THE ACTIVITY OF ε-ADENOSINE DERIVATIVES AS ALLOSTERIC MODIFIERS OF PHOSPHORYLASE b

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1. Introduction

It has recently been shown by Kochetkov et al. [1], as well as by Barrio et al. [2, 3], that chloroacetaldehyde reacts with derivatives of adenine and cytosine to form fluorescent products. In the case of adenosine the product, ε-adenosine (3-β-D-ribofuranosyl-imidazo[2,1-i] purine), has a remarkably high fluorescence intensity, permitting measurements at concentrations as low as 10^{-8} M.

In view of its remarkable properties, it is of interest to examine the biochemical properties of the 5′-nucleotide derivatives of ε-adenosine, in order to determine the extent to which they can replace the natural nucleotides as enzymic substrates and modifiers. In instances where this proves to be the case, these derivatives have the potentiality of serving as fluorescent probes of conformation. In this paper the activities of ε-AMP and ε-ATP as allosteric modifiers of phosphorylase b are described. In brief, ε-AMP has been found to function as an activator and ε-ATP as an inhibitor, although their binding affinities are less than those of the natural nucleotides. The catalytic efficiencies of the ε-AMP and AMP complexes have been found to be similar.

2. Methods

ε-AMP and ε-ATP were prepared by the action of chloroacetaldehyde (2 M) on the disodium salts of 5′-AMP (Boehringer) and 5′-ATP (Sigma), respectively, as described by Barrio et al. [2]. After 24 hr at 37° (pH 4.3), the solution was lyophilized and the product recrystallized from H_2O–C_2H_5OH. A purified preparation of ε-AMP was also provided through the courtesy of Dr. N. Leonard. Homogeneity was verified by paper chromatography using 5% sodium hydrogen phosphate saturated with isoamyl alcohol as the solvent. Only one component was observed. The glycogen used was the rabbit liver preparation of Sigma (Type III). The phosphorylase b preparation used was the twice-crystallized product of Sigma.

3. Results and discussion

Fig. 1 illustrates the dependence on ε-AMP level of the reaction velocity of phosphorylase b. The reaction monitored, which corresponds to the synthesis of glycogen, is the release of inorganic phosphate from glucose 1-phosphate in the presence of a constant (0.71%) level of glycogen, according to the basic procedure of Cori et al. [4]. Aliquots of enzyme solution were added to the incubation mixture containing glucose 1-phosphate, glycogen, and ε-AMP or AMP. Rates were computed from the slopes of the initial (linear) portions of the curves of the release of inorganic phosphate as a function of time. In the absence of AMP or ε-AMP, little enzymic activity is observed, the maximal velocity being less than 0.4% of that attainable in the presence of these activators. It is clear from fig. 1 that ε-AMP functions as an activator. Moreover, the limiting velocity in the presence of saturating levels of ε-AMP is close (110%) to that attained for a saturating concentration of AMP in 0.15 M glucose 1-phosphate. Thus the catalytic efficiency of the ε-AMP complex is similar to that of the complex formed by AMP.

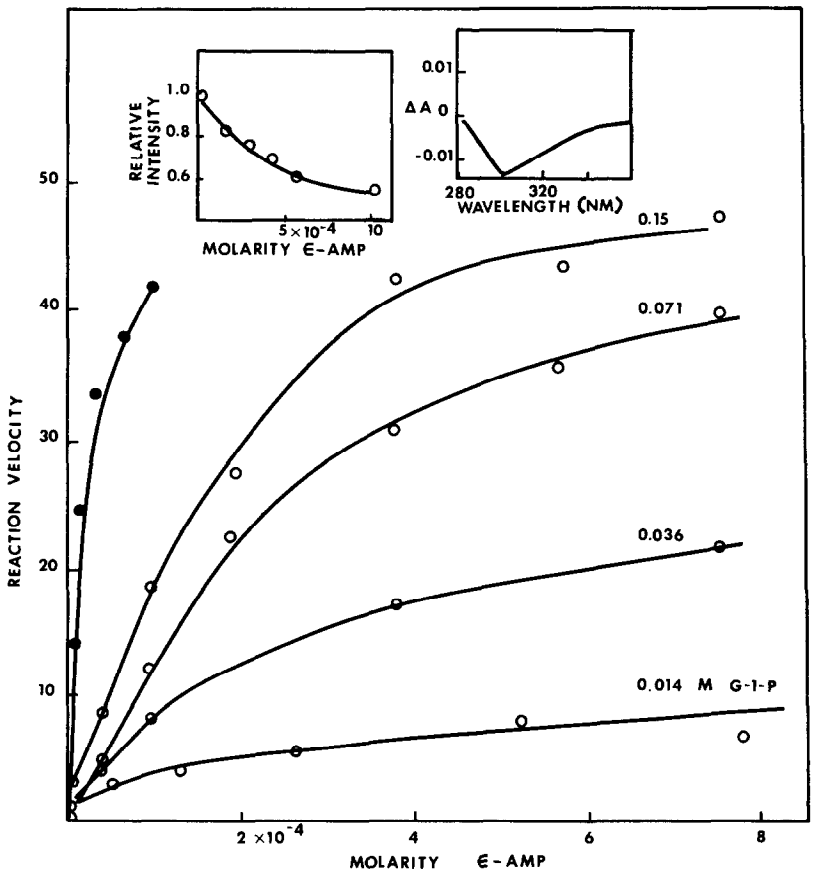

Fig. 1. Dependence of reaction velocity on ϵ-AMP (○–○–○) and AMP (●–●–●) concentration for phosphorylase b (0.06 mg/ml) in the presence of varying levels of glucose 1-phosphate at 33°. The ordinate is the number of μmoles of phosphate released per mg enzyme per min. The glycogen concentration is 0.71%. Here and elsewhere the buffer is 0.1 M glycylglycine, 0.01 M EDTA, 5 mM β-mercaptoethanol, pH 7.0. The AMP curve is for 0.071 M glucose 1-phosphate. Left inset: Dependence of intensity of tryptophan fluorescence of phosphorylase b (0.071 mg/ml) on ϵ-AMP level at 25°. The wavelengths of excitation and emission are 278 nm and 320 nm, respectively. To minimize attenuation of the incident beam by absorption, cuvettes of 3-mm pathlength were used. A correction for attenuation was applied, based on the reduction of the fluorescence intensity of acetyltryptophan by the same levels of ϵ-AMP. Right inset: Differences spectrum developed by phosphorylase b (0.85 mg/ml) in the presence of 1.5×10^{-4} M ϵ-AMP.

However, considerably higher levels of ϵ-AMP are required for equivalent activation. Thus the concentrations of AMP and of ϵ-AMP required for 50% of the maximum activity in 0.071 M glucose 1-phosphate are 1.4×10^{-5} M and 2.3×10^{-4} M, respectively.

Direct evidence for the binding of ϵ-AMP by phosphorylase b is obtained from observations of the quenching of its intrinsic tryptophan fluorescence (fig. 1) in the presence of ϵ-AMP. Since the absorption spectrum of ϵ-AMP overlaps the emission spectrum of tryptophan [2], it would be expected that quenching of the tryptophan fluorescence of phosphorylase b would occur by a radiationless exchange mechanism, if binding is present [5]. Fig. 1 indicates that this is the case. If it is assumed that quenching is confined to the protomer on which the bound ϵ-AMP is located, and that one binding site exists for each of the two protomers, the data in the presence of 0.15 M glucose 1-phosphate may be fitted by a Langmuir absorption isotherm with an intrinsic association constant of 2.6×10^3.

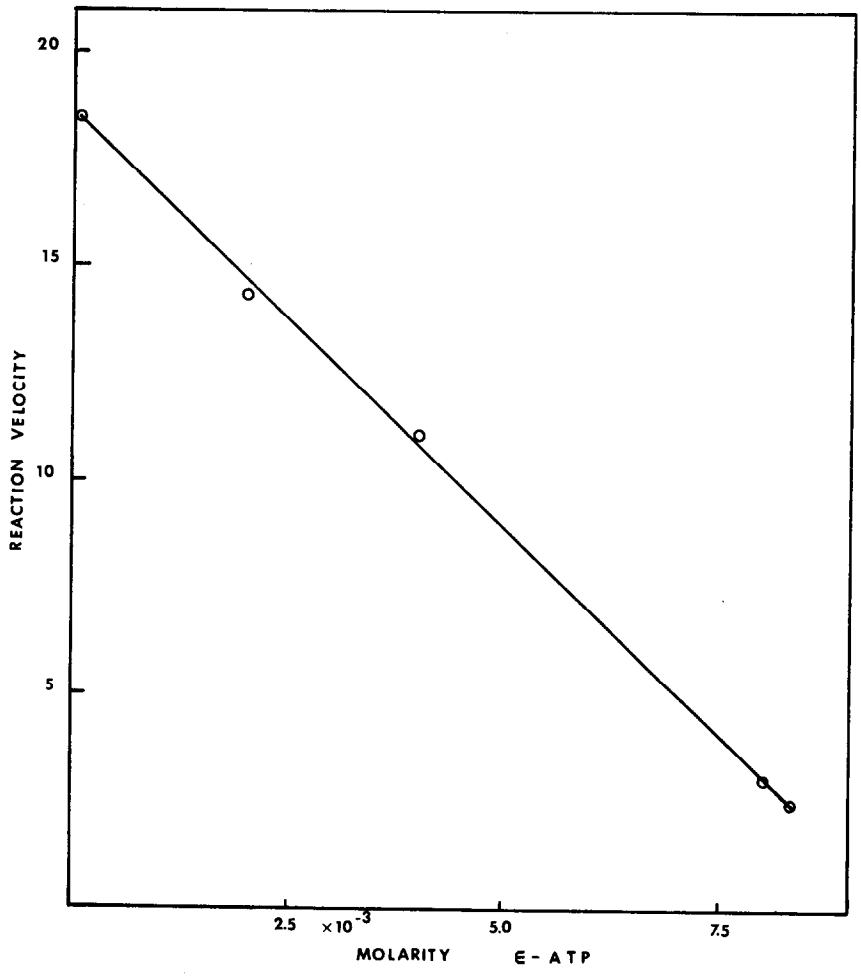

Fig. 2. Dependence of enzymic velocity on ε-ATP level for phosphorylase b (0.042 mg/ml) in the presence of 0.071 M glucose 1-phosphate, 0.71% glycogen, and 1.5 × 10^{-4} M ε-AMP at 33°. The units of the ordinate are the same as for fig. 1.

A difference spectrum is developed upon the binding of ε-AMP (fig. 1), with a negative peak in the 300 nm region.

Fig. 2 shows the dependence on ε-ATP level of the reaction velocity. ε-ATP clearly functions as an inhibitor, by analogy with ATP [6].

These results indicate that the modification of the adenine base involved in the formation of ε-AMP and ε-ATP does not prevent these derivatives from acting as allosteric modifiers of phosphorylase b. In view of the equivalence of the maxima velocities induced by ε-AMP and AMP, it is likely that the same active conformation is stabilized by both.

References

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