Configuration of the active Mg-ATP complex in protein kinase C reaction

Hiroki Kondo*, Junko Kinoshita, Takao Matsuba and Junzo Sunamoto

Laboratory of Bioorganic Chemistry, Department of Industrial Chemistry, Nagasaki University, Nagasaki 852, Japan

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To probe the active site structure of protein kinase C stereochemical studies were carried out by using ATPβS. The enzyme utilizes either one of the diastereomers (S_P and R_P) of ATPβS almost equally well as a substrate. This result contrasts with that for cyclic AMP-dependent protein kinase, suggesting that the topography of the nucleotide-binding site is significantly different between the two kinases.

Phosphorylation *Protein kinase C* *ATPβS* *Stereospecificity*

1. INTRODUCTION

Phosphorylation and dephosphorylation of proteins are one of the major mechanisms by which intracellular events are regulated [1]. Over 30 protein kinase isozymes are now known and they phosphorylate target proteins to modulate their physiological activities. Despite such biological importance, little is known about the mechanism by which these enzymes mediate the phosphoryl transfer to an acceptor substrate. Bolen and collaborators [2] reported that cAMP-dependent protein kinase (protein kinase A) exhibits high stereospecificity for the Δ configuration of Mg-ATP as substrate. An intriguing question is raised as to whether other protein kinases show the same stereospecificity. Here, we have investigated the stereochemical preference of Ca^{2+}-dependent, phospholipid-activated protein kinase (protein kinase C) [3] for the diastereomers of adenosine 5′-*O*-(2-thiotriphosphate) (ATPβS), phosphorothioate analogue of ATP. It was found that, unlike protein kinase A, this enzyme utilizes both diastereomers almost equally well as substrate, suggesting a considerable difference in the topography of the nucleotide-binding site of both kinases.

2. MATERIALS AND METHODS

2.1. *Materials*

Chemicals and gels used in this work were obtained from the following sources: histone type IIIS, phosphatidylserine and diolein, Sigma; DEAE-cellulose and P81 phosphocellulose paper, Whatman; phenyl-Sepharose and AH-Sepharose, Pharmacia; [γ-^{32}P]ATP, Amersham. [γ-^{32}P]-ATPβS S_P and R_P isomers were prepared as in [4] and [5], respectively. Protein kinase C was purified from rat brain as in [6]. Homogeneous protein kinase A catalytic subunit was prepared from bovine heart as detailed in [7,8].

2.2. *Protein kinase assay*

Protein kinase C was assayed at 30°C essentially as in [6]. The assay mixture contained the following components: 20 mM Tris-HCl (pH 7.5), 1 mM 2-mercaptoethanol, 5 mM magnesium acetate, 200 μg/ml histone IIIS, 0.1 mM calcium chloride, 10 μg/ml phosphatidylserine, 1 μg/ml diolein, 50 μM [γ-^{32}P]ATP or ATPβS. The reaction was started by the addition of enzyme and terminated by placing an aliquot on a piece of P81 filter paper [9].

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3. RESULTS

It was found that a phosphorothioate analogue of ATP, ATPβS, serves as a phosphoryl donor substrate for protein kinase C. The relative effectiveness of the S_P and R_P diastereomers was compared under standard assay conditions with histone H1 as the phosphoryl acceptor substrate. As shown in table 1, both isomers exhibited almost identical reactivity, which is about 15% that of ATP. This result is in sharp contrast to that for protein kinase A, which adopts the S_P isomer as the preferred substrate by a large margin [2]. To rule out the possibility that the data on protein kinase C are due to an artifact of our ATPβS preparations, stereochemical studies were also carried out for protein kinase A. When compared in terms of V_{max}/K_m, the R_P isomer was only 1.3% as active as the S_P isomer. Protein kinase C possesses no activity with Cd^{2+} as the activating metal. This behavior is also different from that of protein kinase A [2].

To gain insight into the origin of the above stereochemical (in)discrimination a detailed kinetic analysis was attempted. However, initial rate data for ATPβS did not follow the Henri-Michaelis-Menten equation closely under the various conditions tested, where similar data for ATP can be analyzed to give a K_m of 6.3 ± 0.1 μM [10,11]. Nonetheless, the K_m for either diastereomer of ATPβS appears to lie in the range 1–10 μM and is of similar magnitude. This suggests that both stereoisomers behave almost identically not only in binding but also in catalytic processes in the protein kinase C reaction.

Table 1

Stereochemical preference of protein kinase C for the diastereomers of ATPβS

Nucleotide	Velocity[a] (nmol/min per mg)	Relative rate (%)
ATP	67.5 ± 6.1 (n = 3)	100
ATPβS S_P	10.5 ± 0.7 (n = 5)	16
ATPβS R_P	8.6 ± 1.7 (n = 5)	13

[a] For assay conditions see section 2

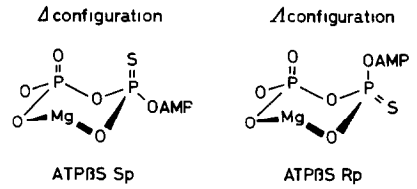

Fig.1. Configurations of the β,γ-bidentate Mg-ATPβS complexes.

4. DISCUSSION

Our results reveal that protein kinase C utilizes either diastereomer of ATPβS equally well as a substrate. Since the magnesium complex of the S_P isomer of ATPβS assumes a Δ configuration, while the corresponding complex of the R_P isomer takes on a Λ configuration (fig.1) [12], these data may imply that the enzyme accepts either geometry of the β,γ-bidentate Mg-ATP complex to its active site. This result is in contrast to that for protein kinase A, which prefers predominantly the Δ configuration as the active substrate [13].

Although the exact K_m values could not be determined for ATPβS, it was suggested that ATPβS S_P and R_P isomers and ATP possess a nearly identical K_m. This indicates that the difference in the overall reactivity of ATP and its analogues (table 1) reflects mainly the catalytic process. Sulfur substitution on the β-P of ATP could diminish the interaction of nucleotide substrate with an active site residue(s), leading to a decreased reactivity relative to ATP.

In summary, the present data suggest that the spatial disposition of the nucleotide-binding site of protein kinases A and C may be considerably different. In line with this notion is the observation that the two enzymes behave distinctly toward isoquinolinesulfonamides which are supposed to bind to the nucleotide-binding site [14]. It would be interesting to see whether protein kinase C retains a sequence like -Gly-X-Gly-X-X-Gly- which is presumed to constitute an ATP-binding site of many proteins including the protein kinase family [15].

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