Expression in *E. coli* of the catalytic domain of rat poly(ADP-ribose)polymerase

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A 2 kilobase pair cDNA coding for the entire C-terminal catalytic domain of rat poly(ADP-ribose)polymerase has been expressed in *E. coli*. The overproduced 55 kDa polypeptide is active in synthesizing poly(ADP-ribose) and the 4 kDa N-terminal region of this domain is recognized by the monoclonal antibody C I,2 directed against the calf enzyme. Also, the minor α-chymotrypsin cleavage site found in the human catalytic domain is not present in the rat enzyme as revealed by the absence of the 40 kDa specific degradation product in the *E. coli* cells expressing the rat domain. The expression of this partial rat cDNA should thus permit the rapid purification and subsequent crystallization of the catalytic domain of the enzyme.

Poly(ADP-ribose)polymerase; *E. coli* expression; α-Chymotrypsin; NAD^+ metabolism; Immunoblotting

1. INTRODUCTION

Poly(ADP-ribose)polymerase (PARP) is a nuclear enzyme that catalyzes the covalent attachment and elongation of poly(ADP-ribose) from NAD^+ on nuclear proteins such as histones, topoisomerase I and PARP (automodification) (for review, see [1]). Poly-(ADP-ribose) is implicated in nuclear events including the relaxation of chromatin, DNA repair and gene expression. PARP can be divided into three functional domains when treated with papain and α-chymotrypsin [2–4]. The N-terminal 42 kDa domain contains 2 zinc-finger motifs that bind specifically to DNA single strand breaks [5,6]. The internal 16 kDa is the automodification domain while the C-terminal 55 kDa domain binds NAD^+ and is the catalytic region.

We have recently cloned a partial rat cDNA coding for the entire catalytic domain of the enzyme [7]. The predicted amino acid sequence is greater than 95% homologous to the same domain of the human [3,8–10] and mouse [11] proteins. Most importantly this domain was shown to be the target of autoantibodies against the PARP in several human rheumatic patients [12]. As a first step toward the purification and crystallization of the catalytic domain, we have expressed in *E. coli* the rat cDNA coding for this region of the PARP.

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Abbreviations: PARP, poly(ADP-ribose)polymerase; kbp, kilobase pair(s); PAGE, polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

2.1. *Expression in* E. coli

We have used a rat cDNA which is one bp (cytosine) longer at the 5′ end than the one described previously [7]. It was inserted in the *Eco*RI site of pTG161 [13] to give the recombinant plasmid, pTGrat55. We have also cloned a *Hin*dIII fragment of the human PARP cDNA [10] in the plasmid pTG920 [13]. This 2.66 kbp fragment (pTGhum87) starts at the nucleotide position +698 [3] and codes for the catalytic domain, the automodification domain and part of the DNA binding domain. The plasmid pTG161 SH1.4 codes for the C-terminal 51 kDa part of the human catalytic domain, starting from the amino acid 572 corresponding to the *Pst*I site [3]. *E. coli* TGE 900 was transformed with the recombinant plasmids, grown at 28°C and shifted to 37°C to induce the transcription of the foreign PARP sequences [13]. Preparation, SDS-PAGE and electrophoretic transfer of the crude extracts on the solid support were as described [14].

2.2. *Detection methods*

The PARP-specific polypeptides were revealed on the nitrocellulose sheet by 2 methods as described [14]. First, an activity blot using [^{32}P]NAD^+ was done in the presence of calf thymus DNA. The immunogenic fragments were then revealed using monoclonal and polyclonal antibodies against the PARP [4] and second antibodies conjugated to alkaline phosphatase [6].

3. RESULTS AND DISCUSSION

We have compared the expression in *E. coli* of the

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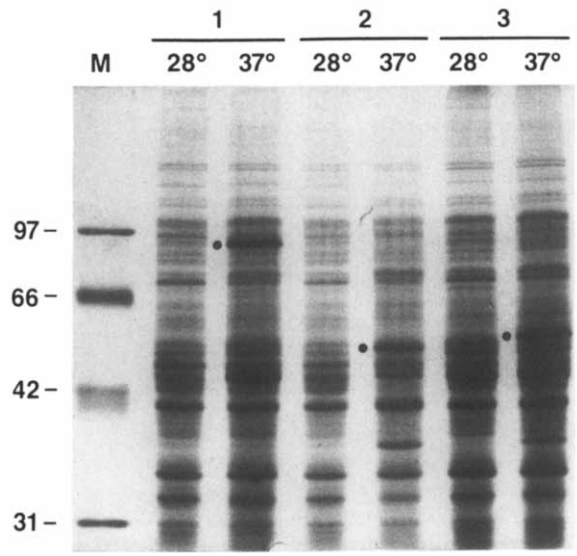

Fig. 1. Expression of the cloned PARP fragments in *E. coli*. Gel electrophoresis of the crude extracts from uninduced (28°C) and induced (37°C) cells transformed with (1) pTGhum87, (2) pTGrat55, (3) pTG161 SH1.4. (M) molecular weight markers in kDa. Black dots indicate the position of the overexpressed proteins.

rat (pTGrat55) and human (PTG161 SH1.4, pTGhum87) cDNA fragments, all encoding the catalytic domain of the PARP. Analysis of the crude extracts showed that these fragments are overexpressed in induced cells and code for polypeptides of the expected molecular weights (Fig. 1). These overproduced proteins (87, 54 and 51 kDa) are recognized on Western blot by a rabbit polyclonal antiserum (Fig. 2A). This antibody also revealed in human HeLa and Rat-1 cellular extracts the 113 kDa PARP as well as many other smaller polypeptides that come from endogenous degradation of the enzyme (Fig. 2A, lanes 1,4) [15]. The expression in *E. coli* of the pTGhum87 plasmid (Fig. 2A, lane 2) as well as other human cDNA frag-

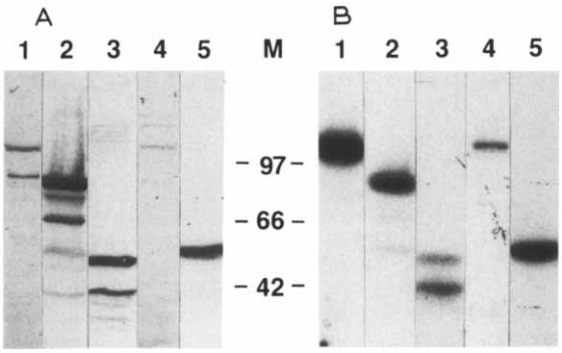

Fig. 2. Analysis of the overexpressed PARP-specific proteins. (A) Immunodetection with a purified polyclonal antibody directed against calf thymus PARP. (B) Activity blot. Lane 1, HeLa cell extract; lane 2, pTGhum87; lane 3, pTG1161 SH1.4; lane 4, Rat-1 cell extract; lane 5, pTGrat55. (M) as Fig. 1.

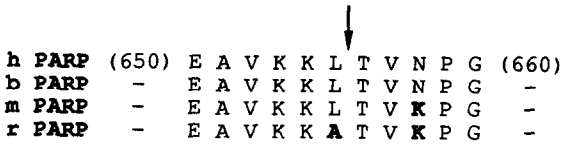

Fig. 3. Analysis of amino acid sequence homology of the minor α-chymotrypsin cleavage site of the PARP. Arrow indicates the cleavage site [6,23]. (h) human [3]; (b) bovine [18]; (m) mouse [11]; (r) rat [7]. Bold letters represents an amino acid variation to the human sequence.

ments [10,16] also produced many lower molecular weight PARP-related polypeptides in addition to the overexpressed proteins. When a small part of the human cDNA (pTG161 SH1.4) coding only for the C-terminal 51 kDa region of the catalytic domain is expressed in *E. coli*, we still observe a major 40 kDa contaminant (Fig. 2A, lane 3). Neither of these fragments nor any other contaminants were ever detected in cells expressing the rat 55 kDa domain (Fig. 2, lane 5). Since the human [10] and rat [7] sequences are homologous around the catalytic domain putative internal start site (not shown) described before [10], we feel that this 40 kDa fragment more likely derives from the cleavage of the human domain by an endogenous α-chymotrypsin-like protease of *E. coli*. α-Chymotrypsin was previously shown to cut in the human [17] and bovine [2,4,18] 55 kDa catalytic domain to generate 40 and 15 kDa fragments. We thus compared the predicted amino acid sequence of the PARP from different organisms at this cleavage site (Fig. 3). The substitution of the leucine 655 for an alanine in the rat sequence could explain the resistance of this region to the hypothesized α-chymotrypsin-like protease of *E. coli*. The activity blot in Fig. 2B reveals that upon incubation with [^{32}P]NAD$^+$,

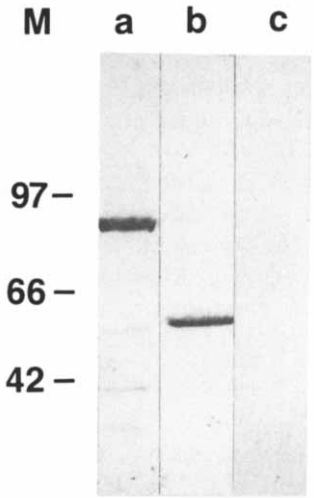

Fig. 4. Immunoreactivity of monoclonal antibody C 1,2. Crude *E. coli* extracts from induced cells transformed with (a) pTGhum87; (b) pTGrat55; (c) pTG161 SH1.4. (M) as in Fig. 1.

the overexpressed rat 55 kDa fragment is active in synthesizing poly(ADP-ribose). Taken together these data show that the rat cDNA is the most suitable choice for the overexpression, purification and crystallization attempts of the PARP catalytic domain.

Finally, the difference in size between the rat 55 kDa fragment (which is recognized by the monoclonal antibody C I,2) and the human 51 kDa fragment (which is not (Fig. 4)), represents the epitope of this antibody. This region is part of the 15 kDa α-chymotrypsic fragment recognized by human autoantibodies [12]. Unlike these antibodies C I,2 does not inhibit the activity of the PARP [4] suggesting that their epitopes are different. However, both map outside the 40 kDa fragment which is still active (Fig. 2B, lane 3) and contains the highly conserved putative nucleotide binding site [7].

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