Formation of formaldehyde from *S*-adenosyl-L-[*methyl*-^{3}H]methionine during enzymic transmethylation of histamine

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The experiments described here show that radiolabelled formaldemethone is formed from the radiolabelled methyl group of *S*-adenosyl-L-methionine in the course of the enzymic conversion of histamine to N^τ-methylhistamine in the presence of dimedone suggesting that the formation of HCHO is probably linked to the enzymic transmethylation of histamine.

Histamine S-*Adenosyl-L-methionine* *Formaldehyde* *Enzymic transmethylation* *Histamine*-N-*methyltransferase* *TLC*

1. INTRODUCTION

Histamine-*N*-methyltransferase (*S*-adenosyl-L-methionine:histamine-*N*-methyltransferase, EC 2.1.1.8) catalyzes the ring methylation of HA in mammalian tissues (e.g. [1,2]). It utilizes SAM as a methyl donor which is then converted into SAH. The latter compound is a potent inhibitor of transmethylation (e.g. [3,4]). HNMT exhibits a high substrate specificity which is almost absolute for HA.

Here we report our findings that following the incubation of HA with partially purified HNMT from rat kidney, and *S*-[*methyl*-^{3}H]SAM, in the presence of an HCHO trapping agent, dimedone [5], ^{3}H-labelled HCHO could be detected as formaldemethone (an adduct of HCHO and dimedone), and the removal of HCHO by dimedone led to a decreased formation of N^τ-me-HA.

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Abbreviations: HA, histamine; SAM, *S*-adenosyl-L-methionine; *S*-[*methyl*-^{3}H]SAM, *S*-adenosyl-L-[*methyl*-^{3}H]methionine; SAH, *S*-adenosyl-L-homocysteine; N^τ-me-HA, N^τ-methylhistamine; N^τ-me-IAA, N^τ-methylimidazoleacetic acid; HNMT, histamine-*N*-methyltransferase

2. EXPERIMENTAL

2.1. *Materials*

Adenosyl-L-[*methyl*-^{3}H]methionine (spec. act. 76 Ci per mmol) and Soluene were purchased from Amersham International (Bucks, England). The compounds, N^τ-me-HA and N^τ-me-IAA were the generous gifts of Dr Granerus (Göteborg, Sweden). Formaldemethone was synthesised by a conventional method [5]. Silica gel 60 F_{254} (0.25 mm) was purchased from Merck (Darmstadt, FRG), and Polygram Sil NHR (0.25 mm) from Macherey Nagel (Düren, FRG).

2.2. *Partially purified HNMT*

Partly purified HNMT was prepared from rat kidney [6]. The purification included homogeniza-

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tion, centrifugation, acid treatment (pH 5) and DEAE-Sephacel anion-exchange chromatography which yielded an approx. 38-fold increase in the specific activity. Protein content was determined by the method of Lowry et al. [7].

2.3. *Histamine-N$^{\tau}$-methylhistamine transformation*

The enzymic conversion of HA to N$^{\tau}$-me-HA was measured according to Taylor and Snyder [8] with minor modifications. The incubation mixture (final volume 80 μl) contained 25 mM phosphate buffer (pH 7.9), 0.2–1 ng HA (0.030–0.150 μM), 0.5 μCi *S-[methyl-*^{3}H]SAM (0.08–0.1 μM) and partially purified HNMT of rat kidney (0.05 mg protein). The formaldehyde trapper, dimedone, was dissolved in water (1 mM) and 20 μl added to the reaction mixture simultaneously with HA (unless otherwise specified in text). The reaction mixture was incubated at 37°C for 60 min and reaction terminated by the addition of 5 μl of 2.4 M $HClO_4$. Under the above circumstances, a linear relationship between the amounts of HA and the counts of N$^{\tau}$-me-HA was observed. Appropriate blanks, with boiled (inactivated) HNMT and also with active HNMT but in the absence of HA, were run concurrently.

2.4. *Extraction of the labelled methylated products of HA*

The methylated products were extracted into chloroform at pH 11 [8], and 100–200 μl portions of extracts were taken for counting the total activity and for separation by TLC.

2.5. *Extraction of formaldemethone*

The chloroform extraction was carried out at pH 1 in order to separate formaldemethone from the methylated products of HA. The chloroform was made peroxide-free by washing with a saturated solution of ferrous sulfate and distilled. The acidic as well as the alkaline chloroform extracts contained only negligible amounts of *S-[methyl-*^{3}H]SAM.

2.6. *Identification of N$^{\tau}$-me-HA*

After evaporation of 200 μl of the chloroform extract, the residue was taken up in 20 μl chloroform : methanol (1 : 1, v/v) containing 1 mM N$^{\tau}$-me-HA as carrier and 10 μl subjected to TLC. 5 μl was applied for counting: total radioactivity of the chloroform extract after evaporation. Chromatograms were developed in chloroform : methanol : NH_3 (60 : 35 : 4) [9]. The radioactive spots on the chromatogram were localized by using standards and visualized with sublimated iodine in a glass tank. R_f values of N$^{\tau}$-me-HA were obtained as 0.35–0.41. The radioactivity of the spot was estimated after placing the fraction into counting vials with 0.1 ml Soluene, heating at 37°C for 60 min, standing overnight and adding 10 ml of scintillation liquid to the fractions.

2.7. *Identification of formaldemethone*

For the identification of formaldemethone [5] chromatograms were developed in benzene : ethylacetate (95 : 5, v/v) [10]. Separation was carried out from 200 μl of acidic chloroform extract after evaporation and resolution in 20 μl methanol : chloroform (1 : 1, v/v) containing 0.1 nM formaldemethone as a carrier. 10 μl sample was applied to TLC separation, and after development, the counts of the formaldemethone spot were estimated. The standard was visualized with UV light. The R_f value of formaldemethone was determined as 0.52 in this system.

3. RESULTS

Fig.1a shows the separation of HA and its methylated metabolites by TLC from an alkaline

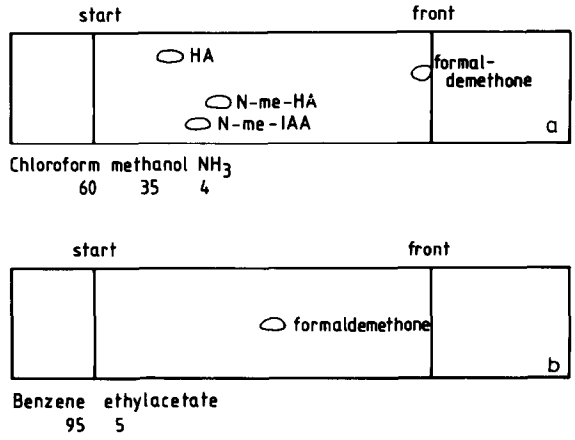

Fig.1. Spots of HA, N$^{\tau}$-me-HA, N$^{\tau}$-me-IAA and formaldemethone on chromatograms, developed in chloroform : methanol : NH_3 (60 : 35 : 4, v/v) or benzene : ethyl acetate (95 : 5, v/v).

chloroform extract. N^{τ}-me-HA was located at 5 cm (R_f = 0.41). When dimedone was added to the reaction mixture, formaldemethone could also be identified (at 0.3 cm) below the front (fig.1a). For a more exact identification of HCHO as formaldemethone by TLC, the benzene:ethyl acetate system was used (fig.1b) with an acid chloroform extract (free from the methylated metabolite of HA).

Addition of dimedone (at the beginning of the incubation period) to a reaction mixture, containing *S*-[*methyl*-^{3}H]SAM and partially purified HNMT of rat kidney in a phosphate buffer (pH 7.9), led to a noticeable formation of labelled formaldemethone. When enzymatic reaction was run in the absence of dimedone, capturing radioactive formaldehyde only at the end of the incubation period, or when dimedone was added to a reaction mixture, containing all constituents but boiled (inactivated) HNMT, only small amounts of radioactive formaldemethone were detected (table 1).

Examining the effect of dimedone on the incorporation of the [^{3}H]methyl group into HA, the addition of dimedone resulted in a decreased incorporation of the labelled methyl into N^{τ}-me-HA (table 2), and increased the counts migrating with the front. Under control conditions, the radioactivity migrated as N^{τ}-me-HA on the chromatogram and only negligible counts were detected below the front (not shown). Thus, decreased formation of N^{τ}-me-HA seems to be due to the effect of dimedone.

To examine further the relationship between inhibition of HA transmethylation and HCHO formation, radioactivity, detected in the absence and presence of dimedone, was compared to that of formaldemethone (table 2), and the loss of the radioactivity of N^{τ}-me-HA in the presence of dimedone was plotted against the counts identified as formaldemethone (fig.2). The loss of the radioactivity of N^{τ}-me-HA was significantly correlated with that estimated as formaldemethone (r = 0.98, n = 4, $p < 0.01$).

Arginine could capture HCHO forming methylol-arginine under conditions described [11]. We also examined whether the arginine residue of HNMT plays a role in the enzymic transformation of HA to N^{τ}-me-HA. Chemical modification of the arginine residue of HNMT was performed by treatment of the protein with 50 mM cyclohexane-1,2-dione in 0.15 M sodium borate buffer (pH 9) for 15 min at 37°C [13]. The data showed a complete inhibition of N^{τ}-me-HA formation when arginine residues of rat kidney HNMT

Table 1

^{3}HCHO, detected as formaldemethone in the course of enzymic formation of N^{τ}-methylhistamine

[^{3}H]Methyl acceptor	Experimental conditions	Formaldemethone (dpm) (100 μl extract)
Histamine (1 ng)	without dimedone	∅
Histamine (1 ng)	with dimedone (added at the beginning of the incubation)	6887 ± 620[a] (4)[b]
Histamine (1 ng)	with dimedone (added at the beginning of the incubation)	2019 ± 188 (4)
Histamine (1 ng)	with dimedone and boiled enzyme (added at the beginning of the incubation)	2107 ± 210 (4)

[a] Mean ± SE

[b] Number of samples in parentheses

[^{3}H]SAM, added to the reaction mixture, was counted as 836000 dpm

Table 2

^{3}HCHO formation from *S*-[*methyl*-^{3}H]SAM in the course of the enzymic conversion of histamine to N^τ-methylhistamine

[^{3}H]Methyl acceptor	^{3}H detected as N^τ-me-HA (dpm) (100 μl extract)		^{3}H detected as formaldemethone (dpm) (100 μl extract)
	Without dimedone	With dimedone	
Water	no	no	1745 ± 189[a] (4)[b]
HA (0.2 ng)	2416 ± 211 (4)	611 ± 50 (4)	3246 ± 290 (4)
	Loss: 1805		Net (extra): 1501
HA (1 ng)	15561 ± 1389 (4)	10460 ± 980 (4)	6887 ± 620 (4)
Water	no	no	2086 ± 188 (4)
	Loss: 5101		Net (extra): 4801
HA (1 ng) with boiled enzyme	no	no	2107 ± 210

[a] Mean ± SE

[b] Number of samples in parentheses

[^{3}H]SAM added to the reaction mixtures was counted as 1200000 and 836000 dpm

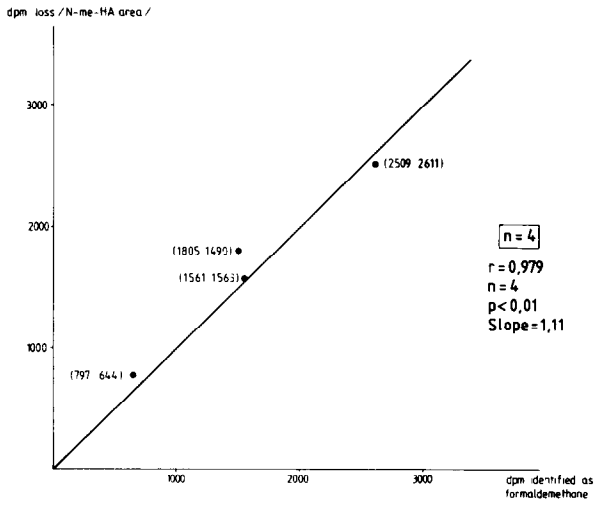

Fig.2. Correlation of loss in N^τ-me-HA counts (in the presence of dimedone) with counts, identified as formaldemethone. Measurements were carried out as described in section 2. Values represent means of four measurements.

were modified by cyclohexane-1,2-dione. 15 min preincubation of HNMT in borate buffer (pH 9) influenced only slightly the basal activity of the enzyme.

4. DISCUSSION

Tithapandha and Cohn suggested that N^τ-methylation of HA proceeded by two half reactions in a ping-pong mechanism with the intermediate formation of a methylated enzyme [14]. This hypothesis could not be fully substantiated because the 'methylated' form of HNMT could not be isolated from the reaction mixture [14,15]. We suggest a more labile product as intermediate instead of the methylated enzyme.

The present experiments provide evidence that HCHO is formed from *S*-[*methyl*-^{3}H]SAM during the enzymic conversion of HA to N^τ-me-HA. On the other hand, the results show that the HCHO trapper, dimedone, produces losses in the counts of N^τ-me-HA without affecting the total counts of

the chloroform extracts (not shown). It has also been stated that the loss in the counts of N^{τ}-me-HA (in the presence of dimedone) could be identified as formaldemethone.

All these statements suggest that the production of HCHO is not a side reaction of an enzymic transmethylation as stated by Meller et al. [16] but rather a process which is involved in a complex mechanism of this enzymic transformation.

HCHO, formed presumably from $-CH_3$, can be readily bound to an NH_2 group of an amino acid, e.g. arginine [17]. Similarly, HCHO, from S-CH_3-^{3}H of SAM might be coupled with an NH_2 group of an amino acid (e.g. arginine) of the active site of HNMT, forming a methylol-protein and then this methylol-protein might give its methylol group to the acceptor molecule. A complete loss of enzyme activity by modifying arginine residues of HNMT seems to confirm this assumption. In the reduction of this methylol a thiol-disulfide exchange reaction is presumable involved as has already been assumed for the catalytic function of HNMT [14].

The present investigation is the first to reveal that the formation of HCHO from SAM is linked to an enzymic transmethylation.

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