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

Pedro Carmona [a,*], Marina Molina [b], Arantxa Rodríguez-Casado [a]

[a] Instituto de Estructura de la Materia (CSIC), Serrano 121, 28006 Madrid, Spain
[b] Escuela Universitaria de Optica, Departamento de Química Orgánica I, Arcos de Jalón s/n, 28037 Madrid, Spain

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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.
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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.

* Corresponding author. Tel.: +34 915616800; fax: +34 915645557.
E-mail address: p.carmona@iem.cfmac.csic.es (P. Carmona).

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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).

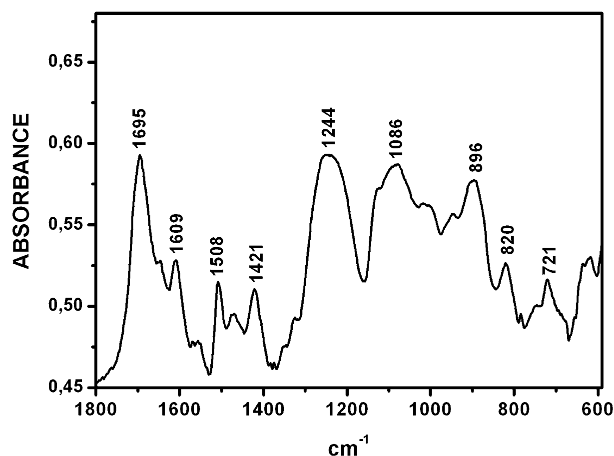

Fig. 1. Infrared spectrum of a dried ATP film.

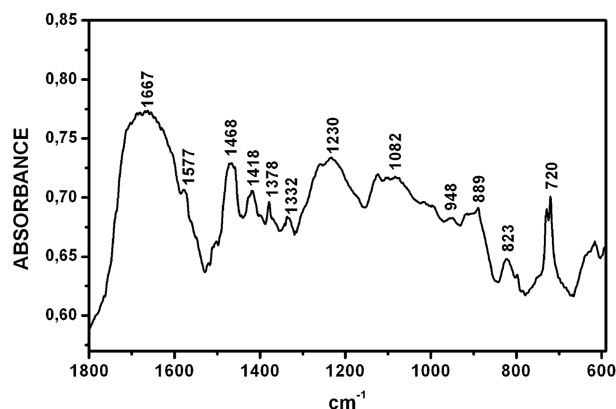

Fig. 2. Infrared spectrum of a film of dried ATP–GBA complex.

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

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