Regulation of RNA Polymerase II-dependent Transcription by Poly(ADP-ribosylation) of Transcription Factors

(Received for publication, September 9, 1998, and in revised form, October 1, 1998)

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Poly(ADP-ribosyl) transferase (ADPRT) is a nuclear protein that modifies proteins by forming and attaching to them poly(ADP-ribose) chains. Poly(ADP-ribosylation) represents an event of major importance in perturbed cell nuclei and participates in the regulation of fundamental processes including DNA repair and transcription. Although ADPRT serves as a positive cofactor of transcription, initiation of its catalytic activity may cause repression of RNA polymerase II-dependent transcription. It is demonstrated here that ADPRT-dependent silencing of transcription involves ADP-ribosylation of the TATA-binding protein. This modification occurs only if poly(ADP-ribosyl)ation is initiated before TATA-binding protein has bound to DNA and thereby prevents formation of active transcription complexes. Specific DNA binding of other transcription factors including Yin Yang 1, p53, NFκB, Sp1, and CREB but not c-Jun or AP-2 is similarly affected. After assembly of transcription complexes initiation of poly(ADP-ribosyl)ation does not influence DNA binding of transcription factors. Accordingly, if bound to DNA, transcription factors are inaccessible to poly(ADP-ribosyl)ation. Thus, poly(ADP-ribosyl)ation prevents binding of transcription factors to DNA, whereas binding to DNA prevents their modification. Considering its ability to detect DNA strand breaks and stimulate DNA repair, it is proposed that ADPRT serves as a molecular switch between transcription and repair of DNA to avoid expression of damaged genes.

EXPERIMENTAL PROCEDURES

Purification of Recombinant Proteins—After overexpression in Escherichia coli cells the human His-tagged ADPRT was purified as described previously (15). Recombinant human His-tagged YY1 (plasmid pHisYY1) produced in E. coli cells was purified as described by Seto et al. (16). Recombinant human p53 was obtained from E. coli cells transformed with the plasmid pET-8c-p53H-47 (17). Recombinant TBP and TFIIIB were purchased from Promega.

Electrophoretic Mobility Shift Assay—32P labeling of the oligonucleotides (YY1 (18), GGTTCCGCGGCCATCTTGGCGGCT; Sp1 (19), ATTCGATCGGGGCAGCGGAC; AP-1 (20), CGCTTGATGCTACCGC-GGA; and TATA (21), GCAGAGCATATAAGGTGAGGTAGGA) and EMSAs with HeLa nuclear extracts (Promega) were performed as described previously (13). In brief, in a binding reaction nuclear HeLa extracts (4 μg of protein) or recombinant proteins were incubated with 5 ng of 32P-labeled duplex oligonucleotide for 20 min at room temperature in 20 mM Tris-HCl, pH 8, 60 mM KCl, 5 mM MgCl2, 1 mM dithiothreitol, 0.05% Nonidet P-40, 10% glycerol, 50 μg/ml bovine serum albumin, and 500 ng of poly (dl-dC) in a final volume of 10 μl. If EMSAs were carried out with recombinant proteins, the binding reaction contained in addition 400 ng of an unrelated oligonucleotide as unspecific competitor DNA to inhibit unspecific oligonucleotide binding activities present in the extracts (13). If EMSAs were carried out with recombinant proteins, the binding reaction contained as unspecific inhibitor 200 ng of a promoter-less plasmid p(CAT)2 (22). Following the binding reaction protein-DNA complexes were separated in a 4% polyacrylamide gel. EMSA with TATA oligonucleotide was performed according to the

NAD+ pool (4). It was concluded that this effect is part of a defense mechanism, because DNA repair after γ-irradiation is stimulated by ADPRT activity (5, 6). The target proteins of poly(ADP-ribosyl)ation include the enzyme itself as well as a limited number of nuclear proteins (reviewed in Ref. 3).

ADPRT has also been shown to participate in the regulation of transcription and appears to exert a dual function. On the one hand, the active component of transcription factor IIC was identified as the enzyme ADPRT (7, 8). In the absence of its substrate, NAD+, ADPRT has been demonstrated to be an enhancer of activator-dependent transcription (9). On the other hand, it was reported recently that protein modification with poly(ADP-ribose) was an efficient means of reversibly silencing general polymerase II-dependent transcription (9, 10). Consequently, the activating or repressing influence of ADPRT on transcription depends on the catalytic activity of this enzyme and appears to be mediated by direct interactions with basal and gene-specific transcription factors. Transcription factors TFII F (11) and p53 (12) have been reported to be acceptors of poly(ADP-ribose) chains. Transcription factors YY1 and Oct-1 were found to tightly associate with ADPRT (13, 14). Because silencing of transcription by poly(ADP-ribosylation) (9, 10) was observed in a system requiring only the basal transcriptional complex, the influence of ADPRT on gene expression may be more general rather than restricted to a small number of genes.

In the present study the molecular mechanism of the ADPRT-dependent silencing of transcription was investigated. It was found that the poly(ADP-ribosyl)ation reaction prevented specific transcription factors from binding to DNA, thus excluding the formation of an active transcription complex. The specific DNA binding of some transcription factors (YY1, TBP, and p53) was prevented by covalent modification with poly(ADP-ribose). No ADP-ribosylation of transcription factors was detected if they had bound to DNA before the modification reaction was initiated. The results suggest that when activated, ADPRT may modify certain general and other transcription factors and thereby prevent their binding to DNA.
protocol of Promega. 20 ng of TBP and 50 ng of TFIIIB were incubated with 5 ng of 32P-labeled TATA duplex oligonucleotide for 15 min at room temperature in 20 mM Tris-HCl, pH 8.0, 7 mM MgCl2, 0.1 mM EDTA, and 10% glycerol in a final volume of 10 μl. Following the binding reaction 10 ng of poly(dI-dC) was added, and samples were subjected to electrophoretic separation on a 6% polyacrylamide gel. After electrophoresis the gels were autoradiographed.

Determination of ADPRT Activity—Recombinant purified ADPRT was incubated in binding buffer (10 mM Tris-HCl, pH 8.0, 7 mM MgCl2, 0.1 mM EDTA, and 10% glycerol) at 25 °C in a final volume of 10 μl. Following preincubation for 20 min at ambient temperature in transcription buffer (10 mM Tris-HCl, pH 8, 80 mM KCl, 10 mM MgCl2, 2 mM dithiothreitol, and 10% glycerol in a final volume of 10 μl. Following the binding reaction 10 ng of poly(dI-dC) was added, and samples were subjected to electrophoretic separation on a 6% polyacrylamide gel. After electrophoresis the gels were autoradiographed.

RESULTS AND DISCUSSION

Because it was the goal of the present study to understand how activation of ADPRT may cause repression of transcription, the effect of poly(ADP-ribosyl)ation on transcription factors was analyzed. The ability of transcription factors to bind to their cognate sequences was tested in EMSAs following incubation with 32P-labeled YY1 oligonucleotide had proceeded for 20 min under conditions indicated in the legends to the figures. Incubations were stopped by the addition of SDS-containing sample buffer. Proteins were separated by SDS-PAGE. After electrophoresis gels were dried, and modification of their cognate sequences was tested in EMSAs following incubation, the effect of poly(ADP-ribosyl)ation on transcription factor activity DNA binding of YY1 was strongly suppressed (Fig. 1A, lane 1), with 500 ng of purified recombinant ADPRT (lanes 2, 3, and 5), or with 4 nmol of ADP-ribose units of isolated ADP-ribose polymers (PAR, lane 4). In lane 3, NAD+ was present at a final concentration of 1 mM. Incubations were carried out for 20 min prior to the binding reaction with 32P-labeled YY1 oligonucleotides containing consensus sites for Sp1 (lanes 1–3) or AP-1 (lanes 4–6). In lanes 1 and 2, 1 mM NAD+ and 1 mM 3-aminobenzamide (3-ABA, lane 3), or poly(ADP-ribose) (4 nmol of ADP-ribose units, lane 4) for 20 min prior to the binding reaction with 32P-labeled YY1 oligonucleotide. In lane 5, 1 mM NAD+ was added after the binding reaction of HeLa nuclear extract with 32P-labeled YY1 oligonucleotide had proceeded for 20 min, followed by a further incubation for 20 min. C, HeLa nuclear extracts (4 μg of protein) were incubated with no further addition (lane 1) or in the presence of 1 mM NAD+ (lane 2), 1 mM NAD+ and 1 mM 3-aminobenzamide (3-ABA, lane 3), or poly(ADP-ribose) (4 nmol of ADP-ribose units, lane 4) for 20 min prior to the binding reaction with 32P-labeled YY1 oligonucleotide. In lane 5, 1 mM NAD+ was added after the binding reaction of HeLa nuclear extract with 32P-labeled YY1 oligonucleotide and unspecific competitor DNA had proceeded for 20 min (post), followed by a further incubation for 20 min. Positions of specific DNA-YY1 complexes (yin-yang symbol) and unmodified oligonucleotide (free) are indicated. B, HeLa nuclear extracts (4 μg of protein) were incubated with no further addition (lane 1) or in the presence of 1 mM NAD+ (lane 2), 1 mM NAD+ and 1 mM 3-aminobenzamide (3-ABA, lane 3), or poly(ADP-ribose) (4 nmol of ADP-ribose units, lane 4) for 20 min prior to the binding reaction with 32P-labeled YY1 oligonucleotide. In lane 5, 1 mM NAD+ was added after the binding reaction of HeLa nuclear extract with 32P-labeled YY1 oligonucleotide had proceeded for 20 min, followed by a further incubation for 20 min. C, HeLa nuclear extracts (4 μg of protein) were incubated in the absence (lanes 1 and 4) or presence (lanes 2 and 5) of 1 mM NAD+ for 20 min prior to the binding reaction. Binding reactions were carried out with 32P-labeled duplex oligonucleotides containing consensus sites for Sp1 (lanes 1–3) or AP-1 (lanes 4–6). In lanes 3 and 6, 1 mM NAD+ was added after the binding reaction of HeLa nuclear extract with 32P-labeled oligonucleotides and unspecific competitor DNA had proceeded for 20 min (post), followed by a further incubation for 20 min. Positions of specific DNA-protein complexes and free DNA probes are indicated.

FIG. 1 Poly(ADP-ribosyl)ation prevents specific DNA binding of transcription factors. EMSAs were performed as described under "Experimental Procedures." A, 250 ng of purified recombinant YY1 was incubated with no further addition (lane 1), with 500 ng of purified recombinant ADPRT (lanes 2, 3, and 5), or with 4 nmol of ADP-ribose units of isolated ADP-ribose polymers (PAR, lane 4). B, HeLa nuclear extracts (4 μg of protein) were incubated with no further addition (lane 1) or in the presence of 1 mM NAD+ (lane 2), 1 mM NAD+ and 1 mM 3-aminobenzamide (3-ABA, lane 3), or poly(ADP-ribose) (4 nmol of ADP-ribose units, lane 4) for 20 min prior to the binding reaction with 32P-labeled YY1 oligonucleotide. In lane 5, 1 mM NAD+ was added after the binding reaction of HeLa nuclear extract with 32P-labeled YY1 oligonucleotide had proceeded for 20 min, followed by a further incubation for 20 min. C, HeLa nuclear extracts (4 μg of protein) were incubated in the absence (lanes 1 and 4) or presence (lanes 2 and 5) of 1 mM NAD+ for 20 min prior to the binding reaction. Binding reactions were carried out with 32P-labeled duplex oligonucleotides containing consensus sites for Sp1 (lanes 1–3) or AP-1 (lanes 4–6). In lanes 3 and 6, 1 mM NAD+ was added after the binding reaction of HeLa nuclear extract with 32P-labeled oligonucleotides and unspecific competitor DNA had proceeded for 20 min (post), followed by a further incubation for 20 min. Positions of specific DNA-protein complexes and free DNA probes are indicated.

Sp1 consensus oligonucleotide (Fig. 1C, lane 2) as well as with a NFκB consensus oligonucleotide (not shown). Using a CREB consensus oligonucleotide the effect of poly(ADP-ribosyl)ation was detectable but less pronounced (not shown). In contrast, retention of AP-1 (Fig. 1C, lane 5) or AP-2 (not shown) oligonucleotides in the EMSA was insensitive to ADPRT activity. Addition of deproteinized ADP-ribose polymers did not influence DNA binding of Sp1 or AP-1 (not shown). As has been observed with YY1 (Fig. 1B, lane 3), repression of Sp1 DNA binding was prevented by the addition of 3-aminobenzamide (not shown). Again, binding of the proteins to their consensus sequences was unaffected if it had taken place prior to the addition of NAD+ (Fig. 1C, lane 3). Importantly, TBP (in complex with TFIIIB) exhibited also a similar response to ADPRT activity, which is of significance with regard to the ADPRT-dependent silencing of transcription (see below).

The interference of ADPRT activity with specific DNA binding as described above has also been observed with purified recombinant p53 (12), a known partner of ADPRT (25) and target protein of modification by poly(ADP-ribose) (12). As opposed to the observations with YY1 (Fig. 1A, lane 4 and B, lane 4) or Sp1, specific p53 DNA binding was also repressed in the presence of small amounts of deproteinized ADP-ribose polymers (not shown). This high affinity of p53 to isolated promoters is in accordance with a recent report by Malanga et al. (26).

Thus, it is concluded that the catalytic activity of ADPRT prevents specific DNA binding of some transcription factors.
However, it is important to point out that initiation of the poly(ADP-ribosyl)ation reaction does not cause the dissociation of preformed complexes between DNA and transcription factors.

The conditions under which the DNA binding of some transcription factors was influenced implicated an interaction of ADPRT with these factors that depended on the functional state of the enzyme. To gain insight into whether transcription factors in turn exert an effect on the catalytic activity of ADPRT, we compared the rate of poly(ADP-ribosyl)ation with or without the addition of transcription factors. Addition of purified recombinant YY1 resulted in a substantial acceleration of the poly(ADP-ribosyl)ation reaction (Fig. 2A). The observed enhancement of [32P]poly(ADP-ribose) synthesis was attributable to both the stimulated auto(ADP-ribose)ylation of the enzyme and the occurrence of heteromodification of YY1 with poly(ADP-ribose) (Fig. 2A). Remarkably, ADP-ribosylation of YY1 was not detectable if this transcription factor bound to DNA before the addition of NAD$. This finding holds important implications for the ability of ADPRT to repress transcription (see also below for TBP). Similarly to YY1, addition of TBP or p53 also resulted in a stimulation of the auto(ADP-ribose)ylation reaction (Table I), although less efficiently. Nevertheless, these effects appear to be specific, because other known partner proteins of ADPRT, XRCC1 (x-ray repair cross-complementing 1) (27) or DNA-dependent protein kinase (28), inhibited the poly(ADP-ribose)ylation reaction.

The AP-1-binding protein c-Jun whose specific DNA binding was insensitive to poly(ADP-ribosyl)ation (cf. Fig. 1C, lane 5) had no effect on the rate of poly(ADP-ribose) synthesis (Table
The stimulating effect of the basal transcription factor TFIIB on ADPRT activity was rather weak. Still, if, in addition to TBP, TFIIB was present, poly(ADP-ribosyl)ation was further enhanced (Table 1).

As mentioned before, TBP (in complex with TFIIB) exhibited a behavior resembling that of YY1 in the assays presented above (Figs. 1 and 2), indicating a specific physical interaction between these transcription factors and ADPRT. This is of considerable interest, because TBP and TFIIB, known to tightly associate for initiation of transcription (29), are essential components of the basal transcriptional machinery. The results are presented in Fig. 3 (A and B). EMSAs were performed using TATA oligonucleotides and recombinant TBP and TFIIB. As shown in Fig. 3A, complex formation of TBP/TFIIB with the TATA oligonucleotide was sensitive to ADPRT activity (Fig. 3A, lane 2). However, the poly(ADP-ribosylation) reaction did not influence preformed complexes of TBP/TFIIB with DNA (Fig. 3A, lane 3). Thus, DNA binding and formation of transcription complexes are only prevented if the poly(ADP-ribosylation) reaction is accomplished before the basal transcription factors have bound to DNA.

Analysis of protein modification with poly(ADP-ribos) revealed that only TBP and not TFIIB is a specific target for poly(ADP-ribosyl)ation (Fig. 3B). Importantly, modification of TBP occurred only under conditions that greatly diminished specific DNA binding of TBP/TFIIB (Fig. 3A, lane 2). That is, when the transcription factors had bound to DNA prior to the addition of NAD+, there was no detectable ADP-ribosylation of TBP (Fig. 3B, lane 3), and complex formation as visualized in EMSA remained unaffected (Fig. 3A, lane 3). These findings provide strong support for the conclusion that TBP may be prevented from binding to its cognate sequence by modification with poly(ADP-ribos). It is proposed, therefore, that the mechanism underlying ADPRT-dependent silencing of transcription (10) includes modification of TBP causing the inability of this essential factor to bind to the TATA box. Indeed, the conditions required to observe ADPRT-dependent silencing of Pol II-dependent transcription (Fig. 3C, lane 2) parallel those found to be crucial for modification of TBP (Fig. 3B, lanes 2 and 4) and prevention of its DNA binding (Fig. 3A, lane 2).

In a recent report (11) the transcription factor TFIIIB was shown to be an acceptor of poly(ADP-ribos), whereas TBP was not modified. It would appear from the data presented here that TBP escaped modification (11), because it had already bound to DNA prior to the initiation of poly(ADP-ribosyl)ation (cf. Fig. 3B, lane 3).

Several lines of evidence demonstrate the specificity of the poly(ADP-ribosyl)ation for some transcription factors. For example, in HeLa nuclear extracts transcriptional silencing caused by poly(ADP-ribosyl)ation was accompanied by selective modification of primarly ADPRT itself and a rather limited number of nuclear proteins (10). In addition, in the presence of other proteins, e.g. albumin or TFIIIB (Figs. 2A and 3B), only ADPRT and the specific acceptor proteins, YY1 or TBP, respectively, were detected to be modified. Furthermore, although TBP and YY1 were readily ADP-ribosylated (Figs. 2A and 3B), modification of c-Jun, which binds specifically to AP-1 sites, was detectable only after prolonged incubation (not shown). The observation that poly(ADP-ribosyl)ation did not affect AP-1 binding in the EMSA (Fig. 1C, lane 5) suggests that the rate of modification by ADPRT might be crucial for prevention of DNA binding. This supposition was corroborated by EMSA using NAD+ analogs that permit only very slow rates of the poly(ADP-ribosyl)ation reaction (30). None of these analogs influenced the retention of oligonucleotides in experiments similar to those presented in Fig. 1 (not shown). In this respect it is important to note that ADPRT-dependent silencing of transcription as shown in Fig. 3C is achieved within less than 5 min following the addition of NAD+ (10).

Finally, this study provides evidence for the suggestion that poly(ADP-ribosyl)ation of TBP is a crucial step in ADPRT-dependent silencing of transcription. It also underlines the important conclusion that when initiated, poly(ADP-ribosyl)ation prevents formation of new transcription complexes without interrupting ongoing transcription. That is, once transcription preinitiation complexes have been formed, the transcription factors are inaccessible for ADP-ribosylation. In essence, poly(ADP-ribosyl)ation prevents their binding to DNA, whereas DNA binding of transcription factors prevents their poly(ADP-ribosyl)ation. According to the role of ADPRT as a survival factor following genotoxic stress, participation of this enzyme in the regulation of transcription may be of importance during recovery from DNA damage. In addition, selective poly(ADP-ribosyl)ation of transcription factors represents a novel means of controlling gene expression by covalent protein modification.

Acknowledgments—We thank Drs. T. Shenk, T. C. Lee, W. M. Yang, B. Lichter, and V. Rotta for kindly providing plasmids for overexpression of recombinant YY1 and p53 and G. Buchlow for expert technical assistance.

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