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# High levels of protein carboxyl methyltransferase in well-differentiated human endometrial carcinoma

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The levels of protein carboxyl methyltransferase, an enzyme that methylates free carboxyl groups of proteins, were determined in normal human proliferative and secretory endometrium and in poorly and well-differentiated endometrial carcinoma. Protein carboxyl methyltransferase activity was 67% higher in the well-differentiated carcinoma than in the normal tissues, while similar enzyme levels were observed in the two normal tissues and in the poorly differentiated carcinoma. The results suggest that in the well-differentiated tumor there is excessive protein methylation and offer a possible biochemical probe for distinguishing between the two types of tumor.

Endometrial carcinoma; Differentiation; Protein carboxyl methylation

## 1. INTRODUCTION

Well-differentiated endometrial carcinomas, in contrast to poorly differentiated tumors, are usually confined to the uterus at the time of diagnosis, and are therefore curable by total abdominal hysterectomy and bilateral salpingo-oophorectomy [1]. The morphological determination of the grade of tumor differentiation is therefore an important prognostic factor in endometrial carcinoma. Recently biochemical methods, such as estrogen and progesterone [2] receptor-binding assays, were successfully used to assist in the distinction between well- and poorly differentiated tumors, and in the proper management of endometrial carcinoma. The determination of tumor-associated biochemical changes is therefore important for the prognosis as well as for the understanding of tumor growth and differentiation.

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*Abbreviations:* PCM, protein carboxyl methyltransferase; [$^3$H]SAM, *S*-[*methyl*-$^3$H]adenosyl-L-methionine

Studies on tumor cell lines such as pituitary mouse tumor AT-t-20 [3], mouse C1300 neuroblastoma [4] and its clone N1E-115 [5] revealed that these cells contain the active protein carboxyl methyltransferase (PCM), an enzyme that post-translationally modifies free carboxyl groups of proteins [6]. Moreover, in the N1E-115 cells it was found that PCM activity is increased under conditions that promote morphological differentiation [5]. These findings led us to consider the possibility that the PCM activity of endometrial carcinoma might differ from that found in the normal endometrium. We therefore examined the activity of this enzyme in poorly and well-differentiated tumors as well as in normal endometrium.

## 2. EXPERIMENTAL

Endometrial tissues were obtained from 10 patients with endometrial carcinoma and from 14 patients with benign leiomyomata, all of whom underwent abdominal hysterectomy. Four of the endometrial carcinomas were morphologically characterized as poorly differentiated, and six as well-differentiated tumors. Of the 14 normal endometrial samples, six were in the proliferative phase and eight in the secretory phase. The uterus was opened immediately after extirpation and

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endometrial samples obtained by scraping with a surgical knife were extensively washed with saline. In cases of endometrial carcinoma, the sample was scraped from the tumor itself. Tissues were blotted on filter paper, weighed and homogenized (10%, w/v) in 5 mM Na phosphate buffer, pH 7.0, containing 1 mM EDTA and 14 mM 2-mercaptoethanol with a motor-driven teflon-glass homogenizer.

Usually 10-$\mu$l aliquots of the whole tissue homogenate were used for the determination of PCM activity, employing the method of Diliberto et al. [6] and as detailed in [5]. Reaction mixtures (incubated for 10 min at 37°C) contained tissue homogenate, 20 mg/ml gelatin as methyl acceptor protein, 2 $\mu$M *S*-[*methyl*-$^3$H]adenosyl-L-methionine ([$^3$H]SAM, 15 Ci/mmol, NEN) and 100 mM Na acetate buffer, pH 6.0. Each of the tissues were assayed in triplicate together with triplicate samples containing 100 $\mu$M *S*-adenosyl-L-homocystein, a competitive inhibitor of PCM. The latter served as blanks.

## 3. RESULTS

Mammalian PCMs have broad substrate specificity and can thus be determined using various exogenous methyl acceptor proteins [6]. In the present work the exogenous substrate used was gelatin, which is known to be an excellent methyl acceptor protein [7]. With a saturating concentration of gelatin (20 mg/ml) and with 2 $\mu$M [$^3$H]SAM as methyl donor, endometrial PCM activity varied linearly with time (for at least 12 min, fig.1A) and with the amount of tissue homogenate (up to 1.25 mg tissue, fig.1B).

In the initial experiments we determined the $K_m$ and $V_{max}$ values for PCM in normal endometrium and in endometrial carcinoma, using various concentrations of [$^3$H]SAM. The findings revealed no significant differences in the $K_m$ for [$^3$H]SAM between the normal and malignant tissues ($K_m$ = 7 ± 2 and 8 ± 3 $\mu$M, respectively; $n$ = 4, see fig.2). However, the $V_{max}$ appeared to be higher in the endometrial carcinoma. Since the $K_m$ values were similar, all subsequent experiments were performed under standard assay conditions with 2 $\mu$M [$^3$H]SAM, 20 mg/ml of gelatin and 1 mg tissue.

Fig.3A summarizes the results of the experiments performed with homogenates of normal endometrium (14 subjects) and of endometrial carcinoma (10 subjects). The mean level of PCM activity in the endometrial carcinoma (92 ± 22 pmol·min$^{-1}$·g tissue$^{-1}$) was significantly higher than that in the normal endometrial samples (62 ± 19 pmol·min$^{-1}$·g tissue$^{-1}$) as analyzed by the Mann Whitney U test ($p < 0.005$). Comparison of the means of the four groups of endometrial tissues (proliferative endometrium, secretory endometrium, poorly differentiated and well-differentiated carcinoma) by analysis of variance (ANOVA) yielded the value $F = 6.13$, $p < 0.005$. This indicated that at least two of the four individual groups differ significantly from one another. The differences between the means of the individual groups were then analyzed by the Mann Whitney U test. No difference was observed between the mean PCM activities of the secretory ($n$ = 5) and the proliferative ($n$ = 9) endometrial samples (64 ± 11 and 60 ± 21 pmol·min$^{-1}$·g$^{-1}$, respectively, $p = 0.64$). However, a significant difference in mean PCM activity was found between the well-differentiated carcinoma (103 ±

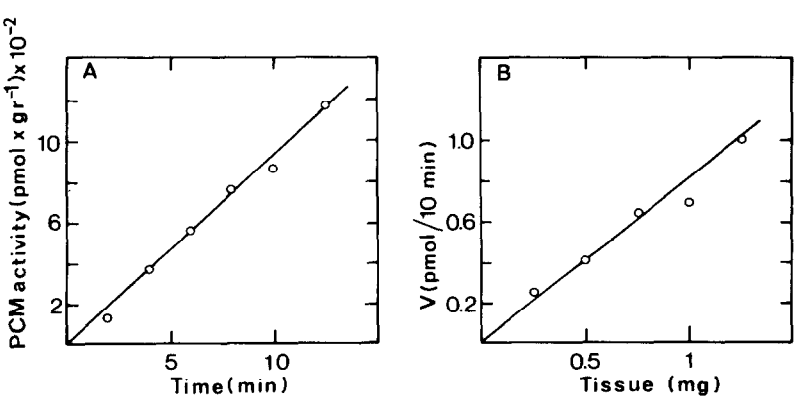


Fig.1. Linearity of PCM activity as a function of time (A) and the amount of tissue (B). Enzyme activity was determined as described in section 2, using 2 $\mu$M [$^3$H]SAM, 20 mg/ml of gelatin, and homogenate equivalent to either 1 mg tissue (A) or 0.25–1.25 mg tissue (B). Data are expressed in pmol [$^3$H]methyl groups transferred to the gelatin per g tissue (A) or per 10 min (B).

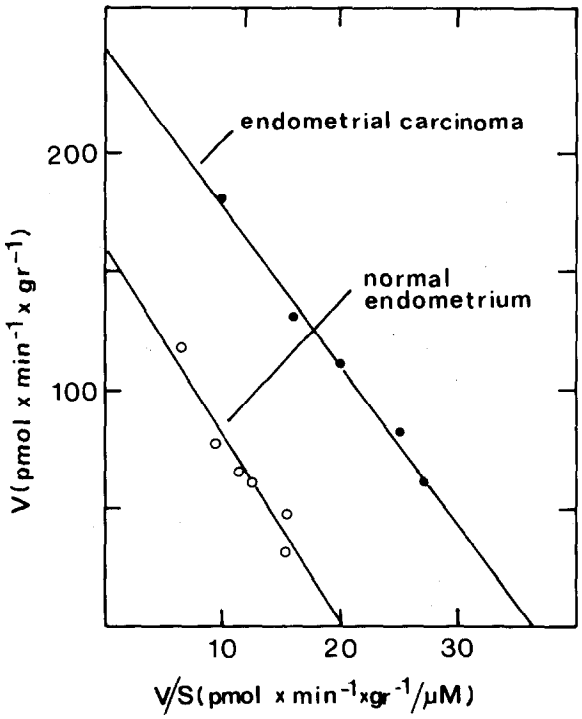


Fig.2. Eadie-Hofstee plot of PCM activity in normal secretory endometrium and in well-differentiated endometrial carcinoma. Enzyme activity was determined as described in section 2. Results are expressed in pmol [$^3$H]methyl groups transferred to gelatin/min per g tissue.

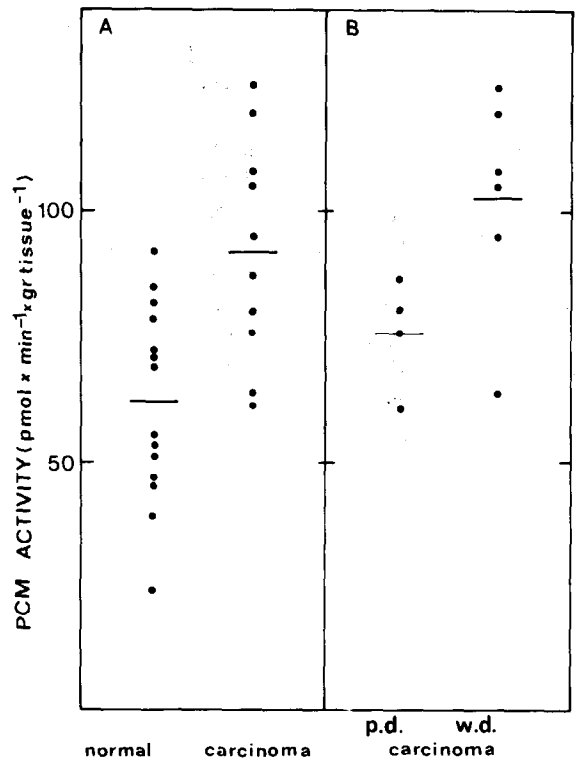


Fig.3. PCM activity in normal endometrium (14 subjects) and in endometrial carcinoma (10 subjects). Enzyme activity was determined in each case with 2 $\mu$M [$^3$H]SAM, 20 mg/ml gelatin and 1 mg tissue. (A) Comparison between the normal and the malignant tissues. (B) Comparison between the poorly differentiated (p.d.) and the well differentiated (w.d.) carcinoma. Horizontal bar represents the mean values of PCM activity. Statistical analysis is described in the text.

22 pmol·min$^{-1}$·g$^{-1}$, $n = 6$) and the normal tissues ($p < 0.02$), and between the well-differentiated and the poorly differentiated carcinomas (103 ± 22 vs 75 ± 11 pmol·min$^{-1}$·g$^{-1}$, $p < 0.05$, see fig.3B). The mean PCM activity of the poorly differentiated carcinoma was not significantly different from that of either of the two normal tissues.

## 4. DISCUSSION

Methylation of proteins in eukaryotic cells has been associated with the control of cellular differentiation, as indicated by the observed elevation in levels of PCM activity during the differentiation of human monocytes and macrophages [8,9] and in the developing rat liver [10]. Here, no difference in PCM activity was observed between the differentiated secretory endometrium and the proliferative tissue, possibly because the detection of such differences would necessitate a correlation between PCM activity and the blood levels of estrogen and progesterone. However, comparison between the PCM activity in the malignant and the normal tissues revealed a 47% higher activity in the carcinoma (fig.3). The small difference in PCM activity between the poorly differentiated carcinoma and the normal tissues was not statistically significant, yet the 67% higher activity in the well-differentiated endometrial carcinoma than in the normal tissues was clearly significant. The PCM activity in the well-differentiated carcinoma was also significantly higher (by 37%) than in the poorly differentiated tumor. It thus appears that the high enzyme activity is primarily associated with the well-differentiated type of tumor. This finding may have important diagnostic as well as

prognostic implications. It is possible that the use of electrophoretic methods for the separation of PCM isozymes [11] in combination with anti PCM antibodies will allow a better distinction between well and poorly differentiated tumors.

The higher PCM activity in the well-differentiated endometrial carcinoma than in the poorly differentiated tumor is reminiscent of the observed increase in PCM activity of mouse neuroblastoma cells cultured under conditions that promote morphological differentiation [5]. It raises the possibility of an association between PCM activity and morphological differentiation of transformed cells.

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