Volume 315, number 2, 173–177 FEBS 11958 January 1993

Effect of metal ions on the activity of casein kinase II from *Xenopus laevis*

Marta Gatica[a], María V. Hinrichs[a], Ana Jedlicki[a], Catherine C. Allende[b] and Jorge E. Allende[a]

[a]*Departamento de Bioquímica, Facultad de Medicina* and [b]*Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Casilla 70086, Santiago 7, Chile*

Received 13 November 1992

Casein kinase II purified from the nuclei of *Xenopus laevis* oocytes as well as the recombinant α and β subunits of the *X. laevis* CKII, produced in *E. coli* from the cloned cDNA genes, were tested with different divalent metal ions. The enzyme from both sources was active with either Mg^{2+}, Mn^{2+}, or Co^{2+}. Optimal concentrations were 7–10 mM for Mg^{2+}, 0.5–0.7 mM for Mn^{2+} and 1–2 mM for Co^{2+}. In the presence of Mn^{2+} or Co^{2+} the enzyme used GTP more efficiently than ATP as a phosphate donor while the reverse was true in the presence of Mg^{2+}. The apparent K_m values for both nucleotide triphosphates were greatly decreased in the presence of Mn^{2+} as compared with Mg^{2+}. Addition of Zn^{2+} (above 150 μM) to an assay containing the optimal Mg^{2+} ion concentration caused strong inhibition of both holoenzyme and α subunit. Inhibition of the holoenzyme by 400 μM Ni^{2+} could be reversed by high concentrations of Mg^{2+} but no reversal of this inhibition was observed with the α subunit.

Casein kinase II; Casein kinase II α and β subunits; Divalent metal ion; *Xenopus laevis*

1. INTRODUCTION

Protein kinases play a key role in metabolic regulation and in cell growth by catalyzing phosphorylation reactions that modify the biological activity of many proteins. Among the nearly 100 protein kinases known, several have deserved particular attention due to their ubiquity and the importance of the substrates they modify. Casein kinase II (CKII) is a protein kinase that is present in all the eukaryotic species tested and has been found both in the nucleus and cytoplasm of cells. The active enzyme is a heterotetramer of a $\alpha_2\beta_2$ or $\alpha\alpha'\beta_2$ composition. The α and α′ subunit contain the catalytic center and are active by themselves, while the β subunit plays a regulatory function increasing severalfold the activity of the α-type subunits (for Reviews see [1,2]).

CKII has been implicated in the phosphorylation of DNA and RNA polymerases, DNA topoisomerases, of the oncogenes products of *fos*, *myc*, *myb* and sv-40 large T and of the p53 tumor suppresor gene [1–4]. In addition, several reports have presented evidence linking CKII with the regulation of the cell cycle and with the induction of cell growth [5,6].

In spite of the interest of CKII, there is only limited information regarding its biochemical properties and little is known about the physiological mechanisms that may regulate its activity. CKII has two identifying characteristics, it can use both GTP and ATP as phosphate donors and it is extremely sensitive to heparin inhibition [1,2]. In addition, it is known that CKII can be activated by polyamines and by polylysine and that it requires high ionic strength for its activity [7,8].

No detailed information has been previously reported regarding the effect of divalent metal ions on CKII activity. The requirement of Mg^{2+} for activity has been well documented and its optimal concentration has been reported to be between 5 and 15 mM [9]. There are some conflicting reports regarding the capacity of Ca^{2+} and Mn^{2+} to replace Mg^{2+} [10,11].

In the present work, we describe the effect of several divalent metal ions on the CKII obtained from *X. laevis* oocyte nuclei and on the recombinant α and β subunits of this enzyme produced in *E. coli*. It is shown that Mn^{2+} and Co^{2+} can replace Mg^{2+} in the metal requirement for catalytic activity. It is also demonstrated that replacement of Mg^{2+} for Mn^{2+} significantly changes the kinetic parameters observed with ATP and GTP and the relative activities of these substrates. Similar results are observed with the recombinant α subunit of *Xenopus* CKII expressed in *E. coli*. Several other divalent metal ions tested cannot replace Mg^{2+} but tend to inhibit the enzyme assayed in their presence. Inhibition by Zn^{2+} at concentrations of the metal above 150 μM is observed with the holoenzyme as well as with the recombinant α subunit. However, the inhibition caused by the presence of Ni^{2+} can be reversed by high Mg^{2+} concentrations with the native holoenzyme or reconstituted $\alpha_2\beta_2$ but not with the isolated catalytic α subunit.

2. MATERIALS AND METHODS

2.1. *Purification of casein kinase II from oocyte nuclei*

Ovaries were obtained by surgery from adult female *X. laevis*. Stage 5 and 6 oocytes and their nuclei were prepared by the method of

Correspondence address: E. Allende, Departamento de Bioquímica, Facultad de Medicina, Casilla 70086, Santiago 7, Chile. Fax: (56) (2) 737 63 20.

Burzio and Koide [12]. Highly purified oocyte CKII was prepared from isolated oocyte nuclei by chromatography on DEAE-Sephadex [13] and phosphocellulose [14] as described originally by Hathaway and Traugh [15].

2.2. *Recombinant α and β subunits of X. laevis CKII*

As reported previously [16] the cDNA genes coding for the α and β subunits of CKII from *X. laevis* oocytes have been cloned and sequenced. The cDNA gene coding for the α subunit has been introduced into a pT7-7 expression vector and expressed in *E. coli* and subsequently purified to homogeneity by chromatography on DEAE-cellulose and phosphocellulose, giving a specific activity of 65 pmol/min per μg protein (Hinrichs et al., manuscript in preparation). The cDNA gene for the β subunit was expressed using the PGEX-2T vector and the resulting fused protein was purified with glutathione-Sepharose affinity chromatography. Thrombin hydrolysis liberated a β subunit that was able to stimulate the activity of the α subunit 4 to 5-fold (Hinrichs et al., manuscript in preparation).

2.3. *Assay of casein kinase II*

For the purified nuclear holoenzyme, reactions (50 μl) contained 50 mM HEPES pH7.8, 150 mM KCl, 7mM $MgCl_2$ (or as specified), 0.5 mM dithiotreitol and 50 μM [γ-^{32}P]ATP or GTP (500–1000 cpm/pmol). For the assay of α subunit alone, conditions were identical except for the use of 50 mM KCl. For the assay of reconstituted CKII (α and β subunits) the assay was carried out as for holoenzyme, with a molar ratio of 1:1 of the subunits without prior preincubation. Assays for holoenzyme activity contained 20–30 U/ml of CKII [14] purified from *X. laevis* oocyte nuclei or 0.1–0.2 μg of the recombinant proteins, and 5 mg/ml of dephosphocasein. Incubation times were routinely 15 min at 30°C and the reaction was linear for at least 30 min. The reaction was terminated by removal of an aliquot to a 2 × 1 cm Whatman P81 phosphocellulose paper which was immersed in 75 mM phosphoric acid and washed 3 times in the same acid, dried and counted. Values reported have been corrected for controls performed without enzyme. All assays were performed in duplicate and the results presented are representative of 2–4 experiments.

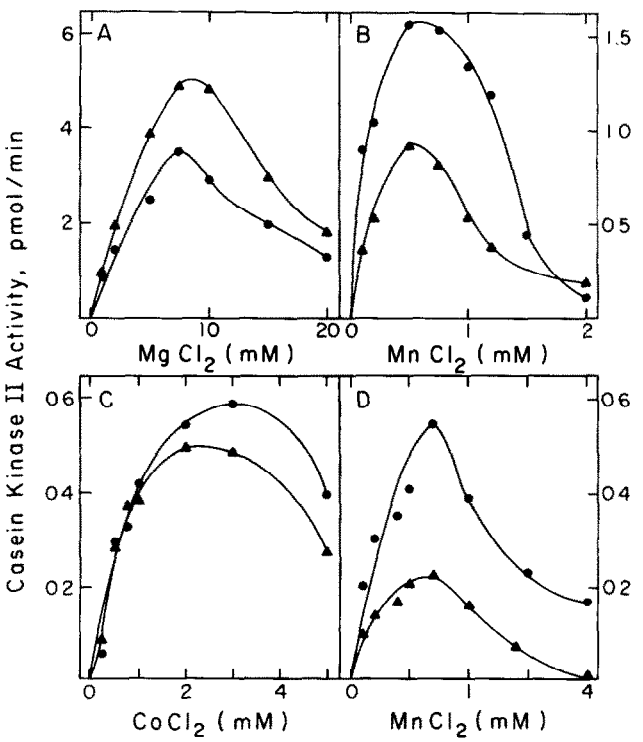

Fig. 1. Comparison of activity of casein kinase II holoenzyme and recombinant subunits with ATP and GTP in the presence of different metals. A. and B. Holoenzyme from oocyte nuclei was assayed under standard conditions with ATP (▲) or GTP (●) in the presence of varying concentrations of magnesium or manganese chloride. C. Recombinant purified α subunit was assayed under standard conditions with ATP (▲) or GTP (●) in the presence of varying concentrations of cobalt chloride. D. Recombinant purified subunits α and β in a 1:1 ratio were assayed with ATP (▲) or GTP (●) in the presence of different concentrations of manganese chloride.

3. RESULTS

When CKII is assayed under standard conditions with ATP as phosphate donor and in the presence of different divalent ions, activity is observed with Mg^{2+}, Mn^{2+} and Co^{2+}. No activity is detectable when Ca^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} are tested at 1 mM concentration (not shown). The highest relative activity is obtained with Mg^{2+}, while Mn^{2+} and Co^{2+} present 20 to 30% as much activity.

In Fig. 1 the effect of different metal concentrations are tested using ATP or GTP as substrate for holoenzyme. Comparing Fig. 1A with 1B, it is clear that the optimum Mn^{2+} concentration (0.5 to 0.7 mM) is more than 10-fold lower than the required optimum concentration of Mg^{2+} (7–10 mM). Also it is noteworthy that the ratios of the activities obtained with ATP and GTP are reversed, the guanosine triphosphate being the better substrate with Mn^{2+}. In the presence of Co^{2+} the results are qualitatively similar to those found with Mn^{2+} with respect to the relative activity of the phophate donors with the holoenzyme.

Similar experiments were carried out with recombinant α and β subunits of *Xenopus* CKII produced in *E. coli* and purified to homogeneity. Fig. 1C shows the activity of the isolated α subunit in the presence of different concentrations of Co^{2+} and ATP and GTP. It is observed that an optimum activity is achieved at about 2 mM Co^{2+} and that GTP is a slightly better substrate than ATP under these conditions. Very similar results were obtained with the recombinant reconstituted enzyme with Co^{2+}. Fig. 1D shows the relative activity of the reconstituted holoenzyme produced by recombinant α and β subunits tested with Mn^{2+} and the

Table I

Apparent K_m values of casein kinase II holoenzyme and α subunit for ATP and GTP in the presence of different metals

Casein kinase II	ATP-Mg^{2+}	GTP-Mg^{2+}	ATP-Mn^{2+}	GTP-Mn^{2+}
Holoenzyme	11.0 ± 0.4	20.8 ± 2.1	1.3 ± 0.3	4.6 ± 0.9
α Subunit	6.9 ± 0.6	19.2 ± 1.9	2.7 ± 0.2	1.8 ± 0.1

The experiments were performed with purified casein kinase II from *X. laevis* oocyte nuclei or with the purified recombinant catalytic α subunit. Values are averages of two to four experiments as described in section 2.

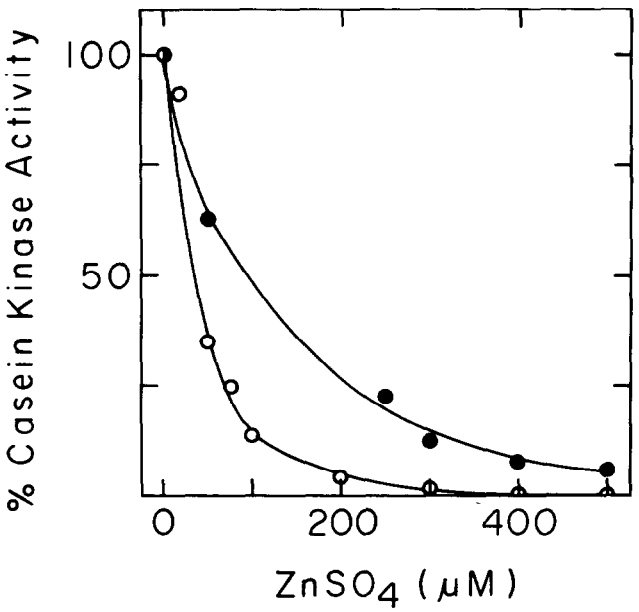

Fig. 2. Inhibition of casein kinase II activity by zinc sulfate. Holoenzyme (●) or α subunit (○) was assayed under standard conditions in the presence of different concentrations of zinc sulfate.

two nucleotide triphosphates. It is clear that the reconstituted recombinant enzyme also uses Mn^{2+} and with this metal ion yields about twofold higher activity with GTP than with ATP.

It is clear from these data that the purified holoenzyme and the recombinant α and α plus β subunits show similar behaviour with respect to the nucleotide triphosphates. The dependence of activity of the metal concentration is also similar although the recombinant α plus β activity in general shows broader concentration optima.

The apparent K_m of the CKII holoenzyme and α subunit for ATP and GTP was measured with Mg^{2+} and Mn^{2+}. The results are presented in Table I. It is evident that the apparent K_m for both ATP and GTP are greatly reduced when the assay is carried out in the presence of Mn^{2+} instead of Mg^{2+}. No significant differences in the apparent K_m values for these substrates is observed when the recombinant subunit is compared with the holoenzyme purified from oocyte nuclei.

We have observed that certain divalent metal ions are inhibitory when they are added to the enzyme that is assayed in the presence of Mg^{2+}. The addition of zinc sulfate at concentrations over 150 μM is strongly inhibitory. Fig. 2 shows that this metal ion inhibits both the activity of the holoenzyme as well as the activity of the isolated α subunit. The chloride and acetate salts are equally inhibitory. Fig. 3 shows the effect of 400 μM Ni^{2+} when the recombinant holoenzyme and the alpha subunit are assayed in the presence of different concentrations of Mg^{2+}. In this case, an interesting difference is observed between the two forms of the enzyme. It is apparent that in the case of the recombinant holoenzyme, high concentrations of Mg^{2+} can reverse the inhibitory effect of Ni^{2+}. A similar result has been obtained with the naturally occurying holoenzyme purified from *X. laevis* oocytes. In the case of the isolated alpha subunit, the inhibition caused by the Ni^{2+} is

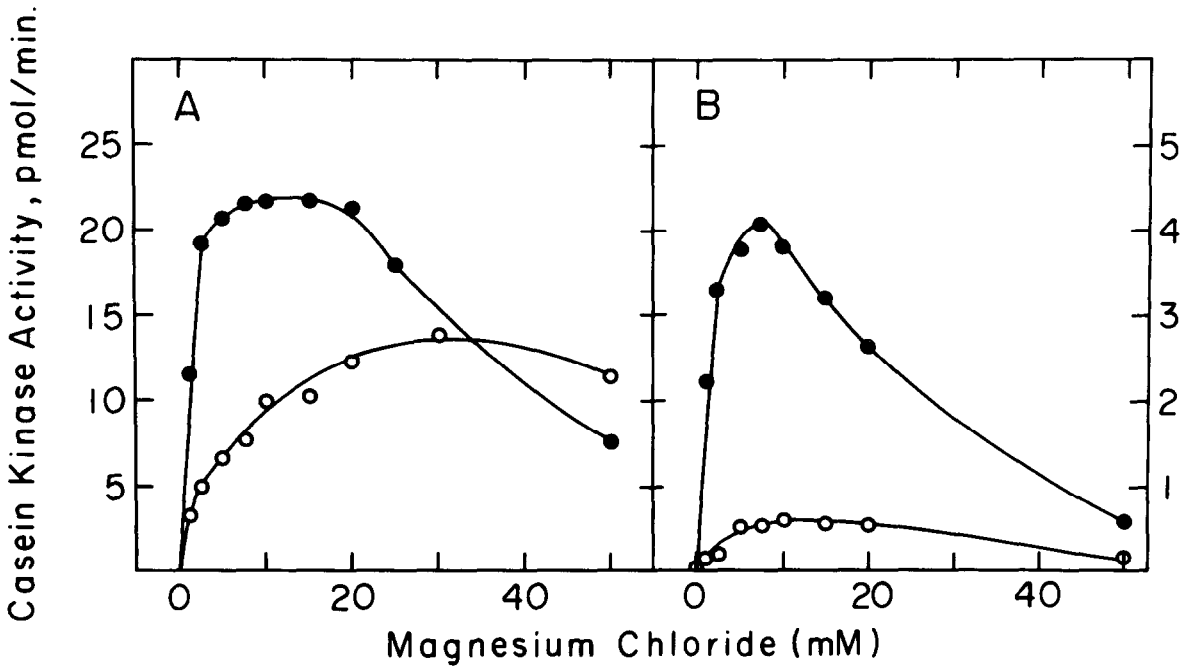

Fig. 3. The effect of nickel chloride on casein kinase II activity. Assays were performed using standard conditions and (A) reconstituted enzyme from equimolar amounts of recombinant α and β subunits or (B) α subunit, in the presence (○) or absence (●) of 400 μM $NiCl_2$.

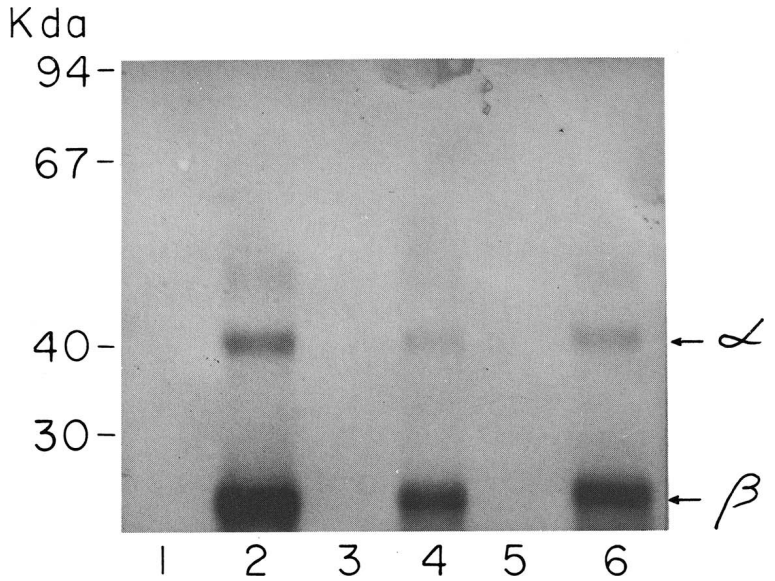

Fig. 4. Autophosphorylation of casein kinase II in the presence of metal ions. Recombinant α subunit, 0.5 μg, was incubated as in the standard assay conditions but in the absence of casein, alone (lanes 1,3,5), or in the presence of 0.5 μg of recombinant β subunit (lanes 2,4,6) with 10 mM $MgCl_2$ (lanes 1 and 2) with 0,75 mM $MnCl_2$ (lanes 3 and 4) and with $CoCl_2$ 0,5 mM (lanes 5 and 6). The KCl concentrations were 80 and 150 mM for the α' and α + β systems respectively. The reactions were stopped by the addition of Laemmli gel sample buffer and electrophoresis carried out on a 10% SDS-polyacrylamide gel, followed by autoradiography of the dried gel.

stronger and high concentrations of Mg^{2+} are not effective in reversing this effect.

Optimum CKII activity requires the presence of high concentrations of monovalent cations [17]. We have carried out experiments with the *X. laevis* purified enzyme and with the recombinant subunits to test the effects of different concentrations of K^+. The results obtained (not shown) confirm the reports of others [18] that indicate that the isolated α subunit has a lowered requirement for monovalent cations, reaching a maximum activity between 30–80 mM K^+ while the holoenzyme shows a broad optimum around 150 mM KCl.

4. DISCUSSION

This work has established that CKII is active when either Mn^{2+} or Co^{2+} are added to replace Mg^{2+} in the assay system. Activity maxima with Mn^{2+} or Co^{2+} are obtained with much lower concentrations of the divalent cation than with Mg^{2+} but these activities only reach 20–30% of the maximum observed with Mg^{2+}. The finding that CKII can use Mn^{2+} and Co^{2+} is particularly interesting because it indicates that this enzyme is similar to tyrosine protein kinases that are known to be able to use these two divalent ions [20,21]. Previously, work from Pinna's laboratory and from our own [22,23] had demonstrated that CKII is able to recognize the presence of tyrosyl residues within acidic peptides. Recently a novel group of ambivalent protein kinases that can phosphorylate both serine/threonine and tyrosine residues have been described [24]. On the basis of its use of metal ions and of its recognition of tyrosine residues, it may be argued, therefore, that CKII is a serine/threonine kinase that has taken some evolutionary steps towards this ambivalency.

Another finding described in this work is the fact that replacement of Mg^{2+} for Mn^{2+} very significantly changes the kinetic parameters for the use of ATP and GTP as substrates of CKII. With Mn^{2+} the apparent K_m values are reduced several fold and GTP becomes a better substrate than ATP. These observations are confirmed by the use of the recombinant α subunit of CKII synthesized in *E. coli*.

The inhibition of the holoenzyme observed with concentrations of Zn^{2+} above 150 μM was interesting because this enzyme has been shown to interact with nucleic acid [25] and because the β subunits contain a cysteine rich segment reminiscent of a 'zincfinger' structure [26]. However, the isolated α subunit which lacks this type of structure is also inhibited by zinc at somewhat lower concentrations.

An interesting difference is found with regard to the inhibition by Ni^{2+} of the holoenzyme and of the catalytic subunit of CKII. In the case of the holoenzyme, high Mg^{2+} concentrations can displace the inhibition caused by Ni^{2+}, while little reversal is observed with the alpha subunit. This observation suggests that the regulatory beta subunit affects the relative affinity of α for different metal ions. In this respect, it must be remembered that the α subunit is monomeric, while the holoenzyme is a heterotetramer of $\alpha^2\beta_2$ structure [27]. This fact may also help to explain the difference in the

concentration requirements observed with the monovalent K^+ ion on the activity of holoenzyme and the α subunit.

Acknowledgements: This work was supported by grants from The Council for Tobacco Research, the International Centre for Genetic Engineering and Biotechnology and from FONDECYT-Chile.

REFERENCES

[1] Tuzaon, P.T. and Traugh, J.A., in: Advances in Second Messenger and Phosphoprotein Research (P. Greengard and G.A. Robinson, Eds.) Vol. 23 Raven Press, New York, 1991, pp. 123–164.
[2] Pinna, L.A. (1990) Biochim. Biophys. Acta 1054, 267–284.
[3] Luscher, B., Christenson, E., Lichfield, D.W., Krebs, E.G. and Eisenman, R.N. (1990) Nature 344, 517–522.
[4] Meek, D.W., Simon, S., Kikkawa, Y. and Eckhart, W. (1990) EMBO J. 9, 3253–3260.
[5] Ackerman, P. and Osheroff, N. (1989) J. Biol. Chem. 264, 11958–11965.
[6] Klarlund, J.K. and Czech, M.P. (1988) J. Biol. Chem. 263, 15872–15875.
[7] Cochet, C. and Chambaz, E.M. (1983) Mol. Cell. Endocrinol. 30, 247–266.
[8] Meggio, F., Boldyreff, B., Marin, O., Marchiori, F., Perich, J.W., Issinger, O.G. and Pinna, L.A. (1992) Eur. J. Biochem. 205, 939–945.
[9] Hathaway, G.M. and Traugh, J.A. (1984) Arch. Biochem. Biophys. 233, 133–138.
[10] Rose, K.M., Bell, L.E., Siefken, D.A. and Jacob, S.T. (1981) J. Biol. Chem. 256, 7468–7477.
[11] Yutani, Y., Tei, Y., Yukioka, M. and Inoue, A. (1982) Arch. Biochem. Biophys. 218, 409–420.
[12] Burzio, L.O. and Koide, S.S. (1976) Arch. Biol. Med. Exp. 10, 22–27.
[13] Leiva, L., Carrasco, D., Taylor, A., Veliz, M., Gonzalez, C., Allende, C.C. and Allende, J.E. (1987) Biochem. Int. 14, 707–717.
[14] Taylor, A., Allende, C.C., Weinmann, R. and Allende, J.E. (1987) FEBS Lett. 226, 109–114.
[15] Hathaway, G.M. and Traugh, J.A. (1983) Methods Enzymol. 99, 308–317.
[16] Jedlicki, A., Hinrichs, M.V., Allende, C.C. and Allende, J.E. (1992) FEBS Lett. 297, 280–284.
[17] Hathaway, G.M. and Traugh, J.A. (1984) J. Biol. Chem. 259, 7011–7015.
[18] Lin, W.J., Tuzaon, P.T. and Traugh, J.A. (1991) J. Biol. Chem. 266, 5664–5669.
[19] Grankowski, N., Boldyreff, B. and Issinger, O.G. (1991) Eur. J. Biochem. 198, 25–30.
[20] Nakamura, S., Yanagi, S. and Yamamura, H. (1988) Eur. J. Biochem. 174, 471–477.
[21] Swarup, G., Dasgupta, J.D. and Garbers, D.L. (1983) J. Biol. Chem. 258, 10341–10347.
[22] Meegio, F. and Pinna, L.A. (1989) Biochim. Biophys. Acta 1010, 128–130.
[23] Tellez, R., Gatica, M., Allende, C.C. and Allende, J.E. (1990) FEBS Lett. 265, 113–116.
[24] Kosako, H., Gotoh, Y., Matsuda, S., Ishikawa, M. and Nishida, E. (1992) EMBO J. II, 2903–2908.
[25] Gatica, M., Allende, C.C. and Allende, J.E. (1989) FEBS Lett. 255, 414–418.
[26] Berg, J.M. (1986) Science 232, 485–489.
[27] Hu, E. and Rubin, C.S. (1990) J. Biol. Chem. 265, 20609–20615.