ARTICLES

Saccharomyces boulardii Produces in Rat Small Intestine a Novel Protein Phosphatase that Inhibits *Escherichia coli* Endotoxin by Dephosphorylation

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ABSTRACT: Using a polyclonal antibody raised against a highly conserved sequence of 38 amino acids containing the activation site (VTDSAAGAT) common to mammalian and yeast alkaline phosphatases (AP), we identified in decapsidated *Saccharomyces boulardii* a protein phosphatase detected by autoradiography as a single signal (63 kD). Using an affinity chromatography column, the protein phosphatase could be concentrated 39.1-fold and presented as a doublet of two subunits. Compared with rat and bovine purified intestinal AP, the enzyme from *S. boulardii* had a greater ability to dephosphorylate the lipopolysaccharide (LPS) of *Escherichia coli* 055B5. When tested *in vivo*, intraperitoneal injection of intact LPS to rats produced, after 9 h, 100 ng/mL of circulating tumor necrosis factor-α with inflammatory lesions and apoptotic bodies in the liver and the heart, whereas rats injected with partially dephosphorylated LPS produced only 40 ng/mL tumor necrosis factor-α without organic lesions. In conclusion, *S. boulardii* is able to inhibit toxicity of *E. coli* surface endotoxins by the release of a protein phosphatase exhibiting a great capacity of dephosphorylation. (*Pediatr Res* **60: 24–29, 2006**)

Saccharomyces boulardii, a nonpathogenic yeast, exerts therapeutic properties in acute and chronic enterocolopathies, antibiotic-associated diarrhea, and enterotoxigenic *Clostridium difficile* infections (1–4). In human volunteers (4,5) and in growing rats (4), several studies have documented that oral treatment with a lyophilized preparation of *S. boulardii* produces trophic intestinal effects, including increases in the activities of BBM enzymes (4,5) and enhanced secretion of s-IgA in intestinal fluid (6). After oral treatment of rats with *S. boulardii*, there is a marked stimulation of sodium-dependent D-glucose uptake into BBM vesicles with a corresponding accumulation of the sodium D-glucose co-transporter-1 (SGLT-1) (7). These trophic effects are, at least in part, mediated by endoluminal release of polyamines, as yeast cells contain substantial amounts of spermine and spermidine (4,8,9). In a recent work (10), we found that *S. boulardii* enhanced N-terminal peptide hydrolysis in suckling rat small intestine by endoluminal release of a zinc-binding metalloprotease. In the present study, we have analyzed whether oral treatment with *S. boulardii* could enhance the endoluminal activity of IAP. We also have purified a protein phosphatase secreted by *S. boulardii* in the rat intestinal lumen and have compared some of its properties with rat and bovine IAP. Finally, we have assessed whether the protein phosphatase released from *S. boulardii* can inhibit the toxicity of LPS from O55B5 *Escherichia coli* by dephosphorylation of its two phosphorylation sites.

METHODS

Media and culture conditions. S. boulardii cells were inoculated in YPD (yeast extract, 0.5%; peptone, 2%; glucose, 2%; DIFCO, Detroit, MI) media and were grown at 30°C with moderate shaking to exponential growth as described (10).

To disrupt the external capside, yeast cells were concentrated (1.45–1.50 × 10^10 cells/mL) and shaken with beads (0.45-μm diameter) under cold CO_2 flux by using an MSK pulse device (Braun, Paris, France) (10). After stabilization in 50 mM Tris-Hcl buffer (pH 8), particulate components were removed by centrifugation (500 × *g*) for 15 min at 0°C and the supernatants were stored at –170°C in liquid nitrogen until analyzed.

Animals and treatments. The present study was approved by the Animal Welfare Committee of the Catholic University of Louvain, Faculty of Medicine. Litters of Wistar rats were reduced to six pups per lactating mother to equalize conditions of nursing and feeding. *S. boulardii* was prepared in a lyophilized form (100 mg per flask, biologic activity 2.9 × 10^9 viable cells/mL) by the manufacturer (Biocodex, Gentilly, France). As reported, we used a dose of 0.5 mg of lyophilized yeast cells per gram body weight per day (10). The appropriate dose was administered in 0.5 mL saline by nasogastric intubation twice daily from d 28–32 postpartum. Control groups were treated according to the same schedule and received equal volumes of saline. Six to 10 animals per group were studied during the weaning period because, at that

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Abbreviations: BBM, brush border membrane; **IAP,** intestinal alkaline phosphatase; **LPS,** lipopolysaccharide; **PMSF,** phenylmethanesulfonylfluoride

time, rat BBM alkaline phosphatase activity is low, whereas during the suckling period the activity is very high (11) .

Collection of endoluminal fluid. On d 32 postpartum, rats were killed by decapitation, and the small intestine from the pylorus to the ileocecal valve was immediately excised. The total length was measured and divided into two equal segments. The proximal half was considered the jejunum and the distal half the ileum. For collection of intestinal fluid, jejunal and ileal segments were flushed with 2 mL of cold 0.9% saline. The collected fluid was centrifuged (500 × g, 5 min), and the supernatants were pooled and filtered through a 0.2-μm membrane filter (Millipore Corp., Bedford, MA) to discard yeast cells in suspension.

Enzyme assays. Alkaline phosphatase activity was assayed on suspensions of *S. boulardii* cells, intestinal fluid, and purification buffers using *p*-nitrophenylphosphate (2.15 mM) in ethyl-amino-2-ethanol buffer (150 mM, pH 9.3) as described by Forster *et al.* (12). Unless otherwise indicated, assays were performed at 37° for 30 min in the presence of 1 mM MgSO$_4$. One unit equals 1 μmol of *p*-nitrophenol formed per minute and per gram protein. Protein content was determined by the method of Lowry *et al.* (13).

Immunoprecipitation and immunoblotting. To demonstrate the production by *S. boulardii* of a protein phosphatase, a peptide corresponding to a highly conserved sequence of 38 amino acids between alkaline phosphatase of humans, rats, and *S. cerevisiae* (HFIGSSRTRSSDSLVTDSAAGATA FA-CALKSYNGAI) was synthesized. This sequence contains the activation site (VTDSAAGAT) common to *Saccharomyces cerevisiae* and *Saccharomyces pombe*. Rabbits were immunized with the above peptide corresponding to a sequence of alkaline phosphatase of *S. cerevisiae*. A polyclonal antiserum was generated, and IgG were purified.

One hundred microliters of protein A–Sepharose-4B beads (50% suspension) (Pharmacia, LKB, Antwerp, Belgium) was mixed with 1.5 mL of IgG purified antisera during 4 h at 4°C under gentle continuous rotation. After centrifugation (500 × g, 3 min), the supernate was discarded and the beads coated with the antibodies (100 μL) were mixed with 1 mL of decapsided *S. boulardii* cell suspension (viable cells, 1.5 × 10^{10}/mL), 1 mL of 0.1 N NaOH, and 2 mL of immunoprecipitation buffer containing Tris-HCl 100 mM (pH 7.5), PMSF 1 mM, leupeptin 1 μg/mL, and aprotinin 1 μg/mL. After overnight incubation at 4°C, the beads were recovered by centrifugation (500 × g, 3 min) and washed once with a washing buffer [Tris-HCl 50 mM (pH 7.4), NaCl 100 mM, SDS 0.1%, Triton-X, 0.1%], followed by six washes with the same buffer without NaCl. After aspirating the excess of buffer, immunoprecipitated proteins were solubilized in 30 μL of Laemmli's buffer and boiled at 100°C for 5 min. Immunoprecipitated proteins were separated by electrophoresis (7.5% SDS-PAGE) and electrotransferred to polyvinylidene difluoride membranes as described by Towbin *et al.* (14). Using ^{125}I-labeled protein A (Amersham, Gent, Belgium), bound alkaline phosphatase protein was detected by autoradiography using 24 × 30 cm Fuji film (St. Nicolas, Belgium) as previously described (15).

Purification of alkaline phosphatase from S. boulardii. To purify alkaline phosphatase in native conditions, broken *S. boulardii* cells suspended in 5 mM Tris-HCl (pH 8.5), Triton-X 0.5%, and antiproteases (PMSF, leupeptin aprotinin) were ultracentrifuged at 0°C (105,000 × g, 60 min) (Beckman Ultracentrifuge, Namur, Belgium). This step yielded three phases: a fat cake, a clear yellow supernatant, and a brownish pellet. After discarding the upper fat cake, the supernatant was collected. Proteins were precipitated by the addition of nine volumes (vol/vol) of cold acetone at 4°C overnight. The white precipitate (420 mg protein per gram of powder) was thereafter dried at vacuo (Speed Vac, Savant, Inc, Holbrook, NY), aliquotted, and stored at –20°C until use.

A column for affinity chromatography (15 × 1 cm) was filled with 10 mL of 4% agarose beads in 1 N NaCl (CNBR activated, Sigma Chemical Co.-Aldrich, Steinheim, Germany) bound to a ligand consisting in L-histidyldiazobenzyl phosphonic acid. The matrix is specific for the binding of bovine alkaline phosphatase and yields between 100 U of alkaline phosphatase per milliliter of elution buffer (16). Ten milligrams of proteins dissolved in 5 mL of 5 mM Tris-HCl (pH 8) buffer or 10 mL of clear yellow supernatant, pretreated with 200 μL of RNase and DNase (1 mg/mL, Roche Molecular Biochemicals, Mannheim, Germany), were slowly applied to the column. Samples (1–85) collected as 1-mL fractions were monitored at an O.D. of 280 μm. The elution buffer consisted in 5 mM of Na$_2$ HPO$_4$, which was applied to the column at sample 162. Protein concentration and phosphatase activity (pH 7.5) were measured in each fraction and samples that exhibited the highest activity were pooled and concentrated 10-fold (Amicon filter of 50 kD cut-off; Millipore). This procedure yielded a 39.1-fold enrichment in phosphatase activity (803.5 U/mL) compared with the initial activity measured in the yellow supernate (20.5 U/mL).

To compare alkaline phosphatase (AP) from yeast cells and IAP from cow and from suckling rats, rat IAP was purified from BBM vesicles prepared according to the method of Schmitz *et al.* (17). The P2 fractions were solubilized in the buffer used to measure IAP activity. Thereafter, specific,

phosphatidyl-inositol-phospholipase C was added to the P$_2$ fraction to hydrolyze the phosphatidyl inositol anchor (18). IAP activity was measured in the pellet at pH 8 and at 37°C after a brief centrifugation. Samples (1 mL) contained more than 8000 units of enzyme per milliliter of buffer.

To demonstrate purification to homogeneity, the concentrated IAP from suckling rats and from cows and the protein phosphatase from *S. boulardii* were run on a 7.5% SDS-PAGE. After separation, proteins were stained with Coomassie blue, according to Laemmli (18).

Dephosphorylation of LPS endotoxin from O55B5 E. coli using purified protein phosphatase of S. boulardii and purified alkaline phosphatase from rat and bovine intestinal mucosae. Some enterotoxigenic *E. coli* produce an endotoxin called LPS, which is a constituent of the external cellular membrane layer of the bacteria. LPS is composed of lipid A, containing two phosphorylated sites that are essential for its toxicity—the central polysaccharide and the 0 chain—the composition of which varies in each bacteria. In this experiment, we have compared the dephosphorylation activity of *S. boulardii* alkaline phosphatase and of rat and bovine IAP by measuring *in vitro* the release of inorganic phosphorus (P$_i$) at several values of pH. The method used was that of Chandrarajan *et al.* (20), which allows measurement of the release of inorganic phosphorus (P$_i$) in the presence of organic phosphorus (P$_o$) and of a high protein concentration. Optical densities were recorded after 15 min at 870 nm and were expressed as a linear reference plot corresponding to the O.D. of KH$_2$ PO$_4$ at increasing concentrations of P: 1–5 μg (20–100 μL). Blanks were made as samples and contained distilled water in place of KH$_2$ PO$_4$.

Effect of LPS dephosphorylated on serum tumor necrosis factor (TNF)-α circulating levels (in vivo). To determine *in vivo* that dephosphorylation of LPS inhibited toxicity of *E. coli*, three groups of rats were made. The control group, including six rats of 140 g, was injected intraperitoneally with 250 μL NaCl 0.9%. After 90 min, rats were sacrificed. Blood samples were collected, left at room temperature, and allowed to coagulate. Thereafter, samples were centrifuged and the sera were collected and stored at –20°C until used for TNF-α measurement.

A second group (LPS) of six rats (140 g body weight) was injected intraperitoneally with 750 μg per 100g body weight of LPS. The stock solution consisted in 5 mg LPS diluted in 1 mL of NaCl 0.9%. Rats of this group were killed after exactly 90 min, when TNF-α levels peak in the serum (preliminary observations). Blood samples were collected and after coagulation, and the sera were separated and frozen at –20° until used for TNF-α measurement.

The third group (LPS + PP) was also injected with LPS that was partially dephosphorylated. Therefore, purified protein phosphatase from *S. boulardii* was diluted in 50 μL Tris-HCl buffer containing traces of ZnCl$_2$ and MgCl$_2$. The enzyme was added to 1 mg (200 μL) LPS (5 mg of LPS in 1 mL of NaCl 0.5%). The samples were thereafter incubated at 37°C in a water bath during 1 h to allow dephosphorylation of the toxin. To stop the reaction, samples were placed on ice. The next step was performed to discard the enzyme from the solution to be injected to rats so that only dephosphorylated LPS was given to the animals. Therefore, Sepharose beads were coated with specific antiphosphatase (*S. boulardii*) IgG antibodies. The samples containing the enzyme were incubated under slow rotation overnight with the coated beads. After brief centrifugation, the supernatant was injected individually to six rats (140 g) intraperitoneally. After 90 min, blood samples were collected, coagulated at room temperature, and centrifuged to obtain the sera, which was frozen at –20°. Injection of LPS that had been incubated exactly as dephosphorylated LPS but without protein phosphatase in the reaction produced similar effects on TNF-α as did intact LPS. TNF-α was quantified in the sera using an immunoassay kit (Quantikine, R & D Systems Europe, Oxford, UK). Values were expressed in nanograms per milliliter of serum.

Statistical analysis. Statistical analysis was carried out using an ANOVA followed by a multiple comparison test (Newman-Keuls) using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). Differences between means were considered to be statistically significant when $p < 0.05$.

RESULTS

Production of protein phosphatase by yeast cells in culture. The specific activity (55 mU/mg protein) and the total activity (4800 mU per total cells of culture 2.5 × 10^{10} cells) of the phosphatase protein were measured in the culture media (YPD). Addition of small quantities of EDTA (0.1 mM) complexed the co-factors (Mg^{++} and Zn^{++}) of the reaction and decreased significantly the specific and total activities by about 40%. When EDTA concentration was increased to 20

mM, activities were virtually abolished, suggesting that the protein phosphatase produced by *S. boulardii* cells is a met-alloprotease activated by the co-factors (Zn^{++}, Mg^{++}).

Effect of oral treatment of S. boulardii on rat IAP activity. Oral administration of *S. boulardii* to growing rats ($n = 9$) produced no change in body weight gain nor in intestinal mucosal parameters compared with control rats treated with 0.9% saline ($n = 9$).

Figure 1 shows that rats treated with *S. boulardii* for 8 d had a moderate increase (15%) of IAP activity in the jejunal fluid filtered to discard *S. boulardii* cells. By contrast, we found a marked and significant increase in IAP activity in the ideal fluid (+56%, $p < 0.01$ *versus* controls). Likewise, protein contents in jejunal and ileal fluids were significantly ($p < 0.05$) higher in *S. boulardii*–treated rats than in controls.
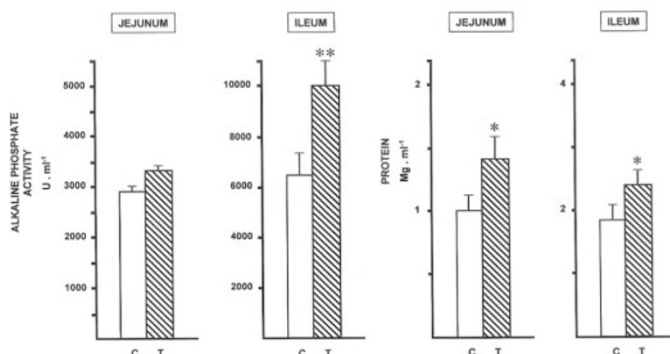
Characterization of the phosphatase protein from S. boulardii. To determine whether *S. boulardii* cells are able to release a protein phosphatase, we synthesized a 38-amino acid polypeptide corresponding to a highly conserved domain of AP produced by *S. cerevisiae* and containing the activation site (*VTDSAAGAT*) common to *S. cerevisiae* and *S. pombe*. Using the purified IgG fraction of the corresponding anti-serum, we immunoprecipitated the protein with protein A–Sepharose beads. After being separated from the antibody, the enzyme protein was run on SDS-PAGE 7.5% and trans-ferred to a PVDF membrane. After immunoblotting and wash-ings, the enzyme was revealed with the same IgG purified antibody used for autoradiography (protein A–I[125]).
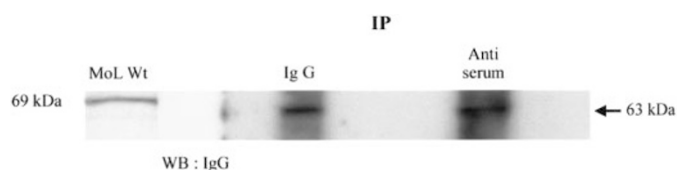
Figure 2 shows that a signal was detected with both the purified IgG fraction as well as with the whole antiserum and corresponded to a protein of 63 kD. The signal was detected by autoradiography as a single band, whereas, after purifica-tion of the enzyme by affinity chromatography, SDS-PAGE (7.5%) revealed two subunits with molecular weight very close each to the other (see Fig. 4*A*).

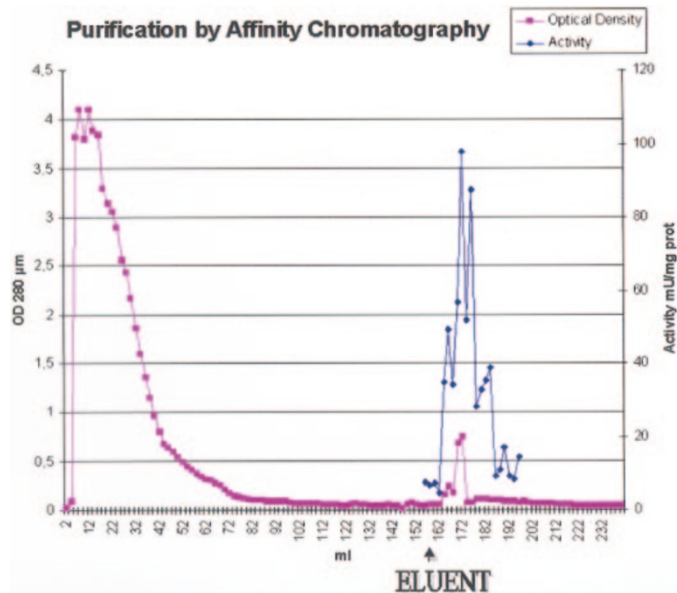***Purification of the phosphatase protein from S. boulardii and comparison with rat and bovine IAP.*** As shown in Figure 3, the protein phosphatase of *S. boulardii* was purified by an affinity chromatography column. Proteins of the supernatant fraction containing decapsidated *S. boulardii* cells were eluted

Figure 2. Autoradiography of the protein phosphatase of *S. boulardii* after immunoprecipitation with a specific antibody, SDS-PAGE, and Western blotting (see "Methods"). Only one single signal was detected at 63 kD using either the purified IgG fraction of the antibody or the whole antiserum. *Left side,* molecular weight marker of 69 kD.

Figure 3. Purification of the phosphatase protein from *S. boulardii* by affinity chromatography. As shown in the figure, the phosphatase protein was eluted specifically with 10 mM $Na_2H\ PO_4$ at 162 mL. This represented an increase in enzyme concentration of 39.1-fold.

from the column between 2 and 72 mL of buffer, whereas phosphatase protein was eluted specifically with Na_2HPO_4 (10 mM) at 162 mL. The enrichment steps in the enzyme protein yielded an increase in purification of 39.1-fold over the initial activity measured in the supernate after ultracentrifugation.

To verify its purity, the enzyme protein was run on an SDS-PAGE of 7.5% and was compared with purified rat and bovine IAP.
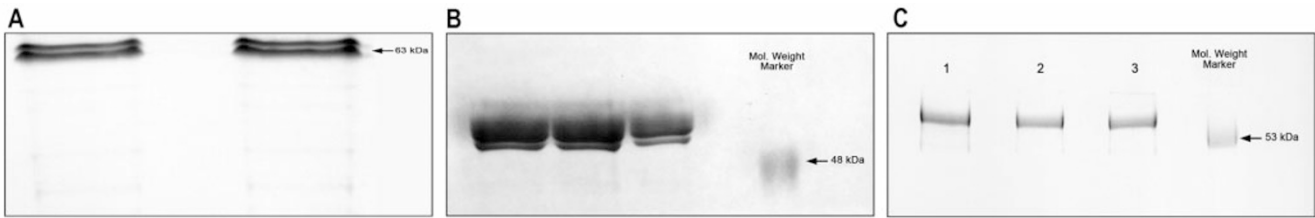
The protein phosphatase from *S. boulardii* was detected as a doublet composed of two subunits of about 63 kD (Fig. 4*A*). Likewise, as shown in Figure 4*B*, bovine intestinal phospha-tase run on SDS-PAGE 7.5% showed a similar doublet of two subunits of molecular weight estimated to be 60 kD. Figure 4*C* shows rat IAP that was purified by cutting enzymatically the phosphoinositol anchor resulting in an enzyme protein of lower molecular weight (55–58 kD).
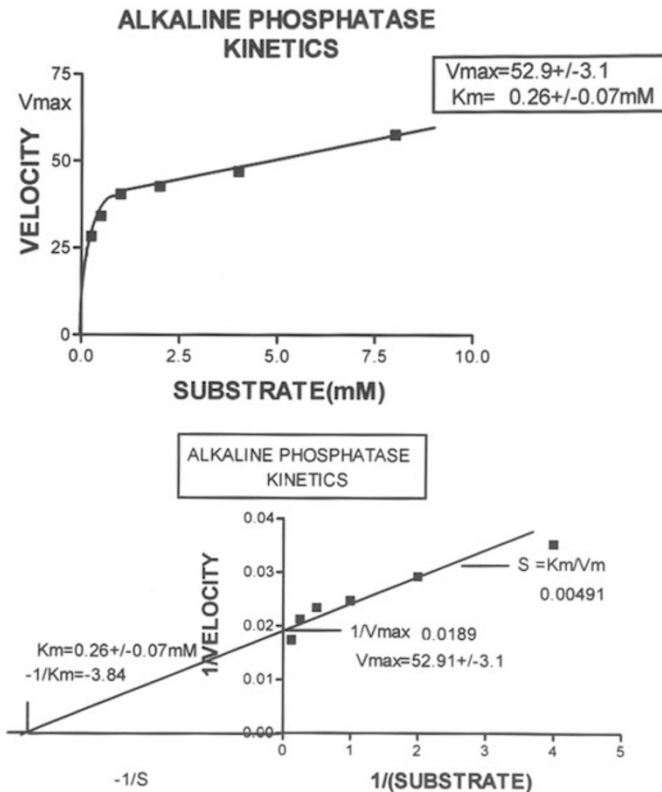
Kinetic properties. The kinetic properties of the protein phosphatase purified from *S. boulardii* are depicted in Figure 5. Using as substrate para-nitrophenylphosphate, V_{max} was 52.9 ± 3.1 ($n = 5$) and the K_m was 0.26 ± 0.07 mM ($n = 5$). These values are close to those reported for rat and for bovine IAP (Brenda enzyme program).

Soluble and particulate enzymes. Because AP from *S. cerevisiae* exists as two intravesicular enzyme proteins—a

Figure 1. Changes in the specific activity of phosphatase protein in the jejunum and ileum of *S. boulardii*–treated rat during growth (after waning) and changes in protein concentration. Changes in the jejunum were very mild and not significant, whereas in the ileum, there was an increase of 40% in the protein phosphatase activity (*$p < 0.05$, **$p < 0.01$, $n = 9$–10 for each group).
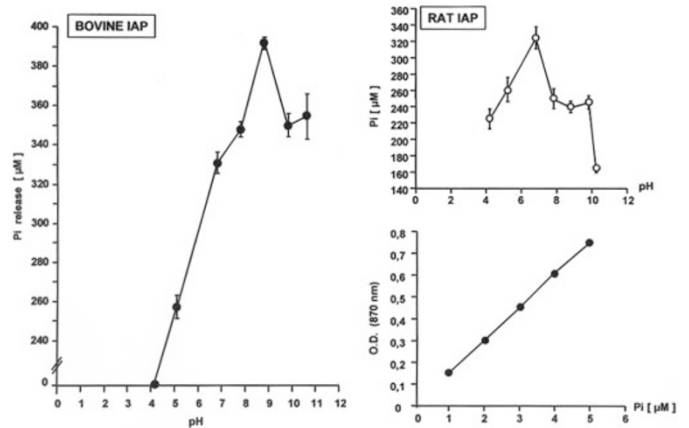
Figure 4. (*A*) *S. boulardii* protein phosphatase was detected on SDS-PAGE 7.5% as a doublet composed of two submits of 63 kD. (*B*) Purified bovine intestinal alkaline phosphatase stained with Coomassie blue. Staining revealed an important protein composed of two submits of molecular weight estimated to be 60 kD. (*C*) Rat intestinal alkaline phosphatase purified by cutting the glycosyl phosphoinositol (GPI) anchor from the BBM resulting in an enzyme protein of lower molecular weight (55–50 kD).

Figure 5. Kinetic properties of the protein phosphatase purified from *S. boulardii* ($n = 5$). V_{max} (52.9 ± 3.1) and K_m (0.26 ± 0.07 mM) were close to the values reported for rat and bovine intestinal alkaline phosphatase.

Figure 6. Dephosphorylation activity of LPS by bovine ($n = 4$) and rat intestinal phosphatases ($n = 4$) according to variations in pH. The amount of enzyme was identical for all the assays (25 mU). Peak of LPS dephosphorylation occurred at pH 9 and pH 7.8, respectively, and decreased thereafter sharply. The *right lower graph* shows linear plot of O.D. at 870 μm *vs* P_i concentration in μM.

was slightly greater for the bovine enzyme (390 μM) than for the rat enzyme (P_i 330 μM). By contrast, the phosphatase protein produced from *S. boulardii* exhibited a much broader dephosphorylation activity ranging from pH 2 up to pH 10 with a peak at pH 4 (430 μM) (Fig. 7).

In vivo toxicity of LPS. Three groups (n = 9 each) of growing rats received intraperitoneally either NaCl 0.9% (controls) either LPS (group LPS or nondephosphorylated LPS) or

soluble and a particulate one—which differs each from the other by pH and by temperature, the particulate form being resistant to heating up to 100°C, we assessed whether the phosphatase activity from *S. boulardii* could also be the result of two different enzyme proteins. The responses of the soluble (supernatant) and particulate (pellet) enzymes to variations in pH (optimal, 6.8) and in temperature (optimal, 40°C) appeared to be similar. Thus, we presumed that both the soluble form and the particulate form corresponded to a unique protein.
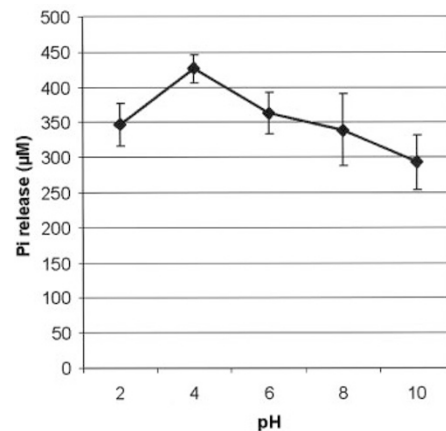
In vitro dephosphorylation activity of bovine and rat IAP and dephosphorylation activity of phosphatase protein from S. boulardii. Figure 6 shows the dephosphorylation activity of LPS by bovine and rat IAP according to variations in pH. The amount of enzyme in the reaction was identical for all the assays (25 mU). Both bovine and rat IAP showed a peak of dephosphorylation activity at pH 9 and pH 7.8, respectively, which decreased sharply thereafter. P_i release in the reaction
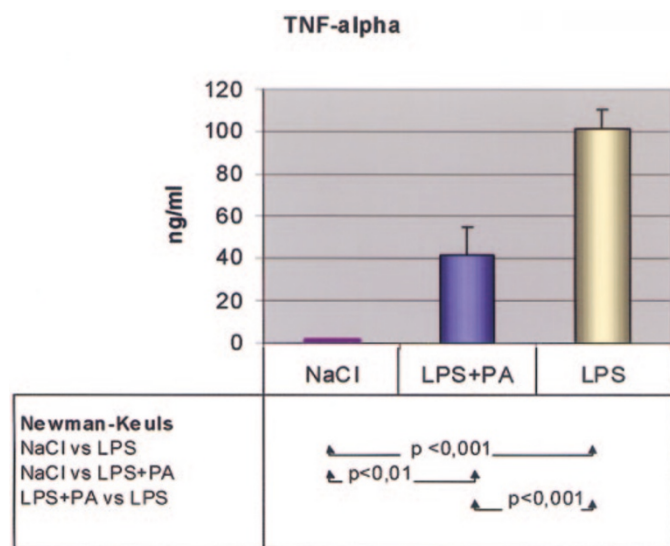

Figure 7. Phosphatase protein from *S. boulardii* exhibited a broad dephosphorylation activity ($n = 4$) over the whole pH range with a peak at pH 4. P_i release (430 μM) was higher than for the rat and for bovine alkaline phosphatase.

LPS dephosphorylated with the phosphatase protein of *S. boulardii* as described above. After 90 min, rats were killed and the sera were collected and frozen at –20°C until use.

Each serum was used for determination of circulating TNF-α by RIA. The results are presented in Figure 8. The control group exhibited practically no serum TNF-α, whereas the group of rats receiving intact LPS had an increase of TNF-α to about 100 ng/mL. The group of rats that received dephosphorylated LPS (partially inactivated) showed a mean value of 40 ng/mL. After ANOVA, the multiple-comparison Newman-Keuls test confirmed statistical differences between the three groups. In addition, all rats treated with active LPS showed after 9 h at autopsy inflammatory lesions and apoptotic bodies of the portal zones of the liver and of the myocardial muscles of variable importance, whereas the animals treated with NaCl 0.9% or dephosphorylated LPS showed no lesion.

DISCUSSION

Alkaline phosphatases are metalloenzymes (EC 3.1.3.1) that are found in nearly all living microorganisms, with the exception of some plants. IAP are glycoproteins (20% carbohydrates) attached to the apical intestinal membrane by a GPI anchor. In mammals, there are four different isoenzymes: placental, placental-like, intestinal, and tissue nonspecific (liver/bone/kidney). IAP is a dimmer of two proteins, differing slightly in molecular mass, likely by differences in glycosylation. Similarly, in bovine IAP we found two enzymes differing by their molecular mass. *S. cerevisiae* and *S. pombe* also produce AP whose properties and functions have been studied very little and whose sequence has been deduced from analysis of the coding genes. In *S. cerevisiae*, AP presents as a soluble and a particulate enzyme, located in lysosome-like vacuoles. They differ by some properties like as their resistance to high temperatures. The particulate form is especially thermostable exhibiting full activity up to 80°C degrees,

whereas the soluble form is more thermolabile (45°C). Our data indicate that *S. boulardii* also produces a soluble and pellet AP that exhibited similar activity curves in response to variations in pH and temperature. Therefore, we presumed that they were the same protein. *S. boulardii* had no GPI anchor because preincubation with specific phosphatidyl inositol-phospholipase C did not lower its molecular weight as with IAP of the rat.

In the presence of small concentrations of EDTA, activity of the phosphatase protein in yeast growth culture (YPD) was decreased and was virtually abolished at 20 mM EDTA, suggesting that the yeast enzyme is also a metalloprotease .

After immunoprecipitation of soluble proteins from 2.5×10^{10} decapsidated cells with a specific antiserum or with the IgG purified fraction, Western Blot, and autoradiography of the protein revealed a single band of 63 kD. Treatment of growing rats with 50 mg/kg body weight per day of the lyophilized preparation of *S. boulardii* increased moderately the specific activity of AP in the jejunal fluid but markedly and significantly ($p < 0.05$) the activity in the ileal fluid. Both endoluminal fluids had been filtered to discard *S. boulardii* cells.

This confirms that *S. boulardii* enhances in the intestinal lumen the activity of AP by releasing a protein phosphatase, mostly in the distal small bowel and colon as a reflection of the metabolism of yeast cells during their intestinal transit.

The main finding of the present study is that the protein phosphatase from *S. boulardii* is able to dephosphorylate endotoxins such as LPS of *E. coli* O55B5 and to partially inactivate its cytotoxic effects. We, however, have no evidence that the phosphate groups released from LPS derived from the lipid A portion of the LPS molecule. Circulating serum levels of TNF-α were significantly reduced by 60% when LPS had been preincubated with the protein phosphatase (25 mU) purified from *S. boulardii* compared with the TNF-α levels measured in rats treated with active nondephosphorylated LPS. Compared with rat and bovine IAP, the protein phosphatase of *S. boulardii* exhibited a pronounced dephosphorylation activity over a broad range of pH (2–10).

These findings suggest that endoluminal endotoxins from *E. coli* can be efficiently inactivated by dephosphorylation of their activation sites by IAP and by a phosphatase protein secreted by *S. boulardii*. If these experimental data can be transposed to humans, they suggest that the yeast phosphatase protein could exert a protective effect in young infants in whom intestinal *E. coli* endotoxins may translocate in blood and cause severe sepsis, toxic shock, and fatal issue.

Figure 8. Reponses of TNF-α measured in the serum of rats receiving intraperitoneal NaCl 0.9% ($n = 9$), LPS pure, or LPS dephosphorylated ($n = 9$) with the protein phosphatase of *S. boulardii*. Differences between mean levels of each group were statically significant ($p < 0.01$)

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