equation

$$\mathrm{H}_2A + S\mathrm{H} \rightleftharpoons S\mathrm{H}_2^+ + \mathrm{H}A^-, \qquad 2$$

where HS denotes a molecule of solvent. Since the solutes involved in the ionization are charged, of the two basic steps in solute solvent interactions, *i.e.* charge transfer and charge separation [35], only the first one will be dominant. Therefore, although the difference in permittivity between pure water and the water-ethanol mixture is appreciable (78.3 *vs.* 49.0 at 25 °C), it will have little effect on the protonation constants of the solutes. Also, the solvation in a mixed ethanol-water solvent should not differ much from that in water, since the structures of the two solvents are similar. Consequently, the two effects will result in relatively small values of $\Delta G_{(\mathrm{ion})}$.

Generally, it was recongized that properties of solvents such as permittivity and acidic or basic strength play a major but not exclusive role. Other factors such as stabilization of the different species existing in equilibrium by hydrogen bonding together with ion-solvent interaction and dispersion forces contribute to the ionization behaviour of weak acids. Accordingly, the observed slight changes in pK_{a1} and pK_{a2} values of *AMP* and *ATP* upon enrichment of the medium in methanol or ethanol can be mainly interpreted as resulting from the following two factors:

(*i*) the relatively high stabilization of the conjugate bases $HAMP^{-1}$ and $HADP^{-1}$ by a donor hydrogen bond in a pure aqueous medium relative to that in presence of methanol or ethanol. This is due to the greater tendency of water molecules to donate hydrogen relative to the other solvent molecules [36]. Thus, an increase in the methanol or ethanol proportion in the aqueous medium will result in an increase in the activity coefficient of the conjugate base, thereby causing a slight increase in the pK_a values.

(*ii*) the greater stabilization of protons in methanol or ethanol-water mixtures relative to pure water by ion-solvent interactions [37, 38]. This generates a low activity coefficient of the proton in alcohol-water mixtures compared to that in pure aqueous medium. A slight decrease in pK_a values is observed.

Effect of dipolar aprotic solvents. The observed constancy in the pK_{a1} and pK_{a2} values of *AMP* and *ADP* in the presence of varying amounts of *DMF* and *DMSO* can mainly be explained on the basis of the following two opposing effects:

(*i*) *DMF*-water or *DMSO*-water mixtures are considered to be more basic than water [39]. This behaviour is based on the formation of a strong acceptor hydrogen bond from the aromatic $-\overset{+}{N}H$ group of the adenine moiety or the $-OH$ group of the phosphate residue of the adenosine-5′-mono- and -diphosphates in the former medium as compared to the latter one, thus facilitating the ionization process, *i.e.* lowering the pK_{a1} and pK_{a2} values.

(*ii*) The stabilization of the conjugate adenosine-5′-mono- and -diphosphate bases by hydrogen bonds donated from solvent molecules in *DMF*- or *DMSO*-water mixtures is low compared to that obtained in pure aqueous medium, resulting in high pK_a values.

Effect of acetone. The presence of acetone as solvent exerts a pronounced effect on pK_{a1} and pK_{a2} of *AMP* and *ADP* as compared to the effect of the other organic solvents studied. The observed increase in the pK_a values when the amount of the organic solvent acetone in the medium is increased can be mainly be attributed to the following effects:

(*i*) Solvent basicity: Acetone is considered to be a very weak basic solvent [40–43]. Therefore, one can expect that the basicity of the solvent-water mixtures is lower than that of a pure aqueous medium [39]. This results in a lower ionization of the phosphate group in the former media as compared to that in the latter one, *i.e.* higher pK_{a2} values.

(*ii*) Solute-solvent interactions effect: Acetone is considered to be both a poorer acceptor and donor of hydrogen bonds compared to water [44]. Thus, one may expect a low stabilization of the free *AMP* or *ADP* bases by hydrogen bonding interactions in presence of this solvent, leading to higher pK_a values in such media.

(*iii*) Proton-solvent interaction effect: It was recognized that the special type of solvation of the H^+ ion plays a vital role in acid-base equilibria [43]. Since acetone is a weakly basic solvent, one must expect that the H^+ ion becomes less stabilized in presence of this solvent. Thus, increasing the acetone proportion in the aqueous medium results in a high activity coefficient of the proton, *i.e.* a high pK_{a2} value.

Effect of dioxane. The observed increase in the pK_{a1} and pK_{a2} values of *AMP* and *ADP* as the solvent is enriched in dioxane may be attributed to a decrease of the permittivity which increases the fraction of associated ions to form *Bjerrum* ion pairs [45] and higher aggregates such as triple ions and dipole aggregates [46]. In this aprotic nonionizing medium, the concentration of free ions is very low, and acidity phenomena are governed largely by ionic association reactions, as *Kolthoff* and *Bruckenstein* have shown convincingly [47].

Experimental

Chemicals and solutions. Adenosine-5′-monophosphoric acid disodium salt ($C_{10}H_{12}N_5Na_2O_7P \cdot H_2O$, ($Na_2AMP \cdot H_2O$)) and adenosine-5′-diphosphoric acid disodium salt ($C_{10}H_{12}N_5Na_2O_{10}P_2 \cdot 2H_2O$, ($Na_2ADP \cdot 2H_2O$)) were purchased from Sigma Chemical Co. and were used without further purification. The amount of free phosphate initially present in the nucleotides was determined [22]. A stock solution could not be kept for extended periods due to hydrolysis. Thus, appropriate samples were weighed individually before each titration and dissolved to obtain the desired concentration. The organic solvents ethanol, methanol, dimethylformamide, dimethylsulfoxide, acetone, and dioxane were of high purity (a.r. or spectrograde). All other chemicals used were of a.r. grade. Sodium hydroxide stock solutions were prepared by diluting concentrated solutions with freshly boiled double-deionized water, which was saturated with purified nitrogen before use. They were systematically checked by *Gran* titrations [23] against standard potassium hydrogen phthalate and proved to be carbonate-free. The molarity of HNO_3 was checked by titration with standard NaOH solution.

Procedure. Potentiometric measurements were performed in a double-walled glass vessel at 25 ± 0.1 °C with a commercial Fisher combined electrode. The *pH* was measured with a Fisher Accumet *pH*/ion meter model 2301 with a precision of ±0.002 *pH* units. The temperature was controlled by a VEB model E3E ultrathermostat bath and maintained constant within ±0.1 °C. Purified nitrogen was bubbled through the solution in order to maintain an inert atmosphere. Efficient stirring of the solution was achieved with a magnetic stirrer. All solutions were prepared in a constant ionic medium of 0.1 *M* KNO_3 by mixing the appropriate amounts of ligand, nitric acid, and potassium nitrate solution. The concentration of *AMP* and *ADP* in solution was 5×10^{-4} *M*. All aqueous solutions of organic solvents were prepared by adjusting the total volume to 50 cm^3 with double-distilled water. The concentration of hydrogen ions was decreased by the addition of sodium hydroxide, prepared in the ionic medium used for the solution. At each solvent percentage, at least 4 titrations (50 data points each) were performed. In all cases the titration was completed in a short time to avoid errors due to dephosphorylation.

The pH-meter readings have been corrected in accordance with the method described by *Douheret* [24, 25]. This was carried out to account for the differences in acidity, basicity, permittivity and ion activity in partially aqueous solutions relative to the pure solvent.

The concentration of free hydrogen ions, *h*, at each point of the titration was calculated from the measured *emf*, *E*, of the cell *RE*/*TS*/*GE* (*RE* and *GE* denote the reference and glass electrodes, respectively) from the *Nernst* equation

$$E = E^0 + Q \log h, \qquad 3$$

where E^0 is a constant which includes the standard potential of the glass electrode and Q is the slope of the glass electrode response. The value of E^0 for the elecrode was determined from a separate titration of nitric acid with potassium hydroxide, both of known concentration, under the conditions of the test solution titrations. The data obtained were analyzed by the program MAGEC [26]. During the MAGEC calculation, the autoprotolysis constant of water, K_w, was refined until the best value for Q was obtained (58.2 mV at 25 °C). This indicates the reversible response of the glass electrode used.

Initial estimates of pK_a values were refined using the ESAB2M computer program [27] by minimizing the error squares sum

$$U_v = \Sigma_i W_i(V_i - V_{calc,i})^2, \qquad 4$$

where V_i and $V_{calc,i}$ are experimental and calculated volumes of the titrant for every point i of the titration curve, respectively. The weight is calculated by

$$\frac{1}{w_i} = S_i^2 = S_v^2 + (\delta V_i/\delta E_i)^2 \cdot S_E^2, \qquad 5$$

where S_i, S_v, and S_E are estimated variance, estimated standard deviation in titrant volume and estimated standard deviation in titrant potential, respectively. The titrant volume V_{calc} can be calculated from Ref. [28, 29]. The program ESAB2M minimizes equation 4 by using the *Gauss-Newton* nonlinear least squares method [30] with the very efficient *Levenberg-Marquardt* algorithm [31, 32]. Our calculations have been performed with the assumption that $S_v = 0.005$.

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