The interaction between the archaeal elongation factor 1α and its nucleotide exchange factor 1β

Gennaro Raimo, Mariorosario Masullo, Vincenzo Bocchini*

Dipartimento di Biochimica e Biotecnologie Mediche, Università di Napoli Federico II, Via S. Pansini, I-80131 Naples, Italy

Received 4 March 1999; received in revised form 15 April 1999

Abstract In *Sulfolobus solfataricus* the binding of the exchange factor 1β (*Ss*EF-1β) to *Ss*EF-1α·GDP displaces the nucleotide and the *Ss*EF-1α·*Ss*EF-1β complex is formed. The complex itself is stable, but it dissociates upon the addition of GDP or Gpp(NH)p but not ATP. Since the rate of the formation of the *Ss*EF-1α·*Ss*EF-1β complex is significatively slower than the rate of the nucleotide exchange catalyzed by *Ss*EF-1β it can be inferred that in vivo the GDP/GTP exchange reaction proceeds via an *Ss*EF-1α–*Ss*EF-1β interaction without involving the formation of a stable binary complex as an intermediate.

Key words: Elongation factor 1β; Exchange factor; *Sulfolobus solfataricus*; Protein–protein interaction; Archaea

1. Introduction

Eukaryotic and archaeal EF-1α and EF-1β are the functional analogues of eubacterial EF-Tu and EF-Ts respectively [1]. In the course of protein synthesis EF-1β plays a key role since it accelerates the regeneration from the inactive EF-1α·GDP of the active EF-1α·GTP complex that carries the aa-tRNA onto the ribosome. The role of EF-1β is essential since the affinity of EF-1α for GDP is higher than that for GTP [2]; therefore the GDP/GTP exchange is rate limiting in the formation of the active EF-1α·GTP complex (for a review see [3]).

The elongation factors 1α (*Ss*EF-1α) and 1β (*Ss*EF-1β) have been purified and characterized from the archaeal hyperthermophile *Sulfolobus solfataricus* [2,4]. *Ss*EF-1α is a GTP binding protein with a relative molecular mass of 49 000 [5]. *Ss*EF-1β stimulates the rate of the GDP/GTP exchange on *Ss*EF-1α·GDP [4]. It is a homodimer with an M_r of 20 000, made of two identical subunits of 90 amino acid residues each [4,6]. The amino acid sequence shows homology with the C-terminal portion of eucaryal EF-1β [4] which contains the region involved in the nucleotide exchange activity [7]. Both *Ss*EF-1α and *Ss*EF-1β possess a remarkable resistance against denaturation by chemical and physical agents [4,7].

This paper reports the interaction between *Ss*EF-1α and *Ss*EF-1β, the kinetics of the process, the stoichiometry and the stability of the complex. The results allow a hypothesis on the mechanism of action of *Ss*EF-1β in *S. solfataricus* cells.

2. Materials and methods

2.1. Chemicals, chromatographic media and buffers

All chemicals used were of analytical grade. Superdex 75 HR 10/30 and HiLoad Superdex 75 26/60 were from Pharmacia. [^{3}H]GDP was purchased from Amersham; GDP, GTP, Gpp(NH)p and ATP were from Boehringer Mannheim. The following buffers were used: A, 30 mM Tris/HCl, pH 8.0, 200 mM $(NH_4)_2SO_4$, 1.5 M NaCl and 0.5 mM DTT; B, 20 mM Tris/HCl, pH 7.8; C, 20 mM Tris/HCl, pH 7.8, 10 mM $MgCl_2$, 50 mM KCl.

2.2. Production of nucleotide free SsEF-1α, SsEF-1α·GDP and SsEF-1α·Gpp(NH)p

To prepare nucleotide free *Ss*EF-1α (*Ss*EF-1α_{free}) 1 mg of *Ss*EF-1α·GDP was incubated at 60°C in the presence of 5 μg of alkaline phosphatase (Sigma) in 2 ml of buffer A. After 5 h incubation, the mixture was dialyzed against buffer B and then loaded onto a DEAE-Sephadex A-50 (Pharmacia) column (1 cm×15 cm) equilibrated with buffer B and operating at room temperature with a flow rate of 1 ml/min. *Ss*EF-1α was collected in the flow through and then analyzed for the nucleotide content using the HPLC method described by Tucker et al. [8]. The yield of *Ss*EF-1α_{free} was 98% of the initial amount; stored at −20°C in buffer C supplemented with 50% (v/v) glycerol *Ss*EF-1α_{free} was stable for at least 12 months.

To prepare either *Ss*EF-1α·GDP or *Ss*EF-1α·Gpp(NH)p, 20 μM *Ss*EF-1α_{free} was incubated in buffer C with 25 μM GDP or Gpp(NH)p, for 30 min at 60°C; under these conditions the titration of *Ss*EF-1α was complete.

2.3. Preparation of the SsEF-1α·SsEF-1β complex

Five nmol of *Ss*EF-1α·GDP was incubated in 2 ml buffer C with a 10-fold molar excess of *Ss*EF-1β for 20 h at 60°C. The reaction mixture was then loaded onto a HiLoad Superdex 75 26/60 column, connected to an FPLC apparatus (Pharmacia) operating at a flow rate of 2 ml/min at room temperature. The fractions (2 ml each) containing the *Ss*EF-1α·*Ss*EF-1β complex were pooled and stored at 4°C. The final yield of *Ss*EF-1α·*Ss*EF-1β complex was the same if *Ss*EF-1α_{free} was used instead.

2.4. Determination of the relative molecular mass of SsEF-1α·SsEF-1β

The M_r of the *Ss*EF-1α·*Ss*EF-1β complex was determined using a Superdex 75 HR 10/30 gel filtration column, equilibrated at 0.5 ml/min with buffer C and calibrated by running separately 10 μg in 110 μl of the following proteins: *Ss*EF-1α (M_r 49 000, [2]), *Ss*EF-1β (M_r 20 000, [4]), *Ss*EF-2 (M_r 82 000, [10]), ribonuclease p2 (M_r 14 000, [11]), all of them isolated from *S. solfataricus*, and the glutaredoxin-like protein (M_r 24 800, [12]) isolated from *Pyrococcus furiosus*.

2.5. Determination of the equilibrium dissociation constant and the association rate constant of the SsEF-1α·SsEF-1β complex

200–250 pmol of *Ss*EF-1α in the GDP or Gpp(NH)p bound form was incubated in 120 μl buffer B with *Ss*EF-1β ranging between 0 and 14 μM. Equilibrium was reached after 20 h incubation at 60°C, the reaction mixture was then cooled and loaded onto a Superdex 75 HR 10/30 column, connected to a computer assisted FPLC apparatus, controlled by the *FPLC director* program (Pharmacia). The amount of the *Ss*EF-1α·*Ss*EF-1β formed was determined on a column previously calibrated by running separately different amounts of *Ss*EF-1α; a linear relationship was found between the area of the peak and the amount of the *Ss*EF-1α loaded. The quantity of *Ss*EF-1α·*Ss*EF-1β complex formed was calculated from the difference between the area of the peak corresponding to the initial amount of *Ss*EF-1α and the

*Corresponding author. Fax: +39 (81) 746.3653.
E-mail: bocchini@unina.it

PII: S0014-5793(99)00560-8

area of the residual unbound *Ss*EF-1α at the equilibrium. The data were then analyzed according to the Scatchard equation: $r = n + K_d \cdot (r/[Ss\text{EF-1}\beta_{free}])$ in which r is the [*Ss*EF-1α·*Ss*EF-1β]/[*Ss*EF-1α] ratio at the equilibrium, n is the number of *Ss*EF-1β binding sites on *Ss*EF-1α, K_d is the apparent dissociation equilibrium constant of the *Ss*EF-1α·*Ss*EF-1β complex and *Ss*EF-1β_{free} is the unbound *Ss*EF-1β at the equilibrium. The apparent second order rate constant of the association reaction was calculated according to the equation $[1/(b-a)]\cdot \ln[a(b-x)/b(a-x)] = k_{+1}\cdot t$, where a and b are the initial concentrations of *Ss*EF-1β and *Ss*EF-1α respectively and x is the concentration of the *Ss*EF-1α·*Ss*EF-1β complex formed after the time t.

3. Results

3.1. Binding of SsEF-1β to SsEF-1α

The binding of *Ss*EF-1β to *Ss*EF-1α was followed by gel filtration. Fig. 1A shows the elution profile of *Ss*EF-1α·[^{3}H]GDP and *Ss*EF-1β at zero time incubation: all the collected radioactivity was bound to *Ss*EF-1α (retention time 20.2 min). Fig. 1B shows that after 20 h incubation at 60°C a peak with a retention time of 19.3 min was observed and all the radioactivity was collected as free [^{3}H]GDP. The retention time of the unbound *Ss*EF-1β remained the same as in Fig. 1A. Under identical conditions *Ss*EF-1α·[^{3}H]GDP incubated in the absence of *Ss*EF-1β did not dissociate thus showing that the shift of the radioactive peak toward low molecular weights was due to [^{3}H]GDP released from the *Ss*EF-1α·[^{3}H]GDP following the addition of *Ss*EF-1β. The peak eluted at 19.3 min accounted for an M_r of about 70 000; this finding indicated the formation of the binary *Ss*EF-1α·*Ss*EF-1β complex, being 49 000 and 20 000 the relative molecular mass of *Ss*EF-1α and *Ss*EF-1β respectively.

The binding of *Ss*EF-1β to *Ss*EF-1α followed second order kinetics (Fig. 2); the association rate constant k_{+1} raised from 0.015 M^{-1} h^{-1} at 40°C to 0.068 M^{-1} h^{-1} at 60°C. The thermophilicity of the binding reaction was also confirmed by the fact that the amount of *Ss*EF-1α·*Ss*EF-1β formed after 2 h incubation increased at increasing temperature and reached a maximum at 80°C (Fig. 3). The decreased amount of the complex at temperatures above 80°C was probably due to the heat inactivation of *Ss*EF-1α [8,9].

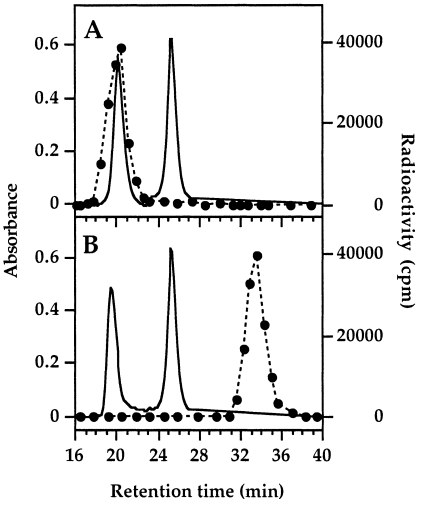

Fig. 1. Formation of the *Ss*EF-1α·*Ss*EF-1β complex. 400 μl buffer C containing 500 pmol of *Ss*EF-1α·[^{3}H]GDP were incubated at 60°C in the presence of 2400 pmol of *Ss*EF-1β. Immediately after mixing (panel A) or after 20 h incubation (panel B) 100 μl aliquots were cooled on ice and immediately loaded onto a Superdex 75 HR 10/30 gel filtration column equilibrated as described in Section 2.5. Proteins eluted were monitored at 280 nm (continuous line). 250 μl fractions were collected and the radioactivity counted on 100 μl aliquots (●).

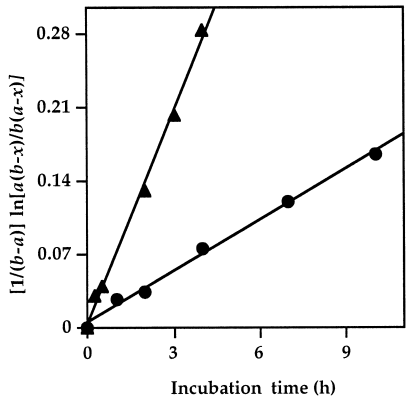

Fig. 2. Effect of temperature on the kinetics of the *Ss*EF-1α·*Ss*EF-1β complex formation. In 1 ml of buffer C, 1200 pmol of *Ss*EF-1α·GDP was incubated with 6000 pmol of *Ss*EF-1β at 40 (●) or 60°C (▲). At the times indicated 100 μl aliquots were withdrawn, cooled on ice and the amount of the formed *Ss*EF-1α·*Ss*EF-1β complex was determined. The data were treated according to second order kinetics as described in Section 2.

3.2. Equilibrium dissociation constant of the SsEF-1α·SsEF-1β complex

*Ss*EF-1α·GDP was incubated at 60°C for 20 h with *Ss*EF-1β added at increasing concentration up to a molar excess of about 9-fold. The amount of formed *Ss*EF-1α·*Ss*EF-1β and the residual *Ss*EF-1α were evaluated by gel filtration (see Section 2). The data analyzed by the Scatchard equation gave a 1:1 molar stoichiometry of the *Ss*EF-1α·*Ss*EF-1β complex and a value of K_d equal to 4.6 μM (Fig. 4). The dissociation rate constant k_{-1} at 60°C was calculated as 0.313 h^{-1}. Under the same experimental conditions, but starting from *Ss*EF-1α·Gpp(NH)p a K_d of 1.1 μM was calulated. This result

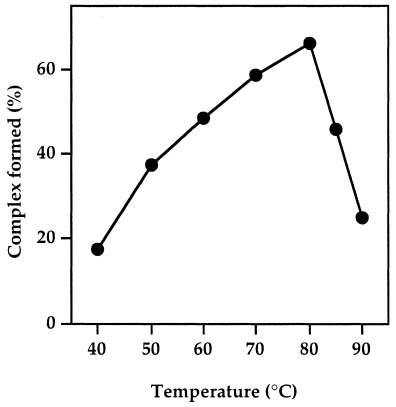

Fig. 3. Effect of temperature on the amount of the *Ss*EF-1α·*Ss*EF-1β complex formed. 110 μl buffer C contained 210 pmol of *Ss*EF-1α and 1760 pmol of *Ss*EF-1β. The reaction mixture was incubated for 2 h at the indicated temperatures, then cooled on ice and analyzed by gel filtration. The amount of *Ss*EF-1α·*Ss*EF-1β complex formed was evaluated as described in Section 2 and reported as percentage of the maximum obtainable.

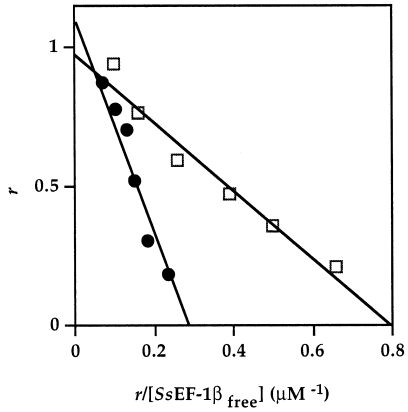

Fig. 4. Scatchard plot for the dissociation of the *Ss*EF-1α·*Ss*EF-1β complex. 200 pmol *Ss*EF-1α·GDP (●) or *Ss*EF-1α·Gpp(NH)p (□) was incubated in 120 μl buffer C at 60°C in the presence of 0–1700 pmol of *Ss*EF-1β. After 20 h of incubation each reaction mixtures was cooled on ice and analyzed by gel filtration. The amount of the residual *Ss*EF-1α not bound to *Ss*EF-1β was determined as described in Section 2. Data were analyzed according to the Scatchard equation (see Section 2).

indicated that in the *Ss*EF-1α·nucleotide complex the Gpp(NH)p was displaced by *Ss*EF-1β more easily than GDP. On the other hand, *Ss*EF-1α_{free} bound *Ss*EF-1β at a very fast rate and no unbound *Ss*EF-1β was detected unless it was added at a concentration higher than that of *Ss*EF-1α_{free}; under these conditions an equilibrium state was not reached and therefore the evaluation of K_d of the *Ss*EF-1α·*Ss*EF-1β complex could not be done.

3.3. Stability of SsEF-1α·SsEF-1β

Analyzed by gel filtration the *Ss*EF-1α·*Ss*EF-1β was stable for at least 24 h at 60°C or 3 months at 4°C. Incubation of *Ss*EF-1α·*Ss*EF-1β for 1 h at 60°C in the presence of a 10-fold molar excess of [^{3}H]GDP provoked the release of the exchange factor (Fig. 5A, retention time 24.7 min). A similar result was observed when Gpp(NH)p was used instead (not shown). Incubated for 20 h at 60°C in the presence of a 100-fold molar excess of ATP *Ss*EF-1α·*Ss*EF-1β did not dissociate (Fig. 5B) thus confirming that *Ss*EF-1α binds specifically guanosine nucleotides but cannot bind guanosine nucleotides and *Ss*EF-1β simultaneously.

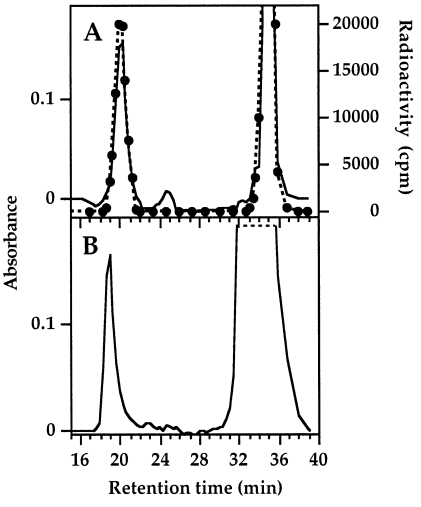

Fig. 5. Effect of [^{3}H]GDP and ATP on the dissociation of the *Ss*EF-1α·*Ss*EF-1β complex. 52 pmol of *Ss*EF-1α·*Ss*EF-1β complex was incubated in 130 μl buffer C with 35 μM final concentration of [^{3}H]GDP (s.a. 1300 cpm/pmol) (panel A) or 380 μM ATP (panel B). After 1 h of incubation at 60°C, 100 μl of the reaction mixture was analyzed on Superdex 75 HR 10/30 as described in Section 2. Proteins eluted were monitored at 280 nm (continuous line). Radioactivity was counted on 100 μl aliquots of 250 μl fractions (●).

4. Discussion

In this paper evidence is reported that an *Ss*EF-1α·*Ss*EF-1β complex is formed. However, in order to be entirely converted into *Ss*EF-1α·*Ss*EF-1β, *Ss*EF-1α·[^{3}H]GDP needs a long incubation at 60°C in the presence of at least 5-fold molar excess of *Ss*EF-1β (Fig. 1); this last condition is quite different compared to that occurring in the *S. solfataricus* cell extract where the EF-1α/EF-1β ratio is around 15 [2,4]. A similar result was observed with eubacterial and eucaryal cells in which the concentration of EF-Tu [13] or EF-1α [14] is significatively higher than that of their respective nucleotide exchange factor. In *E. coli* a stable EF-Tu·EF-Ts complex was purified even from the cell extract [13], at least when the concentration of GDP or GTP was maintained low [15], thus indicating that under these conditions in eubacteria the formation of the complex takes place even at a very high EF-Tu/EF-Ts ratio [13,16]. The observation that *Ss*EF-1β binds rapidly to *Ss*EF-1α_{free}, suggests that in the absence of the nucleotide *Ss*EF-1α assumes a more appropriate conformation for its interaction with *Ss*EF-1β.

*Ss*EF-1α·*Ss*EF-1β complex is constituted by one molecule of *Ss*EF-1α and one molecule of the homodimer *Ss*EF-1β; this finding is different from what was found in eubacteria in which a heterotetrameric (EF-Tu·EF-Ts)$_2$ structure is reported [17,18]. In addition, it has been demonstrated that in *Thermus thermophilus* the dimerization of EF-Ts is essential to obtain the EF-Tu·EF-Ts and to accelerate the GDP/GTP exchange rate on EF-Tu·GDP [19].

The formation of a ternary complex involving EF-1α, EF-1β and a guanosine nucleotide is an event that does not occur in *S. solfataricus* because *Ss*EF-1α·*Ss*EF-1β dissociates in the presence of a guanosine nucleotide (Fig. 5). Since the interaction of the *Ss*EF-1β with *Ss*EF-1α·[^{3}H]GDP causes the displacement of the bound nucleotide (Fig. 1) it can be stated that the binding to *Ss*EF-1α of *Ss*EF-1β and guanosine nucleotide is mutually exclusive. This result is opposite to what was found in eubacteria and in eucarya in which one of the intermediates of the EF-Tu/1α cycle was the ternary complex constituted by EF-Tu/1α, EF-Ts/1β and GDP or GTP [20–23].

In *S. solfataricus* the GDP/GTP exchange rate on *Ss*EF-1α·GDP was accelerated by the presence of *Ss*EF-1β even at a concentration comparable to that of *Ss*EF-1α [4]. This observation, together with the finding that the formation of *Ss*EF-1α·*Ss*EF-1β occurs at a very low rate (Fig. 2) and at a concentration of *Ss*EF-1β in a great excess compared to *Ss*EF-1α (Fig. 1), allows the hypothesis that in vivo the GDP/GTP exchange reaction proceeds via a transient *Ss*EF-1α–*Ss*EF-1β interaction probably because *Ss*EF-1β lacks part of the region(s) responsible for its anchorage to *Ss*EF-1α. The mechanism proposed is in agreement with the results of a site directed mutagenesis on *E. coli* EF-Tu in which the H118G

mutation destabilizes the EF-Tu·EF-Ts complex without hindering the exchange activity of EF-Ts [24].

Studies on the exchange activity of C-terminal fragments of human EF-1β showed a presumable relationship between the size of the truncated exchange factor and the rate of the nucleotide exchange reaction [25]. This observation confirms our previous hypothesis [4] that being the length of the polypeptide chain of *Ss*EF-1β about one half of that of eucaryal EF-1β, the archaeal exchange factor needs, in addition to the presence of specific binding site(s), a homodimeric structure to reach an appropriate size for a correct interaction with *Ss*EF-1α, at least to ensure an efficient nucleotide exchange activity.

In conclusion, the data reported in this article indicate that even though the sequence of the steps of the elongation cycle is the same in all the living organisms, the mechanism of specific intermediate reactions may be different among the different species.

Acknowledgements: This investigation was supported by the European Community Human Capital and Mobility Programme Contract ERBCHRXCT940510 and partially by MURST (PRIN 1997).

References

[1] Klink, F. (1985) in: C.R. Woese and W. Wolfe (Eds.), The Bacteria, Vol. VIII, pp. 379–410.
[2] Masullo, M., Raimo, G., Parente, A., Gambacorta, A., De Rosa, M. and Bocchini, V. (1991) Eur. J. Biochem. 199, 529–537.
[3] Moldave, K. (1985) Annu. Rev. Biochem. 54, 1109–1149.
[4] Raimo, G., Masullo, M., Savino, G., Scarano, G., Ianniciello, G., Parente, A. and Bocchini, V. (1996) Biochim. Biophys. Acta 1293, 106–112.
[5] Arcari, P., Gallo, M., Ianniciello, G., DelloRusso, A. and Bocchini, V. (1993) Nucleic Acids Res. 21, 1666.
[6] Arcari, P., Raimo, G., Ianniciello, G., Gallo, M. and Bocchini, V. (1995) Biochim. Biophys. Acta 1263, 86–88.
[7] van Damme, H., Amons, R., Jansen, G.M.C. and Möller, W. (1991) Eur. J. Biochem. 197, 505–511.
[8] Masullo, M., Raimo, G. and Bocchini, V. (1993) Biochim. Biophys. Acta 1161, 35–39.
[9] Tucker, J., Sczakiel, G., Feuerstein, J., John, J., Goody, R.S. and Wittinghofer, A. (1986) EMBO J. 5, 1351–1358.
[10] Raimo, G., Masullo, M., Dello Russo, A., Parente, A., Gambacorta, A. and Bocchini, V. (1993) It. J. Biochem. 42, 1–11.
[11] Fusi, P., Tedeschi, G., Aliverti, A., Ronchi, S., Tortora, P. and Guerritore, A. (1993) Eur. J. Biochem. 211, 305–310.
[12] Guagliardi, A., de Pascale, D., Cannio, R., Nobile, V., Bartolucci, S. and Rossi, M. (1995) J. Biol. Chem. 270, 5748–5755.
[13] Arai, K. and Kawakita, M. (1972) J. Biol. Chem. 247, 7029–7037.
[14] Crechet, J.B., Canceill, D., Bocchini, V. and Parmeggiani, A. (1972) J. Biol. Chem. 247, 7029–7037.
[15] Leberman, R., Antonsson, B., Giovanelli, R., Guariguata, R., Schumann, R. and Wittinghofer, A. (1980) Anal. Biochem. 104, 29–36.
[16] Arai, K., Ota, Y., Arai, N., Nakamura, S., Henneke, C. and Oshima, T. (1978) Eur. J. Biochem. 92, 509–519.
[17] Jiang, Y., Nock, S., Nesper, M., Sprinzl, M. and Sigler, P.B. (1996) Biochemistry 35, 10269–10278.
[18] Kawashima, T., Berthet-Colominas, C., Wulff, M., Cusack, S. and Leberman, R. (1996) Nature 379, 511–518.
[19] Nesper, M., Nock, S., Sedlak, E., Antalik, M., Podhradsky, D. and Sprinzl, M. (1998) Eur. J. Biochem. 255, 81–86.
[20] Arai, K., Arai, N., Nakamura, S., Oshima, T. and Kaziro, Y. (1978) J. Biol. Chem. 256, 5591–5596.
[21] Chau, V., Romero, G. and Biltonen, R.L. (1981) Eur. J. Biochem. 92, 521–531.
[22] Janssen, G.M.C. and Möeller, W. (1988) J. Biol. Chem. 263, 1773–1778.
[23] Negrutskii, R.S. and El's Kaya, A.V. (1998) Prog. Nucleic Acids Res. 60, 47–78.
[24] Jonak, J., Anborgh, P.H. and Parmeggiani, A. (1998) FEBS Lett. 422, 189–192.
[25] Perez, J.M.J., Kriek, J., Dijk, J., Canters, G.W. and Möller, W. (1998) Prot. Expr. Purif. 13, 259–267.