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for

The Human Histone Acetyltransferase P/CAF is a Promiscuous Histone Propionyltransferase

Hans Leemhuis, Len C. Packman, Karl Nightingale and Florian Hollfelder*

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Further measurements and reaction specificity

Butyryl transfer to histone H3 peptide - While AcCoA and ProCoA are efficient acyl donors, the slightly larger butyryl is transferred at a 100-fold lower rate. When 500 µM H3 peptide and 500 µM butyryl-CoA were incubated with 1.6 µM P/CAF (100 mM MES buffer, pH 7.5, 25 °C) CoA was formed at a higher rate than in the analogous incubation in the absence of the H3 peptide. This indicates the transfer of butyryl to the H3 peptide. The rate was, however, only 50% above the rate of spontaneous hydrolysis of butyryl-CoA, corresponding to a rate of 0.12 min^{-1} only. Nevertheless, MALDI-TOF MS (Figure S2) confirmed that the H3 peptide was butyrylated by P/CAF.

Propionyl transfer to histone H4 peptide - Since P/CAF is known to acetylate histone H4 with a low rate[1] we tested whether P/CAF could also transfer propionyl moieties to the histone H4 peptide. Incubating 1,000 µM H4 peptide with 200 µM ProCoA and 8.3 µM P/CAF (100 mM MES buffer, pH 7.5, 25 °C) resulted in a faster formation of CoA compared to the analogous incubation in the absence of the H4 peptide. The rate was, however, only 40% above the rate of spontaneous hydrolysis of propionyl-CoA, corresponding to a rate of 0.04 min^{-1}. Under identical conditions the rate of acetyl transfer to the H4 peptide was 0.3 min^{-1}. The low activity observed did not allow more precise measurement of the kinetic parameters. The much lower rates of propionylation and acetylation with the H4 peptide, compared with the H3 peptide, demonstrates that the specificity of P/CAF for the peptide substrate is not affected by the identity of the acyl donor.

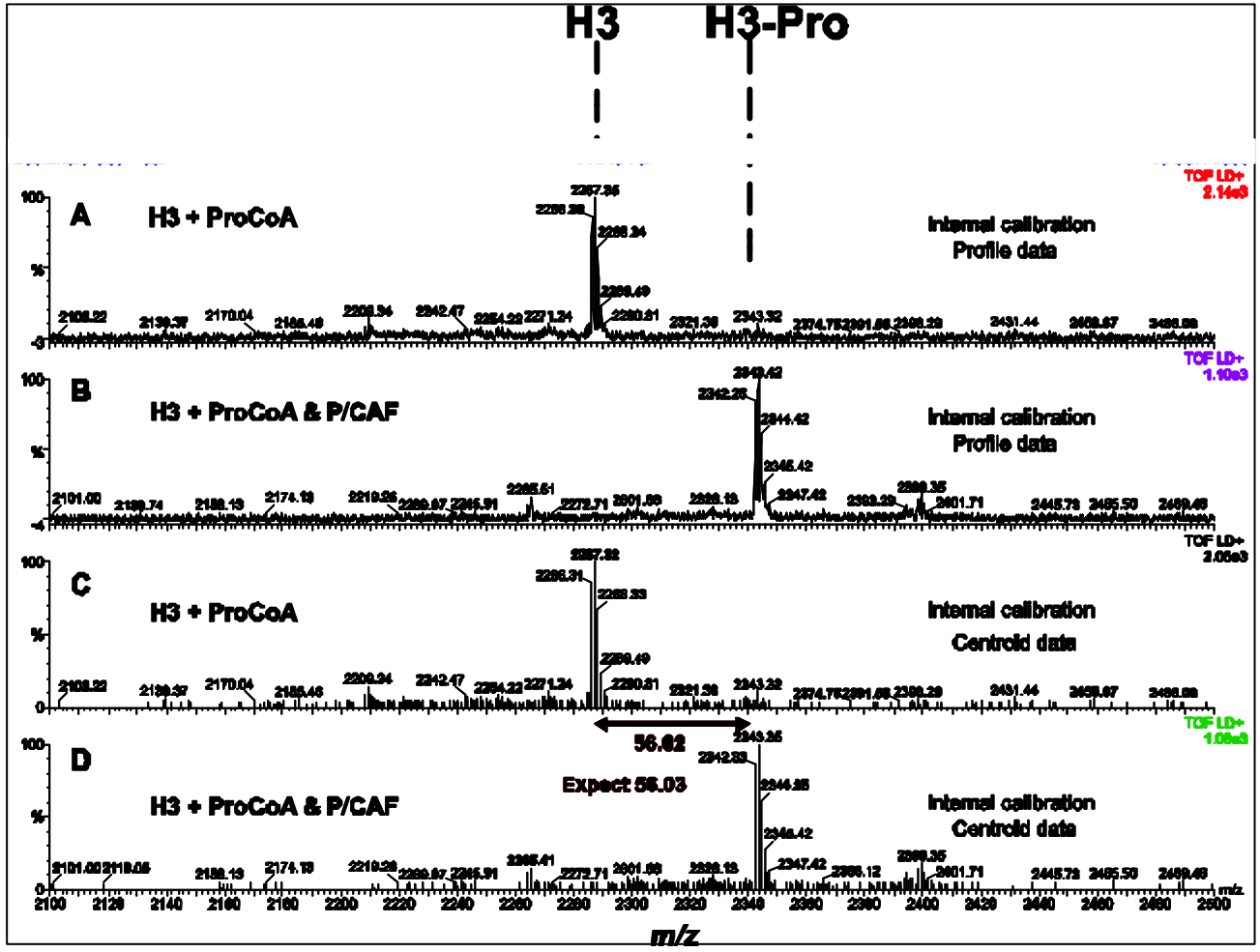

Figure S1. MALDI-TOF MS spectrum of the histone H3 tail peptide (ARTKQTARKSTGGKAPRKQLC) incubated with propionyl-CoA and (B & D) P/CAF HAT enzyme and (A & C) without the enzyme. *Conditions:* 1 h at pH 7.5 and 25 °C, 3.8 μM P/CAF, 300 μM H3 peptide and 400 μM propionyl-CoA. Shown are the profile and centroid data, both with internal calibration. A small amount of di-propionylation can be detected at *m/z* 2398.3; this site of labelling was not determined.

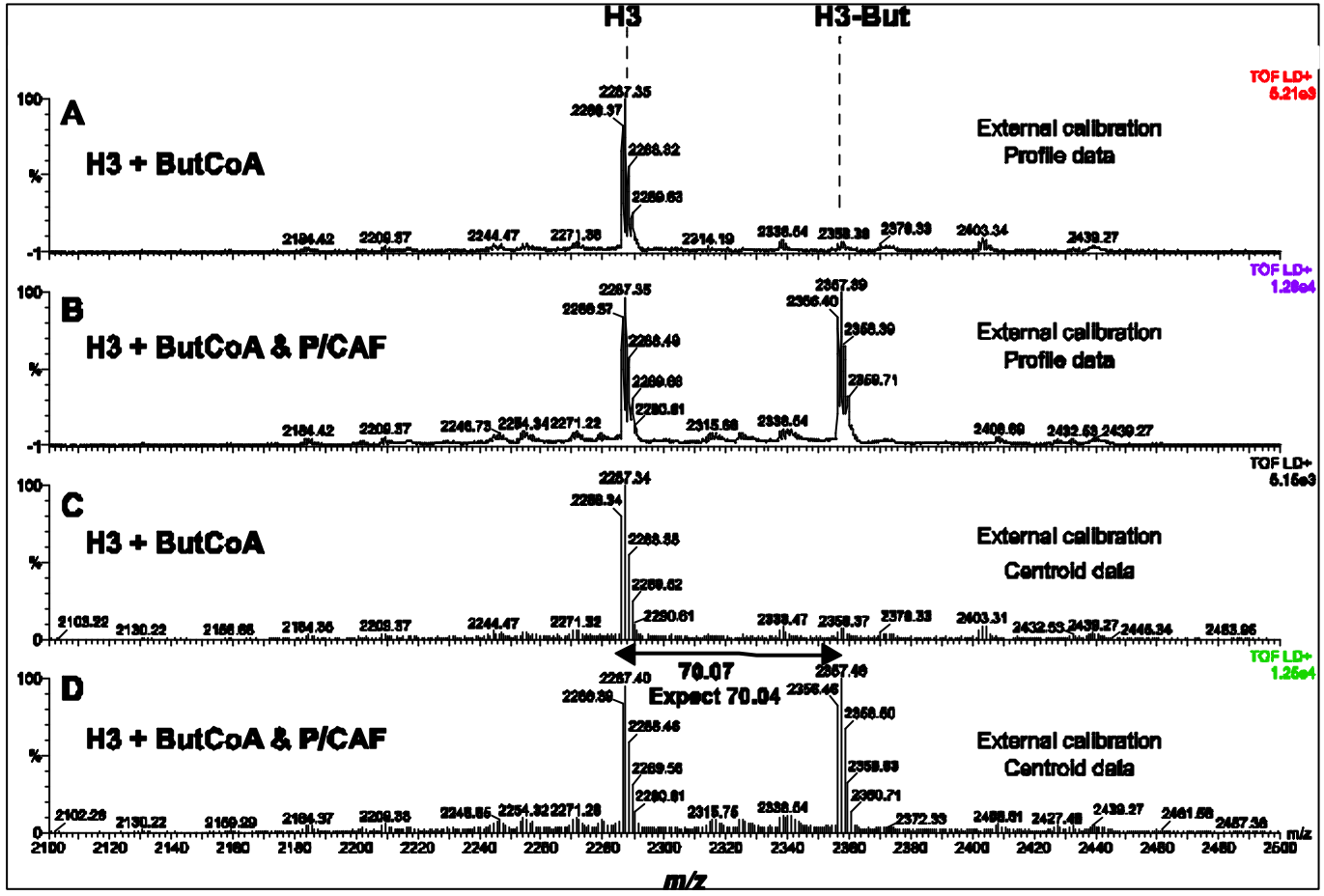

Figure S2. MALDI-TOF MS spectrum of the histone H3 tail peptide (ARTKQTARKSTGGKAPRKQLC) incubated with butyryl-CoA and (B & D) P/CAF HAT enzyme and (A & C) without the enzyme. Profile and centroid data are shown, both with close external calibration. Note that not all of the H3 peptide is butyrylated which is in agreement with the low activity measured for butyryl-CoA as acyl donor; there is also a trace of dibutyrylation (*m/z* 2426.48). The control (-P/CAF) shows a trace of butyrylation, whose site is undetermined. *Conditions*: 4 h at pH 7.5 and 25 °C, 10 µM P/CAF, 300 µM H3 peptide and 600 µM butyryl-CoA.

Acyl-lysine substrate tolerance of histone deacetylase was tested using a small synthetic substrate, Boc-L-Lys(N^6-acyl)-MCA; were MCA=7-amino-4-methylcoumarin. The acyl groups acetyl, propionyl, butyryl, pivalinyl and isobutyryl were tested as substrates with rat liver histone deacetylase (from Calbiochem) and recombinant human histone deacetylase HDAC 8. Both histone deacetylases showed activity with the acetyl substrate. The rat histone deacetylase, in addition, displayed activity with the propionyl substrate. The other compounds were not accepted as substrates by any of the enzymes.[2]

References

[1] Roth, S. Y.; Denu, J. M.; Allis, C. D., Histone acetyltransferases. *Ann. Rev. Biochem.* **2001,** 70, 81-120.

[2] Riester, D.; Wegener, D.; Hildmann, C.; Schwienhorst, A., Members of the histone deacetylase superfamily differ in substrate specificity towards small synthetic substrates. *Biochem. Biophys. Res. Commun.* **2004,** 324, 1116-1123.