MECHANISM OF PYRIDINE INHIBITION OF iA, ANTI-iA BINDING

C. JAYABASKARAN, M. SUGUMARAN AND T. M. JACOB*

Department of Biochemistry and ICMR Centre for Cell Biology and Genetics, Indian Institute of Science, Bangalore 560 012 (*India*)

ABSTRACT

Study of the pyridine inhibition of isopentenyl adenosine (iA), anti-iA binding revealed non-competitive inhibition in the case of both anti-iA anti serum and anti-iA Fab fragments.

INTRODUCTION

MOST of the methods used for the isolation of specific antibodies make use of the interaction between the antigen and the antibody. The success of these methods depends on the efficient dissociation of the antigen-antibody complexes under mild conditions. Humayun and Jacob[1] showed that low concentration of pyridine inhibit the interaction of isopentenyl adenosine (iA) and deoxyadenylic acid (dpA) with the respective antibodies. The concentrations of pyridine needed for the complete inhibition of the hapten antibody interaction were found not to affect the antibodies when tested after removal of the pyridine. The inhibitory effect of pyridine has been taken advantage for the elution of anti-iA and anti-dpA antibodies[1] and iA containing tRNAs[2–4] from affinity columns and for the dissociation of antibody-hapten complexes[3]. In spite of the wide applicability of this method the mechanism of pyridine inhibition of antigen-antibody interaction has remained unexplored. In this communication an attempt is made to understand the mechanism of pyridine inhibition by kinetic studies on the interaction of iA and anti-iA, in presence of different concentrations of pyridine.

MATERIALS AND METHODS

$N^6(\Delta^2$-isopentenyl) adenosine (iA) was from Sigma Chemical Co., U.S.A. (^{3}H)-$NaBH_4$ (sp. act. 12·4 μ/m mole) was from Radio Chemical Centre, Amersham. Nitrocellulose membrane filters (0·45 μ, MDI filters) were from Advanced Microdevices, Ambala, India. (^{3}H)-Isopentenyl adenosine was prepared by the periodate oxidation and borohydride reduction[5] of iA and is represented as (^{3}H) -iA^{ox-red}. The preparation of (^{3}H)-$iA^{ox\ red}$ had a specific activity of 6000 cpm/p mole. Pyridine was purified by refluxing with *p*-toluene sulfonyl chloride and fractional distillation[1]. Anti-Isopentenyl adenosine antibodies were raised in rabbits by injecting isopentenyl adenosine-bovine serum albumin conjugate[1]. Fab-fragments were prepared from anti-iA IgG by papain digestion and purified by affinity chromatography and gel filtration (C. Jayabaskaran and T. M. Jacob, unpublished work).

The hapten antibody binding was studied by nitrocellulose membrane filter assay[6].

RESULTS AND DISCUSSION

The kinetics of the inhibition of the binding of (^{3}H)-iA^{ox-red} to anti-iA antiserum by pyridine is given in Fig. 1 as double reciprocal plots. At different concentrations of pyridine the slope as well as the intercept are different[7] showing that the pyridine inhibition is of non-competitive type. The secondary plots[7] are given in the inset of Fig. 1. The slope replot is a parabolic function of varying pyridine concentration while the intercept replot is a linear function, suggesting that pyridine inhibition belongs to I-linear S-parabolic non-competitive type. The results of a similar study with the purified anti-iA Fab-fragments given in Fig. 2, further confirms the nature of pyridine inhibition.

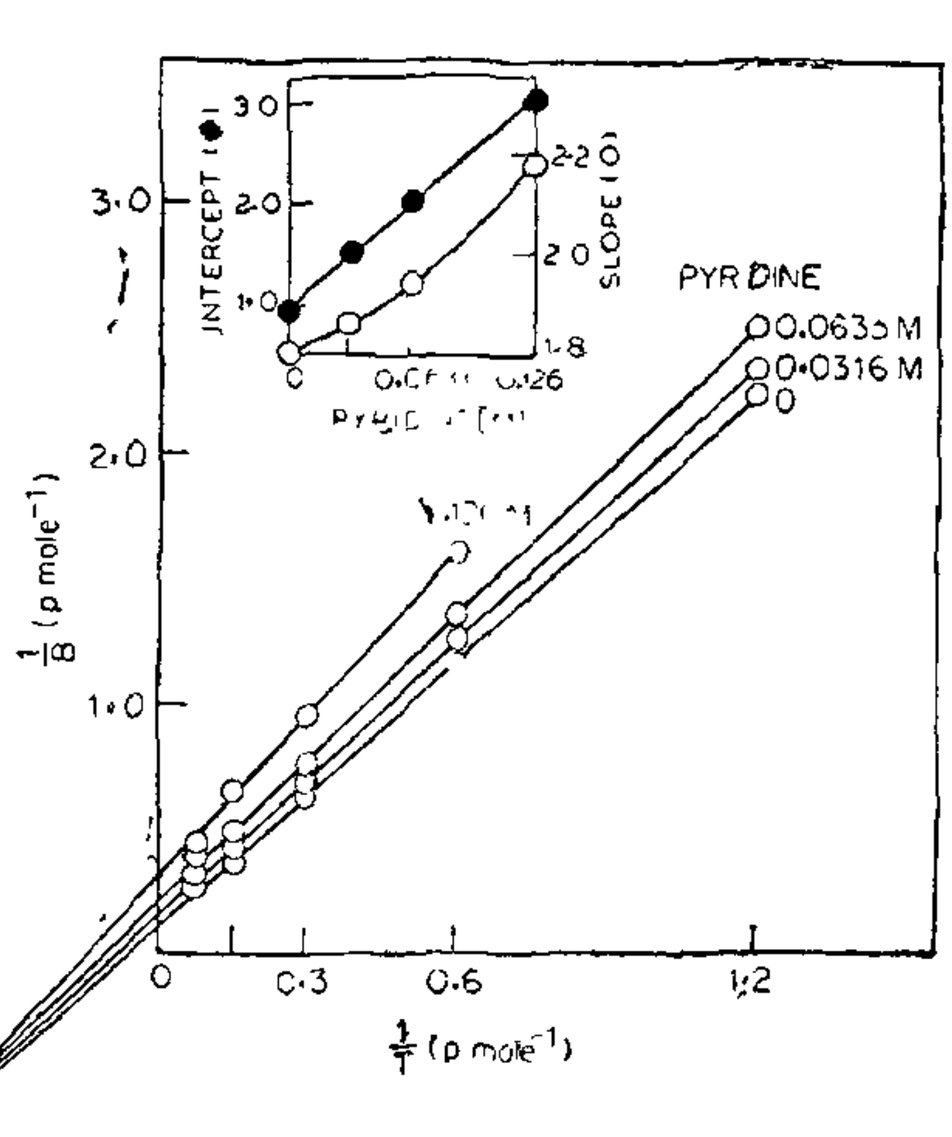

FIG. 1. Kinetics of pyridine inhibition of iA binding to anti-iA serum.

The reaction mixture contained anti-iA serum (1 : 500 diluted; 0·1 ml), (^{3}H)-iA^{ox-red} (0·1 ml, 0·833 to 13·26 p moles) and TBS (0·01 M Tris-HCl pH 7·5, 0·14 M NaCl 0·2 ml) containing different

* For correspondence.

amounts of pyridine so as to give 0·0316 M, 0·0633 M or 0·126 M solutions. The antiserum was added last and incubated at 37° C for 10 minutes. The mixture was then filtered on MDI filters, washed with TBS, dried and radioactivity monitored. Reciprocal plots of the varying concentrations of total (T), (^{3}H)-iA^{ox-red} used and antiserum bound (B), (^{3}H)-iA^{ox-red} for fixed concentrations of pyridine are given in the figure. The inset gives replots of slope and intercept values *versus* varying pyridine concentration.

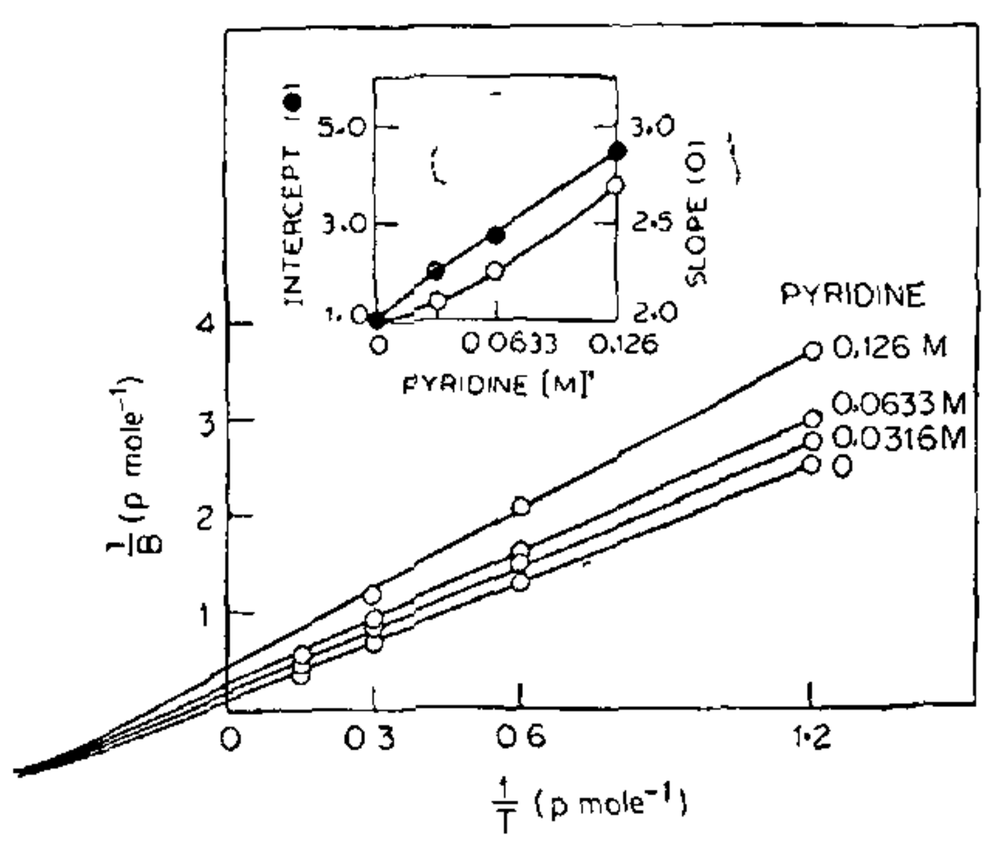

FIG. 2. Kinetics of pyridine inhibition of iA binding to Fab-fragments of anti-iA.

The experiment was done as described under Fig. 1 except that anti-iA Fab-fragments (3 μg) was used instead of anti-iA serum.

Since there are some structural features common to pyridine and iA, a competitive type of inhibition could not be ruled out before this study. It is unlikely that pyridine serves as a hydrogen bond breaker at the concentration employed in this study because reagents such as urea which are known to be good hydrogen bond breakers[8] do not dissociate antigen antibody complexes as efficiently as pyridine at similar concentrations. The inhibition may be due to the protein conformational changes that accompany the shift from aqueous to pyridine environment. This necessitates the binding of more than one inhibitor molecule per molecule of antibody. The above mechanism is in conformity with the parabolic non-competitive type inhibition observed.

A consequence of the mechanism postulated is that pyridine inhibition of antigen-antibody interaction can be expected to be of general applicability. It is also to be expected that eventhough pyridine may inhibit the formation of precipitate, it may not be able to dissociate the antigen-antibody precipitate after its formation.

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KARYOLOGICAL STUDIES ON FOUR SPECIES OF LIZARDS FROM PENINSULAR INDIA

S. KRISHNA RAO AND N. V. ASWATHANARAYANA
Department of Zoology, University of Mysore, Manasa Gangotri, Mysore 570 006, *India*

ABSTRACT

The karyotypes of four species of lizards, *viz.*, *Mabuya trivittata* (Hardwicke and Gray) and *M. carinata* (Schneider) (Family: Scincidae); *Psammophilus dorsalis* (Gray) and *Calotes versicolor* (Daudin) (Family: Agamidae) are described. The morphometric data on the chromosomes are presented. Geographic variation in the karyotype of *M. carinata* is described. The karyotypes of these species are compared with those of the other karyologically analysed lizards.

KARYOLOGICAL data are sparse for lizards of the families Scincidae and Agamidae and many more species are to be worked out. So far, only seven genera of the family Scincidae and seven genera of the family Agamidae have been analysed[1]. In the present communication detailed karyological data on *Mabuya trivittata* (Hardwicke and Gray) and *M. carinata* (Schneider) of the family Scincidae; *Psammophilus dorsalis* (Gray) and *Calotes versicolor* (Daudin) of the family Agamidae are presented. Among these four species, the chromosomes of *M. trivittata* and *P. dorsalis* have not been reported