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Influence of histone acetylation on the modification of cytoplasmic and nuclear proteins by ADP-ribosylation in response to free radicals

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Abstract

Inhibition of histone deacetylase by addition of 5 mM *n*-sodium butyrate to the growth medium increases the utilization of [^{32}P]NAD$^+$ and ADP-ribosylation (ADPR) of total cellular proteins of V79, HeLa, mouse B16, mouse Fib/T and human T1 kidney cells by a factor of 1.2–2.3. When the ADP-ribosylase is challenged by exposing cells to damage by ·OH radicals (25 μM $CuSO_4$ 2.8 mM H_2O_2) ADPR increases by factors of 5.7–6.0 and 3.2–4.0 in normal and butyrated cells, respectively. Operation of the free radical generator is supported by the response to EDTA and radical scavengers. Densitometric analysis of autoradiographs from SDS-gels show that butyrate exposure increases basal ADPR-modification of histones from T1 cells by factors of 1.1–1.9. Addition of ·OH radicals increases the ADPR modifications of histones 4.4–8.7-fold in normal cells and 3.2–6.7-fold in butyrate exposed cells. Butyrate exposure elevates base level ADPR-modification and reduces subsequent ADPR-modification initiated by DNA damage. The results are consistent with the view that ADPR-modification and histone acetylation have overlapping functions and probably induce similar structural changes in chromatin.

Keywords: Histone acetylation; Histone ADP-ribosylation; Free radical; Haber-Weiss reaction

1. Introduction

ADP-ribosylation (ADPR) is a posttranslational modification which adds ADP-ribose units and negative charges to the target protein. This results in profound changes in the structure of the protein and induces alterations in macromolecular functions. The subject has been extensively reviewed by Hayaishi and Ueda [1], Williams and Johnstone [2], Shall [3], Althaus and Richter [4], and Boulikas [5,6]. Synthesis and degradation of ADP-ribose are rapid events with half lives of seconds to minutes [7] and are linked to DNA replication [8], cell differentiation [9–11] and gene expression [4]. It is also well established that the ADP-polymerase is activated by DNA strand breaks [12] and that this modification process is an important event in DNA repair [6]. ADPR of nuclear proteins alters chromatin structure [13] and renders DNA accessible to repair enzymes [14,15]. We have previously shown [16,17] that factors which regulate interparticle interaction and chromatin condensation, i.e., histone H1, Mg^{2+} and acetylation of core histone

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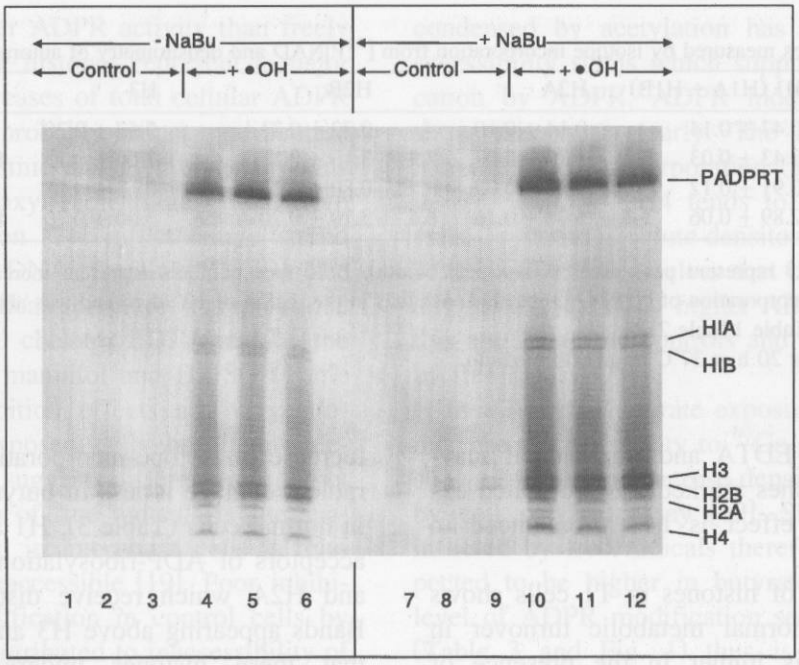

Fig. 1. Autoradiogram showing [^{32}P]ADPR of proteins of non-butyrated (lanes 1–6) and butyrated (lanes 7–12) T1 cells with $+OH^-$ (lanes 4–6 and 10–12) and without $-OH^-$ (lanes 1–3 and 7–9) treatment. Triplicate lanes of each group represent three independent experiments. PADPRT = PolyADP-ribosyltransferase.

cell diameter. Control cells reached confluency at $1.3–3.0 \cdot 10^6$ cells per culture tube depending upon cell type. Butyrate added 20 h before harvesting caused cells to remain subconfluent and cell numbers to be lower by 11–33% depending on cell type and cell duplication time. In butyrate exposed cells the diameter increased by 22–57% in V79 cells and by 10–14% in T1 cells. B16, HeLa and Fib/T cells showed increases ranging between 18 and 26%. Increase in nuclear diameter correlates with histone acetylation [19] and this was taken as an indication that the inhibition of the histone deacetylase by butyrate was effective. Hyperacetylation of H3 and H4 histones was also confirmed by urea gels (not shown).

Table 1 shows that ADPR in butyrated cells is higher by a factor of 1.2–2.3 than in control cells. When the two groups of cells were exposed to free radicals, isotope incorporation in control cells (A) was generally higher than in butyrated cells (B), (Table 2). The B/A ratio of ADPR modification was found to be 0.70 in T1 cells and 0.54 in V79 cells. This shows that butyrate exposure diminishes ADPR stimulated by free radicals. A similar trend is seen in the ADPR modification of histones (Table 3).

Table 1
Effect of sodium butyrate on ADPR of total cellular proteins of various cell lines (mean ± S.D.)

Cell line	ADPR (% of control)
V79	230 ± 9.8
HeLa	192 ± 5.4
B-16	118 ± 12.6
Fib/T	159 ± 16.0
T1	210 ± 19.0

^{32}P-incorporations in TCA precipitate were in the region of 10^4 cpm or approximately 0.5% of the activity offered as [^{32}P]NAD.

Table 2
Effect of free radical damage upon ADPR of total cellular proteins in normal and sodium butyrate exposed cells (mean ± S.D.)

Cell line	*n*	ADPR (% of control) non-butyrated A	ADPR (% of control) butyrated B	B/A
T1	7	573 ± 19	401 ± 9	0.70
V79	5	596 ± 23	320 ± 60	0.54

^{32}P-incorporations in TCA precipitate were in the region of 10^4 cpm or approximately 0.5% of the activity offered as [^{32}P]NAD.

Table 3
ADP-ribosylation of histones measured by isotope incorporation from [^{32}P]NAD and densitometry of autoradiograms

Conditions	H1 (H1A + H1B)		H2A		H2B		H3		H4	
− Nabu [a]	0.47 ± 0.14		0.14 ± 0.10		0.72 ± 0.31		0.57 ± 0.20		0.18 ± 0.18	
− Nabu + Cu^{2+}/H_2O_2	2.43 ± 0.03	**5.2**	1.10 ± 0.07	**7.9**	3.13 ± 0.11	**4.4**	2.66 ± 0.73	**4.7**	1.57 ± 0.08	**8.7**
+ Nabu	0.91 ± 0.12		0.22 ± 0.02		0.80 ± 0.15		0.63 ± 0.05		0.30 ± 0.05	
+ Nabu + Cu^{2+}/H_2O_2	2.89 ± 0.06	**3.2**	1.44 ± 0.16	**6.6**	3.70 ± 0.20	**4.6**	3.00 ± 0.70	**4.8**	2.02 ± 0.04	**6.7**

Values given (mean ± S.D.) represent peak areas (OD × peak width). Bold face numbers represent increase of histone modification induced by free radicals. Incorporation of [^{32}P]NAD into histones was in the region of 10^4 dpm and was about 50% of the total activity taken up by the cells (see Table 1Table 2).
[a] 5 mM sodium butyrate for 20 h at 37°C in growth medium.

In the presence of EDTA and free radical scavengers, ADPR diminishes markedly in butyrated exposed cells but this effect is less pronounced in control cells (Table 4).

The autoradiogram of histones in T1 cells shows that ADPR due to normal metabolic turnover in undamaged controls is higher in the presence of butyrate than in the absence of the inhibitor (Fig. 1, lanes 1–3 and 7–9). This is particularly clear in the densitometric tracings (Table 3) which show a near 2-fold higher isotope incorporation in the H1 group of histones as well as in H2A and H4 compared with the non-butyrated samples. Upon addition of free radicals to control cells the isotope incorporation increases markedly (Fig. 1, lanes 4–6). Butyrate exposed cells show an even higher level of ^{32}P-incorporation after the radical damage (Fig. 1, lanes 10–12).

Densitometry of peak areas further indicate that free radical damage causes prominent modification in all histones in the controls and in butyrate exposed cells. Butyrate exposed cells generally show a higher level of ADPR (Table 1). However, the relative increase of isotope-incorporation in response to free radical damage is less in butyrate exposed cells than in normal cells (Table 3). H1 and H2B are the major acceptors of ADP-ribosylation, followed by H3, H4 and H2A which receive distinctly less (Table 3). Bands appearing above H3 and H4 (Fig. 1) indicate that these histones undergo various levels of poly(ADP)-ribosylations but these modifications were not quantitated.

Table 4
ADP-ribosylation of total proteins in T1 cells in percent of control (mean ± S.D., $n = 7–15$) under various conditions

Conditions	25 μM $CuSO_4$ and 2.8 mM H_2O_2 [a]			
		EDTA [a] (100 μM)	Mannitol [a] (50 mM)	DMSO [a] (50 mM)
− Nabu	331 ± 32	302 ± 20	354 ± 97	276 ± 11
+ Nabu [b] (5 mM)	219 ± 10	84 ± 20	115 ± 7	108 ± 8

^{32}P-incorporations in TCA precipitate were in the region of 10^4 cpm or approximately 0.5% of the activity offered as [^{32}P]NAD.
[a] 30 min of 37°C in growth medium.
[b] 5 mM sodium butyrate for 20 h at 37°C in growth medium.

4. Discussion

The experimental system used here differs from that commonly used in that the manipulations are carried out with intact and attached cells, and that any kind of trauma arising from trypsinisation, scraping, centrifugation or isolation of nuclei is avoided. These manipulations markedly stimulate ADPR and often lead to erroneous results [22,36].

The problem of overstimulation or spurious stimulation of the enzyme systems thus has been avoided. Increase of the cold NAD concentration in the incubation medium from 1 μmol to 5 μmol did not alter the ^{32}P-incorporation indicating that NAD levels were not limiting (not shown). The low absolute level of isotope incorporation (~ 0.5% of the offered activity) also indicates excess of substrate.

We found 5 mM butyrate to be mildly toxic in V79 cells as indicated by the fact that cell proliferation in V79 cells was depressed and cell duplication time was increased by factor of 3–5. This is in agreement with other studies [19]. Butyrate exposed cells which essentially reside in G_0 utilize 1.2–2.3-times more [^{32}P]NAD^+ than control cells (Table 1). Observations on G_1 arrested erythroleukemic cells

show up to 4-fold higher ADPR activity than freely cycling cells [11]. Since histones represent a major cellular constituent, increases of total cellular ADPR (TCA precipitate) most probably reflect modification of histones and cytoplasmic and membrane proteins are lesser targets. Hydroxyl radicals generated from the Haber-Weiss reaction [24] inflict single strand breaks in DNA [25] and DNA-crosslinks [26,19]. The operation of the free radical generator was examined by addition of the metal chelator EDTA and by the free radical scavengers mannitol and DMSO (Table 4). The fact that inhibition effects are very pronounced in butyrate exposed cells but barely detectable in control cells, suggests differences in target accessibility. Production of free radicals is site specific [24] and chromatin in interphase cells is relatively condensed and inaccessible [19]. Poor inhibition of the ADPR-modification in control cells by EDTA and mannitol is attributed to inaccessibility of the metal binding DNA-sites and the short half life of the free radical. The fact that the inhibitors varied in effectivity (Table 4) also supports this view. In isolated chromatin 50 mM mannitol has been found to reduce the effects of Cu^{2+}/H_2O_2 on DNA-protein crosslinks by 50% [34]. The maximum inhibition achievable by 50 mM mannitol under conditions of unrestricted access measured by the in vitro oxidation of 2 hydroxybenzoic acid has been found to be 87% [35]. Intact cells cultured in butyrate and displaying relaxed chromatin show a level of 60% inhibition (Table 4). Viewed in the context of target accessibility, the inhibition experiments support the conclusion that the ADPR responses seen are due to free radical damage.

Following exposure to free radicals, ADPR in the total cellular TCA precipitate showed a 5.7–6.0-fold increase and a 3.2–4.0-fold increase in butyrated cells (Table 2). That butyrate exposure depresses the free radical induced ADPR strongly suggest that nuclear ADPR targets play a role. Butyrate inhibits histone deacetylase and results in histone hyperacetylation [37]. A consequence of this modification is poising and relaxation of chromatin structures [27]. ADPR modified chromatin remains decondensed even in the presence of Mg^{2+} [28]. Our observation that free radical induced ADPR modifications of all cellular proteins in control cells are higher than in butyrate exposed cells (Table 2) suggests that chromatin decondensed by acetylation has already acquired an accessibility status which suppresses further modification by ADPR. ADPR modification of histones show this very clearly. The free radical induced increase of ^{32}P-incorporation (Table 3), particularly in H1, H2A and H4 tends to be less in butyrated cells. From the absolute densitometry readings (Table 3) it is nevertheless clear that butyrated cells generally have a distinctly higher ADPR-modification and this applies to the controls and to the cells damaged by free radicals.

In V79 cells butyrate exposure has been found to increase the sensitivity to ^{60}Co-gamma-irradiation as shown by alkaline sucrose density centrifugation and by cell survival assay [19]. Single strand damage inflicted by free radicals therefore may also be expected to be higher in butyrated cells. The higher level of ADPR modification seen in butyrated cells (Table 3 and Fig. 1) thus is most likely due to increased demand for repair.

Autoradiograms further illustrate the differential effect of butyrate exposure and free radical damage. Free radical damage generates a marked increase of ADPR modification in non-butyrated and butyrated cells (Fig. 1), much higher modification levels being displayed in the butyrated group. Additional bands decreasing in mobility or labelling intensity appearing between H1 and H3 and between H4 and H2A are due to poly-ADPR-ribosylated H3, H2B and H4 species and such modifications have previously been observed in response to dimethyl sulfate [30], nucleases [31], and mitogens [32]. Free radical damage resembles damage by alkylating agents [33] in that histone H2B emerges as the most strongly modified histone (Fig. 1). Increased ADPR-modification resulting from radical attack during butyrate exposure is less enhanced for histone H1 (Table 3). Histone H1 in conjunction with the core histone N-terminal domains [29] control chromatin condensation [16] and regulate DNA accessibility [27]. The results on ADPR-modification of histones in butyrated and non-butyrated cells suggest that chromatin which is already poised and decondensed by acetylation may require less ADPR modification because part of the specific effect of ADPR on chromatin structure has already been met by histone acetylation. It is possible that the two structural modifications have a similar objective.

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