DISTRIBUTION OF N^G,N^G-DIMETHYLARGININE IN NUCLEAR PROTEIN FRACTIONS

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Received November 29,1976

SUMMARY: Nuclear proteins have been fractionated into five distinct classes according to their extractability from rat liver nuclei at different pH and salt concentrations. The fractions have been analyzed for their amino acid composition which shows the presence of N^G,N^G-dimethylarginine , in sizable amount , in non-histone nuclear proteins (NHNP) . This modification is most prominent in proteins which are found associated with rapidly-labeled heterogeneous RNA (HnRNP proteins).

Methylation of arginine , lysine and histidine residues is one of the major post-synthetic modifications which occur in nuclear proteins (1). Histones normally contain measurable amounts of N^ϵ-methyllysine and N^ϵ,N^ϵ-dimethyllysine , while 3-N-methylhistidine and methylated arginine have been detected only by labeling with radioactive precursors (2 , 3). Conversely methylated arginine has been reported to be the only methylated amino acid present in non-histone nuclear proteins (4).

The results presented in this paper show that :

1) N^G,N^G-dimethylarginine is the main methylated arginine derivative found in NHNP while N^G-monomethylarginine is present in trace amounts.
2) Proteins associated with rapidly-labeled heterogeneous nuclear RNA (HnRNA) contain about 65% of the total N^G,N^G-dimethylarginine found in the cell nucleus.

Abbreviations used: NHNP, non-histone nuclear proteins; HnRNP proteins, nuclear proteins associated with HnRNA in 40S particles; HnRNA, rapidly-labeled heterogeneous nuclear RNA; PMSF, phenylmethylsulfonyl fluoride.

MATERIALS AND METHODS

Liver nuclei were isolated from male Wistar rats (150-200 gm) as previously described (5). Purified nuclei were suspended in 0.32 M sucrose , 3 mM $MgCl_2$ and aliquots were taken for protein determination and amino acid analysis (total nuclear proteins) . Nuclei were recovered by centrifugation at 1000xg for 10 minutes and washed 3 times with 5 volumes of 100 mM NaCl, 10 mM triethanolamine-HCl pH 7.0 , 3 mM $MgCl_2$, 1 mM dithiothreitol and 0.1 mM PMSF. The wash solutions were combined, lyophilized , dissolved in a small volume of water and dialyzed extensively against water. Aliquots were used for amino acid analysis and protein determination (nuclear wash pH 7). The nuclei were then suspended in 30 volumes of 100 mM NaCl , 10 mM triethanolamine-HCl pH 8.0 , 3 mM $MgCl_2$, 1 mM dithiothreitol and 0.1 mM PMSF and immediately centrifuged as before. The pellet was suspended in one volume of the same solution stirred gently for 30 min. at 4 C^{o}, and centrifuged , saving the extract. Three more 30 min extractions were carried out and the four extracts were combined , clarified by centrifugation at 2000xg. Aliquots of the extract were used for protein determination and amino acid analysis (nuclear extract pH 8) and the remaining suspension was used for the isolation of HnRNP particles . Acid soluble proteins were extracted from the pH 8 extracted nuclei with 0.25 M HCl and residual nuclear proteins were prepared following the procedure already described (6).

40S and 4-20S particles were obtained from the pH 8 extract essentially by the procedure of Samarina et al. (7).

Protein content in nuclear protein fractions was determined by the Lowry procedure (8).

Standard amino acid analyses were conducted in a model 120 C Beckman amino acid analyzer and methylated amino acids were separated following the method already described (3).

In isotope-labeling experiments , rats (150-200 gm) were injected with 0.5 mC/body weight of [methyl-^{3}H]-L-methionine (specific activity 3 C/mmole) or of [3-^{3}H]-L-arginine (specific activity (30C/mmole) and killed after one hour.

Radioactive amino acids were obtained from New England Nuclear, Boston,Mass. . Methylated amino acid standards were obtained from Calbiochem, La Jolla, California.

RESULTS

Identification of methylated arginines . The presence of N^{G}-methylated arginine has been already reported in acid hydrolyzates of nuclear proteins (4 , 9) and ribosomal proteins (10), but reports on nuclear proteins do not distinguish among the three naturally occurring methylated arginines (N^{G}-monomethylarginine, N^{G},N^{G}-dimethylarginine and N^{G},N'^{G}-dimethylarginine) (1).

Our experiments prove that NHNP contain N^{G},N^{G}-dimethylarginine as a major methylated derivative. Standards of methylated amino acids were separated as previously reported (3) by a technique

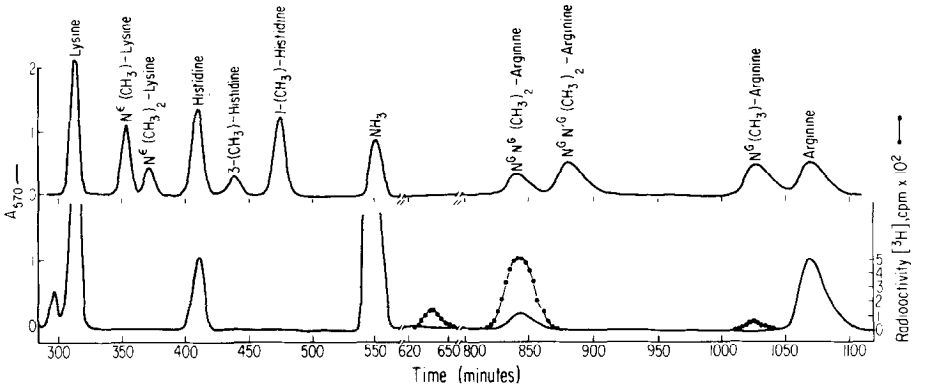

Fig.1 (top) Elution profile of a standard amino acid mixture containing the methylated derivatives of lysine, histidine and arginine separated under the conditions described by Gershey et al. (3); (bottom) chromatographic separation under the same conditions of the amino acids of rat liver 40S HnRNP proteins labelled in vivo with [methyl-^{3}H] -L-methionine , ninhydrin color intensity (*solid line*) and radioactivity (*dotted line*) are plotted against the time of elution.

that permits the simultaneous analysis of all methylated basic amino acids (Fig.1). The acid hydrolyzates of all NHNP fractions show the presence of a ninydrin positive peak coemerging with the peak of the authentic N^G,N^G-dimethylarginine marker. Fig.1 shows analysis of a HnRNP protein hydrolyzate. In a parallel set of experiments rats were injected with (^{3}H-methyl) labeled methionine (a known donor of methyl groups in the enzymatic methylation of proteins (11)).When NHNP fractions were analyzed for their methylated amino acids a peak of radioactivity was found in the position of N^G,N^G-dimethylarginine and a smaller peak was coincident with N^G-monomethylarginine (see Fig 1). No radiactivity was detectable in other regions of the chromatogram where methylated derivatives of lysine and histidine would have appeared, but a small unidentified radioactive peak appears between ammonia and N^G,N^G-dimethylarginine. NHNP fractions were also obtained from livers of rats that had been previously injected with radioactive arginine.

TABLE I : AMINO ACID COMPOSITION OF NUCLEAR PROTEIN FRACTIONS
moles / 100 moles total amino acid *

AMINO ACID	TOTAL NUCLEAR PROTEINS	NUCLEAR WASH pH 7	NUCLEAR EXTRACT pH 8	ACID SOLUBLE PROTEINS	RESIDUAL NUCLEAR PROTEINS	40S PARTICLES	4-20S PARTICLES
Aspartic Acid	8.52	9.99	11.43	6.34	7.90	10.83	11.17
Threonine	5.07	5.39	4.69	4.36	4.12	3.90	4.78
Serine	5.29	5.82	6.10	5.22	5.60	4.97	6.18
Glutamic Acid	11.04	12.84	12.66	11.27	11.33	10.94	14.04
Proline	7.36	5.94	3.53	4.65	4.09	6.81	7.87
Glycine	10.65	9.86	13.03	8.74	14.75	17.88	10.45
Alanine	8.53	7.49	6.98	11.23	6.17	5.35	7.51
Valine	6.21	6.57	6.91	6.77	7.04	5.68	3.95
Methionine	2.07	2.52	1.81	1.80	3.69	0.41	3.81
Isoleucine	3.59	4.31	3.89	3.99	5.43	2.70	3.84
Leucine	7.23	8.25	7.20	7.43	7.85	4.68	7.69
Tyrosine	2.02	2.26	2.87	1.62	3.41	3.33	2.30
Phenylalanine	2.31	3.45	4.10	1.41	3.16	3.26	3.29
Lysine	9.12	7.48	6.20	11.41	5.59	7.71	6.60
Histidine	2.16	2.43	1.72	2.07	1.87	2.51	1.86
Arginine	8.00	5.37	6.40	11.10	7.67	7.97	4.54
N^{ϵ}-monomethyl-lysine	n.d.@	n.d.@	n.d.@	0.14@	n.d.@	n.d.@	n.d.@
$N^{\epsilon},N^{\epsilon}$-dimethyl-lysine	traces	n.d.@	n.d.@	0.45@	n.d.@	n.d.@	n.d.@
N^{G},N^{G}-dimethyl-arginine	0.23	0.08	0.46	n.d.@	0.23	1.07	0.12

* Values not corrected for hydrolytic losses

@ n.d. = not detectable

These samples were hydrolyzed and analyzed as above. We have found (data not shown) that a large peak of radioactivity emerges in the position of N^G,N^G-dimethylarginine while a small peak is coincident with N^G-monomethylarginine.

N^G,N^G-dimethylarginine content in nuclear protein fractions .

Proteins from rat liver nuclei were fractionated according to the successive extraction procedures given in Materials and Methods. Each fraction was analyzed for standard amino acid composition and for its content of methylated amino acids (Table I) . It can be seen that only histones contain methylated lysines, which are not detectable in other protein fractions. Conversely, histones do not appear to be methylated at their arginine residues, while the remaining NHNP fractions contain different amounts of N^G,N^G-dimethylarginine. In particular , the pH 8 extract , which is known to contain HnRNP particles is considerably enriched in methylated arginine as compared to the total nuclear proteins. If 40S HnRNP particles are purified from the pH 8 extract by sedimentation in sucrose gradients, their proteins contain more than 1% N^G,N^G-dimethylarginine.

Table II lists the amount of methylated arginine in total nuclear proteins as compared to different nuclear protein fractions Most of the N^G,N^G-dimethylarginine (71%) is found in the pH 8 extract which is then almost totally recovered in the 40S HnRNP particles. It should be pointed out that this fraction represents only a small portion of the NHNP. Our data also indicate that on the average 12% of the arginine residues in the total HnRNP proteins are methylated (Table II).

DISCUSSION

We have presented evidence that NHNP are methylated only at the arginine residues and that no other basic amino acids are

TABLE II : DISTRIBUTION OF N^G,N^G-DIMETHYLARGININE IN NUCLEAR PROTEIN FRACTIONS

FRACTION ANALYZED	PROTEIN mg	N^G,N^G-DIMETHYL-ARGININE µmoles	ARGININE µmoles	N^G,N^G-DIMETHYL-ARGININE * %
Total nuclear proteins	227.5	3.23	164.7	1.92
Nuclear wash pH 7	29.3	0.20	14.3	1.38
Nuclear extract pH 8	51.0	2.28	23.9	8.96
Acid-soluble proteins	109.0	n.d.@	108.3	0
Residual nuclear proteins	37.8	0.80	26.7	2.91
40S particles	22.6	2.07	15.0	12.10
4-20S particles	23.7	0.26	9.9	2.57

* (µmoles N^G,N^G-dimethylarginine) / (µmoles N^G,N^G-dimethylarginine) + (µmoles arginine) x 100

@ n.d.= not detectable

modified in vivo . Although there are previous reports (4 , 9) indicating the presence of methylated arginine in nuclear proteins from rat liver we have shown that N^G,N^G-dimethylarginine is the main component. N^G-monomethylarginine can also be detected by radioactive tracer experiments and it may be the precursor of the dimethylated derivative.

By fractionating nuclear proteins into different classes we have also shown that most of the N^G,N^G-dimethylarginine found in the nucleus is in the proteins which are known to be complexed with HnRNA. The occurrence of dimethylarginine in HnRNA-associated proteins has been observed in other laboratories[1,2] but we have observed that this modification is not common to all proteins of nuclear 40S particles. Data to be published elsewere (12) indicate that a major HnRNP protein , purified by chromatography, contains 32% of the arginine residues as dimethylated amino acid. These observations open the question as why some HnRNP-associated proteins are specifically methylated at the arginine residues and to such a large extent.

ACKNOWLEDGEMENTS : This research was supported by grants from the National Foundation March of Dimes (1-440), the Rockefeller Foundation Program in Reproductive Biology and by U.S.Public Health Service (CA-14908)

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