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DIFFERENT CONFORMATION OF PURIFIED HUMAN RECOMBINANT INTERLEUKIN 1β FROM *ESCHERICHIA COLI* AND *SACCHAROMYCES CEREVISIAE* IS RELATED TO DIFFERENT LEVEL OF BIOLOGICAL ACTIVITY

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SUMMARY: Human recombinant interleukin 1β produced in *Escherichia coli* and in *Saccharomyces cerevisiae* was purified to homogeneity by a combination of ion exchange, gel filtration and hydroxylapatite column chromatography. The two proteins, both expressed in the mature form, differ in that the protein secreted from yeast is glycosylated and lacks the first four amino acids. The biological activity of IL-1 obtained from *E. coli* is comparable to that of the natural protein, while the protein produced from yeast showed very low specific activity. The analysis of the state of oxidation of the two cysteine residues present in the IL-1 molecule and the evaluation of the immunoreactivity of the two proteins have proved that a different conformation is at the basis of the different biological activity of the two proteins.

Human interleukin 1β is the predominant form of human IL-1, a term used to describe a family of polypeptide hormones mainly produced by activated macrophages, affecting many different cell types involved in immune and inflammatory responses (1). The cDNA coding for this protein has been cloned and expressed both in *E. coli* as soluble protein within the cytoplasmic space (2) and in *S. cerevisiae* as secretion product in the medium (3). The recombinant protein from *E. coli* was expressed in the mature form, i.e. the sequence 117-269 of the precursor molecule (4), while the recombinant protein produced in yeast was the mature form lacking the first four amino acids and glycosylated at Asn 123 (3).

In this work these two recombinant proteins have been purified and characterized chemically and immunologically. In order to obtain information on the structure-function relationship in hu IL-1β, the biological activity of the two proteins was assessed and related to their structural characteristics.

Abbreviations
hu rIL-1β, human recombinant interleukin 1β; SDS, sodium dodecylsulfate; EDTA, ethylenediaminetetraacetic acid; DTNB, 5,5'-dithiobis (2-nitrobenzoic acid).

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MATERIALS AND METHODS

Purification of IL-1β from S. cerevisiae: Human recombinant IL-1β was purified from crude concentrated yeast culture supernatant using a modification of a previously described technique (5). The concentrated supernatant was chromatographed on a DEAE-Sepharose column equilibrated in 10 mM phosphate buffer pH 7.5. The unabsorbed fraction was loaded directly onto a hydroxylapatite column equilibrated in the same buffer. IL-1β was then eluted by increasing the phosphate buffer concentration. Fractions containing IL-1β were pooled, concentrated by ultrafiltration using a YM10 membrane (Amicon, Lexington, MA) and applied on a Sephadex G-75 column equilibrated with 10 mM sodium phosphate pH 7.0. The final purified protein solution was freeze-dried.

Purification of IL-1β from E. coli: Human recombinant IL-1β from *E. coli* was purified with a previously described protocol (6), modified as follows. After cell disruption and centrifugation, the supernatant was applied to a DEAE-Sepharose column previously equilibrated with 50 mM Tris-HCl pH 8.5. The unabsorbed fraction, containing IL-1β, was concentrated by ultrafiltration using a YM10 membrane, then chromatographed on a Sephadex G-50 column equilibrated with 10 mM sodium phosphate pH 7.0. The final purified protein solution was freeze-dried.

SDS-polyacrylamide gel electrophoresis: Electrophoresis was performed on a 15% acrylamide slab gel according to Laemmli (7). Samples were dissolved, heated at 100°C for 1 min in the sample buffer containing 0.18 M 2-mercaptoethanol and applied to the gel. Gels were fixed and stained with Coomassie brilliant blue.

Isoelectric focusing: Isoelectric focusing was performed utilizing a Phast System apparatus (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. A Phast gel sheet (Pharmacia) with a pI range of 5-8 was used.

Amino acid analysis: Amino acid analysis was performed by hydrolyzing the samples at 110°C for 24 h with 6 N HCl containing 0.05% phenol. A Chromakon 500 amino acid analyzer (Kontron, Zurich, Switzerland) was employed. This analysis was also used to determine the concentration of purified IL-1 protein.

Biological assay of IL-1: The biological activity of IL-1 was assayed in the murine thymocyte proliferation assay as previously described (8). Specific activity was expressed as half maximal units (U)/mg IL-1 by comparing titration curves of samples to that of a standard hu rIL-1β preparation (1.3×10^7 U/mg; Biogen, Geneva, Switzerland).

Sulphydryl analysis: The sulphydryl groups were tested both by direct analysis as described by Ellman (9) and after reduction of the disulphide bridges. In the first case, the protein was dissolved in 100 mM Tris-HCl pH 8.0, containing 1% SDS and 5 mM EDTA, and titrated with DTNB. In the second case, the denatured protein was reacted with sodium borohydride as described (10), then titrated with DTNB as above.

Radioimmunoassay: The monoclonal antibody BRhG3, a murine IgG_1 raised against yeast-derived hu rIL-1β, was produced by standard techniques and purified from ascitic fluid. Competitive radioimmunoassay was performed as described in detail elsewhere (12), by coating microtiter wells with 25 μg of antibody and incubating them with 150,000 cpm/well of ^{125}I labeled yeast-derived IL-1β (by the chloramine T method; specific activity 175 Ci/mmole) and increasing doses of unlabeled hu rIL-1β from yeast or *E. coli*.

RESULTS

Human recombinant IL-1 expressed in *E. coli* and in *S. cerevisiae* was highly purified using modifications of previously described protocols (5, 6). The degree of purity of the proteins was similar to that obtained with the previous methods in that they showed a homogenous band by SDS-gel electrophoresis (Fig. 1) and their amino acid composition

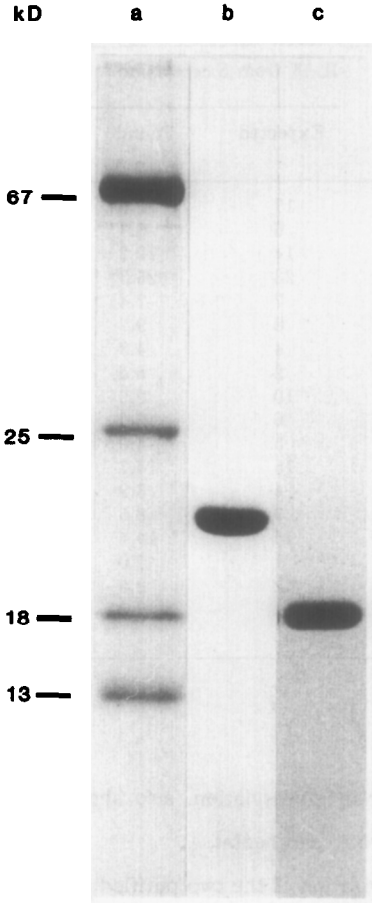

Figure 1. SDS-gel electrophoresis of recombinant IL-1β.
(a) Molecular weight standards; (b) purified IL-1β from yeast; (c) purified IL-1β from *E.coli*.
The apparent molecular weight of IL-1β from yeast is about 22 kD instead of the expected 17.4 kD, due to the effect of glycosylation.

was consistent with that deduced from the cDNA sequence (Table 1). Furthermore, the purified IL-1β from *E. coli* gave a single band having pI 6.8 by analytical isoelectric focusing, a value in agreement with that of the natural protein (12). The purified product from yeast gave a single band by the same analysis but having pI 6.0, probably because of the carbohydrate and of the missing arginine.

When the two recombinant IL-1β were assayed for biological activity, the specific activity of IL-1β produced from *E. coli* was 1.4×10^7 U/mg, i.e. identical to that of the commercially available recombinant IL-1β preparation used as reference standard (1.3×10^7 U/mg) and fully comparable to that of human natural IL-1β (13). In contrast, the biological activity of IL-1β produced in yeast proved to be very low (mean of 340 U/mg in six experiments). Moreover, IL-1β produced in yeast grown in the presence of

Table 1. Amino acid composition of purified IL-1β

Amino acid	IL-1β from *S.cerevisiae*		IL-1β from *E. coli*	
	Expected	Found	Expected	Found
Asx	17	16.6	17	16.3
Thr	6	6.3	6	5.9
Ser	14	12.2	14	12.0
Glx	23	25.3	23	23.7
Pro	7	7.4	8	7.5
Gly	8	9.7	8	8.6
Ala	4	4.3	5	5.4
Cys	2	n.d.	2	n.d.
Val	10	9.5	11	10.8
Met	6	6.0	6	5.6
Ile	5	4.6	5	4.8
Leu	15	14.7	15	15.4
Tyr	4	3.9	4	4.3
Phe	9	8.5	9	9.4
Lys	15	14.4	15	15.0
His	1	1.0	1	1.0
Arg	2	2.5	3	3.6
Trp	1	n.d.	1	n.d.

n.d.: not determined.

tunicamycin, an inhibitor of glycosylation, also showed very poor biological activity (mean of 507 U/mg in two experiments).

Upon analytical characterization of the two purified proteins, it resulted that in the *E. coli*-derived molecule both of the two sulphydryl groups present were reactive to Ellman's reagent by direct analysis. This indicates that the cysteine residues are in the reduced form. In contrast, they form an intramolecular disulphide bond in the IL-1β molecule produced in yeast since they were reactive to sulphydryl reagent only in reducing conditions (Table 2).

Finally, the ability of a monoclonal antibody to recognize the two recombinant IL-1β molecules was tested. This monoclonal antibody, BRhG3, was generated against the

Table 2. Sulphydryl groups determination

Sample	*-SH groups/molecule*	
	Direct analysis	Analysis after reduction
IL-1β from *E. coli*	2.1	2.5
IL-1β from *S. cerevisiae*	0.2	1.9

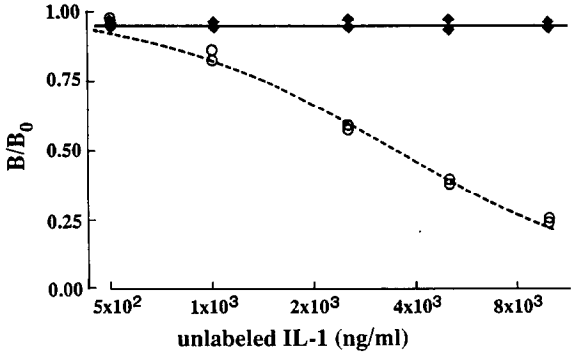

Figure 2. Competetive radioimmunoassay for IL-1β.
Binding of radiolabeled yeast-derived hu rIL-1β by the monoclonal antibody BRhG3 was measured in the presence of unlabeled hu rIL-1β either produced in yeast (o- -o) or in *E.coli* (◆—◆). B/Bo is bound/maximal binding.

yeast-derived IL-1β protein and could recognize an epitope within the region in position 218-243 of hu IL-1β (determined by immunoblotting of human recombinant IL-1β fragments, as described in ref. 14). As shown in Fig. 2, binding of BRhG3 to radiolabeled yeast-derived IL-1β could be effectively inhibited by yeast-derived IL-1β but not by the *E. coli*-derived protein.

DISCUSSION

In this report, two different forms of human recombinant IL-1β have been compared for their structural and biological characteristics. The fully active IL-1β protein expressed in *E. coli* corresponds to the mature human IL-1β polypeptide 117-269, it is not glycosylated, and it presents the two cysteine residues with free -SH groups. The inability of the two cysteines to form a disulphide bond is probably due to their location within the molecule, hidden in an hydrophobic environment which protects them from oxidation. Indeed, other groups have reported that in biologically active human recombinant IL-1β the two cysteines only react with the Ellman's reagent in the presence of agents (SDS, guanidine-HCl) which cause protein unfolding (15, 16). In contrast, the yeast-derived human recombinant IL-1β is a truncated mature polypeptide (121-269), which lacks the first four N-terminal amino acids (3), it is glycosylated at Asn 123 (3), and it presents the two cysteine residues in positions 124 and 187 oxidized in a disulphide bridge. This protein is virtually inactive *in vitro* in the thymocyte proliferation assay (about 340 U/mg IL-1). Glycosylation of the yeast-derived protein does not seem to be responsible for the loss of biological activity. In fact, the unglycosylated protein secreted by yeast in the presence of tunicamycin is poorly active altogether (507 U/mg IL-1). On the other hand, the lack of the first four N-terminal residues (117-120) may be of key importance for the IL-1β biological activity. In fact, it has been reported that deletion of residues 117-120 and substitutions in

position 120 could heavily impair both the *in vitro* biological activity and the receptor-binding capacity of *E. coli*-derived human IL-1β (17, 18). It is thus hypothesized that the lack of N-terminal residues may be responsible for incorrect protein folding, which then allows formation of the disulphide bridge and brings about loss of biological activity. The conformational difference between the two recombinant IL-1β proteins is further stressed by the notion that a monoclonal antibody raised against yeast-derived IL-1β is unable to bind the *E. coli*-derived protein. Thus, the epitope recognized by the antibody, located within the region 218-243, is possibly hidden within the structure of *E. coli*-derived IL-1β, while well exposed in the conformationally-altered IL-1β produced in yeast.

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