EXTRACTION AND PARTIAL PURIFICATION OF TWO HISTONE-SPECIFIC TRANSACETYLASES FROM RAT LIVER NUCLEI.

Dieter Gallwitz

Physiological Chemical Institute
University of Marburg (Lahn), Germany

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SUMMARY

Two histone-specific transacetylases with an approximate molecular weight between 80,000 and 90,000 were solubilized from rat liver nuclei by sonication in the presence of 1 M ammonium sulfate and 20% glycerol followed by precipitation with 3.5 M ammonium sulfate. A 50-fold purification was achieved by DEAE-cellulose and Sephadex G-200 chromatography. The acetate transfered by the enzymes from acetyl coenzyme A to histones is N-linked. The pH-optimum of the enzymatic acetylation is approximately 8.6.

The occurence of NH_2-terminal acetylserine residues in histones has been known for some time (1,2). In addition, ε-N-acetyllysine has been identified in histones after *in vitro* incubation of isolated thymus nuclei in the presence of labelled acetate (3). From the recent sequencing studies it is evident that in the arginine-rich histone IV (f $2a_1$) from calf thymus and pea seedlings a specific lysine residue is partially acetylated in the ε-N-position (4,5,6). This modification of a specific lysine residue seems to be an intranuclear process and takes place on already completed protein molecules (7,8). Methylation and phosphorylation of specific amino acid residues are other modifications occuring in the highly basic portion of histone IV and may have some influence on the binding of the histone to DNA.

To establish a basis for an understanding of the biological

meaning of these modification reactions the study of the enzymes involved should prove helpful. It has already been shown that acetone fractionation of pigeon (9) and rat liver (10) yields proteins catalyzing the transfer of acetate from acetyl coenzyme A to histones more or less unspecifically.

The extraction of two histone-specific transacetylases and some properties of the partially purified enzymes are reported here.

EXPERIMENTAL

Liver nuclei were prepared from male 120-180 g BR II Wistar rats initially by a modified procedure of Chauveau et al. (10,11), in the later stage of the study by use of the detergent Triton X 100 (12). The nuclei were homogenized in medium A (Tris-HCl, 75 mM, pH 7.6, $MgCl_2$, 1 mM, EDTA, 0.25 mM, 2-mercapto-ethanol, 5 mM, ammonium sulfate, 1 M, 20% glycerol, v/v), the viscous homogenate immersed in an ice bath was sonicated in 40 ml aliquots with 25 2 sec bursts (Branson sonifier, 20 kHz, 125 Watt) and subsequently stirred at 2° for 2 hrs. Crystalline ammonium sulfate was slowly added to yield a final concentration of 3.5 M. After stirring for 1 hr in the cold the extract was centrifuged for 90 min at 120,000 x g_{av}. The protein pellets were suspended in medium B (Tris-HCl, 15 mM, pH 7.6, $MgCl_2$, 1 mM, EDTA, 0.25 mM, NH_4Cl, 10 mM, 2-mercaptoethanol, 5 mM, 20% glycerol, v/v) and stirred for 60 min. To remove the ammonium sulfate the supernatant obtained after centrifugation at 40,000 x g_{av} for 10 min was either dialyzed for 40 hrs against medium B or passed over Sephadex G-25 equilibrated with medium B. After this procedure about 20% of the extracted proteins remained in solution and were adsorbed onto DEAE-cellulose previously equilibrated with medium B. Proteins were eluted from the column

with a linear 0.01-0.3 M NH_4Cl gradient. The fractions having enzymatic activity were pooled, the proteins precipitated with 3.5 M ammonium sulfate and passed over Sephadex G-200 equilibrated with medium B.

Enzyme activity was determined as the incorporation of ^{14}C-acetate from acetyl coenzyme A into hot trichloroacetic acid precipitable material. The incubation medium contained 15 µmoles of Tris-HCl, 0.35 µmole of $MgCl_2$, 0.1 µmole of EDTA, 3.5 µmoles of NH_4Cl, 25 µmoles of KCl, 2 µmoles of 2-mercaptoethanol, 15% glycerol (v/v), 0.025 µC of ^{14}C-1-acetyl coenzyme A (spec. act. 56.6 mC/mM), 50 µg substrate and 2 to 25 µg enzyme protein in a total volume of 0.5 ml. At the end of the incubation time (20 min at 37°) 1 mg of albumin was added as carrier and 0.2 ml aliquots of the incubation mixture were precipitated onto filter paper discs in ice cold 25% trichloroacetic acid. The filters were treated and counted as described (10).

Histones were prepared from purified rat liver or thymus nuclei after extensive washing with 0.14 M NaCl. Extraction was achieved by 0.25 N HCl and subsequent precipitation in 10 vol of acetone. Protein was estimated according to Lowry et al. (13). ^{14}C-1-acetyl coenzyme A (spec. act. 56.6 mC/mM) was purchased from New England Nuclear Corporation.

RESULTS AND DISCUSSION

The DEAE-cellulose elution profile of the soluble enzyme preparation from rat liver nuclei is shown in Fig. 1. There is one major peak of activity with an intimate shoulder eluting at about 0.09 M NH_4Cl. The purification in this step was 6- to 8-fold.

The enzyme could be further purified by chromatography on Sephadex G-200 as seen in Fig. 2. There are two distinct peaks of activity which may point to the existence of at least two

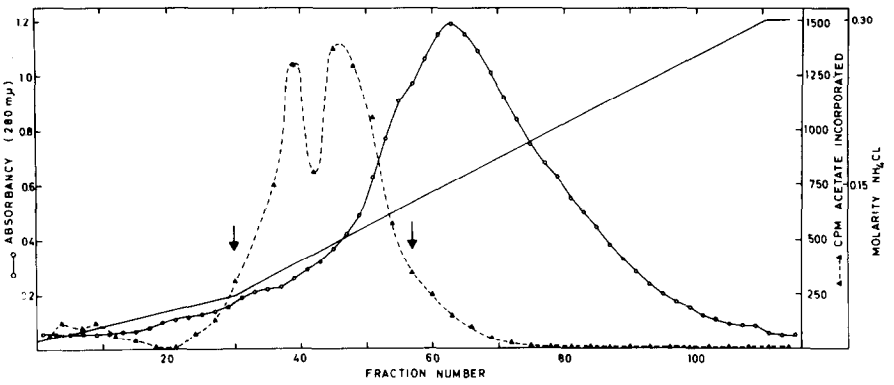

Fig. 1. DEAE-cellulose chromatography of soluble nuclear histone transacetylase. 150 mg protein were adsorbed to a 2 x 9 cm DEAE column and eluted with a linear 350 ml 0.01 - 0.3 M NH_4Cl gradient at a flow rate of 40 ml/hr.

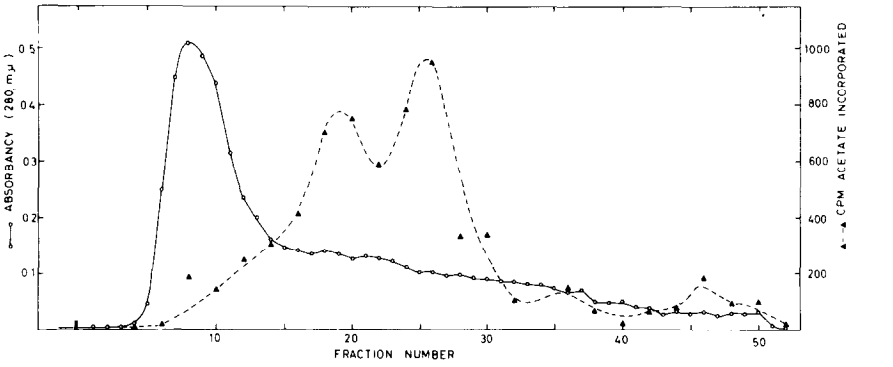

Fig. 2. Sephadex G-200 chromatography of nuclear histone transacetylase. 25 mg protein previously chromatographed on DEAE-cellulose (fractions pooled as indicated by arrows in Fig. 1) were chromatographed on a 1.8 x 90 cm Sephadex G-200 column at a flow rate of 5 ml/hr.

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different histone acetylating enzymes. According to gel filtration the molecular weight of the two enzymes is approximately 80,000

to 90,000. The final purification compared with the extract adsorbed to DEAE-cellulose was about 50-fold.

The enzymes from Sephadex G-200 transfer acetate from acetyl coenzyme A specifically to histones and predominantly to the arginine-rich ones. Other basic as well as nonbasic proteins tested were not acetylated (Table 1). Under the test conditions used no absolute specificity of the two enzymes has yet been found for one specific histone fraction.

Table 1. Specificity of nuclear histone transacetylase. 5 µg of the enzyme purified on Sephadex G-200 were incubated at pH 8.6 with 50 µg acceptor protein as described in Methods.

Acceptor protein	µµmoles acetate incorporated
	50 µg acceptor protein
Total histone (rat liver)	22
Total histone (rat thymus)	25
Hemoglobin (bovine)	-
Cytochrome C (horse heart)	-
Lysozyme	-
RNase	-
Albumin (bovine)	-

In earlier studies it was observed that by raising the pH from neutral to alkaline histones were acetylated spontaneously by acetyl coenzyme A (8,10). This is obviously a chemical reaction and can be prevented by KCl at a final concentration of 50 mM. Under these test conditions pH 8.6 was optimal for the histone acetylating enzymes (Fig. 3).

In concentrations of 10^{-3} to 10^{-8} M adenosine 3',5'-cyclic monophosphoric acid, known to activate histone phosphorylating enzymes (14,15) was without any effect on the histone transacetylases.

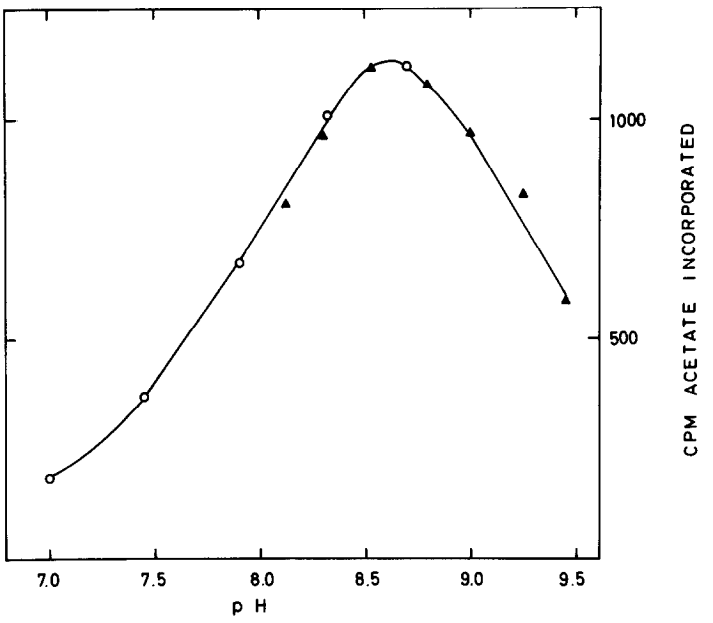

Fig. 3. pH-dependence of nuclear histone transacetylase. Tris-HCl buffer (o——o) and glycine-NaOH buffer (▲——▲) were used.

The stability of the incorporated acetate to the treatment in hot trichloroacetic acid shows that the acetyl groups are N-linked. The identification of the amino acid(s) acetylated is in progress.

This work has clearly established that enzymes which specifically acetylate histones are located in the cell nucleus. Since histone synthesis takes place in the cytoplasm (16,17,18) and is probably initiated with N-acetyl-seryl-tRNA (19) it is very likely not the N-terminal amino acid but an amino acid positioned internally in the histone molecule which is acetylated by the nuclear enzymes.

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REFERENCES

1. Phillips, D.M.P., Biochem. J. 87, 258 (1963).
2. Phillips, D.M.P., Biochem. J. 107, 135 (1968).
3. Gershey, E.L., Vidali, G. and Allfrey, V.G., J. Biol. Chem. 243, 5018 (1968).
4. DeLange, R.J., Fambrough, D.M., Smith, E.L. and Bonner, J., J.Biol. Chem. 244, 319 (1969).
5. Ogawa, Y., Quagliarotti, G., Jordan, J., Taylor, C.W., Starbuck, C.W. and Busch, H., J. Biol. Chem. 244, 4387 (1969).
6. DeLange, R.J., Fambrough, D.M., Smith, E.L. and Bonner, J., J. Biol. Chem. 244, 5669 (1969).
7. Allfrey, V.G., Faulkner, R. and Mirsky, A.E., Proc. Nat. Acad. Sci. U.S. 51, 786 (1964).
8. Gallwitz, D. and Sekeris, C.E., Z. Physiol. Chem. 350, 150 (1969).
9. Nohara, H., Takahashi, T. and Ogata, K., Biochim. Biophys. Acta 154, 529 (1968).
10. Gallwitz, D., Biochem. Biophys. Res. Commun. 32, 117 (1968).
11. Chauveau, J., Moule, Y. and Rouiller, C., Exptl. Cell Res. 11, 317 (1956).
12. Beato, M., Homoki, J. and Sekeris, C.E., Exptl. Cell Res. 55, 107 (1969).
13. Lowry, O.H., Rosebrough, J.N., Farr, A.L., and Randall, R.J., J. Biol. Chem. 193, 265 (1951).
14. Langan, T.A., Science 162, 579 (1968).
15. Jergil, B. and Dixon, G.H., J. Biol. Chem. 245, 425 (1970).
16. Robbins, E. and Borun, T.W., Proc. Nat. Acad. Sci. U.S. 57, 409 (1967).
17. Gallwitz, D. and Mueller, G.C., Science 163, 1351 (1969).
18. Gallwitz, D. and Mueller, G.C., J. Biol. Chem. 244, 5947 (1969).
19. Liew, C.C., Haselett, G.W. and Allfrey, V.G., Nature 226, 414 (1970).