ORGAN SPECIFICITY OF HISTONE ACETYLTRANSFERASES

D. GALLWITZ

Physiologisch-Chemisches Institut der Universität Marburg, 355 Marburg, Germany

Received 26 January 1971

1. Introduction

Enzymatic modification of specific amino acid residues of histones, such as acetylation, methylation and phosphorylation, have been implicated to be of functional significance since these structural changes occur on or near the potent binding sites of histone molecules to DNA [1–5].

The arginine-rich histone $f2a_1$ (IV) of calf thymus is acetylated in the *ε-N*-position of the lysyl residue 16 [2] and in pea seedlings this histone, in addition to residue 16, is partially acetylated at a second lysyl residue at position 5 or 8 [4]. Sung and Dixon have recently demonstrated multiple sites of acetylation (lysines 5, 8, 12 and 16) occurring in the same histone $f2a_1$ from trout testis [5]. Furthermore, two out of 14 lysyl residues of calf thymus histone f3 (III) are partially modified by acetylation [6]. Clearly, multiple sites of acetylation at specific positions within histone molecules very likely require a specific enzymatic machinery located in the cell nucleus where these modification reactions take place [1, 7, 8, 9].

For the first time we have isolated and partially purified enzymes from rat liver nuclei active in transferring acetate from acetyl coenzyme A specifically to histones [10, 11]. These studies gave the first indication of the existence of more than one histone acetyltransferase in the cell nucleus.

In the present communication we describe multiple histone acetylating enzymes isolated from purified nuclei of three different rat organs, liver, thymus, and kidney. Two enzymes from liver and kidney and three from thymus have been separated on DEAE-cellulose. The differing chromatographic patterns of the histone acetyltransferases from organ to organ indicate a certain organ specificity of these enzymes.

2. Materials and methods

1-^{14}C-Acetyl coenzyme A (spec. act. 56.6 mCi/mmole) was purchased from New England Nuclear Corporation.

Nuclei from liver, thymus and kidney were prepared from male 100–120 g BR II rats by a modified Chauveau procedure [12] as described earlier [10]. All three organs were taken from the same animal (usually 15 for one experiment) and the nuclei were purified simultaneously.

The acetyltransferases were extracted from the nuclear preparations in principle as described recently [11]. Briefly, the isolation steps consisted of homogenization in the presence of 1 M ammonium sulfate, sonication, ammonium sulfate precipitation (3.5 M) and desalting on Sephadex G-25. The three protein extracts after Sephadex G-25 were adsorbed on three 2.2 × 6 cm DEAE-cellulose columns packed simultaneously under the same pressure and equilibrated with medium A (tris-HCl pH 7.9, 15 mM, NH_4Cl 10 mM, EDTA 0.25 mM, 2-mercaptoethanol 5 mM, glycerol 20%). The proteins adsorbed to DEAE-cellulose were then eluted simultaneously from the columns with the same linear 0.01–0.3 M ammonium chloride gradient in medium A at a flow rate of 35 ml/hr.

Enzyme activity was estimated at pH 8.7 as described previously using total histone from rat thymus if not otherwise stated [11]. Histones from different rat organs were isolated from purified nuclei as des-

North-Holland Publishing Company – Amsterdam

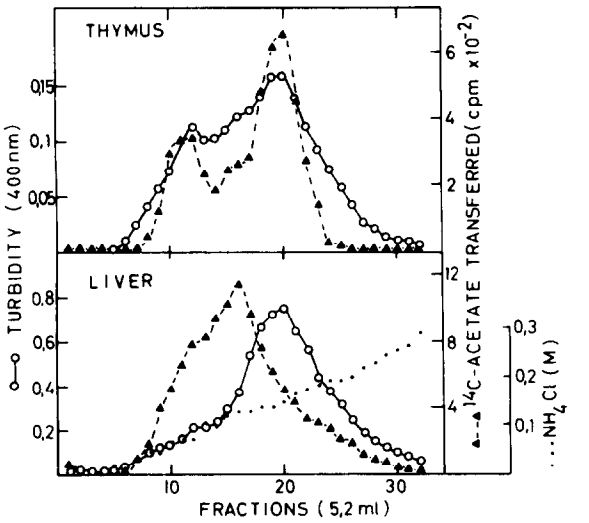

Fig. 1. Separation on DEAE-cellulose of histone acetyltransferases from rat thymus and liver nuclei. Proteins adsorbed onto DEAE-cellulose (2.2 × 6 cm) were eluted with the same linear 0.01–0.3 M NH_4Cl gradient at a flow of 35 ml/hr.

cribed [11] and histone fractions were prepared from calf thymus according to Johns' method I [13]. Protein was estimated either according to Lowry et al. [14] or by measuring the turbidity in 1.1 M TCA at 400 nm [15].

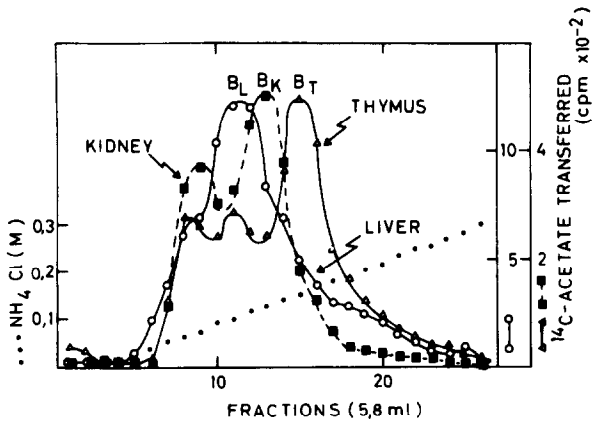

Fig. 2. Elution patterns from DEAE-cellulose of histone acetyltransferases from rat thymus, liver and kidney nuclei. The three DEAE-columns were packed, loaded and eluted simultaneously. For a beter comparison, the profiles of enzyme activity are superimposed.

Table 1
Substrate specificity of acetyltransferases B from different rat organs.

Source of acetyltransferase B	^{14}C-acetate transferred (cpm) 50 μg of histone fraction	
	f2a	f3
Liver	2,230	2,645
Kidney	1,170	256
Thymus	1,132	22

In a final volume of 0.5 ml 50 μg of either calf thymus histone fraction f2a or f3 were incubated with 10 μg of enzyme protein (after DEAE-cellulose chromatography) in the presence of 0.025 μCi 1-^{14}C-acetyl coenzyme A. Incubation time 20 min at 37°.

3. Results and discussion

The elution patterns of proteins and histone acetyltransferase activities of rat liver and thymus nuclei are presented in fig. 1. In the liver, one major peak of activity at 0.11 M NH_4Cl with a reproducible shoulder at 0.075 M NH_4Cl is obtained, as already described [11]. From thymus three peaks of histone acetylating activity are detectable at ammonium chloride concentrations of 0.065, 0.10 and 0.15 M. All enzymes from rat thymus have been further purified, details will be reported in a forthcoming paper.

Another experiment is depicted in fig. 2 in which the patterns of histone acetylating activities from rat liver, thymus and kidney are superimposed. Again, three thymus and two liver and kidney enzyme peaks are obtained, the kidney acetyltransferases eluting at 0.075 and 0.125 M NH_4Cl, respectively. All enzymes from liver, kidney and thymus showed approximately the same affinity for either liver, kidney or thymus total histones.

The enzymes from the three tissues eluting last from the DEAE-cellulose columns and having the highest activities have been designated as histone acetyltransferases B. The B-enzymes from the three organs exhibit different specificities for calf thymus histone fractions f2a (in which histone f2a$_1$ amounts to about 60%) and f3 which are known to be the histone fractions containing acetylated lysyl residues. From table 1 it can be seen that the B-enzymes from thymus and kidney have a definite specificity for

histone fraction f2a whereas for the liver enzyme both, histone fraction f2a and f3, serve as substrates nearly equally well. It is possible that histone acetyltransferase B from liver is a mixture of two enzymes, one specific for histone fraction f2a, the other for fraction f3. No pronounced preference for different histone fractions has been detected for the other enzymes at this stage of purification. However, the enzyme from thymus eluting first from DEAE-cellulose after extensive purification preferentially acetylates histone f3 (our unpublished results).

The following conclusions which can be drawn from these experiments: purified nuclei from different rat organs contain at least two or three histone acetyltransferases which are separated by DEAE-cellulose chromatography. The enzymes from different organs exhibit a different elution pattern from the DEAE-column suggesting structural differences. One enzyme from thymus as well as from kidney shows specificity towards acetylation of histone fraction f2a. Since the primary structure of most of the corresponding histones from different species and organs are most likely identical [2, 4, 16], except perhaps histones I [17, 18], differences of the enzymes acetylating the same histones from different organs may possibly be explained by different sites in corresponding histones acetylated by these enzymes. In accordance with this assumption are the findings, already mentioned in the introduction, that histone $f2a_1$ from calf thymus, pea and trout testis, having the same primary structure at the NH_2-terminal region in which acetylation occurs, can be acetylated at one, two or four different lysyl residues respectively [2, 4, 5]. In this respect it will be of interest to explore whether different enzymes are required to acetylate two or more sites in the same histone molecule of a given organ.

Organ specificity of histone acetyltransferring enzymes would also be compatible with the proposed role of histone acetylation in the complex process of gene regulation in higher organisms.

Acknowledgements

The author is grateful to Prof. Dr. P. Karlson for his interest and support and to Mrs. G. Fröhlich for excellent technical assistance. This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

References

[1] V.G. Allfrey, Federation Proc. 29 (1970) 1447.
[2] R.J. DeLange, D.M. Fambrough, E.L. Smith and J. Bonner, J. Biol. Chem. 244 (1969) 319.
[3] Y. Ogawa, G. Quagliarotti, J. Jordan, C.W. Taylor, W.C. Starbuck and H. Busch, J. Biol. Chem. 244 (1969) 4387.
[4] R.J. DeLange, D.M. Fambrough, E.L. Smith and J. Bonner, J. Biol. Chem. 244 (1969) 5669.
[5] M.T. Sung and G.H. Dixon, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1616.
[6] R.J. DeLange, E.L. Smith and J. Bonner, Biochem. Biophys. Res. Commun. 40 (1970) 989.
[7] E.L. Gershey, G. Vidali and V.G. Allfrey, J. Biol. Chem. 243 (1968) 5018.
[8] D. Gallwitz and C.E. Sekeris, Physiol. Chem. 350 (1969) 150.
[9] D. Gallwitz, Physiol. Chem. 351 (1970) 1050.
[10] D. Gallwitz, Biochem. Biophys. Res. Commun. 32 (1968) 117.
[11] D. Gallwitz, Biochem. Biophys. Res. Commun. 40 (1970) 236.
[12] J. Chauveau, Y. Moule and C. Rouiller, Exptl. Cell. Res. 11 (1956) 317.
[13] E.W. Johns, Biochem. J. 92 (1964) 55.
[14] O.H. Lowry, J.N. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[15] J.M. Luck, P.S. Rasmussen, K. Satake and A.N. Tsvetikov, J. Biol. Chem. 233 (1968) 1407.
[16] R.H. Stellwagen and R.D. Cole, Ann. Rev. Biochem. 38 (1969) 951.
[17] J.M. Kinkade, Jr., J. Biol. Chem. 244 (1969) 3375.
[18] M. Bustin and R.D. Cole, J. Biol. Chem. 244 (1969) 5286.