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pH-dependent changes in structure and RNA-binding activity of casein kinase 2 from *Rana temporaria* oocytes

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It is demonstrated by filter-binding assay that casein kinase 2 from *Rana temporaria* oocytes binds rRNA in vitro with high affinity. Ligand-blotting shows that rRNA-binding activity is inherent to α and α' subunits of the enzyme. Increase of pH from 6.5 to 7.5 has little effect on casein kinase but completely suppresses rRNA-binding activity of the enzyme. Sedimentation coefficient of casein kinase 2 also depends on pH: at pH 7.5 it is mainly 10 S, and at pH 6.5 – 18 S. At pH 6.95 the amounts of both forms are equal. The heavy form of casein kinase 2 practically lacks rRNA-binding activity.

Casein kinase 2; RNA-binding; pH

1. INTRODUCTION

Casein kinases 2 are found in all eukaryotic cells studied and account for a great deal of the total phosphoprotein transferase activity of cell extracts. All animal casein kinases 2 share a common polypeptide structure of $\alpha_2\beta_2$ or $\alpha\alpha'\beta_2$, with α/α' having a molecular mass of 35–44 kDa and β of 24–29 kDa [1]. The catalytic centre of the enzyme is located on the α/α' subunit while the functions of the β subunit are presently unknown [2,3].

An important problem is the mode of regulation of casein kinase 2 activity in vivo and in vitro. It is established that this enzyme is regulated on the post-translational level in different cells by steroid and peptide hormones, growth factors, and serum [4–6]. The molecular mechanism of such control is poorly understood now since well-known signal molecules or covalent modifications in vitro have very little if any effect upon casein kinase 2. However, it has been shown that polyanions (heparin, poly(Glu), polynucleotides) heavily suppress casein kinase activity in vitro [7–13]. These polynucleotides and probably RNA may serve as natural effectors for casein kinase 2 because stable complexes of this kinase and RNA (most likely mRNA) have been detected in many eukaryotic cells [14,15] and *Rana temporaria* oocytes in particular [16].

In the process of our work with amphibian oocytes we have noticed a significant but transient post-translational activation of casein kinase 2 during oocyte maturation 7 h after progesterone administration. Another notable event which takes place during oocyte maturation is the rise of intracellular pH from 7.2–7.3 to 7.6–7.7 [17,18]. The physiological meaning of this phenomenon is obscure; however, it may affect a variety of biochemical processes. In the present article we demonstrate that the variation of pH value in the physiological range has little effect upon the protein kinase activity of casein kinase 2 but changes its RNA-binding activity, measured with 16 S rRNA. Moreover, evidence is presented that in this pH interval casein kinase 2 exists in two distinct interconvertable forms with sedimentation coefficients of 10 S and 18 S. RNA-binding activity is inherent mainly to the light form of the enzyme. We suppose that physiological changes of pH may affect binding of casein kinase 2 to different types of cellular RNAs and so alter the compartmentalization and activity of the enzyme.

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2. MATERIALS AND METHODS

rRNA was labelled with [^{14}C]uridine and [^{14}C]uracil in vivo and isolated from *E. coli* MRE 600. Its specific activity was 50000 cpm/μg. Casein kinase 2 was isolated from *Rana temporaria* oocytes as was described earlier [16]. The standard buffer contained 10 mM triethanolamine or MES, 110 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 6 mM 2-mercaptoethanol, pH 5.5–7.9. In some experiments 5–10% glycerol was added.

For determination of RNA-binding activity casein kinase 2 was incubated with [^{14}C]rRNA in 1 ml of standard buffer at 20°C for 10–15 min and filtered through nitrocellulose filters (HAWP, 0.45 μm, 25 mm Millipore). Each filter was washed 4 times with 1 ml of standard buffer, dried and counted. RNA-binding activity was expressed as pmol of radioactive rRNA retained on a filter in the presence of a certain amount of casein kinase 2.

Determination of the sedimentation coefficient of casein kinase 2 at different pH values was provided with the help of centrifugation

in the 10–30% sucrose gradients (prepared on standard buffer) in SW-65 rotor of Beckman L2-65 centrifuge at 60000 rpm and 4°C for 4 h and 15 min. rRNA (23 S, 16 S and 5 S) and aldolase (8.5 S) were used as markers. Usually 150 μl of casein kinase 2 (about 20 μg/ml) was layered on each 4.8-ml gradient.

To provide ligand-blotting the gel, after electrophoresis according to Laemmli [19], was washed for 15 min in 25 mM Tris-HCl, 0.192 M glycine, 20% methanol, pH 7.3, and the proteins were transferred to nitrocellulose sheet (Schleicher & Schuell BA 85) in an electroblotting apparatus at 36 V and 150 mA for 3 h. The nitrocellulose was washed 3 times for 90 min with 50 ml of binding buffer which contained 20 mM MES, 50 mM NaCl, 1 mM EDTA, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll 400, pH 6.5 [20]. Then nitrocellulose sheet was placed into 22 ml of [^{14}C]rRNA (16 S or 23 S) solution in binding buffer (10–15 μg/ml) and incubated overnight at 4°C with light shaking. Nitrocellulose was washed 6 times for 10–15 min with 40 ml binding buffer, dried, and covered with 2,5-diphenyloxazole. Fluorography was provided at −70°C with Kodak X-Omat X-ray film.

Protein kinase activity of the enzyme was measured as earlier [16], protein content – by the method of Schaffner and Weissmann [21], electrophoresis of rRNA and polynucleotides in 6% polyacrylamide gel was provided as in [22] with minor modifications.

3. RESULTS AND DISCUSSION

The filter-binding assay provided with pure casein kinase 2 from *Rana temporaria* oocytes and radioactive rRNA (Fig. 1) demonstrates that this enzyme possesses strong rRNA-binding activity. Casein kinase 2 also binds poly(U) but practically lacks poly(A)-binding activity (results are not shown).

In the next experiment we try to find out which subunit of casein kinase 2 is responsible for its RNA-binding activity. The results of ligand-blotting (Fig. 2) show that radioactive rRNA binds to α and α' subunits and does not bind to β subunit.

Fig. 3 demonstrates that RNA-binding activity of casein kinase 2 measured with 16 S rRNA is very sensitive to pH. It is maximal at pH 6.0–6.5 and falls to background levels when the pH has risen to 7.5. Casein kinase activity of the enzyme does not change much with pH. Self-phosphorylation of casein kinase 2 in vitro does not lead to visual changes of its rRNA-binding activity or sensitivity to pH.

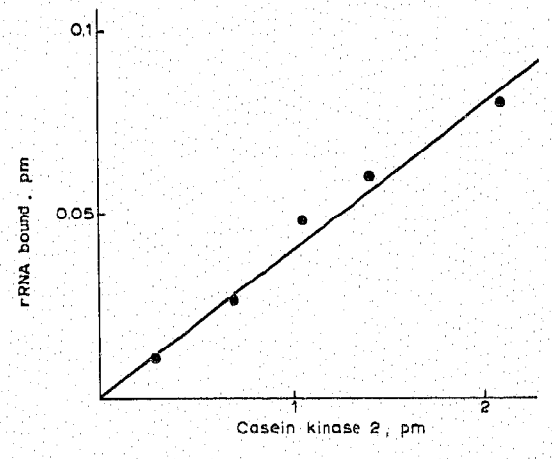

Fig. 1. Binding of casein kinase 2 to 16 S [^{14}C]rRNA. Casein kinase 2 (M_r 140 kDa) was incubated with 0.14 μg (0.25 pmol) of radioactive 16 S rRNA in 1 ml of standard buffer (pH 6.5) for 10 min at 20°C and filtered through nitrocellulose filters.

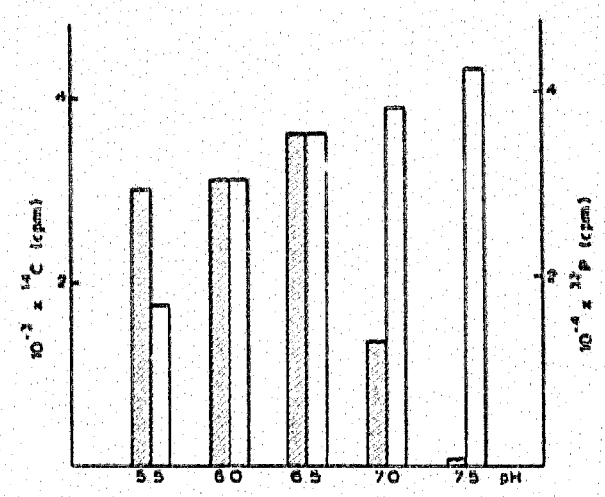

Fig. 2. Identification of RNA-binding subunits of casein kinase 2 by ligand-blotting. (A) Nitrocellulose was stained with Amido black 10B. (B) Nitrocellulose was incubated with [^{14}C]23 S rRNA as indicated in section 2. (C) Self-phosphorylation of casein kinase 2. Each line contains 2.5 μg of casein kinase 2 non-phosphorylated (A and B) or pre-phosphorylated in vitro (C). (B) and (C) are fluorographs. Exposure time – 4 weeks. Arrows indicate the positions of casein kinase 2 subunits.

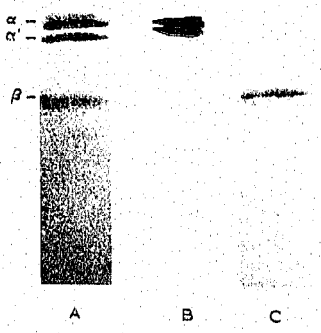

Fig. 3. pH-dependence of casein kinase and RNA-binding activities of casein kinase 2. To measure RNA-binding activity (closed bars) 0.25 μg of casein kinase 2 was incubated with 0.5 μg of 16 S [^{14}C]rRNA in 1 ml of standard buffer with different pH for 10 min at 20°C and filtered through nitrocellulose filters. Casein kinase activity (open bars) was measured in 0.1 ml of standard buffer with different pH which contained 0.1 μg of casein kinase 2, 60 μg of dephosphorylated casein and 0.1 mM of [γ-^{32}P]ATP with specific activity 200 cpm/pm. Incubation lasted for 1 h at 20°C [16].

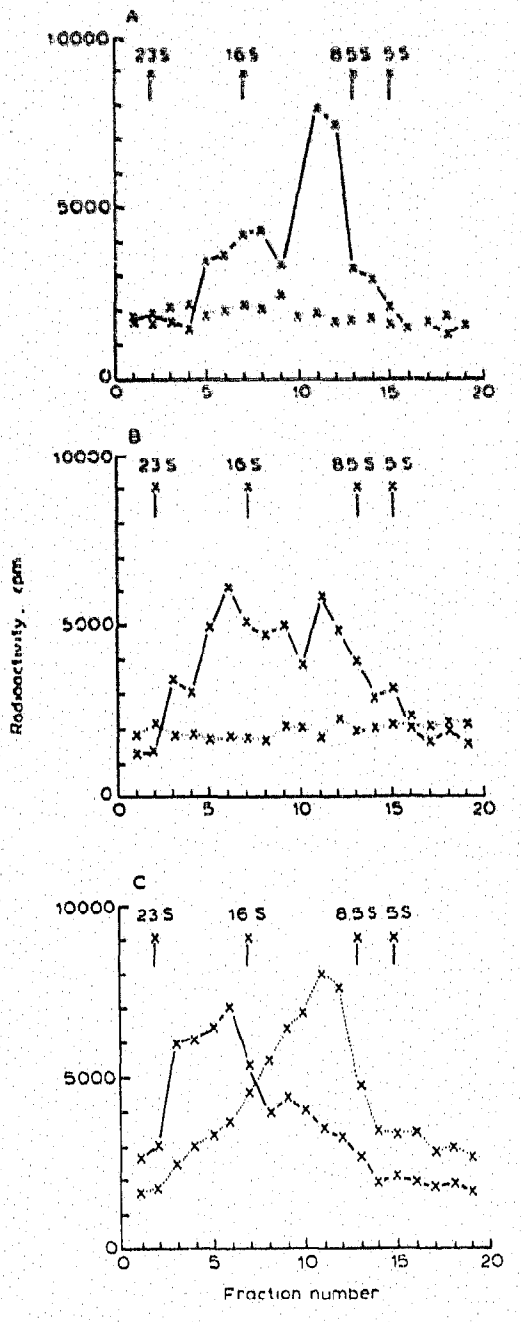

Fig. 4. Centrifugation of casein kinase 2 at pH 7.6 (A), 6.95 (B) and 6.45 (C). Centrifugation was performed as indicated in section 2. For determination of casein kinase activity (solid line) 40 μl of each fraction was incubated with 0.1 mM [γ-^{32}P]ATP (300 cpm/pmol) and 60 μg of dephosphorylated casein in the total volume 0.1 ml for 1 h at 20°C. RNA-binding activity (dotted line) was measured in 0.15 ml of each fraction with 1.5 μg of 16 S [^{14}C]rRNA. Arrows indicate the positions of markers.

Changes in pH do not lead only to the specific alterations of RNA-binding activity of casein kinase 2, but affect the structure of the enzyme as well. Fig. 4 shows that at pH 7.5 the sedimentation coefficient of the main form of casein kinase 2 is about 10 S, but it is increased to 18 S at 6.5. At 6.95 amounts of both forms are equal. RNA-binding activity (which can be measured only at pH 6.5 according to Fig. 3) is essential much more to the low-molecular weight form of the enzyme.

The formation of heavy complexes by casein kinase 2 may have much in common with the polymerisation of this enzyme from *Drosophila* [28]. The absence of RNA-binding activity from such complexes may be a result of masking of RNA-binding sites during aggregation.

It has been demonstrated earlier that casein kinase 2 participates in the formation of mRNP particles in different eukaryotic cells (reviewed in [23]). Regulation of RNA-binding activity of the mRNP proteins is very important because it seems to be connected with the availability of a message to the translational machinery [23]. On the other hand, reversible formation of complexes with RNA gives an opportunity to regulate the activity of casein kinase 2 on the post-translational level, which is also of great interest. Future investigations of these problems may reveal some of their common mechanisms.

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