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On the role of arginine–glutamic acid ion pair in the ATP hydrolysis

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Abstract

The complex between adenosine triphosphate (ATP) and 4-guanidinobutyric acid (GBA) has been studied by infrared spectroscopy dry and hydrated (60% relative humidity). Partial nonenzymic hydrolysis has been detected, as deduced from characteristic bands of adenosine diphosphate (ADP) and inorganic orthophosphate formation. An infrared continuum, which increases upon hydration, demonstrates that the hydrogen bonded system in this complex has a large proton polarizability due to collective proton fluctuation. On this basis, a mechanism for splitting of lytic water molecules is also discussed.

Keywords: ATP hydrolysis; Fourier transform infrared spectroscopy; Hydrogen bonding

1. Introduction

ATP is a nucleotide which is involved in two reactions, ATP hydrolysis and ATP synthesis. The first reaction type occurs when metabolic conditions dictate, generating a proton gradient to support nutrient uptake and locomotion. The second occurs in mitochondria, chloroplasts and bacteria, and is driven by proton gradient energy. In the past, virtually all researchers working on this field have assumed that the two reactions are reversible using the same reaction intermediates although there are some arguments for writing separate, non-reversible schemes [1]. The hydrolysis reaction has been studied in far greater depth and detail because of its comparative ease of manipulation and experimental study. Arginine and glutamic acid are found in crystal structures of ATPase pockets, where they form ionic bonds with one another during the process of ATP hydrolysis [2–5]. The detailed mechanism of ATP hydrolysis is still unclear. Experiments on site-directed mutation of Arg238 and Glu459 in the myosin ATPase pocket [6–9] showed that substitution of Ile, Ala, or Glu for Arg causes a decrease in ATPase activity, and that the substitution of Arg or Ala for Glu also causes a decrease in ATPase activity. However, ATPase activity of R238K mutant myosin was very similar to that of wild-type myosin [7]. Furthermore, the double substitutions of Glu and Arg for Arg and Glu exhibited the same ATPase activity as that of wild-type myosin [8,9]. These experimental findings suggest that the formation of ionic bonds between Arg238 and Glu459 of myosin ATPase is essential for the initiation of ATP hydrolysis. Also, in F_1F_0–ATP synthase there is hydrogen bonding between β-Arg246 and β-Glu181 [3]. Mutagenesis of Glu181 in β subunit of F_1F_0–ATPase has very large impairing effects on catalysis, reducing hydrolysis by three or more orders of magnitude, e.g. in the Gln mutant [10,11]. In addition, deleterious effects on catalysis were found for the β-Arg246 to Cys mutation. These investigations of F_1F_0–ATPase seem to reveal that the above Glu181 and Arg246 amino acid residues are very important for catalysis.

A question arises as to the reasons why hydrogen bonding between arginine and glutamic acid residues plays a significant role in ATP hydrolysis. In this connection, it is of interest to know the detailed molecular interactions of ATP when bound to model molecules having arginine–glutamic acid ion pair, as occurs for 4-guanidinobutyric acid (GBA). Consequently, we report here the properties of hydrogen bonds formed between ATP and GBA as a function of the degree of hydration as studied by infrared spectroscopy.

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2. Experimental

Adenosine 5′-triphosphate disodium salt (99% min. purity), 4-guanidinobutyric acid and deuterium oxide (D_2O, 99.9 at.% D) were purchased from Sigma Co., and $NaH_2PO_4{\cdot}H_2O$ and $CH_3COONa{\cdot}3H_2O$ were acquired from Fluka.

Fourier transform infrared spectra (FTIR) were measured in a Perkin Elmer 1725X spectrometer equipped with a DTGS detector. The liquid samples were placed in home-demountable cells with ZnSe windows with 12 μ spacer, and the infrared cell for the study of solid films at different relative humidities is of the same kind as those described in other works [12,13]. Film specimens of ATP and of equimolar mixtures of ATP with GBA in the solid state were cast from 50 mM aqueous solutions having pH at 5.7 and 7.3, respectively. After being dried under vacuum for 2–3 h, the specimens were equilibrated over saturated salt solutions at the desired humidities. A 25 mm cylindrical cell as described elsewhere [12,13] was prepared to include a small reservoir, which contained saturated salt solution. The films were cast on one of the removable ZnSe windows and equilibrated for at least 2 h. After the spectra were recorded, the sample window was replaced by a ZnSe blank, and the spectra of the moist air were also recorded. These spectra were then subtracted from those of the specimens. To eliminate spectral contributions due to atmospheric water vapour, the spectrometer was purged with dry air. Spectral contributions from residual water were eliminated using a set of water vapour spectra measured under identical conditions. The subtraction factor was varied until the second derivative of the absorption region above 1700 cm^{-1} was featureless, thereby avoiding artificial bands and/or incorrect band positions in the 1700–1400 cm^{-1} region. All spectra were obtained at room temperature with a 2 cm^{-1} resolution by averaging 64 scans. GBA deuterated in both guanidine and carboxyl moieties was prepared by repeated exchanges with D_2O. Infrared analysis showed that deuteration reached levels higher than 99%. Signals obtained were fed to a microcomputer for storage, display, plotting and processing, and the manipulation and evaluation of the spectra were carried out using the GRAMS/AI software (ThermoGalactic).

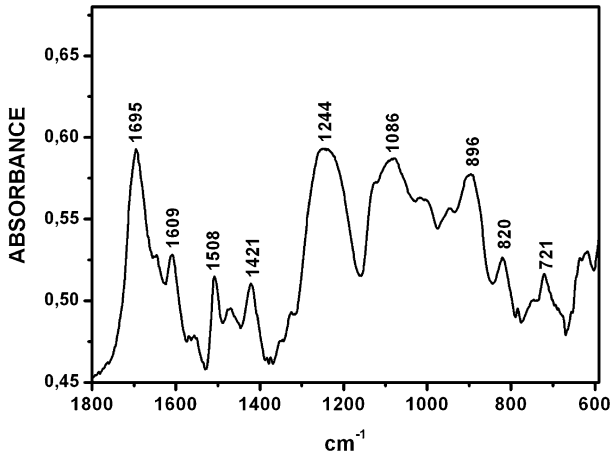

Fig. 1. Infrared spectrum of a dried ATP film.

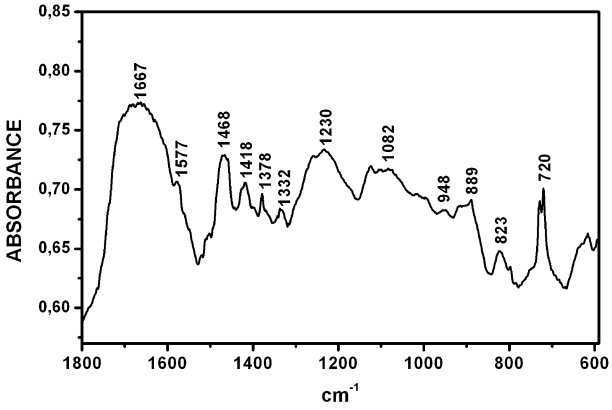

Fig. 2. Infrared spectrum of a film of dried ATP–GBA complex.

Concentration of orthophosphate anion (P_i) resulting from ATP hydrolysis was measured by a spectrophotometric test at 712 nm (kit of the Spectroquant orthophosphate test from Merck, Cat. No. 1.00798). Briefly, in sulfuric solution orthophosphate ions react with molybdate ions to form molybdophosphoric acid. Ascorbic acid reduces this to phosphomolybdenum blue that is determined spectrophotometrically.

3. Results and discussion

Fig. 1 shows the infrared spectrum of a film of ATP. The absorption bands on the wavenumber between 1800 and 1480 cm^{-1} basically belongs to the adenine nucleobase vibrational modes involving in-plane motions of pyrimidine and imidazole rings [14]. A very strong band is located at 1244 cm^{-1}, which is characteristically generated by the $\nu_{as}PO_2^-$ vibration in ATP. By contrast, the analogous vibrational mode of adenosine 5′-diphosphate (ADP) appears usually in the 1215–1230 cm^{-1} range [15,16]. Other typical phosphate bands of ATP appear at 1086 and 896 cm^{-1} which are attributable to the $\nu_sPO_2^-$ and ν_{as}P–O–P vibrations, respectively [15,17].

When ATP is bound to GBA, significant spectral changes occur which are indicative of the presence of ADP resulting from ATP hydrolysis. Thus, the infrared spectrum of an equimolar mixture of these interacting compounds shows that the $\nu_{as}PO_2^-$ vibrational mode is found here at 1230 cm^{-1} (Fig. 2, Table 1), which results from the well known frequency downshifting of the $\nu_{as}PO_2^-$ vibration upon ADP formation from ATP [15,16]. That ATP hydrolysis occurs to some extent in the presence of GBA is also indicated by upshifting of the ν_{as}P–O–P band in going from ATP (896 cm^{-1}) to its complex with GBA (919 cm^{-1}), as described elsewhere for nonenzymic hydrolysis of ATP [15]. The absorption increasing near 950 cm^{-1} relative to the 919 cm^{-1} band has been also reported to be indicative of the presence of ADP [15,17]. Other noticeable spectral change occurring upon ATP–GBA complex formation concerns the intensity decreasing of the 1508 cm^{-1} band, assigned to in-plane adenine ring vibration, and the concomitant intensity increase of the band located near 1468 cm^{-1}. The ATP absorption increase at this frequency can not be explained by the GBA band near 1465 cm^{-1}, which shows weak intensity (Fig. 3, Table 1). In addition, this change is also

Table 1
Infrared bands (cm^{-1}) of ATP, GBA and their complex in the solid state

ATP[a]	GBA[b]	ATP–GBA	Tentative assignment
1695 s	1692 s (1230 s)	1695 sh	δNH_2
		1667 vs, br	δNH_2
1646 w			$\nu C{=}C$, $\nu C{=}N$
	1624 vs (1590 s)		$\nu_{as}CN$
1609 m			ν (ring) (Pyr)
	1598 s (1560 m)		$\nu_{as}CN$
1565 w		1577 sh	ν (ring)
1508 m		1508 sh	ν (ring)
1468 w		1468 m	δCH_2 (ribose), ν (ring)
	1465 w (1477 m)		δCH_2
	1426 m (1426 m)	1426 sh	δCH_2
1421 m		1418 w	ν (ring) (Im)
	1397 s (1392 vs)	1400 sh	$\nu_s(COO^-)$
	1375 m (1373 m)	1378 m	$\nu_s(COO^-)$
	1324 m	1332 w	νCN, tCH_2
1244 vs		1230 s	$\nu_{as}PO_2^-$
	1240 w		rNH_2
	1185 m		
	1156 m (1156 m)		tCH_2
1126 sh		1126 m	δCH (ring)
	1112 w		rNH_2
1086 vs		1082 s	$\nu_sPO_2^-$
1015 m		1015 m	νCC, νCO (ribose)
1002 sh		997 sh	νCC, νCO (ribose)
	983 w (985 vw)		νCC
945 w		948 m	$\nu_{as}P{-}(OH)_2$, $H_2PO_4^-$
	925 w (925 w)		νCC
896 s		919 m	$\nu_{as}P{-}O{-}P$
	871 m (772 w)	889 m	tNH_2
	835 m (823 m)		wNH_2
820 w		823 w	$\nu_sP{-}O{-}P$
	748 w (723 w)		δCOO^-
721 m		729 m	ν (ring)
		720 m	ν (ring)

[a] Assignments based on Refs. [14–18].
[b] Assignments based on group frequencies and *N*-deuteration.

accompanied by the appearance of the 729 cm^{-1} band in the ATP–GBA complex, which can be assigned to the ν_{10} ring breathing vibration of adenine residue [14]. Frequency downshifting of this band is observed upon protonation on the N1 atom of adenine pyrimidine ring. Therefore, these spectroscopic results can be interpreted in terms of losing of the proton

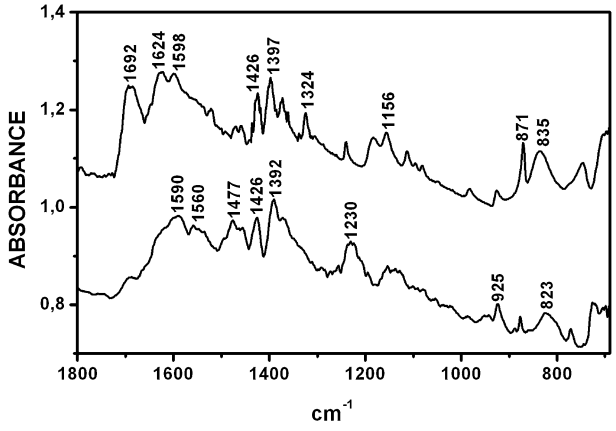

Fig. 3. Infrared spectra of dried films of GBA (*upper*) and *N*-deuterated GBA (*lower*).

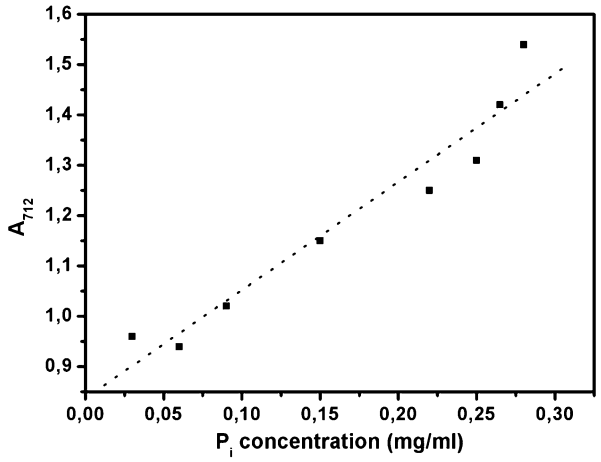

Fig. 4. Calibration plot of absorbance at 712 nm against P_i concentration. Linear regression equation: $A_{712}=2150P_i+0837$. $R=0.97$, SD=0.061.

located on the N1 site of adenine ring, because the pK_a of this adenine proton acceptor is lower for ADP (~3.9) as compared with ATP (~4.1) [17]. Accordingly, this proton moves likely to the terminal phosphate of ADP, which is consistent with the relative increasing of the infrared absorption near 950 cm^{-1} generated by $\nu_{as}P{-}(OH)_2$ motions. No significant spectral features indicative of ADP and inorganic phosphate (P_i) formation are visible for equimolar mixtures of ATP with butyric acid sodium salt. This suggests that the said spectral changes can be attributable to the arginine–carboxylate ion pair of GBA. Finally, the fact that ATP hydrolysis occurs under the influence of GBA is also supported by the P_i that we have detected according to the photometric test described above (Fig. 4). The amount of P_i detected is about 20% of the expected from an equimolar aqueous mixture of ATP and GBA. This may be due to the lack of appropriate orientation for all of the interacting molecules leading to hydrolysis, unlike what occurs in the enzyme pockets.

As to the mechanism by which orthophosphate splitting in ATP occurs, this must involve obviously hydrogen bonding which leads to splitting of a water molecule. Fig. 5 shows the

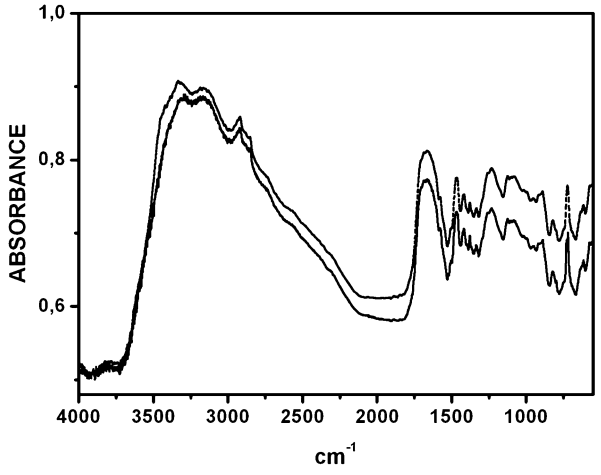

Fig. 5. Infrared spectra of ATP–GBA films thoroughly dried (solid line) and at 60% relative humidity (dashed line).

spectra of the ATP–GBA system. The solid line is the dry, and the dashed line the hydrated system. There can be several kinds of hydrogen bonds in this system. One corresponds to POH···$^{-}$OP ⇆ PO^{-}···HOP hydrogen bonds, where donor and acceptor are the same type of groups and a largely symmetrical double minimum proton potential is present due to the relatively small difference of pK_a ($\Delta pK_a < 3$) between acceptor and donor [17]. These hydrogen bonds have been reported to have large proton polarizability [17,18] which generates broad absorption continua in the infrared spectra, as occurs for the dry system included in Fig. 5. These infrared continua have been found also for POH···$^{-}$OOC ⇆ PO^{-}···HOOC hydrogen bonds [18,19], which may be present in the ATP–GBA system. A third type of hydrogen bonding in this system involves guanidinium moiety of GBA whose positive charge is attracted by the phosphate and carboxylate negative charges (Fig. 6). Both guanidinium–phosphate and guanidinium–carboxylate hydrogen bonds are not expected to cause absorption continua because their proton potentials are very asymmetrical ($\Delta pK_a > 7$) [18]. However, addition of water leads to a largely symmetrical system such as that included in Fig. 6, for the following reasons. The acidity of water is comparable to that of the protonated guanidine group, and the same can be said for the acidity of the ATP phosphate groups that interact with water and guanidinium moiety. Although the acidity of groups within the hydrogen bonded system of Fig. 6 is not the same, the whole system can show a large proton polarizability due to the strong coupling of the proton motion. Of course the symmetry of the above system would be similar if an additional water molecule is inserted between phosphate and guanidinium moieties. This is demonstrated by the infrared spectrum of the hydrated ATP–GBA system which shows a stronger absorption continuum as compared with the dry system (Fig. 5). Thus, due to the large proton polarizability of this system, a proton can easily be shifted by changes of the local electrical fields or by specific interactions, resulting in a protonation of the phosphate group acceptor and formation of a hydroxyl group which is inserted into the terminal phosphate (Fig. 6). The said changes of the electrical fields may be generated either by appropriate conformational changes to configure the active site for facile hydrolysis of ATP and by magnesium ion which is present in the enzyme active site [1–5]. Our spectroscopic results are consistent with the role of arginine–glutamic acid ion pair that has been suggested by other authors [5]. Thus, in the ATPase pocket of myosin there is a gate for release of phosphate, a product of ATP hydrolysis, and for trapping of water molecules. This gate is closed and opened with the formation or disappearance of ionic bonds between the side chains of Arg238 and Glu459. When ATP and myosin combine, water molecules are trapped which subsequently interact with Arg238 and Glu459 side chains and with the phosphate groups of the ATPase pocket, and this triggers the formation of ionic bonds between Arg238 and Glu459 by electrostatic interactions. As a consequence, the ATP hydrolysis reaction starts without any influence of bulk water molecules [5]. This spectroscopic finding is consistent with other investigation in relation to the role of Arg-789 of GTP-binding protein Ras on the hydrolysis of GTP, where this amino acid residue is considered a key charged residue which favours the hydrolytic phosphate β–γ bond cleavage [20].

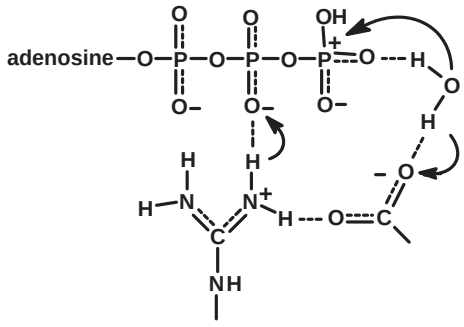

Fig. 6. Structure of the hydrated relay system, having a large proton polarizability, which may be involved in the ATP hydrolysis.

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