Molecular interactions between poly(ADP-ribose) polymerase (PARP I) and topoisomerase I (Topo I): identification of topology of binding

Pal I. Bauer[a,c], Hui-Je Chen[b], Erzsebet Kenesi[a], Istvan Kenessey[a], Kalman G. Buki[c], Eva Kirsten[c], Alaeddin Hakam[c], Jaulang I. Hwang[b], Ernest Kun[c,*]

[a] *Department of Medical Biochemistry, Semmelweis University, Budapest, Hungary*
[b] *Department of Molecular Biology, Taipei University, Taipei, Taiwan*
[c] *Department of Anatomy and Cellular and Molecular Pharmacology, University of California-San Francisco, San Francisco, CA, USA*

Received 3 August 2001; revised 13 September 2001; accepted 13 September 2001

First published online 24 September 2001

Edited by Judit Ovádi

Abstract The molecular interactions of poly(ADP-ribose) polymerase I (PARP I) and topoisomerase I (Topo I) have been determined by the analysis of physical binding of the two proteins and some of their polypeptide components and by the effect of PARP I on the enzymatic catalysis of Topo I. Direct association of Topo I and PARP I as well as the binding of two Topo I polypeptides to PARP I are demonstrated. The effect of PARP I on the 'global' Topo I reaction (scission and religation), and the activation of Topo I by the 36 kDa polypeptide of PARP I and catalytic modifications by poly(ADP-ribosyl)ation are also shown. The covalent binding of Topo I to circular DNA is activated by PARP I similar to the degree of activation of the 'global' Topo I reaction, whereas the religation of DNA is unaffected by PARP I. The geometry of PARP I–Topo I interaction compared to automodified PARP I was reconstructed from direct binding assays between glutathione *S*-transferase fusion polypeptides of Topo I and PARP I demonstrating highly selective binding, which was correlated with amino acid sequences and with the 'C clamp' model derived from X-ray crystallography. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Poly(ADP-ribose) polymerase I; Topoisomerase I binding; Topoisomerase I regulation by poly(ADP-ribose) polymerase I; Binding site; Topology interaction

1. Introduction

Poly(ADP-ribose) polymerase I (PARP I) is known to associate with itself [1] and with numerous nuclear proteins [2], consisting of enzymes, cell specific transcriptional factors [3] and other transcriptional regulators (cf. [4]), suggesting that the cellular function of PARP I, besides signaling single strand DNA cuts [5,6], most likely includes a control of specific gene expressions. It was first reported in 1983 [7,8] that PARP I in the presence of NAD^+ poly(ADP-ribosyl)ates topoisomerase I (Topo I) resulting in enzyme inhibition. In the absence of NAD^+ a direct binding of PARP I to Topo I with concomitant enzymatic activation of Topo I occurs, as assayed by the conventional DNA unwinding test [9,10]. Kinetic analyses indicate a binding between Topo I and PARP I, as well as with circular DNA [9], which is supercoiled by PARP I association [11], thus favoring its substrate function in the Topo I catalyzed process [9]. Since the enzymatic mechanism of Topo I has been significantly clarified [12–14], further questions relevant to the activation of Topo I by PARP I could be asked. These are: (a) what portion of the PARP I polypeptide is required for Topo I activation, as well as (b) what domains of Topo I can bind to PARP I, and (c) can the assay for covalent adduct formation between circular DNA and Topo I or the religation test for Topo I [14] demonstrate activation by PARP I? Experimental analyses of these questions, as described here, permit formulation of a preliminary structural interpretation of the PARP I/Topo I association.

2. Materials and methods

2.1. Isolation of PARP I and Topo I from calf thymus

This technique and the ultimate separation of the two proteins from calf thymus are reported in [2,9].

2.2. Western overlay analyses of the binding of PARP I to Topo I

^{32}P labeled Topo I was prepared by first dephosphorylating Topo I [9] with alkaline phosphatase bound Sepharose 4B resin (Sigma) by incubation of Topo I for 3 h at 25°C in 0.1 M Tris–HCl, pH 8.0. After centrifugal sedimentation of the resin the buffer was changed to 0.1 M Tris–HCl (pH 7.4) containing 10% glycerol in a Centricon 30 filter. The dephosphorylated Topo I was rephosphorylated by protein kinase C_α as reported for PARP I [15] with [^{32}P]ATP, resulting in ^{32}P labeled Topo I with a specific activity of 1500 dpm/μg protein. The overlay assay was carried out as reported [2].

2.3. Binding of ^{125}I labeled PARP I or ^{32}P labeled PARP I to Topo I and its polypeptides

Topo I polypeptides were generated by digestion of Topo I with chymotrypsin [9,16], followed by their electrophoretic separation and transblotting. Polypeptides were incubated either with ^{125}I labeled PARP I (cf. [2]) (4×10^6 dpm) or with ^{32}P labeled PARP I (2×10^6 dpm, cf. [2]) at 25°C, and autoradiographed [2].

2.4. Poly(ADP-ribosyl)ation of Topo I and its peptide fragments

This operation was carried out in situ, after electrophoretic separation and transblot to nitrocellulose membranes, followed by incubation with 50 μCi [^{32}P]NAD^+ and 200 μM NAD^+ in 10 ml medium (100 mM Tris–HCl, pH 8.0), 7 mM 2-mercaptoethanol, 0.1 M NaCl, 20 μg/ml co-enzymic DNA (cf. [21]) and 2 μg PARP I [9] for 30 min at 25°C. This incubation was followed by 3×10 ml wash with Tris–HCl (pH 7.5) buffer, twice with 10 ml of the same buffer containing 0.1 M NaCl and 0.02% SDS, then with 10 ml Tris–HCl (pH 7.4) buffer without NaCl or SDS (each wash for 5 min), and then dried and autoradiographed overnight.

*Corresponding author. Fax: (1)-415-388 7024.
E-mail address: ernestkun@mac.com (E. Kun).

Abbreviations: PARP I, poly(ADP-ribose) polymerase I; Topo I, topoisomerase I; GST fusion polypeptides, fusion products of Topo I polypeptides with glutathione *S*-transferase

0014-5793/01/$20.00
PII: S0014-5793(01)02919-2

2.5. Preparation of the 36 kDa and 52 kDa polypeptide fragments of PARP I

The polypeptide fragments were prepared as reported previously [16].

2.6. Religation assay

The religation assay for Topo I was done as published [14].

2.7. Preparation of glutathione S-transferase (GST) fusion proteins of Topo I domains

These were prepared as described [17] and the pull down assay of GST fusion peptides by PARP I was done by standard techniques.

2.8. Poly(ADP-ribose) polymers

Poly(ADP-ribose) polymers were prepared as described earlier [19,20]

3. Results and discussion

As the results of overlay of isolated polypeptides with either ^{32}P or ^{125}I labeled PARP I or ^{32}P labeled Topo I, Fig. 1 shows the binding of ^{32}P labeled Topo I to PARP I (Fig. 1A) and ^{125}I labeled and ^{32}P labeled PARP I to Topo I as well as of two of its proteolytic polypeptides to Topo I (Fig. 1B). The lower band in Fig. 1A represents traces of proteolytic degradation products of PARP I which also bound ^{32}P labeled Topo I. The poly(ADP-ribosyl)ation of Topo I and two of its chymotryptic peptides is illustrated in Fig. 1C. Only two out of five major chymotryptic polypeptides of Topo I (75 and 60 kDa) were poly(ADP-ribosyl)ated (cf. [9]) indicating modification of the N-terminus of Topo I.

The activity of Topo I, assayed by the unwinding–religation test as reported [9], exhibits characteristic variations depending on specific additions as shown in Fig. 2. In lane 1 the base activity of Topo I is shown as 100%, and lane 2 illustrates 800% activation by added 0.2 μg PARP I. The 36 kDa proteolytic fragment of PARP I activates Topo I by about 50% compared to intact PARP I (lane 3), whereas the 52 kDa PARP I fragment has lost nearly all activating action. The 36 kDa polypeptide of PARP I also binds to DNA [2], therefore the coincidence of DNA binding and Topo I activation suggests that Topo I activation by PARP I also involves DNA binding, as found experimentally [9]. Poly(ADP-ribosyl)ated Topo I has very low activity (lane 5), confirming earlier reports [7,8], and poly(ADP-ribosyl)ated PARP I is also inhibitory to Topo I (lane 6). Preincubation of Topo I with PARP I+NAD^++co-enzymic DNA abolishes the activation of Topo I by PARP I (lane 7), but an inhibitor of PARP I (1 mM benzamide) largely restores the activation of Topo I. The poly(ADP-ribose) polymers do not inhibit Topo I when added in the form of free polymers (lane 9).

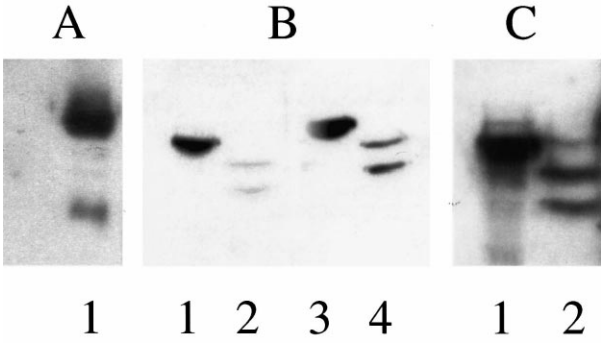

Fig. 1. Binding of PARP I to Topo I and its chymotryptic polypeptide fragments. PARP I (1.2 μg, A) or Topo I (1 μg, lanes 1 and 3 in B and lane 1 in C) and chymotryptic fragments (lanes 2 and 4 in B, and lane 2 in C) were separated by electrophoresis, transblotted and renatured (cf. [2]). Polypeptide fragments of Topo I were obtained by digestion of 3 μg of Topo I (cf. [2]). The PARP I containing blots were overlaid (see Section 2) with ^{32}P labeled Topo I (A). The Topo I containing blots (B) were incubated either with [^{32}P]PARP I (lanes 1 and 2, B) or with [^{125}I]PARP I (lanes 3 and 4, B). C: Autoradiography of in situ poly(ADP-ribosyl)ated Topo I and two of its chymotryptic fragments. In lane 2 of C there are traces of undigested Topo I (top line). Identical results were obtained in triplicate separate experiments.

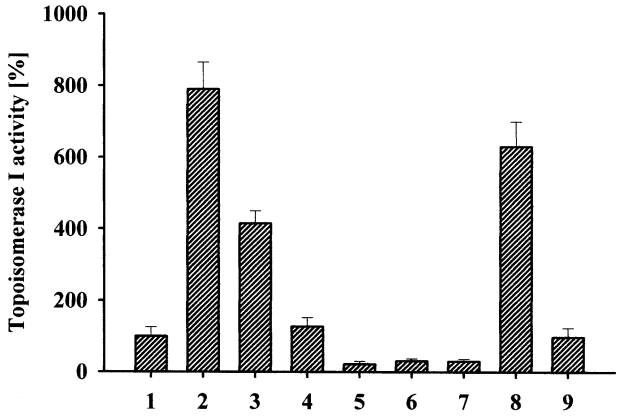

Fig. 2. Effects of PARP I and its poly(ADP-ribosyl)ation and the 36 and 52 kDa PARP I fragments and of the free poly(ADP-ribose) polymer on Topo I activity. Topo I activity was assayed quantitatively as reported [9]. One unit of activity was defined as 100% activity (i.e. the amount of Topo I required to relax 50% of the plasmid, cf. [9]). This is shown in lane 1. Lane 2 illustrates the activation by 0.2 μg PARP I, lane 3 the effect of 0.3 μg of 36 kDa PARP I fragment, lane 4 that of 0.4 μg of the 52 kDa PARP I fragment. Inhibition of Topo I (0.2 μg) by its poly(ADP-ribosyl)ation is illustrated in lane 5, whereas inhibition of Topo I by 0.2 μg poly(ADP-ribosyl)ated PARP I is shown in lane 6. The inhibitory action of in situ poly(ADP-ribosyl)ation of 0.2 μg PARP I (carried out for 10 min) is demonstrated in lane 7, and the rescue by 1 mM benzamide in lane 8. Lane 9 illustrates the absence of inhibitory effect of 1 μg (long chain (cf. [19,20]) free poly(ADP-ribose). Results are the average of four experiments with S.D. error bars shown.

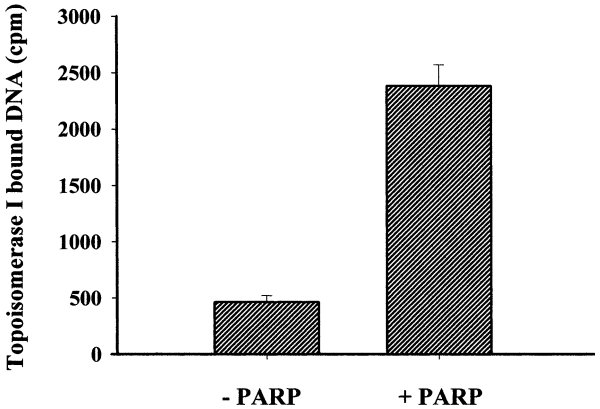

Fig. 3. Effects of PARP I on the covalent Topo I–DNA complex formation step of Topo I catalysis. The binding of Topo I (1 μg) to circular DNA was assayed by the filtration technique as reported (cf. [11]). The DNA–Topo I covalent complex was stabilized by 20 μM camptothecin. Ordinate (cpm): [^3H]DNA (SV3 neo plasmid, 100 ng) bound to Topo I; abscissa: no PARP I added, +1 μg PARP I added. Results show the average of three separate experiments with S.D. error bars shown.

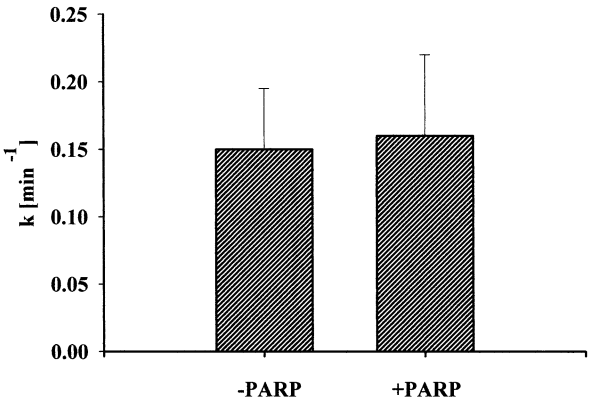

Fig. 4. The effect of PARP I on the religation reaction of Topo I catalysis. Each assay contained 10^5 dpm ^{32}P labeled double stranded DNA (cf. [14]) with covalently attached Topo I (0.2 μg) with or without added PARP I (0.4 μg). The reaction was started by addition of 100-fold excess of displacing single stranded oligo-DNA [14] and incubation continued at 25°C. Aliquots were withdrawn at 1, 5, 15, 30, and 45 min and quenched with 0.5% SDS, then treated with proteinase K (0.5 mg/ml) at 52°C (60 min), heated to 95°C (1 min) in formamide containing loading buffer and electrophoresed in a 20% PAGE gel containing 8 M urea. The location of religated oligo-DNA was done by autoradiography and quantitated by liquid scintillation. The figure shows first order rate constants calculated from two parallel assays.

The activation mechanism of PARP I on Topo I was further studied by determining the binding of ^{3}H labeled supercoiled circular DNA to Topo I in the presence of PARP I (cf. [9]) and camptothecin (20 μM), which stabilizes the Topo I–DNA adduct as shown in Fig. 3. There is a six-fold increase of DNA binding to Topo I in the presence of 1 μg PARP I demonstrating that the rates of scission of the phosphodiester bond and binding of Topo I to DNA are accelerated by PARP I.

The effect of PARP I on the DNA religation assay (cf. [13]) is illustrated in Fig. 4. In contrast to the activation of Topo I by PARP I, as assayed by either the global reaction (scission and religation) where velocity is defined as the time required for 50% unwinding of plasmid DNA, or by the binding of Topo I to plasmid DNA the DNA adduct being stabilized by camptothecin (Fig. 3), the rates of religation of DNA by Topo I are unaffected by PARP I (Fig. 4). These results suggest that religation may not be rate limiting in topoisomerization that is probably determined by rates of preceding steps (scission of phosphodiester bond in DNA and binding to DNA itself), which are directly activated by the binding of PARP I.

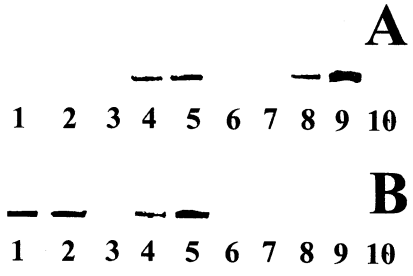

Fig. 5. The binding of free and poly(ADP-ribosyl)ated PARP I to Topo I fragments expressed as GST fusion proteins. In both A and B the abscissa shows the identification of GST polypeptides, which defines localization of binding (in terms of amino acid residues). The ordinate indicates separation of bound PARP I (A) as identified by Western immunoblot or poly(ADP-ribosyl)ated PARP I (B). The abscissa shows the number of GST fusion peptides. Lane 10: GST.

The identification of the Topo I domains that specifically bind to PARP I was done with the aid of GST fusion peptides (cf. [17]) by the pull down technique. Results are summarized in Fig. 5A,B. In the pull down assay, 0.2 μg Topo I fragments (10–40 μl gel slurry), containing bound GST polypeptides expressed in XL-1 blue cells (cf. [17]) were incubated with 0.2 μg PARP I (panel A) or 0.25 μg poly(ADP-ribosyl)ated PARP I (panel B) and then processed (see Section 2) to identify PARP I containing complexes by immunoblots (cf. [2]). Polypeptides of Topo I present in GT-4, 5 and GT-8, 9 bound specifically to PARP I. The binding of poly(ADP-ribosyl)ated PARP I to the GST fusion fragments is shown in Fig. 5B. GT fragments 1, 2 and 4, 5 bind to poly(ADP-ribosyl)ated PARP I. The unconjugated GST protein exhibited no binding to PARP I or poly(ADP-ribosyl)ated PARP I (lanes 10 in A and B).

Based on the X-ray crystallographic analysis [12–14] of Topo I, a 'C clamp' model has been formulated [18]. The topology scheme of PARP I–Topo I interactions (Fig. 6) takes advantage of the X-ray model and is reconstructed from bind-

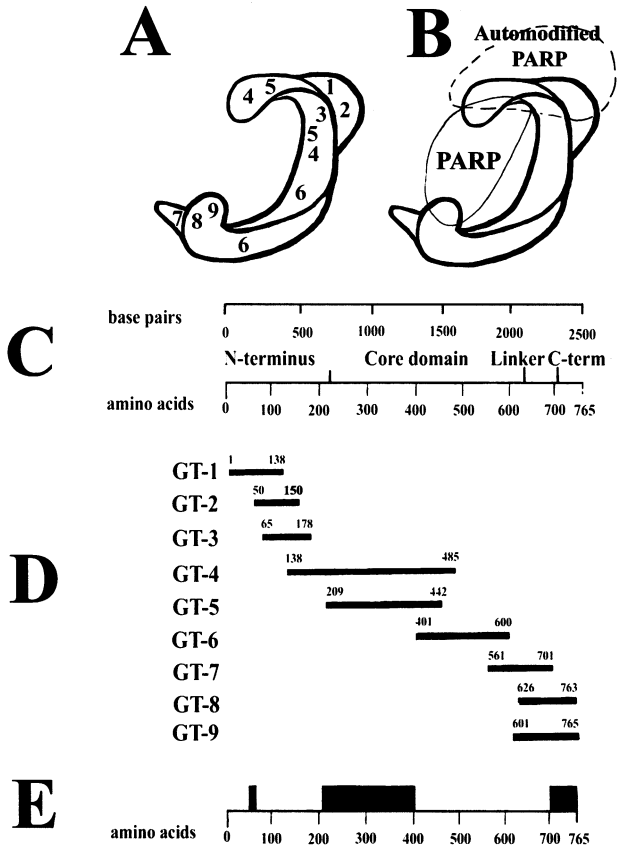

Fig. 6. Summary of topologic interpretation of Topo I–PARP I interactions. PARP I–Topo I and $(ADPR)_n$–PARP I–Topo I binding sites, deduced from the 'C clamp' model and results shown in Fig. 4. A and B illustrate the 'C clamp' conformation, with numbered location of GST–Topo I fragment fusion proteins as projected from D, which is a linear sequence of GST–Topo I fragments correlated with amino acid sequences given in C. B depicts the binding of PARP I (solid ellipsoid) and poly(ADP-ribosyl)ated PARP I (dashed ellipsoid) to Topo I. E is a summary of amino acid sequences identified for the binding sites of PARP I (second and third bars) and for $(ADPR)_n$–PARP I (first and second bars).

ing results (Fig. 5) between PARP I and GST fusion polypeptides of Topo I (see Fig. 6A,C,D). The truncated N-terminal domain of Topo I (aa 1–214) corresponding to GT-1–3 lacks ordered structure, is highly positively charged [13], and is responsible for the binding of several proteins (e.g. nucleolin, p53 and modified PARP I). The core domain (GT-3–6, aa 215–635) clamps DNA and contains amino acids Arg-488, Arg-590, and His-632 (cf. [14]), which contribute to the catalytic site of Topo I (Fig. 6), whereas the C-terminus (aa 712–765 corresponding to GT-8, 9) contains Tyr-723, which is thought to exert a nucleophilic attack on the scissible phosphodiester bond of DNA. The linker (aa 636–712) separates the core and C-terminal domains and corresponds to GT-7 and in part to GT-8 and GT-9.

The binding of PARP I (solid ellipsoid line in Fig. 6B, identified as PARP) and that of the poly(ADP-ribosyl)ated PARP I (dashed ellipsoid line in Fig. 6B, identified as automodified PARP) are shown as amino acid sequences in Fig. 6E. This interpretation is derived from results given in Fig. 5A,B. The binding sites of the unmodified PARP I may explain the activation by providing a more 'tight fisted' Topo I DNA structure [9] that promotes rates of enzymatic catalysis (scission of phosphodiester in DNA and binding to DNA). On the other hand, the poly(ADP-ribosyl)ated PARP I binds to GT-1, 2 and GT-4, 5, which are not in the vicinity of the catalytic center, yet this binding may countermand the conformation changes required for catalytic function. In agreement with this mechanism, it is apparent from results shown in Fig. 2, notably lane 6, that poly(ADP-ribosyl)ated PARP I inhibits Topo I. The free poly(ADP-ribose) polymer has no effect on the enzymatic process of Topo I (Fig. 2, lane 9), whereas poly(ADP-ribosyl)ation of Topo I most probably repels DNA binding, as is also known for certain other DNA binding proteins (cf. [4,21]).

The topology of PARP I–Topo I binding and its relationship to enzymatic activity complements present enzymatic theories [13,18,22,23] inasmuch as it provides a possible mechanism to improve the contact of the decatenated DNA loop with Topo I by way of PARP I binding, a process specifically relevant to eukaryotes.

As we have shown [9] that even highly purified Topo I of calf thymus origin or PARP I preparations from the same source are cross contaminated with each other, it is not surprising that the quasi co-enzymic function of PARP I to Topo I as proposed here has been overlooked.

Acknowledgements: This work was supported by grants (to P.I.B.) TO 23959 and 29620 from OTKA (Hungarian Science Foundation) and by UC A2511 (from Syn-X Inc. Canada) obtained through Dr. George Jackowski (to E.K.). The stimulating discussions with Professor Charles Ordahl (Department of Anatomy, UCSF) are acknowledged with appreciation. The entire research project was originated while P.I.B., K.G.B., A.H., E.K. and E.K. were working in the Romberg Tiburon Centers of San Francisco State University (Tiburon, CA, USA) and completed by collaboration of the laboratories shown on the title page.

References

[1] Bauer, P.I., Buki, K.G., Hakam, A. and Kun, E. (1990) Biochem. J. 270, 17–26.
[2] Buki, K.G., Bauer, P.I., Hakam, A. and Kun, E. (1995) J. Biol. Chem. 270, 3370–3377.
[3] Butler, A.J. and Ordahl, C.P. (1999) Mol. Cell. Biol. 19, 296–306.
[4] Kun, E. (1998) Int. J. Mol. Med. 2, 131–142.
[5] de Murcia, G., de Murcia, M.-J. and Schreiber, V. (1991) BioEssays 13, 455–462.
[6] de Murcia, G. and de Murcia, M.-J. (1994) Trends Biol. Sci. 19, 172–176.
[7] Ferro, A.M., Higgins, N.R. and Olivera, B.M. (1983) J. Biol. Chem. 258, 6000–6003.
[8] Jongstra-Bilen, J., Ittel, M.-E., Niedergang, C., Vosberg, H.-P. and Mandel, P. (1983) Eur. J. Biochem. 136, 391–396.
[9] Bauer, P.I., Buki, K.G., Comstock, J.A. and Kun, E. (2000) Int. J. Mol. Med. 5, 533–540.
[10] Bauer, P.I. and Kun, E. (2000) Int. J. Mol. Med. 6, 153–154.
[11] Sastry, S.S. and Kun, E. (1988) J. Biol. Chem. 263, 1505–1512.
[12] Stewart, L., Redinbo, M.R., Qiu, X., Hol, W.G. and Champoux, J.-J. (1998) Science 279, 1534–1541.
[13] Redinbo, M.R., Stewart, L., Kuhn, P., Champoux, J.J. and Hol, W.G. (1998) Science 279, 1504–1513.
[14] Stewart, L., Ireton, G.C. and Champoux, J.J. (1999) J. Biol. Chem. 274, 32950–32960.
[15] Bauer, P.I., Farkas, G., Buday, L., Mikala, G., Meszaros, G., Kun, E. and Farago, A. (1992) Biochem. Biophys. Res. Commun. 187, 730–736.
[16] Buki, K.G. and Kun, E. (1988) Biochemistry 27, 5990–5995.
[17] Chen, H.-J., Hwong, C.-L., Wang, C.-H. and Hwang, J. (2000) J. Biol. Chem. 275, 13109–13117.
[18] Berger, J.M. (1998) Biochim. Biophys. Acta 1400, 3–18.
[19] Hakam, A. and Kun, E. (1985) J. Chromatogr. 330, 287–298.
[20] Hakam, A., McLick, J. and Kun, E. (1986) J. Chromatogr. 359, 275–284.
[21] Althaus, F.R. and Richter, C. (1987) Mol. Biol. Biochem. 37, 60–68.
[22] Wang, J.C. (1996) Annu. Rev. Biochem. 65, 635–692.
[23] Champoux, J.J. (2001) Annu. Rev. Biochem. 70, 369–413.