FURTHER STUDIES ON THE QUATERNARY STRUCTURE OF YEAST CASEIN KINASE II*

* Department of Molecular Biology, University of M. Curie-Skłodowska, 20-033 Lublin, Poland and
b Department of General and Organic Chemistry, Medical Academy, 15-230 Białystok, Poland

Received 3 July, 1985

Casein kinase type II were isolated by the same procedure, from rat liver, human placenta, Querin carcinoma and yeast, and characterized. The mammalian enzymes were composed of three subunits $\alpha, \alpha'$ and $\beta$, whereas yeast kinase was composed of two subunits $\alpha$ and $\alpha'$. It was shown that the catalytic activity, substrate and phosphate donor specificity, sensitivity to heparin and spermine were the same for all the kinases tested. The results give additional support to the suggestion [1] that the $\beta$ subunit is not required for optimal activity and specificity of yeast casein kinase II. The quaternary structure of the yeast enzyme of a molecular weight of approximately 150 000 is proposed as $\alpha_2 \alpha'_2$.

It has been firmly established that casein kinase II (ATP: protein phosphotransferase, EC 2.7.1.37) isolated from higher organisms consists of two or three polypeptides with a total molecular mass of about 130 000 - 150 000 and a quaternary structure: $\alpha_2 \beta_2$ or $\alpha, \alpha' \beta_2$ [2, 3, 4, 5, 6]. There are some data [3, 4] indicating that the $\alpha$ subunit has a catalytic site. The $\beta$ subunit seems to play a regulatory role, though, as has been suggested, it is also required for optimal activity of the kinase [7]. A common feature of the casein kinase type II isolated from animal tissues is auto-phosphorylation of their $\beta$ subunits [3].

Recently, casein kinase II from baker’s yeast has been purified to an apparent homogeneity and found to be deprived of the $\beta$ subunit [1]. The isolated enzyme is composed of $\alpha$ and $\alpha'$ subunits present at a molar ratio 1: 1. Its activity was not

* This work was supported by the Polish Academy of Sciences within the Project 09.7.
affected by the addition of the β subunit [1]. This and other observations strongly argue against the suggested requirement of the β subunit for the kinase activity [7]. There is still an unanswered question as to whether the yeast polypeptide β is loosely associated in the oligomeric enzyme molecule and can thus be readily separated by gel filtration, or the purification procedure used renders somehow such an association more labile. The present report shows that the susceptibility of casein kinase II to lose the β polypeptide during purification is an intrinsic feature of the yeast enzyme. The same multistep purification procedure [1] applied for isolation of the corresponding mammalian kinases gave in all cases enzyme preparations containing all three subunits including the β subunit.

MATERIAL AND METHODS

Materials: A diploid, As-10, strain of Saccharomyces cerevisiae was cultivated aerobically and cells were harvested in a late logarithmic phase of growth as described previously [8]. Transplantation of Guerin tumour into rats and isolation of tumour tissues were performed as described by Ratkiewicz & Galasiński [9]. Rats, Wistar strain, were of 150 g in weight. Human placenta was from the Clinics of Perinatology and Obstetrics, Medical Academy in Białystok.

Preparation of the yeast and mammalian tissue postribosomal supernatants. Yeast cell lysates were prepared by using a bacterial 1,3-β-glucanase (Zymolyase) for digestion of the cell wall [10]. Tissue homogenates from rat liver, Guerin tumour, and placenta were prepared by the technique of Moldave & Skogerson [11]. The yeast cell lysates and tissue homogenates were centrifuged for 30 min at 30 000 g, and then the resulting supernatants were ultracentrifuged for 2 h at 150 000 g. The obtained supernatants were stored at −20°C and served as a source of casein kinase II.

Preparation of ribosomes and isolation of acidic proteins of 60S ribosomal subunits. The crude ribosomal preparations (150 000 g, sediment) were washed twice with buffered 0.5 M KCl followed by ultracentrifugation [8]. The acidic protein fraction containing yeast ribosomal proteins L44 and L45 or their mammalian counterparts were isolated from purified 80S ribosomes according to Van Agthoven et al. [12] and then purified by chromatography on carboxymethylcellulose [13].

Polyacrylamide gel electrophoresis. Acidic ribosomal proteins, L44 and L45, were identified electrophoretically as described previously [8]. The purity of protein kinases was verified by slab gel electrophoresis on polyacrylamide performed under denaturing conditions [14]. The same technique was also used for the estimation of molecular weights of highly purified kinase preparations.

Determination of casein kinase II activity. The protein kinase activity was assayed in the presence of [γ-32P]ATP as a phosphate donor and various substrates, including total casein, histone, 80S ribosomes, acidic fraction of 60S ribosome subunits under conditions described previously [15].
Reagents: [γ-32P]ATP (specific activity > 5000 Ci/mmole), prepared according to Glynn & Chappell [16], was purchased from the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences (Warsaw, Poland). DEAE-cellulose (DE-52) and P-cellulose (P-11) were from Whatman Ltd. (England) and Ultrogel (AcA 44) from LKB (Uppsala, Sweden). Molecular weight protein standards were supplied by Pharmacia Fine Chemicals (Uppsala, Sweden). All other reagents were of high purity grade.

RESULTS

Isolation of casein kinase II from mammalian tissues. Kudlicki et al. [1] isolated a casein kinase type II from yeast using a multistep purification procedure which involved the fractionation of postribosomal supernatant protein with ammonium sulphate by chromatography on DEAE-cellulose, P-cellulose and finally the filtration on Ultrogel AcA 44. The resulting enzyme preparation was depleted of the β subunit. The same procedure was applied for the isolation of casein kinase II from several mammalian sources in the experiments reported here. Figure 1 shows the final filtration step of purification of casein kinase II from rat liver (A), Guerin carcinoma (B), and human placenta (C). As a control the cerevisiae enzyme was included (D). As one can see, all enzyme preparations emerged from the column in void volume or close to it which points to their high molecular weight. It is of great interest that all mammalian kinases contained the β subunit as shown by polyacrylamide slab gel electrophoresis under denaturing conditions (Plate 1A, a, b, c). Both, in the previous and present experiments the yeast enzyme was deprived of this polypeptide (Plate 1A, e). The presence of the β subunit in mammalian kinases was additionally identified by autoradiography of the samples previously preincubated with [γ-32P]ATP and then electrophoresed on polyacrylamide. The results (Plate 1B, a, b, c) show that the kinases mentioned above have the autophosphorylated β subunit. The polypeptide β when present alone did not undergo autophosphorylation (Plate 1B, e). However, it was readily phosphorylated after the addition of yeast casein kinase containing the two larger subunits [1]. The applied purification procedure made possible an extensive purification of rat liver and human placenta (Plate 1A, a and c, respectively) casein kinases. The enzyme from Guerin tumour was less purified. The gel filtration preparations contained (Plate 1A, b) several minor protein bands in addition to the 3 main polypeptides, the components of the enzyme molecule. Most of the contaminating proteins underwent phosphorylation (Plate 1B, b). Despite a relatively low purification degree of Guerin tumour kinase the gel electrophoresis distinctly shows the presence of the smallest subunit in the enzyme preparations (Plate 1A, b).

The molecular weights of the subunits of casein kinase II preparations isolated from all four enzyme sources appeared to be slightly different (Table I). Strikingly, the difference in molecular weights between subunits α and α' in all the enzymes examined is the same and equals 4000. There is some evidence indicating that the
α' polypeptide can be a product of the α subunit proteolysis [3]. The data collected in Table 1 might suggest, but not prove, that such a proteolytic cleavage removes the same polypeptide fragment from each α subunit.

![Chromatography of kinase preparations on Ultrogel AcA 44.](image)

Fig. 1. Chromatography of kinase preparations on Ultrogel AcA 44. The kinase preparations from rat liver (A), Guerin carcinoma (B), human placenta (C) and yeast (D), after P-cellulose step, were chromatographed on Ultrogel column pre-washed with 50 mM Tris/HCl buffer, containing 50 mM NaCl, 6 mM mercaptoethanol and 0.5 mM EDTA. The column (1.5×95 cm) was washed with the same buffer and eluate fractions were routinely assayed for kinase activity in the presence of casein and radioactive ATP. In the case of yeast (D), endogenous phosphate acceptor was localized in the eluate in the presence of fraction No. 29 and radioactive ATP (□) as described previously [1]. The column was calibrated with the following standards: a, Blue Dextran 2 000; b, bovine serum albumin (Mr = 67 000); c, horseradish peroxidase (40 000), and d, bovine pancreas ribonuclease (13 700). A_{280} (--.--)

The molecular weights of casein kinase II from several animal sources have been determined as approximately 130 000 [3]. From the molecular weights of the subunits (Table 1), assuming that the enzyme structure is α, α', β₂, the total mass
Plate 1. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of highly purified casein kinase II preparations. A, The Coomassie blue-stained gels and B, the corresponding autoradiograms. The enzyme samples were preincubated with radioactive ATP under standard conditions then subjected to electrophoresis. Lanes: a, rat liver kinase; b, Guerin carcinoma, c, human placenta; d, S. cerevisiae; e, isolated polypeptide β of yeast kinase (Fig. 1D, fractions No. 54 - 58). Protein standards: 1, bovine serum albumin (67 000); 2, ovalbumin (45 000); 3, trypsinogen (24 000) and 4, lysozyme (14 300).

Table 1

Subunit composition of casein kinase II from various sources

Casein kinase were isolated as described in Materials and Methods. The final enzyme preparations were subjected to polyacrylamide gel electrophoresis under denaturing conditions. Molecular weight standard proteins were run simultaneously. Molecular weight of yeast β polypeptide was determined after its separation on Ultrogel AcA 44.

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Molecular mass of casein kinase subunits (×10⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>α</td>
<td>41</td>
</tr>
<tr>
<td>α'</td>
<td>37</td>
</tr>
<tr>
<td>β</td>
<td>23</td>
</tr>
</tbody>
</table>
of the mammalian kinases also approximates 130 000. As it has been mentioned previously, all the kinases tested emerged from the Ultrogel AcA 44 column (resolving range 10 000 - 130 000) in the void volume (Fig. 1). In the case of yeast enzyme this result is unexpected since the kinase composed of two subunits (α, α') with the total molecular mass of 78 000 should have been found in the column eluate close to the serum albumin marker. The molecular weight of yeast and rat liver casein kinases was additionally determined by means of glycerol gradient centrifugation. The $M_r$ values obtained approximated 150 000 and 130 000 for the yeast and rat liver enzyme, respectively (Meggio, F., Grankowski, N. & Pinna, L. - in preparation). All these data may suggest that the quaternary structure of yeast kinase is different from that of animal casein kinase II, and most probably the enzyme exists in the form of a tetramer: $\alpha_2 \alpha'_2$.

![Figure 2](image.png)

**Fig. 2.** Effect of heparin (A) and spermine (B) on the activity of isolated kinases. The activity of casein kinases from rat liver (□), Guerin carcinoma (■), human placenta (●), and yeast (○) was estimated in the presence of increasing concentration of heparin (μg/0.1 ml sample). The effect of spermine was examined in the presence of a constant amount of heparin (0.2 μg/sample)

**Properties of kinase preparations.** The isolated mammalian kinases behaved as a typical type II casein kinase. They were inhibited very strongly by a low concentration of heparin (Fig. 2A), a specific inhibitor of this type of protein kinase [3]. The inhibition could be completely reversed by adding 2 mM spermine (Fig. 2B). It is worth noting that the effects exerted by heparin or spermine were the same for all the enzymes tested, no matter whether they contained (mammalian) or were deprived of (yeast) the β polypeptide.

All four casein kinases phosphorylated acidic protein substrates, i.e. casein, phosvitin, isolated acidic proteins of 60S ribosome subunit, or the same ribosomal proteins in situ. When their activity was assayed in the presence of $[^{32}$P]ATP, the addition of non-radioactive GTP to the incubation mixture appeared to be
strongly inhibitory. This indicates that GTP can serve as a phosphate donor. The enzymes also exhibited the same pattern of amino acid phosphorylation. In each case both phosphoserine and phosphothreonine were detected in the phosphorylated protein substrate (not shown). In short, the isolated mammalian kinases met all the criteria enabling us to classify them as casein kinase of type II.

DISCUSSION

The results reported here support the previous suggestion [1] that casein kinase II from yeast cytoplasm contains only subunits α and α'. The absence of the β subunit in the yeast enzyme preparations is not due to the use of a particular purification procedure leading to the removal of this subunit from the native enzyme molecule, since the same procedure applied for the isolation of mammalian casein kinase II did not separate the β subunit. This may indicate that the existence of an enzyme of a different quaternary structure is an intrinsic feature of yeast cells. However, the β subunit or a polypeptide of the same molecular weight as the true subunit β has been found in yeast postribosomal supernatant and at the early stages of kinase purification [1].

As it was already mentioned, Cochet & Chamaz [7] claimed that the β subunit of casein kinase type II from bovine lungs was required to optimize the catalytic activity of subunit α (α'). In contrast to the yeast enzyme, the lung casein kinase II has the subunit β strongly bound to polypeptide. The separation of the two subunits [4] was possible only under denaturing conditions.

Some recent reports indicate the presence of enzymes similar to yeast kinase in the wheat germ cytosol [17] and in the nuclei of Dictyostelium discoideum [18]. The kinase isolated from the above sources contained one main polypeptide of a molecular weight of 36 000 - 38 000, probably subunit α' and they possessed all the properties classifying them unambiguously as casein kinases type II.

The role, if any, of the β subunit for the discussed a typical casein kinases II remains to be elucidated.

REFERENCES