Fluoroaluminates activate transducin-GDP by mimicking the γ-phosphate of GTP in its binding site

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Received 31 August 1985

Fluoride activation of the cGMP cascade of vision requires the presence of aluminum, and is shown to be mediated by the binding of one AlF₄⁻ to the GDP/GTP-binding subunit of transducin. The presence of GDP in the site is required: AlF₄⁻ is ineffective when the site is empty or when GDP₇S is substituted for GDP. This sensitivity to the sulfur of GDP₇S suggests that AlF₄⁻ is in contact with the GDP. Striking structural similarities between AlF₄⁻ and PO₄⁺ lead us to propose that AlF₄⁻ mimics the role of the γ-phosphate of GTP.

1. INTRODUCTION

Fluorides have long been known to influence the activity of hormone-sensitive adenylate cyclase systems [1] and of the analogous cGMP phosphodiesterase (PDE) system of retinal rod outer segments (ROS) [2]. The target of F⁻ is the α-subunit of the GTP-binding proteins, called G/F, Nₐ, Nᵢ or Gₛ, Gᵢ in hormonal systems, and transducin (T) in the retina [3,4]. These proteins activate the cyclase, or PDE, when loaded with GTP by a GTP/GDP exchange catalysed by liganded hormone receptor, or light-activated rhodopsin. They become inactive when the GTP is hydrolysed to GDP. In a normal activation-deactivation cycle [5], with nucleotides in the medium, the site on Tₐ is virtually always occupied: it is occluded, and the nucleotide is non-exchangeable until Tₐ binds to R* [6]. This binding causes the site to open and allows a fast exchange of the bound GDP for GTP; then Tₐ-GTP quickly dissociates from Tₐβγ and from R*, with the GTP again occluded in the site where it will eventually be hydrolysed to GDP. Tₐ-GTP leaves the membrane and activates PDE by binding to its inhibitor subunit. The nucleotide site may be emptied by removing all guanyl nucleotides in the presence of R*. In this case Tₐ-GDP₇S will not dissociate from R* until its decay to opsin.

Fluorides in the millimolar range confer upon the GDP-bound protein structural and functional properties of the GTP-bound one [4,8]. The requirement of a trace amount of aluminum was recently demonstrated for fluoride activation of hormonal systems and it was suggested that AlF₄⁻ was indeed the active species [9]. We demonstrate that in the retinal system one AlF₄⁻ activates with high affinity one Tₐ-GDP molecule. The presence of GDP is required: AlF₄⁻ has no effects on Tₐ when the nucleotide site is empty or contains GDP₇S, even though Tₐ-GDP₇S is functionally and structurally similar to Tₐ-GDP. The sensitivity of AlF₄⁻ to the sulfur of GDP₇S suggests that AlF₄⁻ binds close to the GDP. Striking structural similarities between AlF₄⁻ and PO₄⁺ lead us to propose that AlF₄⁻ interacts with the GDP in the nucleotide site, where it mimics the role of the γ-phosphate of GTP.

Abbreviations: GDP₇S, guanosine 5'-O-(3-thiodiphosphate); R*, photoexcited rhodopsin.
2. MATERIALS AND METHODS

These studies were conducted with purified bovine ROS membranes [10], in which the native content of transducin (~1/10 rhodopsins) and PDE was preserved, or alternatively with reconstituted systems: purified transducin subunits, in various states [11], and partially purified PDE were added back to ROS membranes that had been washed free of all peripheral proteins and in which rhodopsin was virtually the only protein component left. Tα·GDP (or permanently activated Tα·GTPγS) and Tβγ were purified separately or as an associated Tα·GDP-Tβγ complex [11]. In native or reconstituted systems, functionality was assessed by monitoring cGMP hydrolysis by the pH-metric method [12]. As characterized by Kühn [7,10], inactive Tα·GDP and active Tα·GTP (or Tα·GTPγS) were also differentiated by their binding affinities: Tα·GDP binds strongly to Tβγ and the complex binds to ROS membranes in the dark at moderate (~10⁻¹ M) ionic strength and binds strongly to R* upon illumination; in contrast, Tα·GTPγS dissociates from Tβγ and does not bind to ROS membranes in the dark or to R*. Activation of the system by GTP or GTPγS is therefore always correlated with separation of Tα from the membrane and its solubilization in moderate ionic strength media [7,10]. Structural differences between Tα·GDP and Tα·GTPγS were also observed through their different sensitivity to proteolytic enzymes [13].

3. RESULTS AND DISCUSSION

In the presence of NaF and AlCl₃, it was first observed that purified Tα·GDP acquired the capacity to activate PDE in reconstituted systems in the dark with an efficiency comparable to that of Tα·GTPγS. Under the same conditions, in dark native membranes, Tα·GDP dissociates quantitatively from Tβγ and the membrane. The requirement for aluminum can only be demonstrated when working in clean plastic vessels: as pointed out in [9], common glassware releases sufficient amounts of aluminum to elicit activation in the presence of NaF. The quantitative requirements for AlCl₃ and NaF are shown in fig.1. Maximum efficiency is achieved at an NaF concentration

![Fig.1. Demonstration of the activation of Tα by a stoichiometric amount of AlF₄⁻.](image-url)
where the predominant fluoroaluminate complex ion is $\text{AlF}_4^{-}$ [14,15]. Dose-activation curves were run at various $T_{\alpha}$ concentrations. Full activation was always obtained with total AlF$_4^{-}$ concentrations corresponding to one AlF$_4^{-}$ per $T_{\alpha}$ (fig.1C). This demonstrates a stoichiometry of one AlF$_4^{-}$ bound per $T_{\alpha}$ and a binding constant of the order of 1 $\mu$M or higher. AlF$_4^{-}$-activated $T_{\alpha}$·GDP has the proteolytic sensitivity characteristics of $T_{\alpha}$·GTP$\gamma$S [4].

As observed by Stein et al. [4] illumination appeared to cancel the effects of AlF$_4^{-}$, on PDE activation as well as on the binding properties of $T_{\alpha}$. In contrast to the suggestion of Stein et al., this is not due to the blocking of access to the 'fluoride site' by the previous binding of $R^*$ to $T_{\beta\gamma}$: when AlF$_4^{-}$ is applied first in the dark, solubilization of $T_{\alpha}$ and activation of PDE are reversed upon strong illumination; $T_{\alpha}$ appears to bind quantitatively to $R^*$ in the presence of AlF$_4^{-}$ as it does in the absence of AlF$_4^{-}$. The presence of $T_{\beta\gamma}$ is required for this rebinding to take place, as it is for the binding of $T_{\alpha}$·GDP to $R^*$ [7]. We noticed, however, that the deactivation by strong illumination of AlF$_4^{-}$-activated $T_{\alpha}$ is a slow process, which appears to require a few minutes to develop. A significant observation was then that a few micromolar GDP, which does not interfere with the binding of $T_{\alpha}$ to $R^*$ in the absence of AlF$_4^{-}$, or activate the system in the absence of AlF$_4^{-}$, suffices in the presence of AlF$_4^{-}$ to prevent or reverse the rebinding of $T_{\alpha}$ to $R^*$ and to preserve or restore the PDE activation (fig.2). Our interpretation is that AlF$_4^{-}$-activated $T_{\alpha}$·GDP (as does probably $T_{\alpha}$·GTP) still has a low affinity for $T_{\beta\gamma}$ and $R^*$. A very small proportion of AlF$_4^{-}$-activated $T_{\alpha}$·GDP binds back to $T_{\beta\gamma}$ and to $R^*$, then the site opens, the bound GDP is released and, in the absence of nucleotide in the medium it is not replaced, as the affinity of the site is in the $10^{-5}$ M range [6]. With no nucleotide in the site, $T_{\alpha}$ cannot be activated by AlF$_4^{-}$, the binding of $T_{\alpha}$ to $R^*$ becomes irreversible, and progressively all of the $T_{\alpha}$ becomes bound. A few micromolar GDP in the medium suffices to keep a GDP in the open site of an $R^*$-bound $T_{\alpha}$ and to prevent this phenomenon. AlF$_4^{-}$ alone, without nucleotide in the site, is therefore unable to activate $T_{\alpha}$: binding of a GDP molecule and of an AlF$_4^{-}$ is concurrently required. Indications that the AlF$_4^{-}$-binding site is in close proximity to the
Fig. 3. Structural analogies between PO₄⁻ and AlF₄⁻ and schematic model proposed for the interaction of AlF₄⁻ with the GDP occluded in its site in Tₐ.

nucleotide-binding site are provided by the use of GDP analogs. GDP₆S can be substituted for GDP by washing out the GDP of R*-bound Tₐ, adding GDP₆ and letting R* decay to opsin. Tₐ.GDP₆S seems to retain all of the characteristics of Tₐ.GDP: it binds to T₄₂ and to R*, and exchanges its nucleotide for GTP in the presence of R*, like Tₐ.GDP (T.M. Vuong and M. Chabre, unpublished). Tₐ.GDP₆S also has the proteolytic sensitivity characteristics of Tₐ.GDP (not shown). However, Tₐ.GDP₆ is not activated by AlF₄⁻, as if the binding of the ion complex were directly hindered by the presence of the large sulfur atom on the β-phosphate. The observation that fluorides were inactive in the presence of GDP₆S had previously been done on the hormone sensitive G-protein systems where, by contrast, fluoride activation was effective when the analogous Gₛ and Gᵢ proteins by fluorides results from the binding of an AlF₄⁻ into the guanine nucleotide site, close to the β-phosphate of GDP (fig. 3). F⁻ is known to be able to make hydrogen bonds which could substitute for that made by the oxygens of the phosphate group. The bound AlF₄⁻ simulates the presence of the bound β-phosphate of GTP and therefore confers on the protein the structure of the active Tₐ.GTP state. The binding of AlF₄⁻ to GDP and the protein site must be tighter than that of a phosphate group, which is released from the protein after the hydrolysis of the γ P-O-P bond of GTP. This release is spontaneous, and we have checked that phosphate ions are not activators of the system, even at millimolar concentrations. The high electronegativity of F⁻ may account for this tight binding.

This model could also account for the effect of fluorides, or more likely, aluminofluoride complexes in other enzymatic systems involving phosphate groups, such as phosphatases.

ACKNOWLEDGEMENTS

We acknowledge the excellent technical assistance of Mylène Robert. We thank Dr T.M. Vuong for his help in the preparation of this manuscript and Michèle Rondot for her careful typing. M.C. wishes to thank Dr François Morel (MIT, Cambridge) for a very stimulating discussion which triggered ideas developed in this work.

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