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# PARTIAL AMINO ACID SEQUENCE OF RAT TOPOISOMERASE I: COMPARISON WITH THE PREDICTED SEQUENCES FOR THE HUMAN AND YEAST ENZYMES

Egon Durban*, Massimo Bramucci* and Richard Cook+

*Department of Pharmacology and +Howard Hughes Medical Institute and Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030

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The partial amino acid sequence of rat topoisomerase I was determined by gas-phase microsequencing. Seven tryptic peptides closely matched the sequences deduced from human topoisomerase I cDNA (94.5% homology). Similarity to sequences deduced from baker's yeast and fission yeast genomic DNA were restricted to conserved domains which may represent important sites of interaction with DNA or with other proteins. 

Topoisomerase I is a phosphoserine phosphoprotein (1,2) which catalyzes changes in the topological state of DNA by introduction of transient single-strand breaks followed by religation (3,4). The enzyme appears to play an essential role in the transcription process (5-9). The indirect protein sequences have been derived by DNA cloning techniques for topoisomerase I from baker's yeast (S. cerevisiae; ref.10), fission yeast (S. pombe, ref.11) and human origin (12). However, direct amino acid sequences have not been determined. These are essential for determining post-translational modifications such as methylated or phosphorylated amino acids. Furthermore, they provide definite confirmation for DNA derived amino acid sequences.

We report here the partial direct amino acid sequence of rat topoisomerase I which matched well the predicted sequences of human topoisomerase I. A phylogenetic comparison showed regions of structural conservation and diversity between yeast and mammalian topoisomerase I.

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labelled molecules (13). In cartilage, the majority of incorporated [$^{35}$S]-sulphate is found in the large aggregating PG (14). These are high molecular weight glycoproteins (15) which form large aggregates by binding to hyaluronic acid (16). The intracellular assembly and subsequent secretion of these molecules is a complex multi-stage process. The PG core protein is synthesised in the rough endoplasmic reticulum (17) where it is maintained as an unglycosylated pool (18,19). A series of transferase enzymes catalyse the elongation and sulphation of the glycosaminoglycan (GAG) chains (20) in the Golgi apparatus and the completed molecule is rapidly secreted. This synthetic and secretory pathway may therefore be regulated at a number of different stages. The aim of the current study was to determine the mechanisms by which IL1 decreases the production of cartilage PG, and to determine whether foetal and neonatal cartilage responds in the same way as adult cartilage.

## METHODS

Cartilage explant cultures were set up as previously described (11,13) from adult, foetal and neonatal pigs. For chondrocyte cultures, cartilage was incubated overnight in serum-free medium (10ml/g of cartilage) containing 1mg/ml collagenase and 50µg/ml gentamycin. Chondrocytes were pelleted by centrifugation at 100g for 5 mins. The pellet was washed by resuspending in Iscoves medium containing 5% fcs and 5µg/ml gentamycin. Cells were counted, and plated at $10^6$ cells/well into 24 well plates or 3 x $10^6$ cells/dish into 35mm dishes. Cultures were incubated for 48 hr at 37°C in a humidified atmosphere of 5% $CO_2$/95% air before addition of IL1. Human recombinant IL1α was from Roche, U.K. Newly synthesised GAG and total GAG were determined as previously described (13). Total protein synthesis was determined as uptake of [$^{35}$S]-methionine and DNA synthesis as uptake of [$^{3}$H] thymidine incorporated into TCA precipitable material. For secretion experiments, 35mm plates of chondrocytes were incubated with and without IL1 for 24 hr prior to receiving a 5 min pulse of 50µCi/ml [$^{35}$S]-sulphate in sulphate-free medium. They were then washed with PBS and chased in 1ml medium containing 1mM non-radioactive sulphate. IL1 was present in experimental plates throughout the pulse and chase periods. At various chase times ranging from 0-60 mins, medium was removed from 3 control and 3 IL1-treated plates and frozen. 1ml of 4M GuHCl, 0.05M sodium acetate pH 5.8 was added to the plates and incubated for 15 mins at 4°C. The remaining residue fraction was digested in 1ml papain solution and analysed for labelled GAG content as previously described (13).

Synthesis of GAG in the presence of cycloheximide was determined by incubating chondrocytes with 0.5µg/ml cycloheximide for various times (0-240 mins) before receiving a pulse of 20µCi/ml [$^{35}$S]-sulphate. Pulse medium was removed, cell layers washed in PBS and chased in 1ml medium for a further 60 mins. Experimental plates contained cycloheximide throughout the pulse and chase periods. The incubations were terminated by addition of an equal volume of 8M GuHCl, 0.1M sodium acetate pH 5.8 and agitated for 1 hr at 4°C to extract GAG from a combined cell and medium fraction.

Gel electrophoresis was performed on a continuous system using 4% SDS-polyacrylamide gels. Samples were loaded in buffer containing SDS and 8M Urea to prevent aggregation of PG (21). Gels were processed for fluorography, dried and exposed to preflashed X-ray film at -80° (22).

## RESULTS AND DISCUSSION

Previous experiments demonstrated that natural porcine IL1 caused a reversible,dose-dependent inhibition of PG synthesis in explants of cultured

sequence analysis (Fig.1). Three fractions contained a single peptide each (peptides 1, 2, and 7), while two fractions consisted of a mixture of two peptides each (peptides 3,4 and 5,6). Although these were mixtures, sequences could be assigned since the peptides were present in different amounts. Table 1 shows the partial amino acid sequences of rat topoisomerase I peptides which closely matched the predicted amino acid sequence of human topoisomerase I (12). Out of 73 residues, 69 were

Table 1. Amino acid sequences of tryptic peptides of rat topoisomerase I

| Peptide | Sequence | matching human cDNA sequence[1] |
|---|---|---|
| 1 | Asp-Glu-Pro-Glu-Asp-Asp-Gly-Tyr-Phe-Ala-[Val][2]<br>Pro-(Pro)[3]-(lys) | 118-130 |
| 2 | (Gly)-Pro-Val-Phe-Ala-Pro-Pro-Tyr-Glu-Pro-<br>Leu-Pro-Glu-Gly-Val-(Lys)<br>[Asn] | 224-239 |
| 3 | Val-(Pro)-(Gln)-Pro-Ala-Asp-Gly-His-Lys | 392-400 |
| 4 | Glu-Asp-Trp-Lys | 463-466 |
| 5 | (Val,Ile)[4]-(Glu,Thr)-(His,Val)-(Ile,Ala)-<br>(Asn,Asp)-(Leu,Ile)-His-Pro-(Glu,Pro)-<br>(Leu,Glu)-Asp-(Gly,Asp)-(Gln,Gly)-(Glu,Gln) | 509-522 |
| 6 | Ile-Thr-Val-Ala-(Trp) | 728-732 |
| 7 | (Phe)-(Ala)-(Trp)-Ala-Ile-Asp-Met-Thr-(Thr)-[Ala][Asp]<br>Glu-(Glu)-Tyr<br>[Asp] | 752-763 |

1) from D'Arpe et al. (12).
2) Amino acid residues of human topoisomerase I (derived from cDNA) that are different from rat topoisomerase I, are indicated in parentheses directly below the corresponding amino acid residue in the rat peptide, e.g. residue 127 is valine in the human sequence but alanine in the rat sequence.
3) Residues in parentheses within the rat sequence were tentative.
4) In peptide 5, a mixture of two peptides were sequenced simultaneously. Where assignment of residues in a particular step was not possible, two residues are given in parentheses. Note that the first residue of each pair matches the cDNA derived sequence.

identical corresponding to a homology of 94.5 %. The amino acid directly preceding each peptide is lysine or arginine in the cDNA deduced sequence (Fig.3) consistent with cleavage by trypsin. As shown schematically in Fig.2, the seven peptides were distributed relatively evenly across the topoisomerase I molecule including the carboxy-terminus . Therefore one would expect this high homology to reflect the overall homology between rat and human topoisomerase I.

In contrast to the high homology between the two mammalian type I topoisomerases, homology to yeast type I topoisomerases of Saccharomyces cerevisiae (10) and Schizosaccharomyces pombe (11) was restricted. Relatively good homology existed between the mammalian and yeast type I topoisomerases in peptides 2, 3 and 7 (the carboxyl-terminal peptide). In contrast, peptide 1 (beginning at residue 119) and peptide 5 (beginning at residue 509) had little homology with the yeast sequences (Fig.3). Thus, while the rat peptide 1 topoisomerase I sequence was identical to that of the human enzyme, it exhibited limited similarity with the S. cerevisiae sequence and none with the S. pombe topoisomerase I sequence. Likewise, the peptide 5 sequence showed weak homology to the corresponding yeast sequences while good homology existed among the mammalian topoisomerases and among the yeast topoisomerases (Fig.3). Eventhough these regions (peptide 1 and 5) are diverse between yeast and mammalian

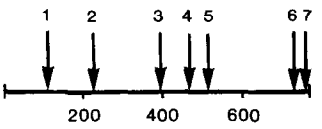


Fig.2. Schematic localization of tryptic peptides 1-7 of rat topoisomerase I within the predicted amino acid sequence derived from human cDNA (from ref.12). The solid line represents the predicted amino acid sequence (765 residues); numbers below indicate residue, arrows indicate the position of tryptic peptides.

PEPTIDE 2

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 223 | | | | | | | | | | | | | | | | |
| human | K | G | P | V | F | A | P | P | Y | E | P | L | P | E | N | V | K |
| rat | | G | P | V | F | A | P | P | Y | E | P | L | P | E | G | V | K |
| | 199 | | | | | | | | | | | | | | | | |
| pombe | N | G | V | I | F | A | P | P | Y | E | P | L | P | K | N | V | K |
| | 151 | | | | | | | | | | | | | | | | |
| cer. | N | G | V | I | F | P | P | P | Y | Q | P | L | P | S | H | I | K |

PEPTIDE 3

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 391 | | | | | | | | |
| human | K | V | P | S | P | P | P | G | H | K |
| rat | | V | (P) | (Q) | P | A | D | G | H | K |
| | 368 | | | | | | | | |
| pombe | P | V | P | E | P | L | P | G | H | Q |
| | 323 | | | | | | | | |
| cer. | P | V | P | P | A | P | E | G | H | K |

PEPTIDE 7

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 751 | | | | | | | | | | | | | |
| human | K | F | A | W | A | I | D | M | A | D | E | D | Y | E | F |
| rat | | F | A | W | A | I | D | M | T | (T) | E | E | Y | | |
| | 799 | | | | | | | | | | | | | | |
| pombe | K | F | N | W | A | A | D | T | P | P | D | W | E | W | |
| | 755 | | | | | | | | | | | | | | |
| cer. | K | F | K | W | A | I | E | S | V | D | E | N | W | R | F |

PEPTIDE 1

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 117 | | | | | | | | | | | | | |
| human | K | D | E | P | E | D | D | G | Y | F | V | P | P | K | |
| rat | | D | E | P | E | D | D | G | Y | F | A | P | P | K | |
| | 43 | | | | | | | | | | | | | | |
| cer. | E | A | E | P | Y | D | S | D | E | A | I | S | K | I | |

PEPTIDE 5

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 508 | | | | | | | | | | | | | | |
| human | R | V | E | H | I | N | L | H | P | E | L | D | G | Q | E |
| rat | | (V) | (E) | (H) | (I) | (N) | (L) | H | P | (E) | (L) | D | (G) | (Q) | (E) |
| | 484 | | | | | | | | | | | | | | |
| pombe | R | Y | E | H | V | T | L | K | P | P | R | T | V | V | F |
| | 439 | | | | | | | | | | | | | | |
| cer. | R | Y | E | H | V | T | L | K | P | P | N | T | V | I | F |

Fig.3. Alignment of partial amino acid sequence of rat topoisomerase I with predicted amino acid sequences derived from human cDNA and yeast genomic DNA. Absolutely conserved residues are boxed.

topoisomerase I, they are conserved among the mammalian species indicating that in an evolutionary sense, these sequences are relatively new and may be essential to mammalian topoisomerase I. Regions of high homology between yeast and mammalian enzyme (for example at peptide 2), may be indicative of domains with

important functional roles such as sites of interaction with DNA or with other proteins.

The specific location of the phosphoserine residue phosphorylated *in vivo* and *in vitro* by nuclear NII kinase remains to be determined; this phosphoserine residue represents a major phosphorylation site of rat topoisomerase I and is situated within a tryptic phosphopeptide 6 residues from the N-terminal cleavage site (2). Ser[52] and ser[74] in the cDNA deduced amino acid sequence of D'Arpe et al. (12) are consistent with such a phosphorylation site since each has a potential lysine cleavage site 6 residues in the amino terminal direction. Further studies are in progress to determine this phosphorylation site unequivocally.

No methylated amino acids were encountered in the limited sequences analyzed here. However, this does not rule out that such modified amino acids exist elsewhere in the topoisomerase I molecule. Besides phosphorylation, ADP-ribosylation was reported in mammalian topoisomerase I as a major post-translational modification which was associated with strong inhibition of topoisomerase I (14). Further work is required to localize the exact site(s) of this modification in the topoisomerase I molecule.

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