Investigation of Fluoroaluminate Complex Formation Using NMR Spectroscorpy

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A number of enzymes or proteins involved in nucleotide binding or phosphate group transfer are sensitive to fluoride anions. Kinases and phosphatases are inhibited, while adenyl cyclases are generally activated.[1] In all cases milimolar concentrations of fluoride are needed. It was also demonstrated that the presence of traces of aluminum or beryllium were required to activated adenyl cyclase with fluoride.[2] In G protein systems it was proposed that AlF_4^- and BeF_3^- might act as phosphate analogues, and that AlF_4^- and BeF_3^- in the form of tetrahedral complexes structurally similar to phosphate, combined with GDP at the position occupied by γ-phosphate of GTP.[3,4] This phosphate analogue model of fluoroaluminate complex has been widely accepted not only for G proteins but also for many other proteins.[5~8] Since the activation was maximal at the fluoride concentration where the proportion of AlF_4^- had been calculated to be maximal, tetrahedral geometry was assumed for the fluoroaluminate complex which would be binding to the active site.

In the case of mitochondrial F_1-ATPase irreversible inhibition was found to occur in the presence of aluminum, fluoride, and ADP, and was proposed to be the result of complexation of AlF_4^- with ADP at the catalytic site.[8] Fluoroberyllatee in the presence of ADP was also found to inhibit irreversibly the mitochondrial F_1-ATPase.[9] Taking advantage of the virtually irreversible nature of the inhibition of F_1-ATPase by fluoroaluminate or fluoroberyllate, the ratio of ADP, berylium or aluminum, and fluoride were measured. In case of aluminium induced inhibition, the bound inhibiting species was found to be ADP-AlF_4, which was suggested to mimic the pentacoordinated 'intermermediate state' in the catalytic sites of F_1. In the case of beryllium-induced inhibition, depending on the initial fluoride concentration, ADP·Be_1F_2 and ADP·Be_1F_3 were found to be bound at the nucleotide binding site of F_1-ATPase. These species were suggested to mimic the condensed state in the pathway of ATP synthesis. Fluorometal complexes representing intermediate state of catalysis can be widely used for the structural investigation of many catalytic pathways involving hydrolysis of nucleotide.[10~12]

Other than activity and fluoride concentration relationship, so far there were no direct experimental approaches for the fluorometal complex formation. We investigated the formation of fluoroaluminate complexes in variety of conditions using ^{19}F and ^{31}P NMR spectroscopy.

Experimental Section

NMR spectra and operating conditions. ^{19}F NMR spectra were obtained at 338.79 MHz. All spectra were taken with 2.5 ml samples in a 10 mm diameter tube. A capillary insert containing D_2O was used as an internal filed frequency lock. Chemical shift of free fluoride was taken as 0 ppm. All the spectra were obtained at 25 °C using pulse-and-collect sequence with 50° pulse and interpulse delay of 0.6 seconds. ^{31}P NMR spectra were obtained at 125.7 MHz. All the spectra were taken with 0.5 mL samples containing 50 μL D_2O in a 5 mm diameter tube. Chemical shift of phosphoric acid was taken as 0 ppm. All the spectra were obtained at 25 °C using pulse-and-collect sequence with 75° pulse and an interpulse delay of 3 seconds.

Fluoroaluminate complex formation. To a solution of 1 mM $AlCl_3$ in Tris/HCl buffer (pH 7.5), NaF, ADP, and $MgCl_2$ was added from a 500 mM, 100 mM, 500 mM stock solution, respectively. 2000 scans were collected for each ^{19}F spectrum.

Results and Discussion

By varying the concentration of NaF at a fixed concentration of $AlCl_3$ (1 mM), the formation of fluoroaluminate complexes was investigated. Figure 1 shows ^{19}F spectra of fluoroalumnate complexes formed at 1 mM, 5 mM, and 10 mM NaF. In this fluoride concentration range only two detectable fluoroaluminate complexes appear at chemical shifts of -32.7 ppm and -33.7 ppm. ^{19}F NMR spectrum at 1 mM NaF concentration showed only one identifiable resonance at -33.7 ppm and a very tiny peak at -32.7 ppm at -32.7 ppm which is difficult to differentiate from noise (Figure 1). As the concentration of fluoride increased in the range of 1 to 10 mM, the relative intensity of the resonance at -32.7 ppm increased. In the calculated distribution curve[13] the only species which keeps increasing as the concentration of NaF increases in the range of 1 to 10 mM is hydrated AlF_4^- which is only a minor species at 1 mM NaF. Thus, ^{19}F resonance at -32.7 ppm is likely to be from AlF_4^-. The comparison of calculated distribution curve[13] and the relative intensity change of the resonance at -33.7 ppm also suggests that the resonance at -33.7 ppm is likey to be from AlF_3, or the sum of $AlF_3 + AlF_3(OH)^-$ in case ^{19}F NMR spectroscopy

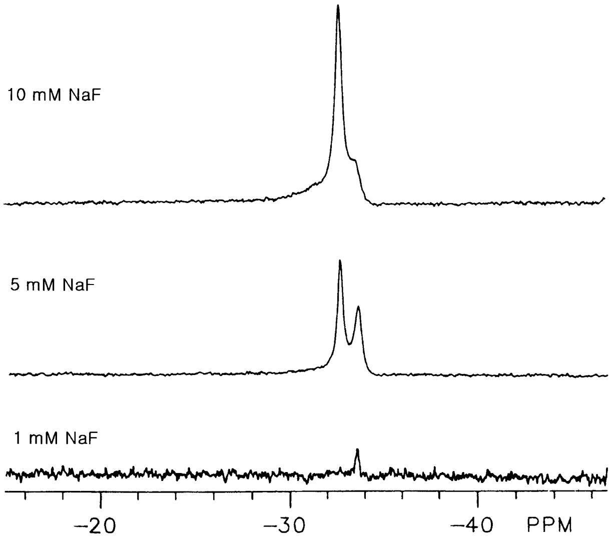

Figure 1. ^{19}F spectra of fluoroaluminate complex formation in the absence of ADP. To a solution of 1 mM $AlCl_3$ in Tris/HCl buffer (pH 7.5) NaF was added from a 500 mM stock solution to make solutions of 1 mM, 5 mM, and 10 mM. ^{19}F spectra were obtained from each solution with pulse-and-collect sequence with 50° pulse and interpulse delay of 0.6 s. 2 K scans were collected for each ^{19}F spectrum.

could not differentiate the number of H_2O or OH^-.

As shown in Figure 2, fluorometal complex formation in combination with 1 mM ADP caused two more resonances to appear in ^{19}F spectrum, a large peak at -29.8 ppm and a tiny peak at -31.6 ppm. Both ^{19}F resonances are presumed to be from ADP-fluoroaluminate complexes in which AlF_4^- or AlF_3 combine with ADP. In contrast to the appearance of two ^{19}F resonances which exhibited similar intensities at biologically active NaF concentration in the absence of ADP (Figure 1), only one dominant ADP-fluoroaluminate species (-29.8 PPM) was observed in the whole range of 1 to 10 mM NaF in the presence of ADP (Figure 2). This result may suggest the existence of only one biologically active ADP-fluoroaluminate complex. The analysis of fluoroaluminate-induced inhibition of F_1-ATPase showed the binding stoichiometry of 4 mol fluoride per mol ADP which favour the complex AlF_4^-.[9,14] Recent x-ray crystallographic analysis of G protein activated with aluminum fluoride also cleary showed the binding of complex $GDP{\cdot}AlF_4^-{\cdot}H_2O$ to G_α.[10] Thus, a new large peak at -29.8 ppm appeared following the addition of 1 mM ADP (Figure 2) may be from $ADP{\cdot}AlF_4^-$. It is known that ADP is a strong complexing agents that compete with F^- as a ligand.[13] However, the comparison of Figure 1 and Figure 2 shows that the presence of ADP rarely affect the overall equilibria of fluoroaluminate complex formation.

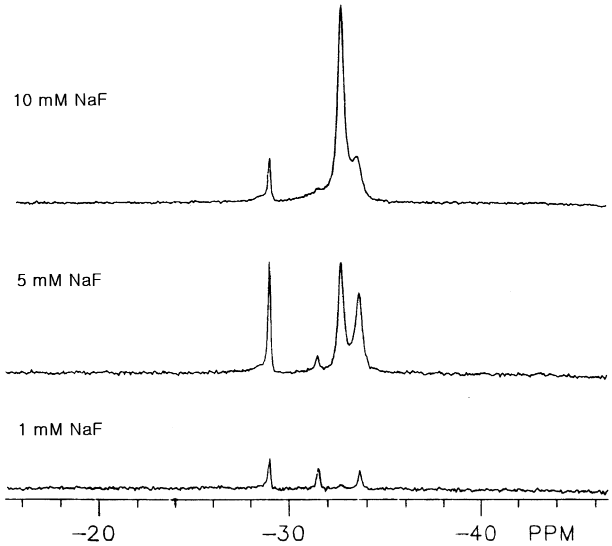

Figure 2. ^{19}F spectra of fluoroaluminate complex formation in the presence of ADP. To a solution of 1 mM $AlCl_3$, 1 mM ADP in Tris/HCl buffer (pH 7.5) NaF was added from a 500 mM stock solution to make solutions of 1 mM, 5 mM, and 10 mM. ^{19}F spectra were obtained from each solution with pulse-and-collect sequence with 50° pulse and interpulse delay of 0.6 s. 2 K scans were collected for each ^{19}F spectrum.

The ^{19}F spectrum of the fluoroaluminate complex in the presence of $MgCl_2$ showed two resonances similar to those in the absence of $MgCl_2$ (Figure 3). However, at 10 mM only a single resonance at an intermediate chemical shift appeared, suggesting rapid chemical exchange between two species. In contrast to ^{19}F spectra of fluoroaluminate in the absence of $MgCl_2$, in which the presence of ADP caused two more resonances to appear, the addition of ADP in the presence of $MgCl_2$ did not show any detectable differences from the spectra for those without ADP. This result suggests that the ADP-fluoroaluminate complex in solution may not occur in the presence of $MgCl_2$.

ADP-fluoroaluminate complex formation was also investigated using ^{31}P NMR spectroscopy (Figure 4). When $AlCl_3$ and NaF was added to a solution of ADP, resonances from α- and β-phosphate of ADP disappeared and the two new resonances appeared. These may indicate the formation of ADP-fluoroaluminate. However, the appearance of two resonances rather than one suggests that fluoroaluminate complex binds between α- and β-phosphate of ADP in solution, rather than at the γ-phosphate position observed inside the

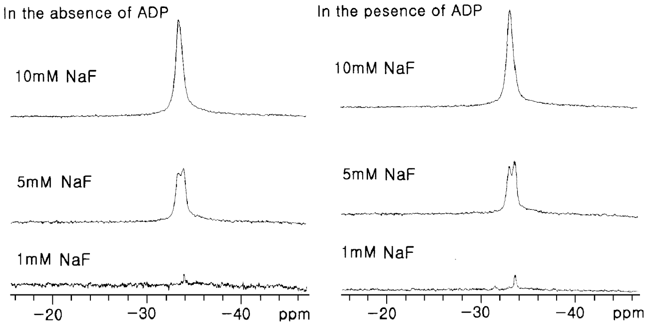

Figure 3. ^{19}F spectra of fluoroaluminate complex formation in the presence of Mg^{2+}. To a solution of 1 mM $AlCl_3$, 1 mM $MgCl_2$, (±1 mM ADP) in Tris/HCl buffer (pH 7.5), NaF was added from a 500 mM stock solution to make solutions of 1 mM, 5 mM, and 10 mM. ^{19}F spectra were obtained from each solution with pulse-and-collect sequence with 50° pulse and interpulse delay of 0.6 s. 2 K scans were collected for each ^{19}F spectrum.

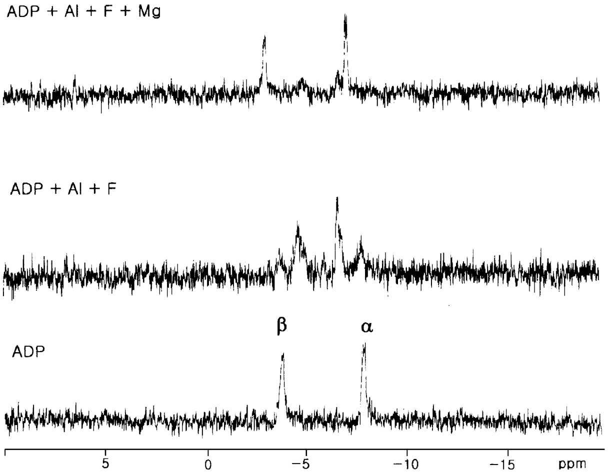

Figure 4. ^{31}P spectra of ADPfluoroaluminate complex formation. ^{31}P spectra of 1 mM ADP, 1 mM ADP + 1 mM $AlCl_3$ + 5 mM NaF (ADP + Al + F), and 1 mM ADP + 1 mM $AlCl_3$ + 5 mM NaF + 1 mM Mg^{2+} (ADP + Al + F + Mg) were compared in Tris/HCl buffer (pH 7.5). ^{31}P spectra were obtained from each solution with pulse-and-collect sequence with 75° pulse and interpulse delay of 3 s. 2 K scans were collected for each ^{31}P spectrum.

protein.[10~12] The addition of $MgCl_2$ to the solution caused these new resonances to disappear, which supports the results of ^{19}F NMR spectroscopy showing that ADP-fluoroaluminate complex cannot be formed in the presence of Mg^{2+}. Mg^{2+} is believed to form much tighter ADP complex than fluoroaluminate between α- and β-phosphate in solution.

The inhibition of F_1-ATPase with fluoride and aluminum or beryllium in combination with ADP is known to require the presence of Mg^{2+}.[9,14] As ADP-fluoroaluminate is formed between α- and β-phosphate only in the absence of Mg^{2+} in solution, the inhibition of F_1-ATPase in the presence of $MgCl_2$, seems to be due to a binding of fluoroaluminate complex, which is isomorphous to phosphate, to a phosphate binding region (γ-position) of a certain catalytic site at which ADP already bound independently rather than the binding of a fluoroaluminate-ADP complex itself to a particular catalytic site.

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