Review

The dynamics of the catalytic site in small GTPases, variations on a common motif

Carsten Kötting, Klaus Gerwert*

Lehrstuhl für Biophysik, Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum, Germany

A R T I C L E   I N F O

Article history:
Received 6 May 2013
Accepted 6 May 2013
Available online 16 May 2013

Edited by Alexander Gabibov, Vladimir Skulachev, Felix Wieland and Wilhelm Just

Keywords:
Time resolved spectroscopy
FTIR
Protein
GTPases
Reaction mechanism

A B S T R A C T

Small GTPases control many cellular processes. Their catalytic downregulation by GTPase activating proteins (GAP) is essential. Many structural models of GTPase GAP complexes obtained by X-ray structural analysis are available nowadays. They reveal important insights into the catalytic site and can suggest important catalytic residues. But this information is static. Time-resolved FTIR spectroscopy can resolve the dynamics of the catalytic site at atomic detail. For the investigation of GAP catalyzed GTPase reactions of small GTPases, the order of events like the action of certain catalytic amino acids, bond breakages and protein conformational changes can be elucidated. This is elaborated for many small GTPases like Ras, Rap, Ran, Rho and Rab and their cognate GAPs. Variations on a common dynamic motif of the catalytic site of small GTPase will be presented.

© 2013 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.

1. Time resolved FTIR spectroscopy of small GTPases

Small GTPases are molecular switches that regulate many cellular events [1,2]. Usually they exist in a GTP bound "on" state and a GDP bound "off" state [3]. The switch-on is accomplished by exchanging GDP by GTP which is catalyzed by guanine nucleotide exchange factors. The switch-off is achieved by GTP hydrolysis. GTP hydrolysis in water is very slow with a life time of about 200 days. Bound to Ras, the intrinsic GTP hydrolysis has a life time of 20 min [4]. The GAP proteins can further accelerate hydrolysis by forming a protein–protein complex, in the case of Ras the life time is then about 50 ms [5]. Mutations which interfere with GTP hydrolysis are frequent in various tumors [6].

In order to understand this catalytic mechanism the determination of the three-dimensional structure is an important milestone [3]. This structure is usually obtained by X-ray structure analysis. X-ray structural analysis is a very powerful technique, however it does not provide full atomic resolution, for example hydrogen atoms are not resolved, and it shows the structure in a crystalized mostly low temperature frozen state. In order to provide not a "picture" but a "movie" of the dynamics of the catalytic site, especially the dynamic interplay with the GAP protein, time-resolved methods need to be applied. Time-resolved FTIR can provide a movie of the molecular reaction mechanism at atomic detail.

Time resolved FTIR spectroscopy is a valuable tool for the investigation of reaction mechanisms [7–10]. It provides marker-free detailed insights into the biochemical reactions and conformational changes within a protein. In order to use FTIR spectroscopy for the investigation of the reaction mechanism of proteins, one has to become selective for the active residues in the catalytic centers. A small GTPase with about 2000 atoms has about 6000 vibrational modes, which produce a large background absorbance as compared to the tiny bands of the few groups involved in the reaction mechanism. Only if the measuring conditions are maintained exactly the same during the whole measurement (temperature control, vibrationally isolated table, vacuum spectrometer) the background will stay unchanged and the very small absorbance changes during a reaction can be separated. To avoid baseline drifts, the reaction needs to be started by a sharp trigger without removing the sample from the apparatus. In the case of GTPases, photolabile caged-GTP, which already binds to the GTPase but cannot be hydrolyzed, can be used [11]. Depending on the necessary time resolution, the 3-1-(2-nitrophenyl)ethyl ester (NPE) [12] or the 3-para-hydroxyphenacyl ester (pHP) [13] of GTP are used. The GTPase loaded with caged-GTP is stabilized within the spectrometer, then GTP is formed by a laser flash and the subsequent hydrolysis reaction can be studied. Finally, the bands found in the difference spectra must be assigned to the functional groups of the protein and the substrate. This can be done by isotopic labeling [14,15] or by site directed mutagenesis [16]. Once a certain IR band is assigned to a specific group it can be used as a marker band. Time-resolved measurements of its absorbance changes will...
provide the mechanism. Here we exemplify this method by the time resolved FTIR measurements of the Ras GTPase reaction catalyzed by the GTPase activating protein (GAP)-domain of NF1 [17,5].

In Fig. 1 the global kinetic analysis of the complete mid-IR spectrum revealed three apparent rate constants; \( k_1 \), \( k_2 \) and \( k_3 \). The time course of the absorbance change of marker bands shows the order of events during the reaction. All marker bands were assigned by isotopic labeling. The first marker band shown is from the carbonyl vibration of the Thr35 backbone, which is a marker of the conformation of the switch I [18]. Thr35 absorbs in this position (1684 cm \(^{-1}\)) only when Ras is in the signal transducing "on" state (usually associated with the GTP state) but not in the "off" state (usually associated to the GDP state). The second marker band is the absorbance change of the most important catalytic residue of the GAP, the arginine-finger [5]. Arginine absorbs in this position (1589 cm \(^{-1}\)) only when it is in a water environment. The third marker band is the phosphate H\(_2\)PO\(_4\), the direct product of hydrolysis, in the hydrophobic internal environment of the protein (1192 cm \(^{-1}\)) [17,19]. Now, we can follow the absorbance changes during the three reaction steps. In the first step proceeding with \( k_1 \), the switch-1 marker band evolves, indicating the "off" to "on" conformational change directly after decaging of GTP. The other marker bands do not change with \( k_1 \). With \( k_2 \) the marker band of switch-1 does not change, indicating that switch-1 stays in the "on" conformation. The absorbance of the arginine-finger in water vanishes with \( k_3 \) due to the movement of the arginine-finger out of the water environment into the catalytic site. With the same apparent rate \( k_2 \) the bond breakage occurs and protein bound P\(_i\) is formed as observed by the appearance of the absorption at 1192 cm \(^{-1}\). Finally with \( k_3 \), switch-1 returns to the "off" state, the arginine-finger leaves the nucleotide binding pocket and the protein bound phosphate is released into the bulk. Thus several new features of the GTPase reaction were revealed by time resolved FTIR label free: The arginine finger is not located within the binding pocket of GTP in the ground state in contrast to the transition state-analog [20]. The movement of the arginine finger into the catalytic site can be observed with \( k_2 \) followed by immediate bond breakage with the same rate \( k_2 \); Ras only returns into the "off" state after P\(_i\) is released into the bulk with \( k_3 \) and this \( P_i \) release is the rate limiting step.

2. Variations of the mechanism among small GTPases

The same approach was applied to several GTPases and GAPs, and the results are summarized in Fig. 2. For Rap a similar protein bound P\(_i\) intermediate is found and its release into the bulk is rate-limiting [21]. On the other hand for Ran, which catalyzes the GTPase reaction without an arginine-finger [22] such an intermediate is not observed and bond breakage is rate limiting [23]. One could speculate that the positive charge of the arginine stabilizes the negatively charged P\(_i\) in the binding pocket. However, for RhoA and Rab1b bond breakage is the rate limiting step despite their arginine finger [24]. The only common feature of all GTPases seems the carboxamide which stabilizes the position of the attacking water molecule [25]. In most cases the carboxamide is provided by the GTPase itself. In the case of Rap1GAP the carboxamide is provided by the GAP, the so called asparagine thumb [26]. Interestingly, there are also dual GAPs, e.g. from the GAP1 family which catalyze both Ras and Rap [27]. Rap does not possess an intrinsic Gln in the usual position 61 and the GAP1 has no catalytically important Asn. Where is the carboxamide? Surprisingly it is another Gln two residues away from the normal catalytic position.

Fig. 1. The GAP catalyzed GTPase reaction of Ras. (A) Reaction scheme with rate constants (at 260 K) for the three apparent rates. (B) Time resolved absorbance changes during the GTPase reaction of three marker bands (in D\(_2\)O). (C) Structural model of a reaction intermediate [19]. The functional groups corresponding to the marker bands in (B) are highlighted.
allow GAPs to be selective for certain GTPases despite the high on top of the arginine finger[24,29]. All these differences help to intrinsic Gln out of the catalytic site, and serves as a second finger Gln and an Asn from the GAP is present. The latter pushes the situation is the opposite for many Rab proteins. Here an intrinsic R105 (Rab1b)
R305 (RhoA)
R789 (Ras)
R371 (dual)
Arg-finger (GAP)

Fig. 2. Comparison of various GTPase GAP combinations. Some GAPs provide an arginine-finger, others not. All reactions are catalyzed by a carboxamide. This can be from the GAPase (e.g. Gln61 in Ras or Gln63 of Rap in the case of GAP1 catalysis) or from the GAP (e.g. the Asn-thumb of Rap1GAP). For some reactions the P, release is rate limiting, for others the bond-breakage is rate limiting.

[28]. The GAP1 can push this Gln63 into the catalytic position. The situation is the opposite for many Rab proteins. Here an intrinsic Gln and an Asn from the GAP is present. The latter pushes the intrinsic Gln out of the catalytic site, and serves as a second finger on top of the arginine finger[24,29]. All these differences help to allow GAPs to be selective for certain GTPases despite the high homology among the GTPases with the common G-domain.

More detailed information on the reaction mechanism can be obtained by combination of trFTIR spectra with QM/MM calculations. The information encoded in the IR spectra can be decoded by these biomolecular simulations. The comparison between experimental and theoretical IR spectra can be used as a very sensitive quality control. The QM/MM simulations provide for the quantum chemical calculated catalytic center of a protein structural details at atomic resolution and in addition the charge distribution. The infrared vibrations are extremely sensitive to structural changes. Shifts of 4 cm⁻¹ correspond to changes in bond length of less than 0.01 Å, well below the resolution of X-ray structural analysis [30]. In the case of Ras it was found that the Ras and the GAP induce conformational changes of GTP (eclipsed versus staggered oxygen atoms) that play a major role for catalysis [31].

In summary trFTIR is a powerful tool to assign the catalytic residues and their dynamics in protein reactions at atomic detail label-free.

Acknowledgments

We thank Till Rudack and Konstantin Gavrlijuk for help with the Figures. We acknowledge the Deutsche Forschungsgemeinschaft (SFB 642, TP A1) for financial support.

References