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Nucleic acids can regulate the activity of casein kinase II

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Casein kinase II purified from nuclei of *Xenopus laevis* oocytes is inhibited by several specific nucleic acids. This kinase, the main phosphorylating activity of the oocyte nucleus, is markedly inhibited by poly U at 10 μg/ml, and this polymer is a competitive inhibitor of the phosporylation of the substrate casein (Kiapp 80 nM). M 13 phage ssDNA and unfractionated yeast tRNA also inhibit between 50 and 200 μg/ml. Poly C, poly A, poly AG, dsDNA and *Escherichia coli* rRNA do not alter activity significantly at similar concentrations. Inhibitions are reversed by RNase (poly U, tRNA) or S_1 nuclease (ssDNA). Oocyte casein kinase I or rabbit cAMP-dependent protein kinase are not inhibited by poly U at 200 μg/ml. The sensitivity of the casein kinase II to these inhibitors suggests a regulatory role for nucleic acids in nuclear phosphorylation reactions.

Protein kinase; Poly U; DNA, single-stranded; tRNA; Nucleus; *(Xenopus laevis)*

1. INTRODUCTION

Protein phosphorylation-dephosphorylation systems are among the most important mechanisms for the regulation of biological activity. A large number of unique members of the protein kinase family have been described, one of the most interesting and ubiquitous being casein kinase II (for review see [1]). Casein kinase II phosphorylates acidic proteins using either ATP or GTP as phosphoryl donor and is the major protein phosphorylating activity detected in the nuclei of amphibian oocytes [2]. The enzyme can be activated by polyamines and polylysine, and it is susceptible to the inhibition by heparin at concentrations of 1 μg/ml. The inhibition by heparin is competitive with the protein substrates [3] and its efficiency depends on the nature of that substrate [4,5]. An explanation for the potency of inhibition by heparin can be deduced from the studies by the groups of Krebs and Pinna [6,7], who established the structural requirements for model peptide substrates of casein kinase II. Their results indicate that this enzyme phosphorylates serines or threonines that are followed by clusters of the acidic side chains of aspartic and glutamic acids, or by previously phosphorylated amino acids. The laboratory of Pinna has also shown that polyglutamic and polyaspartic acids are strong inhibitors of casein kinase II [7].

The function of casein kinase II in the nuclei of cells has not been elucidated. However, there are several lines of evidence that implicate this enzyme in the regulation of the synthesis of nucleic acids [5,8–11]. Zandomeni et al. have obtained results that suggest that casein kinase II may be playing a role in the initiation of transcription by RNA polymerase II [12].

The affinity of casein kinase II for polyanions and its presence in the nucleus raises the question of whether the enzyme could be interacting with nucleic acids and whether this interaction could regulate its activity. Previous reports have implicated that nucleic acids [13–15] or oligonucleotides [16] may inhibit enzymes with proper-

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ties of casein kinase II. In this communication, we present data that demonstrate that poly U, tRNA and single-stranded DNA can act as strong inhibitors of the casein kinase II obtained from the nuclei of *Xenopus laevis* oocytes.

2. MATERIALS AND METHODS

[γ-^{32}P]ATP was prepared by the method of Walseth and Johnson [17] using ^{32}P from Amersham. Yeast tRNA was from Calbiochem, *E. coli* ribosomal RNA and S_1 nuclease were from Boehringer, pGEM-3 from Promega and M13 RF DNA from Bethesda Research Laboratories. Oligo dT (12–18mer) was from Collaborative Research. $tRNA^{Phe}$ was kindly donated by H. Sternbach (MPI, Goettingen). Poly U, poly A, poly C and poly AG (all >100 kDa), nucleotides, nucleases, salmon sperm DNA, spermine, protamine and cAMP-dependent protein kinase (rabbit muscle) were from Sigma. Nucleic acids and nucleotides were dissolved in distilled water and the pH adjusted as necessary. Dephosphorylated casein used in all assays was prepared as described [3].

Ovaries were obtained by surgery from adult female *X. laevis*. Stage 5 and 6 oocytes and nuclei were prepared as described by Burzio and Koide [18]. Casein kinase II was purified from cytosol-free nuclei by chromatography on DEAE-Sephadex [2] and phosphocellulose [5] as described originally by Hathaway and Traugh [19]. Casein kinase I was obtained from the nuclear extracts using DEAE-Sephadex chromatography [2].

Casein kinase II assay: reactions (50 μl) contained 40 mM Hepes, pH 7.7, 180 mM KCl, 10 mM $MgCl_2$, 50 μM dithiothreitol, and 50 μM [γ-^{32}P]ATP (700–900 cpm/pmol). All assays contained 80–100 U/ml of casein kinase II [5] and 0.5 mg/ml dephosphocasein except where indicated otherwise. All additions were made prior to the addition of the enzyme. Incubation times were routinely for 15 min at 30°C and the reaction was linear for at least 30 min. The reaction was terminated by the removal of an aliquot to a 2 × 1 cm Whatman P81 phosphocellulose paper which was immersed in 75 mM phosphoric acid, washed 3 times in the same acid, dried and counted. Values reported have been corrected for controls run with heat denatured enzyme. All assays were performed in triplicate and are representative of 2–4 experiments.

The results obtained for the inhibition of casein phosphorylation in the presence and absence of poly U were analyzed for both mixed and competitive inhibition using the program NATO adapted to an IBM-PC as described by Cornish-Bowden [20]. The best fit fot the experimental data was obtained for competitive inhibition.

3. RESULTS

The addition of single-stranded M13 phage DNA to the standard assay of pure casein kinase II prepared from *X. laevis* oocyte nuclei results in marked inhibition of enzyme activity. As shown in fig.1, the phosphorylation of the exogenous substrate casein was 50% inhibited at a concentration

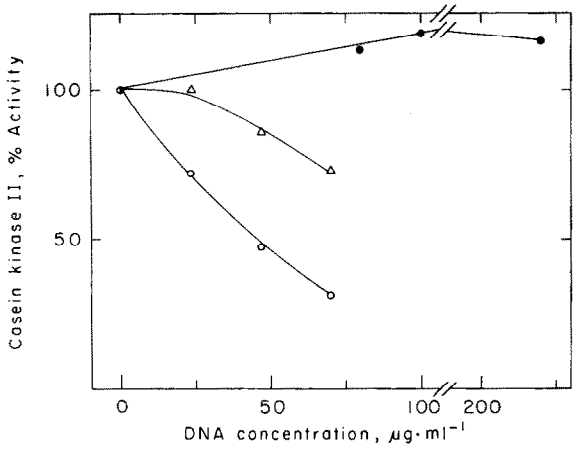

Fig.1. The effect of phage DNA on nuclear casein kinase II from *X. laevis* oocytes. Reactions were carried out using the standard condition described in section 2 and 80 U/ml of casein kinase II, with the following additions: ○, single-stranded M13 DNA; △, double-stranded M13 DNA; and ●, double-stranded pGEM-3 DNA.

of about 50 μg/ml. Double-stranded M13 DNA was much less effective and the DNA of phage pGEM-3 did not cause inhibition at over 200 μg/ml and was found to stimulate 15–20% over control values. Total DNA from salmon sperm did not alter activity.

Unfractionated yeast tRNA which contains a mixture of the different amino acid acceptor species inhibits the enzyme, showing 50% inhibition at 150 μg/ml (fig.2). A similar result was obtained with total oocyte tRNA (not shown). Specific $tRNA^{Phe}$, however, did not inhibit and was even slightly stimulatory at the higher levels tested. *E. coli* 16S and 26S ribosomal RNA, on the other hand, did not alter the kinase activity.

These initial observations suggested that the inhibitory effect was limited to particular nucleic acid structures and not simply related to the polyanionic nature of these compounds. The results obtained with synthetic polynucleotides confirmed this supposition. Fig.3 shows the results obtained with 3 high molecular weight homopolymers. Poly U is seen to be a potent inhibitor of the enzyme, giving 50% inhibition at approximately 10 μg/ml, while poly C is much less effective and poly A and poly AG (not shown) are without effect over the wide range of concentrations tested. The capacity of oligo dT, between 12–18 bases in length, and the trinucleotides ACC and AUU were also tested (not

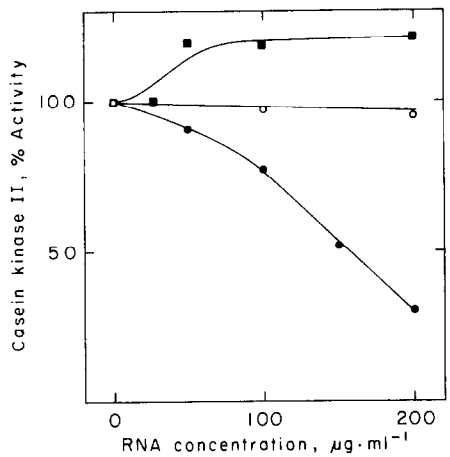

Fig.2. The effect of tRNA and rRNA on nuclear casein kinase II from *X. laevis* oocytes. Reactions were carried out as in fig.1 with the following additions: ■, yeast $tRNA^{Phe}$; ●, yeast total tRNA; and ○, *E. coli* 16S and 23S rRNA.

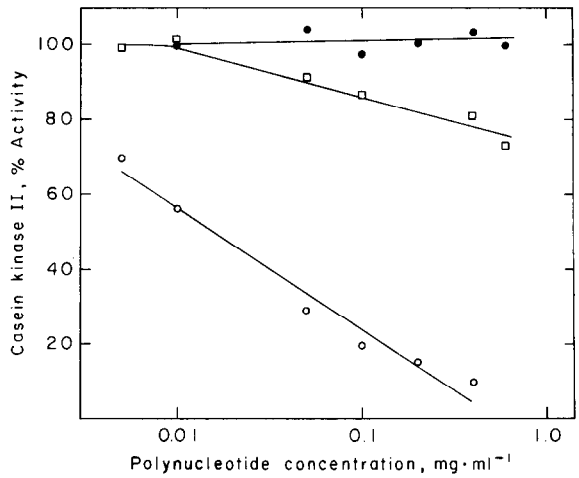

Fig.3. The effect of synthetic polynucleotides on nuclear casein kinase II from *X. laevis* oocytes. Reactions were carried out as in fig.1 with the following additions: ●, poly A; □, poly C; ○, poly U.

shown). Only the triplet AUU had a significant effect, inhibiting 30% at 4 A_{260}/ml.

The nature of the inhibitory effect was analyzed further by the use of specific nucleases. As shown in table 1 the presence of pancreatic ribonuclease added during the 15 min assay period reverses the inhibitory effect of poly U, whereas DNase has no effect. Inhibition by tRNA was also reverted by RNase A. The inhibitory effect of single-stranded DNA from M13 is greatly reduced by S_1 nuclease, whose activity is specific for single-stranded nucleic acids.

The capacity of poly U to inhibit in the presence of polyamines was also evaluated. As shown in table 1, the kinase activity is stimulated in a typical manner by spermine at 1.5 mM but this agent does not override the inhibitory effect of poly U (estimated 100 μM). The lack of complete reversal by the addition of a high concentration of RNase is surprising and may indicate that the poly U is not accessible to the nuclease under these conditions.

The velocity of the casein kinase II phosphorylating reaction was measured using different concentrations of casein in the presence and absence of poly U. The double reciprocal plot shown in fig.4 indicates that the poly U inhibition is competitive with the protein substrate with an apparent inhibition constant of 82 nM.

Casein kinase I is also present in oocyte nuclei and may be separated chromatographically from casein kinase II [2,18]. Such a preparation was tested for the capacity to be inhibited by poly U and was found to have unaltered activity in the presence of 10–200 μg/ml of the polynucleotide (not shown). cAMP-dependent protein kinase was

Table 1

Characteristics of the inhibition of casein kinase II by nucleic acids

Experiment	Addition	Activity (%)
1.	None	100
	Poly U	28
	RNase	94
	Poly U and RNase	86
	DNase	96
	Poly U and DNase	25
2.	None	100
	M13 ssDNA	30
	Nuclease S1	100
	M13 ssDNA and nuclease S1	76
3.	None	100
	Poly U	27
	Spermine	174
	Poly U and spermine	19
	Poly U, spermine and RNase	89

Reactions were carried out under standard conditions described in section 2. The additions were made to give the following final concentrations: poly U and M13 single-stranded DNA (ssDNA), 100 μg/ml; nuclease S1, 60 U/ml; pancreatic RNase and DNase, 20 μg/ml; and spermine, 1.5 mM

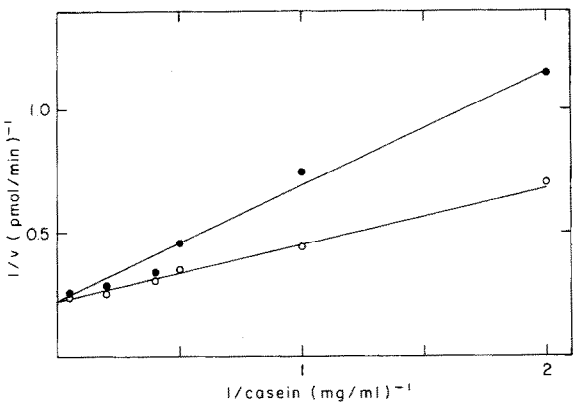

Fig.4. Double reciprocal plot of the inhibition of oocyte nuclear casein kinase II by poly U. Casein was varied from 0.5 to 18 mg/ml and ATP concentration was constant at 50 μM. Incubations were for 10 min, using otherwise standard conditions as given in section 2, in the presence (●) or absence (○) of poly U at 8.5 μg/ml.

likewise unaffected by similar concentrations of poly U when assayed with protamine as substrate.

4. DISCUSSION

The activity of casein kinase II from most sources studied is characteristically affected by two classes of agents, polyanionic compounds such as heparin, a potent inhibitor [3], and polycationic agents such as polylysine and spermine, which activate the enzyme [21]. The results presented in this communication demonstrate that specific nucleic acids can also inhibit casein kinase II.

This observation extends the initial findings of Kandrov and Stepanov [14] who reported that a protein phosphorylating activity obtained from oocytes of *Rana temporaria* and *Xenopus laevis* and with characteristics of a casein kinase could be retained on Poly U-Sepharose columns and that poly U was inhibitory to the phosphorylation of endogenous substrates present in the total oocyte extract [15]. Maenpaa [13] also reported that phosvitin kinase from rooster liver was inhibited by certain tRNAs. The inhibition of rat liver nuclei casein kinase II by an oligonucleotide was also reported [16].

The structural specificity for the inhibition of the nuclear kinase by the various nucleic acids indicates that the polyanionic nature of the polymers is not sufficient to explain this effect. It should be emphasized that all experiments were carried out at high ionic strength and 10 mM magnesium concentrations, since the response of casein kinase II to polyamines and heparin has been shown to be sensitive to salt and magnesium concentrations [21].

The specificity observed with the single-stranded DNA versus the double-helical DNA structure suggests that unpaired bases of the DNA may be important for the inhibition. On the other hand, small oligodeoxinucleotides such as the oligo dT are ineffective. The inhibitory potency of poly U compared to all other nucleic acids tested again points to a high specificity for base structure.

The inhibitory capacity of total oocyte or yeast tRNA is contrasted by the lack of any effect by *E. coli* ribosomal RNA and more interestingly, of purified specific yeast $tRNA^{Phe}$. This might indicate that one or certain of the tRNA species present in the unfractionated preparation are responsible for the inhibition seen. The presence of another type of RNA in the tRNA preparation cannot be ruled out.

The reversal of the effect of poly U by high concentrations of substrate casein is similar to the response of this enzyme to heparin, which has been shown to be a competitive inhibitor in the case of casein kinase II of reticulocytes with casein as substrate [3].

The finding that the nuclear enzyme is sensitive to the addition of RNA, particularly poly U, and also single-stranded DNA, may indicate a yet-unidentified regulatory mechanism. It is noteworthy that two other protein kinases have been shown to interact with nucleic acids, the dsRNA-activated kinase which phosphorylates the α-subunit of initiation factor eIF-2 [22] and a dsDNA-activated kinase present in several animal cell extracts [23]. The particular effectiveness of poly U in inhibiting casein kinase II suggets a role for uridine-rich polynucleotides in the modulation of this kinase activity. Analysis of the effect of the U-rich small nuclear RNAs that participate in RNA processing would be of particular interest, since protein substrates of casein kinase II have been shown to be present in ribonucleoprotein particles [24].

In the light of these observations, and in view of the very high concentrations of RNA in amphibian oocytes (about 4 μg RNA per oocyte) [25], it would be of interest to analyze for the presence of specific inhibitory RNA in the nucleus and whole oocyte.

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