# IDENTIFICATION OF POLY(ADP-RIBOSE) COVALENTLY BOUND TO HISTONE F1 *IN VIVO*

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SUMMARY ADPribose covalently bound to histone F1 has been isolated from rat liver. The mode of attachment is via the serine phosphate moiety of F1.

Histone F1 is known to contain phosphate as serine phosphate *in vivo* (1). It is also known that on incubating nuclei *in vitro* with NAD, poly(ADP-ribose) is found in the histone fractions (2). It was decided, therefore, to investigate the possibility of association between ADPribose and histone F1 *in vivo*.

## MATERIALS AND METHODS

Histone F1 was prepared from rat liver and separated from non-histone contaminants as previously described (3).

Enzymic hydrolysis of F1 by pronase and leucine amino-peptidase was performed by the method of Balhorn *et al.* (4).

Serine and serine-phosphate were estimated by the method of Spackman *et al.* (5).

Ribose was estimated using the orcinol reagent (6).

Phosphate was determined by the method of Bartlett (7) using 60% (w/v) perchloric acid as ashing agent.

Adenine was identified by thin layer chromatography

using the solvent system of Lane (8) and estimated by its absorption at 260nm.

RESULTS

An enzymic hydrolysate of histone F1 was chromatographed on a Dowex 50 column previously equilibrated with 50 mM HCl. Serine phosphate was retained on the column (4) and the effluent was applied to a Dowex-1-formate column (9). Amino acids were washed out with 0.3 M formic acid, and the nucleotide containing material eluted with 3 M formic acid. Ribose and phosphate were determined directly on the 3 M formic acid eluate. Two samples of the eluate were lyophilized and the residues taken up either in 0.5 M KOH and incubated for 30 min at 37° or in 2 M HCl and heated for 1 hr at 100°. The first of these two was used for the estimation of serine and the second for adenine (no other bases were present). The analyses of the eluate are given in Table 1. They indicate the

**TABLE 1**

**Analysis of nucleotide containing material bound to Histone F1.**

| | Rat liver |
|---|---|
| Serine | 0.31 |
| Ribose | 1.73 |
| Phosphate | 1.83 |
| Adenine | 0.89 |

**Figures represent nanomoles per mg protein. Analyses were performed as described in the text.**

presence of ADPribose bound to F1 via serine. If hydrolysis of the eluate by 2 M HCl for 1 hr at 100° is used instead of KOH, serine phosphate is found in place of serine. It is proposed therefore that the ADPribose is associated with F1 as a low molecular weight polymer, covalently bound to the histone via serine phosphate.

The ADPribose is removed quantitatively from the protein by 0.5 M KOH in 30 min at 37°, or by venom phosphodiesterase (9) but is not labile to neutral hydroxylamine (Table 2). It has been pointed out by Greenaway and Murray (10) that nucleotide material is a common contaminant of histone fractions

TABLE 2

**Release of ADPribose by neutral hydroxylamine.**

| | % counts remaining on F1 |
|---|---|
| Control | 100 |
| 0.4 M $NH_2OH$ | 82.5 |
| 4.0 M $NH_2OH$ | 72.4 |

**F1 was prepared from rat liver nuclei labelled with [$^3$H] adenosine NAD (2). It was incubated with hydroxylamine as indicated (pH 7.4) for 30 min at 30°. Counts are those remaining on F1 after dialysis against 0.5 M NaCl, then $H_2O$, each for 8 hrs at 0-4°.**

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and we therefore added the serine phosphate ADPribose to histone F1 which had been isolated from livers of rats injected with $^{32}$P phosphate. Complete separation of the two components was achieved by the chromatographic method used for the

isolation of the original F1 which contained the bound nucleotide.

The evidence presented here indicates that a further micromodification of a histone can be found *in vivo* in nuclei. We have also shown that in the resting normal liver more of this compound is present than in the S phase of regenerating liver, which suggests that it may have some physiological significance.

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