Supplemental data

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Inhibitory effects of Spd(N^1)-CoA (1a) and Spd(N^8)-CoA (1b) on histone acetyltransferase activity in solubilized chromatin. The two positional isomers of Spd-CoA, i.e. Spd(N^1)-CoA or 1a, and Spd(N^8)-CoA or 1b, synthesized following a previously reported regioselective pathway (1) were selected as chemically well-defined HAT inhibitors. As previously reported Spd-CoA synthesized in a non-regioselective manner (thus likely a mixture including 1a and 1b) acts as a potent inhibitor of histone acetylation in chromatin either in isolated nuclei or with solubilized chromatin (2). We thus undertook a comparative study of 1a and 1b with regard to their efficiencies at inhibiting histone acetyltransferase activity in solubilized chromatin.

As apparent in Figure S1(A), the two positional isomers 1a and 1b, display practically identical effects under in vitro conditions using isolated polynucleosomes (PN) in solution as a chromatin substrate. Kinetic enzymatic measurements were carried under well-defined conditions with regard to the cofactor/substrate molar ratio while the concentration, as well as the molecular multiplicity, of the acetylating enzyme remain unknown (endogenous activity associated with the PN material obtained at low ionic strength: see Detailed Methods below).

A preliminary study showed that the endogenous HAT activity in the PN material is time-dependent with a low activity at "0" hour, just after PN preparation, and then becomes optimal at 24-48 h of storage (Fig.S1.B)) and it was maintained for a period of some 27 days (not shown). However, an electron microscopy analysis (not shown; see Detailed Methods below) of the PN material during time showed that the structural integrity of the PN filaments is significantly altered after 14 days of storage (cold room). For this reason we decided to carry out the inhibition measurements with compounds 1a and 1b with a PN material previously kept 24 h or 48 h (in the cold room) before starting the kinetic studies at 37°C.

Detailed Methods.

Preparation of polynucleosomes. Polynucleosomes were prepared from rat liver nuclei as previously described ((3) and references therein), and verified to correspond to some 30-50 nucleosomes in length by electron microscopy using the BAC (benzyldimethyl-n-hexadecylammonium chloride)-method for chromatin spreading (4).

Kinetics of in vitro enzymatic acetylation of the histones in the polynuclesomes. The solubilized polynucleosomes in buffer A (DEA 20 mM, EDTA 0.2 mM, Na-butyrate 20 mM, PMSF 1 mM, at pH 8.7) were radioactively labeled using [1-^{14}C]acetyl-CoA at 37°C as a function of time through enzymatic catalysis in the presence of an endogenous histone acetylating activity in the polynucleosomes. Typically, 3.7 μL of radioactive acetyl-CoA (0.2 μCi) were added to 1 mL of PN at 6 A260 units in buffer A. This corresponds to ~ 5 nmole of acetyl-CoA for ~2.5 nmole of histone octamer, i.e. a molar ratio [acetyl-CoA]/[histone octamer] of ~ 2 (assuming that 28 acetylation sites are present in the histone N tails of the nucleosome octamer this corresponds to a mean molar ratio cofactor per each histone acetylation site of ca. 0.1). At defined times (see Figure S1) aliquots of 100 μL were pipeted from the incubation solution and added with 500 μL of 20% perchloric acid. Each aliquot was applied to a Sartorius filter disc and the latter was rinsed with 2x10 mL of 5% perchloric acid. The dried discs were then counted by standard techniques in Picofluor TM 15 Packard (Frankfurt) scintillation fluid. Data are given in disintegrations per minute (dpm). Under the conditions used (pH ~ 8.7) possible non-specific non-enzymatic acetylation never exceeded 5% of the transferered radioactivity (see Fig.6 in (3)). Kinetic curves were thus determined either in the absence

(control profiles) or in the presence of the inhibitors 1a or 1b at 0.1 mM (Fig.S1) and 1 mM (not shown). The percentage of inhibition is expressed as the ratio 100 x [dpm(0)-dpm(i)]/dpm(0) where dpm(0) and dpm(i) are the disintegrations per minute in the absence and in the presence of the inhibitor, respectively, for a given time of incubation.

1. Roblot, G., Wylde, R., Martin, A., Parello, J. Regioselective synthesis of inhibitors of histone acetyl transferase covalently linking spermidine to the S-terminus of coenzyme A and fragments. Tetrahedron, *49:* 6381-6398, 1993.
2. Cullis, P. M., Wolfenden, R., Cousens, L. S., and Alberts, B. M. Inhibition of histone acetylation by N-[2-(S-coenzyme A)acetyl] spermidine amide, a multisubstrate analog. J Biol Chem, *257:* 12165-12169, 1982.
3. Dumuis-Kervabon, A., Encontre, I., Etienne, G., Jauregui-Adell, J., Mery, J., Mesnier, D., and Parello, J. A chromatin core particle obtained by selective cleavage of histones by clostripain. Embo J, *5:* 1735-1742, 1986.
4. Thoma, F., Koller, T., and Klug, A. Involvement of histone H1 in the organization of the nucleosome and of the salt-dependent superstructures of chromatin. J Cell Biol, *83:* 403-427, 1979.

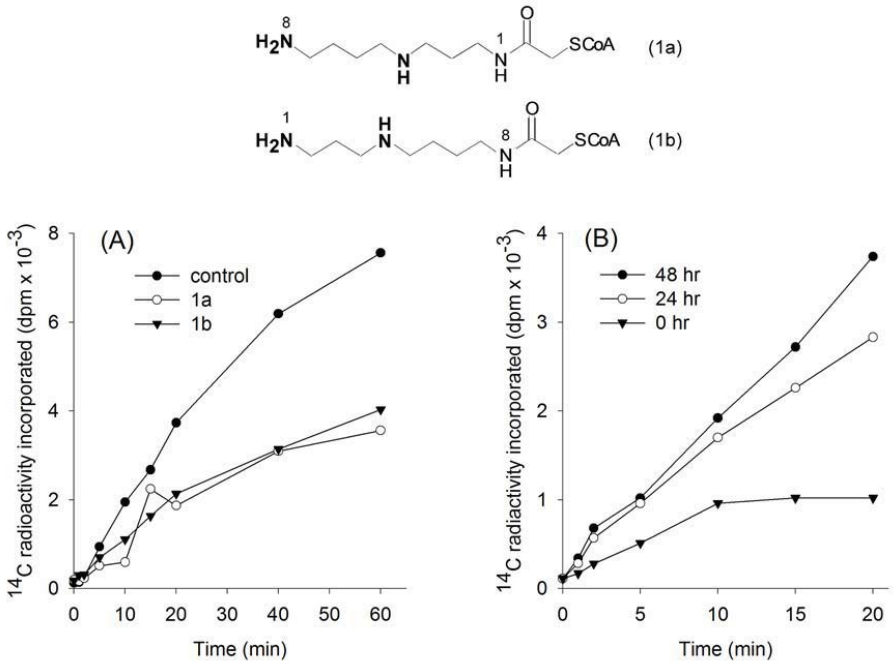

FIGURE LEGEND

Figure S1. (A) Comparison of the inhibitory effects of 1a and 1b on the acetylating enzymatic activity of solubilized chromatin (polynucleosomes with 30-50 nucleosomes including an endogenous HAT activity), assayed 48 hours after its preparation in the absence of inhibitor (control profile) and in the presence of 0.1 mM 1a or 1b (profiles 1a and 1b, respectively). (B) Time-dependence of the endogenous HAT activity as established by kinetic measurements with PN preparations at varying times of storage (cold room), i.e. at 0 hr (immediately after the PN preparation), after 24 hr and after 48 hr. All kinetic measurements (in A and B) were carried out at 37°C (see Detailed Methods below). The ^{14}C radioactivity incorporated in the histones is given in dpm (it corresponds to a transfer of acetyl groups from [1-^{14}C]acetyl-CoA onto the histone substrate of ~ 20% of the total radioactivity used after

60 min incubation in the absence of inhibitor: see control profile in A). Typically, ~ 50% inhibition is observed at 0.1 mM inhibitor (53% and 47%, with 1a and 1b, respectively) and ~90% at 1 mM inhibitor (89% and 87%, with 1a and 1b, respectively) after 60 minutes incubation.