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Polyamines inhibit the yeast histone deacetylase

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n-Butyrate inhibits the histone deacetylase from higher cells, but has little effect on the enzyme activity in *Saccharomyces cerevisiae*. Spermine and spermidine were therefore tested as potential yeast deacetylase inhibitors and found to inhibit fully the enzyme at 2 and 5 mM, respectively. The utility of these inhibitors was demonstrated by showing that 2 mM spermine substantially increased the incorporation of [^{3}H]acetate into histone in a yeast nuclear acetyltransferase assay.

Histone deacetylase; *n*-Butyrate; Spermine; Spermidine; (*Saccharomyces cerevisiae*)

1. INTRODUCTION

Acetate is reversibly incorporated into specific lysyl residues in the N-terminal regions of the core histones [1,2]. Two distinct modes of lysyl residue acetylation are recognized. Prior to deposition onto the DNA, H4 is acetylated in the cytoplasm at two positions by the acetylase B enzyme [3]. After nucleosome assembly, acetylation of all four core histones is catalyzed by the nuclear acetyltransferase A protein [3,4]. Both the cytoplasmic and nuclear enzymes require acetyl-CoA as the acetyl group donor. The amide linkage between the acetyl group and lysyl residue is often subsequently hydrolyzed by a histone deacetylase, and in most higher cells, this hydrolysis is blocked by the short chain fatty acid, *n*-butyrate [5].

In *Saccharomyces cerevisiae*, acetyltransferase A [6,7] and B [7] enzymes have been identified, and a histone deacetylase extracted from isolated nuclei [8]. The nuclear histone deacetylase from yeast uses chromatin bound acetyl-histone as substrate, and is not inhibited by *n*-butyrate [8]. The lack of a known specific inhibitor of the yeast deacetylase makes a variety of experiments difficult to perform. Histone deacetylation occurs during the nuclease digestions required for yeast chromatin fractionation (unpublished), and may occur during acetyltransferase assays in isolated yeast nuclei. Because of the potential utility of a specific yeast deacetylase inhibitor, a number of biochemicals were tested for this capacity. Particular emphasis was placed on the polyamines since they are reported to be capable of enhancing acetyltransferase action in higher cells [9], and at appropriate concentrations, diminishing deacetylase activity [10]. We find 2 mM spermine and 5 mM spermidine to be potent inhibitors of the yeast deacetylase, and 'enhanced' acetyltransferase activity in isolated yeast nuclei in the presence of the polyamines can be attributed to the inhibition of the histone deacetylase.

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Abbreviations: Mes, 4-morpholineethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride

2. MATERIALS AND METHODS

The diploid *S. cerevisiae* strain Y-55 was grown to late log phase (5×10^7 cells/ml) in YPD medium (1% yeast extract, 2% peptone, 2% dextrose) and the cells spheroplasted as described previously

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[11]. Protoplasts were resuspended in NIB (nuclear isolation buffer: 0.25 M sucrose, 60 mM KCl, 15 mM NaCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM 2-mercaptoethanol, 15 mM Mes, 0.1 mM PMSF, pH 6.6) plus 0.8% Triton X-100, pelleted by centrifugation (2000 × *g*) and the pelleted nuclei washed twice in NIB plus Triton X-100.

For extraction of the histone deacetylase, yeast nuclei were resuspended in NIB plus 0.25 M NaCl and centrifuged at 9000 × *g* for 10 min. The supernatant containing the deacetylase activity was either stored at −80°C in 20% glycerol, with retention of full activity, or was dialyzed directly against the appropriate assay buffer. Assays were for 1 h at 37°C using as substrate, [^{3}H]acetate-labeled histone in chicken red blood cell nuclei as described in [8]. Briefly, the assay consists of monitoring the cpm of [^{3}H]acetate released from the chicken histone into the supernatant by the yeast enzyme. The standard deacetylase assay buffer contained 10 mM Tris, 100 mM NaCl, 10 mM *n*-butyrate, 2 mM EDTA, 1 mM 2-mercaptoethanol, 0.1 mM PMSF (pH 7.4). The deacetylase activity in the figures is expressed in relative units where the activity in the standard assay buffer is given as 100%. The actual amount of radioactivity released during a standard incubation is typically 30000 cpm, corresponding to 30% of the total radiolabel. As described in [8], the *n*-butyrate was included in the assay buffer to inhibit the chicken histone deacetylase co-isolated with the chicken erythrocyte nuclei used as substrate.

For yeast histone acetyltransferase assays, yeast nuclei were resuspended in NIB at approx. 5×10^9 nuclei per ml. The NIB also contained 150 μM [^{3}H]acetate (5 Ci/mmol; ICN), 10 μM coenzyme A, 0.04 units/ml acetyl-CoA synthetase (Sigma) and 500 μM ATP. [^{3}H]Acetyl-CoA was generated during the incubation and utilized as substrate by the endogenous yeast nuclear acetyltransferase. After incubation at 37°C, EDTA was added to 10 mM, EtOH added to 90%, samples centrifuged (9000 × *g*), washed twice with 95% EtOH and dried under vacuum. Pellets were either dispersed in 1% SDS for scintillation counting, or resuspended in sample buffer for SDS-polyacrylamide gel electrophoresis. Electrophoreses on SDS 18% polyacrylamide gels and subsequent fluorography were as described [11].

All histone deacetylase and acetyltransferase assays were performed in triplicate with three separate yeast cell preparations. Subsequent graphs present mean values of the data points.

3. RESULTS

The *S. cerevisiae* histone deacetylase is associated with the nuclei during preparation and may be extracted by the addition of NaCl to 0.25 M. The dependence of this histone deacetylase activity on sodium *n*-butyrate and NaCl concentrations is shown in fig.1. The increase in activity in the presence of 0 to 75 mM sodium *n*-butyrate parallels the increase observed in the presence of NaCl. The deacetylase activity peaks at 100 mM for both salts, although the activity in sodium *n*-butyrate is only 80% of that in NaCl. As illustrated in fig.1, whether the assay is performed in the presence of sodium *n*-butyrate or sodium chloride, it appears that an ionic strength effect on enzyme activity is being observed. There is no evidence for the deacetylase inhibitory action ascribed to *n*-butyrate in higher cells.

To verify that an ionic strength effect is being monitored as shown in fig.1, the assay was performed in the presence of Na_2SO_4. As expected for an ionic strength dependence, the peak of activity was at μ = 0.1, corresponding to approx. 40 mM Na_2SO_4.

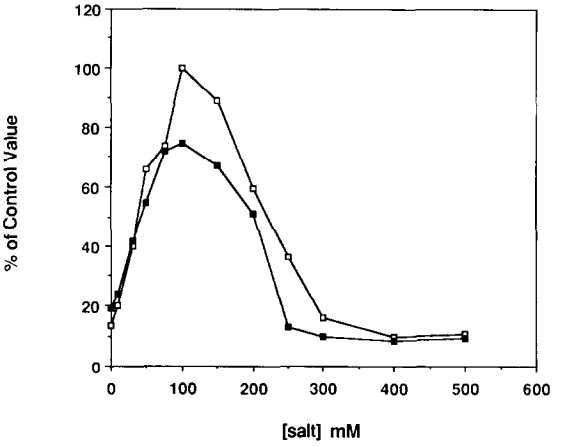

Fig.1. Yeast histone deacetylase activity in the presence of sodium chloride (□) and sodium *n*-butyrate (■). Values are relative to the control value of 100% (30000 cpm of [^{3}H]acetate released) assigned to the activity obtained in the standard incubation buffer (see section 2).

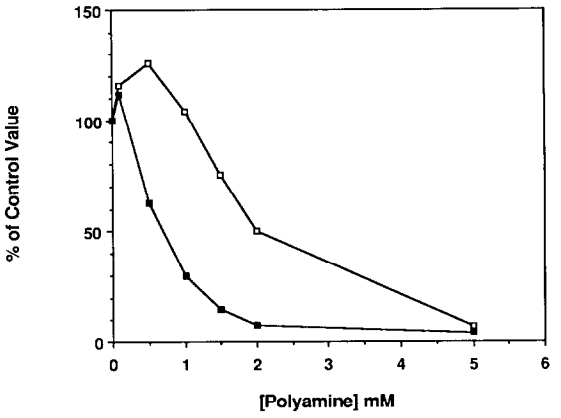

Fig.2. Yeast histone deacetylase activity in the presence of spermidine (□) and spermine (■). A value of 100% is assigned to the activity in standard incubation buffer.

As illustrated in fig.2, the polyamines spermine (*N,N'*-bis-[3-aminopropyl]-1,4-butanediamine) and spermidine (*N*-[3-aminopropyl]-1,4-butanediamine) are quite effective at inhibiting the yeast deacetylase at concentrations of about 2 and 5 mM, respectively. At lower concentrations (0.1 mM spermine and 0.5 mM spermidine), both polyamines slightly stimulate the action of the yeast enzyme.

To test whether the presence of a polyamine might have utility in yeast histone acetyltransferase assays, these assays were performed in the presence, or absence of 2 mM spermine. Isolated yeast nuclei, when incubated in the absence of spermine under conditions which optimize incorporation of [^{3}H]acetate into histone, show very low levels of incorporation, as illustrated in fig.3. The inclusion of 2 mM spermine in the incubation buffer dramatically increases the level of [^{3}H]acetate incorporation, to about 4.5-fold above the control level after 15 min. As shown in fig.4, the incorporation of [^{3}H]acetate is into yeast histone, and the intensity of the bands in lane 3 confirms that incorporation is substantially in-

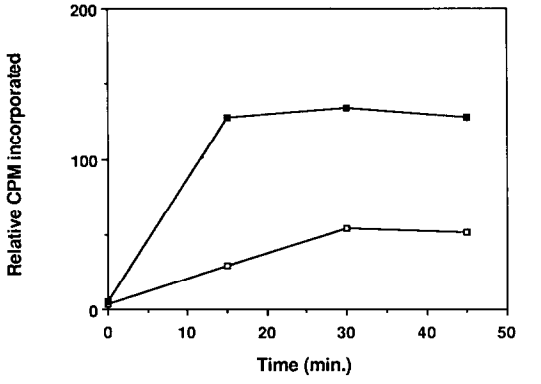

Fig.3. [^{3}H]Acetate incorporation into histone in isolated yeast nuclei by the endogenous histone acetyltransferase. The histone acetyltransferase assay was performed in the absence (□) or presence (■) of 2 mM spermine as described in section 2.

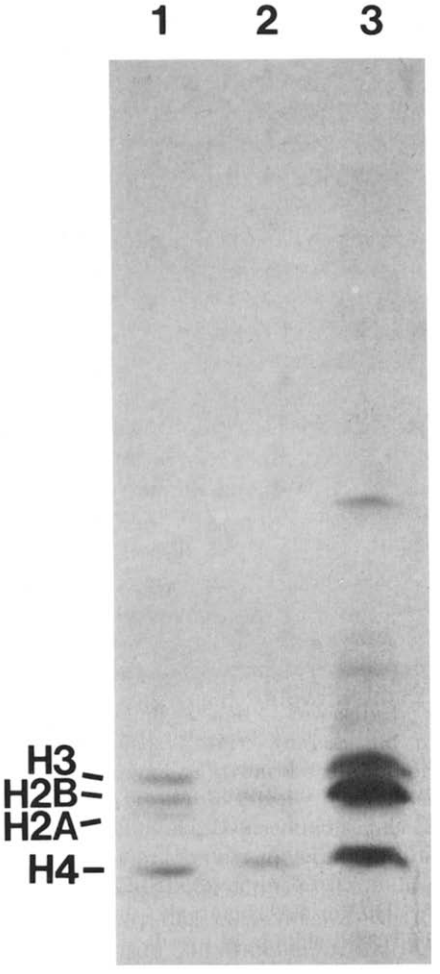

Fig.4. Fluorogram depicting the incorporation of [^{3}H]acetate into histone catalyzed by the endogenous yeast nuclear histone acetyltransferase. Lanes: 1, [^{3}H]acetate-labeled chicken histone standards; 2 and 3, identical yeast samples were incubated as in fig.3 for 20 min in the absence (2) or presence (3) of 2 mM spermine. Samples were electrophoresed on a SDS 18% polyacrylamide gel and a fluorogram prepared.

creased by the presence of 2 mM spermine. The same amount of chromatin was loaded onto lanes 2 and 3 of fig.4 and the samples were treated identically, except for the inclusion of 2 mM spermine in the acetyltransferase assay buffer for the sample in lane 3. A small amount of radiolabel was incorporated into a band in lane 3 electrophoresing near the bromphenol blue dye front (not shown), and may correspond to incorporation into spermine [12].

4. DISCUSSION

Sodium *n*-butyrate, as an inhibitor of higher cell histone deacetylases, has been used in vivo to accumulate hyperacetylated histone forms [13] and to measure rates of histone acetylation [14]. It has been used in vitro to prevent histone deacetylation during nuclear preparation, nuclease digestions [15] and during histone acetyltransferase assays [16]. Analogous to the effect of *n*-butyrate on the histone deacetylase of higher cells, the polyamines inhibit the yeast enzyme, and we have tested whether the presence of spermine, at sufficient concentration to inhibit the yeast deacetylase, might have utility in yeast histone acetyltransferase assays. In the absence of spermine, the endogenous yeast histone acetyltranferase incorporates only low levels of [^{3}H]acetate into histone in isolated yeast nuclei. This could be caused by the loss of the nuclear acetyltransferase during isolation, the high levels of unlabeled histone-acetate at the start of the incubation [11], or due to the presence of an active histone deacetylase co-isolated with the nuclei. At an appropriate concentration, spermine increases the incorporation of [^{3}H]acetate into the histone, suggesting a co-isolated histone deacetylase is responsible for the low levels of incorporation. The yeast acetyltransferase A enzyme is apparently still present and active in the isolated nuclei.

It is possible that in the yeast nuclear acetyltransferase assay, the 2 mM spermine potentiates the acetyltransferase enzyme, rather than simply inhibiting the endogenous histone deacetylase. To directly test the effects of 2 mM spermine on the histone acetyltransferase, nuclei were extracted with 0.4 M NaCl and the yeast deacetylase protein (M_r approx. 1×10^6; unpublished), separated from the acetyltransferase A enzyme (M_r 110000–160000; [6,7]) by agarose A-5m exclusion chromatography. Using purified histone as substrate, we find that the presence or absence of 2 mM spermine has no effect on the activity of the purified nuclear histone acetyltransferase. The single peak of deacetylase activity from the column, however, is fully inhibited by the presence of 2 mM spermine.

We find that the conditions developed in other laboratories for the yeast acetyltransferase assay [6,7] greatly reduce the action of the yeast deacetylase by elevating the ionic strength and pH over those optimal for this enzyme. The inhibition of the yeast deacetylase activity by spermine and spermidine may provide a further tool for assaying acetyltransferase action in isolated yeast nuclei and crude protein preparations, and may have utility in stabilizing histone-acetate content during nuclear preparation and chromatin fractionation.

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