Properties of the yeast nuclear histone deacetylase

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A nuclear histone deacetylase from yeast was partially purified and some of its characteristics were studied. Histone deacetylase activity was stimulated *in vitro* by high-mobility-group non-histone chromatin proteins 1 and 2 and ubiquitin and inhibited by spermine and spermidine, whereas n-butyrate had no significant inhibitory effect. Like the mammalian enzyme, partially purified histone deacetylase from yeast was strongly inhibited by trichostatin A. However, in crude extract preparations the yeast enzyme was not inhibited and treatment with trichostatin *in vivo* did not show any effect, either on the histone acetylation level or on cell viability. At low ionic strength, the enzyme can be isolated

as a complex of high molecular mass that is much less inhibited by trichostatin A than is partially purified histone deacetylase activity. Furthermore, radiolabelled oligonucleosomes were more efficiently deacetylated by the complex than by the low-molecular-mass form of the enzyme. The histone deacetylase activity was separated from a polyamine deacetylase activity and its specificity studied. Using h.p.l.c.-purified core histone species as substrate, histone deacetylase from yeast is able to deacetylate all core histones with a slight preference for H3. Our results support the idea that the yeast histone deacetylase may act as a high-molecular-mass complex *in vivo*.

INTRODUCTION

The amino acid sequences of histones are highly conserved. However, histones are subject to post-translational modifications, most of which are reversible [see (Bradbury, 1992) for review]. Acetylation of the ϵ -amino group of lysine residues in the N-terminal tails has been widely studied, although its exact function still remains unclear [for review see (Csordas, 1990)]. A variety of functions has been proposed for histone acetylation, including transcriptional activation [see (Turner, 1991) for a review], DNA replication and nucleosome assembly (Lin et al., 1989; Talasz et al., 1990), DNA repair (Ramanathan and Smerdon, 1989), histone replacement during differentiation processes (Christensen and Dixon, 1982; Christensen et al., 1984; Loidl and Gröbner, 1986) and recombination events (Schaffhausen and Benjamin, 1976). These processes may be activated or deactivated depending on the acetylation of a total of 26 lysine residues present in a nucleosome that are the target for this modification (Loidl, 1988). Studies using site directed mutagenesis have shown that the N-terminal tail of H4 participates *in vivo* in the repression of the silent mating loci (Johnson et al., 1990; Park and Szostak, 1990; Megee et al., 1990) or in the activation of several inducible genes in yeast (Durrin et al., 1991). These authors have suggested that acetylation or deacetylation of a particular lysine residue of the N-terminal region could be part of a system of gene regulation by which the repressor or activator activities might be drastically altered.

Non-random acetylation of the four N-terminal lysine residues of histone H4 has been reported in some organisms (Pesis and Matthews, 1986; Chicoine et al., 1986, 1987; Couppez et al., 1987; Richman et al., 1988) which supports the idea that histone acetylation is part of a delicately regulated system of signalling and regulation in gene activation (Tordera et al., 1993). The acetylation state of a given lysyl residue of a core histone depends on two different enzymic activities, histone acetyltransferases

and histone deacetylases. The involvement of these enzymes in the resulting pattern of site-specific histone acetylation is currently being studied in our laboratory. The site specificity of a histone acetyltransferase B from pea has recently been determined. The enzyme is able to acetylate lysines 5, 12 and 16 of H4, whereas lysine 8 is not modified at all (Mingarro et al., 1993). A detailed knowledge of the regulation of these two enzyme activities may greatly advance our understanding of the different roles of histone acetylation. In the yeast *Saccharomyces cerevisiae* four histone acetyltransferase activities have been described (López-Rodas et al., 1989). Three enzyme activities are able to acetylate nucleosome-bound histones, whereas only one cytoplasmic activity acetylates free histone H4 (López-Rodas et al., 1991b). Multiple histone deacetylase activities have also been reported in several organisms (Kikuchi and Fujimoto, 1973; Cousens et al., 1979; Sendra et al., 1988, 1991; López-Rodas et al., 1991a, 1992) although less is known about these enzymes. Recently, a regulatory role for histone deacetylase in the initial steps of gene activation has been proposed (López-Rodas et al., 1993).

Histone deacetylase activity was initially detected in yeast by monitoring the change in the level of acetylation during incubation of isolated yeast nuclei (Alonso and Nelson, 1986). The authors found that yeast histone deacetylase was not inhibited by n-butyrate, which is an inhibitor of histone deacetylase of vertebrate cells (Riggs et al., 1977; Candido et al., 1978). Yoshida et al. (1990) reported that trichostatin A (TSA) inhibits mammalian histone deacetylase more strongly and more specifically than n-butyrate, causing an almost complete inhibition of a partially purified enzyme preparation in a nanomolar range. On the other hand, TSA inhibits cell growth by arresting the cell cycle both in G1 and G2 phases; this could be due to the accumulation of acetylated histone isoforms (Yoshida et al., 1990).

Yeast represents a good system for studying histone deacetylase for several reasons: (i) histone acetyltransferases are well charac-

Abbreviations used: AUT, acetic acid/urea/Triton X-100; HMG, high-mobility-group non-histone chromatin proteins; PMSF, phenylmethanesulphonyl fluoride; TSA, trichostatin A.

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terized (López-Rodas et al., 1989; 1991b); (ii) studies on directed mutagenesis of acetyltable lysines in the N-terminal histone domains have been performed (Megee et al., 1990; Park and Szostak, 1990; Durrin et al., 1991); and (iii) as it is a unicellular eukaryote, cellular effects of TSA can be more easily related to the inhibition of histone acetylation.

Preliminary results from our laboratory showed that histone deacetylase activity from yeast protoplasts eluted as a single peak in DEAE-Sepharose chromatography using different extraction procedures (López-Rodas et al., 1989). This activity was located in the nucleus and was able to deacetylate all nucleosomal core histones (López-Rodas et al., 1991b). In this paper we describe some important properties of yeast histone deacetylase, especially that the enzyme seems to act on chromatin in the form of a high-molecular-mass complex, and the effects of TSA both *in vivo* and *in vitro*.

MATERIALS AND METHODS

Preparation of crude enzymic extract

Saccharomyces cerevisiae, diploid strain 1383 CECT (A.T.C.C. 9763) was cultured as previously described (López-Rodas et al., 1985).

Crude preparations of histone deacetylase and acetylspermine deacetylase were obtained by the salt-dissociation/ultracentrifugation method previously described for the histone acetyltransferase activities (López-Rodas et al., 1989). Histone deacetylase complex was prepared from yeast spheroplasts by lysis in hypotonic buffer A [1 mM $MgCl_2$, 0.25 mM EDTA, 5 mM 2-mercaptoethanol, 0.7 mM phenylmethanesulphonyl fluoride (PMSF), 75 mM Tris/HCl, pH 7.9] and ultracentrifugation at 100000 *g* for 1 h. After removal of the floating layer of lipids, the supernatant was used as an extract containing high-molecular-mass complexes of histone deacetylase.

Purification of histone deacetylase

Crude enzymic extracts, prepared by the salt-dissociation/ultracentrifugation method as described above, were purified by DEAE-Sepharose CL-6B chromatography (López-Rodas et al., 1989). Fractions with histone deacetylase activity were pooled, concentrated by ultrafiltration in a Nucleopore cell fitted with an Amicon YM10 membrane, operated under N_2 pressure, and dialysed against buffer B [10 mM NH_4Cl , 0.25 mM EDTA, 5 mM 2-mercaptoethanol, 10% (v/v) glycerol, 15 mM Tris/HCl, pH 7.9]. The solution was loaded on a column (0.8 cm $\times$ 10 cm) of histone-agarose (Sigma) equilibrated with buffer B. Non-retained material was removed by washing with 40 ml of the same buffer. The retained proteins were eluted with 40 ml of a linear gradient of NH_4Cl (0.01–1.0 M) in buffer B. Fractions with histone deacetylase activity were pooled and used as enzyme source.

Size-exclusion chromatography

Ultrogel AcA34 chromatography was performed as previously described (Salvador et al., 1985). To study the effects of increasing ionic strength on the stability of histone deacetylase complexes, Ultrogel AcA34 chromatography was carried out in buffer B containing 35 mM, 200 mM and 500 mM NH_4Cl . In some instances RSB buffer (1 mM $CaCl_2$, 3 mM $MgCl_2$, 10 mM NaCl, 10 mM Tris/HCl, pH 7.5) was used. Sepharose 2B chromatography was carried out on a column (1.8 cm $\times$ 80 cm) equilibrated

with buffer B containing 35 mM NH_4Cl , at a flow rate of 14 ml/h.

Histone deacetylase assay and determination of substrate specificity

Histone deacetylase activity was assayed as described previously (Sendra et al., 1988). Chicken erythrocyte histones were acetylated *in vivo* by incubating a reticulocyte-enriched erythrocyte population with [3H]acetic acid (5.0 Ci/mmol) according to Ferenz and Nelson (1985). To assay histone deacetylase activity, prelabelled histones were incubated with enzymic fractions. After termination of the reaction with acid, the released acetate was extracted with ethyl acetate and its radioactivity measured. The influence of some substances on the histone deacetylase activity was tested by including them in the assay mixture at the desired concentration. Chicken erythrocyte HMG 1 and 2 and HMG 14 were prepared by 5% perchloric acid extraction, followed by chromatography on CM-Sephadex G-25 (Walker and Johns, 1980). Ubiquitin, spermidine and spermine were purchased from Sigma.

To study the substrate specificity, labelled histones were fractionated on an h.p.l.c. Nucleosil 300-5C₄ column with a multistep acetonitrile gradient as described before (López-Rodas et al., 1992). These h.p.l.c.-separated histones were used in the deacetylase specificity assay. For this purpose, samples (200 μ l) of partially purified or high-molecular-mass histone deacetylase were mixed with 5–10 μ g of individual histone species (approx. 15000 c.p.m.) and the released radioactivity was measured.

Several other substrates were used in this study, including chicken oligonucleosomes acetylated *in vivo* with [3H]acetate, and BSA, polylysine, spermine, spermidine and putrescine, all chemically acetylated with [3H]acetic anhydride. These substances were prepared as described previously (Sendra et al., 1988). When oligonucleosomes were used as substrate the final histone concentration was adjusted to that of free histones in the routine assay. The deacetylase activity towards polyamines was assayed using radiolabelled polyamines instead of the histone substrates.

In vitro and *in vivo* TSA effect

The influence of TSA on histone deacetylase activity was analysed in partially purified enzyme fractions, crude extract obtained by the salt-dissociation/ultracentrifugation method, nuclei and lysed spheroplasts.

The *in vivo* effect of TSA on the acetylation level was investigated in whole cells and spheroplasts. Cultures of 15 ml of cells in logarithmic phase were incubated at 28 °C for 3 h in the presence or absence of the inhibitor (2 μ M). The acid-extracted histones were analysed by acetic acid/urea/Triton X-100 (AUT) electrophoresis (Alfageme et al., 1974). The spheroplasts were incubated with 1 mCi/ml of [3H]acetate (Alonso and Nelson, 1986) with or without 2 μ M TSA. The level of histone acetylation was analysed by AUT electrophoresis and fluorography was performed according to López-Rodas et al. (1989). The TSA effect on deacetylation in isolated nuclei was investigated by incubating radiolabelled yeast nuclei at 37 °C for 1 h (Alonso and Nelson, 1986) in the presence or absence of 2 μ M TSA in the incubation medium. To investigate the cytotoxicity of TSA on yeast cells, culture media containing different concentrations of TSA (up to 1 mM) were prepared in a multiwell plate (370 μ l/well). Cells in logarithmic phase (1×10^5 per well) were added and growth was followed by measuring absorbance at 600 nm.

Purification of acetylspermine deacetylase

Crude enzymic extracts, prepared as described above, were purified by DEAE-Sepharose CL-6B chromatography, using the method described for histone deacetylase (López-Rodas et al., 1989). Fractions with acetylspermine deacetylase activity were pooled, concentrated to 5 ml and purified by Ultrogel AcA34 chromatography as previously described (Salvador et al., 1985). Acetylspermine deacetylase activity was assayed with [^3H]acetylspermine as substrate using the method described for histone deacetylase (see above).

Fractionation of histone deacetylase complex on sucrose gradients

Samples (1 ml) of crude enzymic extract were layered on top of a 10 ml linear sucrose gradient (5–20%) in RSB buffer with or without 500 mM NaCl and centrifuged at 35000 rev./min for 14 h in a Beckman SW41 Ti rotor. The gradients were fractionated and histone deacetylase activity was determined.

DNAase I digestion

The crude enzymic extract was dialysed against RSB buffer and diluted with RSB buffer to obtain an absorbance at 280 nm of 1.0. Then 150 μl of DNAase I (Worthington Biochemicals) (2 units/ μl) were added to 1 ml of sample and digestion was carried out for 30 min at 37 °C. Digestion was terminated by adding 200 μl of 100 mM EDTA.

RESULTS AND DISCUSSION

Partial purification of histone deacetylase

Previous results from our laboratory using different extraction methods (López-Rodas et al., 1989, 1991b) indicated the presence of a histone deacetylase activity in yeast. For this study histone deacetylase was obtained from lysed spheroplasts by ultracentrifugation in the presence of 0.5 M NaCl. The enzyme was partially purified by DEAE-Sepharose chromatography followed by gel filtration (López-Rodas et al., 1989) and subsequent chromatography on histone-agarose (Figure 1). The procedure yielded around 2000-fold purification of histone deacetylase activity as compared with lysed spheroplasts extract. The histone deacetylase eluted as a single peak in each chromatographic step. The partially purified enzyme had a molecular mass of 150 kDa, as estimated from size-exclusion chromatography. The enzyme displayed a maximum activity at an ionic strength of around 200 mM NH_4Cl . The effect of some substances on histone deacetylase activity is shown in Table 1. Some of the characteristics of the partially purified enzyme were similar to those observed in intact yeast nuclei (Alonso and Nelson, 1986). The enzyme was almost insensitive to n-butyrate, showing only a 20% inhibition in the presence of 50 mM n-butyrate. Similar findings have been reported in pea (Sendra et al., 1988) and maize (López-Rodas et al., 1991a) in contrast with histone deacetylase from cells of vertebrates which is almost totally inhibited in the presence of 5 mM n-butyrate (Candido et al., 1978; Cousens et al., 1979). Alonso and Nelson (1986) showed that yeast histone deacetylase can deacetylate chicken histones when chicken erythrocyte nuclei were directly used as substrate. Under their experimental conditions the histone deacetylase activity was completely inhibited by 2 mM spermine or 5 mM

spermidine (Vu et al., 1987). Our results, using the partially purified enzyme and free histones from chicken erythrocyte, also indicate an inhibitory effect of polyamines. However, the inhibition was 70% in the presence of 5 mM spermine, suggesting that the inhibition in nuclei was not exclusively due to a direct action of polyamines on the enzyme. On the other hand, it has been previously reported that high-mobility-group non-histone

Figure 1 Purification of yeast histone deacetylase by histone-agarose chromatography

A column (0.8 cm $\times$ 10 cm) was loaded with a concentrated histone deacetylase activity peak from DEAE-Sepharose CL-6B. Elution was carried out with a linear gradient of NH_4Cl (0.01–1.0 M) in buffer B at a flow rate of 10 ml/h and fractions (1 ml) were collected. —, mg of protein per ml determined by Bradford method; $\circ$, histone deacetylase activity in d.p.m.; dotted line, NH_4Cl gradient.

Table 1 Effect of various compounds on yeast histone deacetylase activity

A value of 100% deacetylase activity corresponds to the activity in the standard assay (see the Materials and methods section).

Substance	Concentration	Activity (%)
Butyrate	10 mM	108
	20 mM	83
	50 mM	80
Sodium acetate	10 mM	86
	20 mM	68
	50 mM	56
HMGs 1 and 2	10 $\mu\text{g}/\text{ml}$	115
	20 $\mu\text{g}/\text{ml}$	129
	40 $\mu\text{g}/\text{ml}$	175
HMG 14	10 $\mu\text{g}/\text{ml}$	113
	20 $\mu\text{g}/\text{ml}$	117
	40 $\mu\text{g}/\text{ml}$	110
Ubiquitin	10 $\mu\text{g}/\text{ml}$	105
	20 $\mu\text{g}/\text{ml}$	133
	40 $\mu\text{g}/\text{ml}$	195
Spermidine	1 mM	88
	5 mM	36
Spermine	1 mM	92
	5 mM	31

Figure 2 *In vitro* effect of TSA on histone deacetylase activity

(a) Partially purified histone deacetylase (from Figure 1) was assayed in the presence of increasing concentrations of TSA. (b) Comparison of inhibitory effect of TSA on different histone deacetylase activity samples; (+) with 0.5 μ M TSA, (–) without TSA.

chromatin proteins (HMGs) can modulate histone deacetylase activity (Reeves and Candido, 1980); a slight stimulation in the presence of HMGs 1 and 2 and an inhibition in the presence of HMGs 14 and 17 were observed. Our results are in line with the stimulatory effect of HMGs 1 and 2, which led to an almost 2-fold increase of activity; no apparent effect was observed in the presence of HMG 14 (Table 1). The presence of HMG-like proteins in yeast is still an open subject (Weber and Isenberg, 1980; Estruch et al., 1986; Kolodrubetz and Burgum, 1989), so the interpretation of these results must be considered with caution.

Effect of TSA on yeast histone deacetylase

Partially purified histone deacetylase from yeast was inhibited by TSA over a nanomolar range (Figure 2a). This extent of inhibition was also found for mammalian (Yoshida et al., 1990) and plant histone deacetylase (P. Martínez-Alepuz and R. Sendra, unpublished work). Strikingly, we found that TSA did not cause any histone deacetylase inhibition when prelabelled yeast nuclei were used as substrate in the assay system of Alonso and Nelson (1986). This lack of inhibition was also seen when crude extracts were used as histone deacetylase sources (Figure 2b). When we administered TSA (2 μ M) to whole cells or spheroplasts, no accumulation of acetylated histone subspecies was observed, as analysed by AUT/PAGE (results not shown). Moreover, no effect was seen on the viability or cell growth when TSA was administered in the culture medium at concentrations higher than 1 mM, a value 2000-fold greater than the concentration leading to complete inhibition of the partially purified enzyme. These results show that TSA has no effect on histone deacetylase activity *in vivo*. We obtained similar results when *Schizosaccharomyces pombe* was used as biological material. However, this is not a general effect in unicellular eukaryotes because

proliferation of *Chlamydomonas reinhardtii* was completely inhibited by 0.5 mM TSA, and histone deacetylase activity of this alga was strongly inhibited by TSA *in vitro* (R. Sendra, unpublished work).

The different effect of TSA on partially purified histone deacetylase and crude extracts could be explained in two possible ways: (i) the drug may be sequestered or metabolized by an unknown activity present in yeast. It has been shown that trichostatic acid, which is a product from TSA after hydrolysis of its hydroxamate group, is biologically inactive and has no effect on mammalian histone deacetylase *in vitro* (Yoshida et al., 1990). To check this possibility we incubated a yeast crude extract with TSA and then added partially purified histone deacetylase to measure its activity. The results showed that the inhibitory effect of TSA was still present after such an incubation and it was even maintained if TSA was preincubated with crude extract for prolonged periods. Therefore the drug can not be degraded or modified by any component of the crude extract. (ii) A second possibility is that the TSA-binding site of deacetylase is not accessible *in vivo* or in crude enzymic preparations. During enzyme purification the native organization of histone deacetylase could be altered and this change may allow TSA to bind to the enzyme. This second possibility is more plausible because histone deacetylase seems to be organized as a high-molecular-mass complex (see below). Some of the proteins of the complex may protect the enzyme from TSA inhibition. These results suggest that the physiological form of yeast histone deacetylase *in vivo* could be different, and better protected against TSA inhibition, in comparison with partially purified histone deacetylase activity.

High- and low-molecular-mass forms of yeast histone deacetylase

In some organisms histone deacetylase activity has been described to form complexes of high molecular mass (Hay and Candido, 1983a,b; Mold and McCarty, 1987). In order to check this possibility in yeast, crude enzyme extract was loaded on a gel filtration column and equilibrated with different concentrations of NH_4Cl . The profile of one of these chromatographies, at 200 mM NH_4Cl , is shown in Figure 3(a). At this salt concentration the histone deacetylase activity eluted in two peaks, a high-molecular-mass peak in the exclusion volume (molecular mass > 350 kDa), and a low-molecular-mass peak (approx. 150 kDa). When the ionic strength of the buffer was decreased to 35 mM NH_4Cl , the size of the low-molecular-mass peak decreased in favour of the high-molecular-mass peak (Figure 3b). At an NH_4Cl concentration of 500 mM, only the low-molecular-mass peak appeared. When both low- and high-molecular-mass peaks were subjected to DEAE-Sepharose chromatography, identical profiles were obtained with a single peak of histone deacetylase activity at a concentration of approx. 0.25 M NH_4Cl .

Several experiments were performed to learn more about the biochemical properties of the high-molecular-mass complex. The molecular mass of the complex was determined by ultracentrifugation on sucrose gradients (Figure 3c) and by Sepharose 2B chromatography (results not shown). In both cases a molecular mass of around 500 kDa was obtained for the complex and around 150 kDa for the low-molecular-mass form. When the crude extract was extensively treated with DNAase I and chromatographed on Ultrogel AcA34 the profile of histone deacetylase activity was identical with an untreated control and still contained nucleic acids, as determined by spectrophotometric analysis.

Hay and Candido (1983a,b) described a histone deacetylase activity from HeLa cells associated with high-molecular-mass

Figure 3 High-molecular-mass and low-molecular-mass histone deacetylase (HD) activities at different ionic strengths

(a) Chromatography of crude extract on Ultrogel AcA34 equilibrated at 200 mM NH_4Cl . Aliquots of 5 ml of crude extract were loaded on a 1.8 cm $\times$ 120 cm column and eluted at a flow rate of 14 ml/h. —, Absorbance at 280 nm; $\circ$, histone deacetylase activity in d.p.m. (b) Percentage of high- (open column) and low-molecular-mass (hatched column) histone deacetylase forms at different salt concentrations in the chromatography on Ultrogel AcA34. Enzymic activities were calculated and expressed as percentages of total histone deacetylase

Figure 4 Effect of TSA on high- (●) and low-molecular-mass (○) histone deacetylase activities

Fractions from the separated peaks on Ultrogel AcA34 chromatography (Figure 3a) were used as enzymic sources and assayed for deacetylase activity in the presence of increasing concentrations of TSA.

complexes, which was able to deacetylate endogenous polynucleosomes more efficiently than free histones. However, the complexes of HeLa cells were more stable at high ionic strength than those of yeast and they remained intact even at concentrations of NaCl in the range of 1–2 M. The histone deacetylase from Chinese hamster ovary cells (Mold and McCarty, 1987) was associated with mononucleosomes containing, among other proteins, histone H1 and HMGs 1 and 2, but no HMG 14 nor 17. The presence of HMGs 1 and 2 in the same complex could activate the histone deacetylase *in vivo*. The complexes from Chinese hamster ovary cells were also stable at a NaCl concentration of 1 M. The lower stability in the case of the yeast complex could be explained by the fact that this organism has no histone H1, which could cause a reduced stability not only of the nucleosome, but also of protein–DNA complexes. This reduced stability may also explain why any further purification step of the high-molecular-mass complex was unsuccessful.

We studied the effect of TSA on high- and low-molecular-mass histone deacetylase activity. Figure 4 shows that the complexed form is much less sensitive to TSA than the low-molecular-mass histone deacetylase activity. The behaviour of the complexed form toward TSA is similar to that of histone deacetylase activity in lysed spheroplasts or crude extract, whereas the low-molecular-mass form resembles the properties of partially purified enzyme (see Figure 2). These results indicate that such complexes with histone deacetylase activity are not artifactual.

Furthermore, we investigated the activity of the low-molecular-mass and complexed forms of histone deacetylase against free histones and oligonucleosomes. As shown in Figure 5, the low-molecular-mass histone deacetylase has a similar activity towards

activity in each chromatography. (c) Sucrose gradient ultracentrifugation (5–20%) of crude extracts in the presence of 10 ($\circ$) or 500 mM ($\square$) NaCl. The molecular masses of high- and low-molecular-mass HD forms (arrows in a and c) were determined using as standard molecular-mass markers: lysozyme (14.3 kDa), haemoglobin (64 kDa), aldolase (158 kDa), catalase (240 kDa), apoferritin (443 kDa) and thyroglobulin (669 kDa).

Figure 5 Activity of low- and high-molecular-mass histone deacetylase forms with oligonucleosomes and free histones

Fractions corresponding to the peaks obtained in Figure 3(a) were incubated at 30 °C as indicated in the Materials and methods section. At the indicated times the released [3 H]acetate was measured. —, activity of low-molecular-mass form; ---, activity of high-molecular-mass form; closed symbols, oligonucleosomes as substrate; open symbols, free histones as substrate.

Table 2 Activities of histone deacetylase and acetylspermine deacetylases towards different acetylated substrates

Activities are expressed as percentages relative to the maximum level achieved for each enzyme. Abbreviation: n.d., not determined.

Substrate	Activity (%)	
	Histone deacetylase	Spermine deacetylase
Chicken erythrocyte histones	100.0	5.1
Acetylspermine	0.0	100.0
Acetylputrescine	0.8	3.7
Acetylpolylysine	9.3	0.0
Acetylspermidine	n.d.	0.0
AcetylBSA	0.0	0.0

both substrates. In contrast, oligonucleosomes, presumably the *in vivo* substrate, are much more efficiently deacetylated than free histones by the complexed histone deacetylase form. Therefore our results support the idea that yeast histone deacetylase may act *in vivo* in the form of a high-molecular-mass complex.

Yeast histone deacetylase specificity

We tested the specificity of yeast histone deacetylase for different substrates and compared it with the specificity of yeast acetylspermine deacetylase. Comparison of substrate specificities of the two enzyme activities is important because some histone acetyltransferases may also acetylate polyamines (Libby, 1978, 1980; Sures and Gallwitz, 1980). Table 2 shows that histone and acetylspermine deacetylases are entirely different to each other with respect to their substrate specificity; no significant overlap

Table 3 Histone specificity of yeast high-molecular-mass and partially purified histone deacetylase

Values are expressed as percentages of the total histone deacetylase activity $\pm$ S.D. within three independent experiments

	Histone deacetylase activity (%)	
	High-molecular-mass form	Partially purified form
H2A	9.6 $\pm$ 1.2	13.8 $\pm$ 1.9
H2B	34.1 $\pm$ 4.5	25.9 $\pm$ 2.5
H3	36.9 $\pm$ 3.1	43.1 $\pm$ 2.4
H4	19.4 $\pm$ 1.0	17.2 $\pm$ 0.9

could be observed. In this context it should be mentioned that acetylspermine deacetylase eluted at lower ionic strength in DEAE-Sephacel chromatography; moreover, it showed a molecular mass of 40 kDa in Ultrogel AcA34 chromatography, which is clearly lower than that of histone deacetylase. Acetylspermine deacetylase is highly specific for spermine, because it was not able to deacetylate other polyamines, such as putrescine or spermidine (Table 2).

To analyse the histone specificity of histone deacetylase, we used h.p.l.c.-purified individual core histone species as substrate. Table 3 shows that the partially purified enzyme, as well as the complex, was able to deacetylate all core histones with a slight preference toward H3. Histone acetylation is frequently attributed to activation and inactivation of several essential processes of the cell. Furthermore, it has been proposed that acetylation of core histones, and the level of the engaged enzymes, could directly be involved in the regulation of several nuclear processes (Loidl, 1988; Georgieva et al., 1991; López-Rodas et al., 1993). The presence of probably a single histone deacetylase obtained in yeast together with its ability to deacetylate all four core histones may suggest that histone acetyltransferases rather than histone deacetylase are responsible for maintaining the pattern of histone acetylation in this organism.

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