Mutation Research, 283 (1992) 131–136

© 1992 Elsevier Science Publishers B.V. All rights reserved 0165-7992/92/$05.00

MUTLET 0713

Efficiency of DNA–histone crosslinking induced by saturated and unsaturated aldehydes in vitro

Jim R. Kuykendall and Matthew S. Bogdanffy

Haskell Laboratory for Toxicology and Industrial Medicine, E.I. du Pont de Nemours and Company, P.O. Box 50, Elkton Road, Newark, DE 19714, USA

(Received 2 April 1992)
(Revision received 18 May 1992)
(Accepted 28 May 1992)

Keywords: DNA–protein crosslinks; Formaldehyde; Aldehyde; Acrolein; Glutaraldehyde

Summary

Using a filter-binding assay based on precipitation of pUC13 plasmid DNA bound to calf-thymus histones, we have determined the efficiency of formation of DNA–protein crosslink formation induced by several aldehyde compounds in vitro. Formaldehyde, glutaraldehyde and acrolein were the most potent, causing 1 crosslink per 2.7 kbp of DNA at 1.5, 8 and 150 μM, respectively. All other compounds tested gave 1 crosslink per plasmid molecule in the mM concentration range as follows: acetaldehyde, 115 mM; propionaldehyde, 295 mM; butyraldehyde, 360 mM; crotonaldehyde, 8.5 mM; *trans*-2-pentenal, 6.3 mM. Significant decreases in the efficiency of DPXL formation were observed with monofunctional aldehydes of higher carbon chain length. For example, the concentration of formaldehyde needed to give 1 crosslink per molecule was almost 10^5 times less than that of acetaldehyde. Acetaldehyde differs from formaldehyde only by one saturated carbon. The presence of an unsaturated bond between the 2–3 carbons improved the potential for crosslink formation. For example, acrolein was over 500-fold more potent than propionaldehyde. Glutaraldehyde was almost as potent as formaldehyde, indicating that the bifunctional nature of this 5-carbon saturated aldehyde may be crucial to its high efficiency of DNA–protein crosslinking.

Volatile aldehydes are thought to represent a potential risk to human health. Primary sources of exposure include: tobacco smoke (for review see Heck et al., 1986), ethanol (Majchrowicz, 1975), automotive and diesel exhaust (Auerbach et al., 1977), medical disinfectants and fixatives (Gordon, 1972; Milner et al., 1977; Nethercott et al., 1988), and the chemical and plastics industries, where workers can be exposed to a variety of aldehydes used as intermediates in chemical synthesis reactions. The genotoxic effect of several aldehydes has been reported (Natarajan et al., 1983; Goldmacher and Thilly, 1983; St. Clair et al., 1991), and the mutagenic and carcinogenic potential of several of these compounds is thought to be due to their reactivity with DNA (Feldman,

Correspondence: Dr. Matthew S. Bogdanffy, Haskell Laboratory for Toxicology and Industrial Medicine, E.I. du Pont de Nemours and Company, P.O. Box 50, Elkton Road, Newark, DE 19714, USA.
Tel. (302) 366-5011; Fax (302) 451-4829.

1973; Rosenkranz, 1977; Sasaki and Endo, 1978; Kerns et al., 1983; Chung and Hecht, 1983; Sellakumar et al., 1985; Craft et al., 1987). The primary adduct formed by formaldehyde in cellular DNA was shown to be DNA–protein crosslinks (Casanova et al., 1983; Solomon et al., 1988; Casanova et al., 1989; Miller et al., 1990; Costa, 1991). Another related compound, acetaldehyde, was also found to cause formation of DNA–protein crosslinks (Lam et al., 1986), as well as DNA–DNA crosslinks (Lambert et al., 1985). On the other hand, glutaraldehyde is a potent crosslinking agent, which is known to induce protein–protein crosslinks (Milner et al., 1977). Unfortunately, little is known about the structure–activity relationships of these aldehydes and their DNA–protein crosslinking abilities. A central goal of the present work was to determine the relative crosslinking efficiency of each compound. These experiments are based on filter-binding assays which detect the formation of covalent crosslinks between calf-thymus histones and ^{3}H-labeled pUC13 plasmid DNA by protein precipitation (Trask et al., 1984).

Material and methods

Plasmid isolation

A 1-liter culture of *E. coli* strain HB101 carrying a pUC13 plasmid was labeled during a 4-h incubation with 3 mCi of [^{3}H]thymidine (specific activity of 20 Ci/mmole, New England Nuclear, Boston). Plasmid DNA was isolated using Qiagen columns (Qiagen, Inc., Chatsworth, CA). The specific activity of the plasmid DNA approached 3.8×10^4 dpm/μg DNA.

Chemicals

Acetaldehyde, formaldehyde (37%), propionaldehyde and *trans*-2-pentenal (95%) were purchased from Sigma (St. Louis, MO), while crotonaldehyde, acrolein and butyraldehyde were purchased from Aldrich (Milwaukee, WI). Glutaraldehyde was purchased as an EM grade 50% aqueous solution from Electron Microscopy Sciences (Ft. Washington, PA). All aldehydes were of the highest grade and purity available, and were above 99% pure unless stated otherwise.

DNA–protein crosslink assay

A modified filter-binding assay (Trask et al., 1984), based on SDS–KCl precipitation of protein and covalently attached DNA was used us to study the kinetics of plasmid–histone crosslink formation. In an assay volume of 330 μl, 0.25 μg of ^{3}H-labeled plasmid DNA and 1 μg of calf-thymus histone (Sigma, St. Louis, MO) were incubated for 1 h at 37°C in 0.1 M phosphate (pH 7.4) and 1 mM EDTA. The plasmid and histones were mixed in their stock forms of 0.75 and 1 μg/μl, respectively, and allowed to interact for 15 min on ice prior to dilution with phosphate buffer. The polypropylene reaction vials were placed on ice during addition of reaction components and sealed prior to incubation. Sealed reaction vials were used to reduce the loss of aldehydes from the assay through volatilization. The reaction was terminated by transferring the sample to a 1.5-ml Eppendorf tube on ice and adding 5 μl of 1.0 M Tris (pH 7.4), 2.5 μl of 0.2 M EDTA, 5 μg of calf-thymus histone, 5 μg of calf-thymus DNA, and 35 μl of 10% SDS. The samples were mixed and allowed to stand for 2 min at room temperature. By addition of 35 μl of 2.5 M KCl and incubation on ice for 10 min, the proteins and DNA–protein complexes were precipitated. Precipitates were collected on prewetted Whatman GF/F 25-cm filters (VWR Scientific) in a vacuum Millipore model 1225 sampling manifold. The filters were washed three times with 1 ml of 10 mM Tris–HCl (pH 7.5), 1 mM EDTA and 100 mM KCl and twice with 95% ethanol. Radioactivity present on dried filters was determined by standard scintillation methods.

The control assays, containing only histone and DNA in the buffer, routinely gave a DPXL/PM of 0.1–0.2. By removing the histone from the assay, counts decreased to background. In addition, digestion of control or aldehyde-treated samples was able to reduce the counts precipitated on the filter to background levels.

Determination of DNA–protein crosslinking efficiency

Calculations are based on the use of the Poisson formula, $n = -\ln x$, where n is the average number of DNA–protein crosslinks per plasmid molecule (DPXL/PM) and x is the fraction of

DNA remaining non-crosslinked ([1 − (cpm on filter after wash/total cpm placed on filter]). This assumes that DNA–protein crosslinks are formed randomly in DNA.

Results

The names and chemical structures of all aldehydes studied are shown (Table 1). In preliminary experiments, a five-log concentration curve was used to determine the lower limit of detectable DNA–protein crosslink formation at 37°C by these aldehydes (data not shown). Secondary experiments were carried out in a two-log concentration range, to determine the efficiency of formation of crosslinks by each compound in the filter-binding assay (Fig. 1A–G). Crosslink formation varied by several orders of magnitude across the class of aliphatic aldehydes studied. Formaldehyde, glutaraldehyde and acrolein were the most potent, with detectable DNA–protein crosslinking efficiencies of 0.6396 DPXLs/PM/μM formaldehyde, 0.0934 DPXLs/PM/μM glutaraldehyde and 0.0059 DPXLs/PM/μM acrolein (Figs. 1A, 1B and 1E, respectively; Table 1). *trans*-2-Pentenal and crotonaldehyde induced crosslink formation at the low mM concentration range, with efficiencies of crosslinking of 0.1594 DPXLs/PM/mM and 0.1153 DPXLs/PM/mM (Figs. 1G and 1F; Table 1). Acetaldehyde, propionaldehyde and butyraldehyde were weak crosslinking agents, inducing crosslink formation only at the high mM range. The efficiencies of crosslink formation were 0.0086 DPXLs/PM/mM acetaldehyde, 0.0034 DPXLs/PM/mM propionaldehyde, and 0.0028 DPXLs/PM/mM butyraldehyde (Figs. 1B, 1C and 1D, respectively; Table 1).

Conclusions

The formation of DNA–protein crosslinks has been studied previously using either alkaline elution (Ewig and Kohn, 1978; Craft et al., 1987; St. Clair et al., 1991) or differential solubility of ^{3}H-labeled DNA between aqueous and organic phases (Heck et al., 1986; Lam et al., 1986). Those studies showed that DNA–protein crosslinking is a biologically relevant property of several aldehydes. However, an attempt to determine the chemical efficiency of crosslink formation between DNA and protein in cells and tissues is difficult. Possible differences in metabolism and uptake of these compounds into the

TABLE 1

CHEMICAL STRUCTURE AND REACTIVITY OF A SERIES OF ALDEHYDES

Compound	Chemical structure	CAS No.	DPXL/PM/[aldehyde]	DPXL/PM/[aldehyde]$^{-1}$
A. Monofunctional				
1. Saturated				
Formaldehyde	CH_2=O	50-00-0	0.6396×10^{-6} M [a]	1.6×10^{-6} M [b]
Acetaldehyde	CH_3-CH=O	107-29-9	0.0086×10^{-3} M	116.3×10^{-3} M
Propionaldehyde	CH_3-CH_2-CH=O	123-38-6	0.0034×10^{-3} M	294.1×10^{-3} M
Butyraldehyde	CH_3-CH_2-CH_2-CH=O	123-72-8	0.0028×10^{-3} M	357.1×10^{-3} M
2. Unsaturated				
Acrolein	CH_2=CH-CH=O	107-02-8	0.0059×10^{-6} M	169.5×10^{-6} M
Crotonaldehyde	CH_3-CH=CH-CH=O	123-73-9	0.1153×10^{-3} M	8.7×10^{-3} M
trans-2-Pentenal	CH_3-CH_2-CH=CH-CH=O	1576-87-0	0.1594×10^{-3} M	6.3×10^{-3} M
B. Difunctional				
Glutaraldehyde	O=CH-CH_2-CH_2-CH_2=O	111-30-8	0.0934×10^{-6} M	10.7×10^{-6} M

[a] Efficiencies of DPXL formation by each compound were calculated by linear regression from curves of concentration vs. DPXL/PM (Figs. 1A–H), the data points of which were the means of triplicate assays.

[b] This value is the inverse of the preceding column, and represents the concentration necessary in this assay to achieve a level of 1 DPXL/PM.

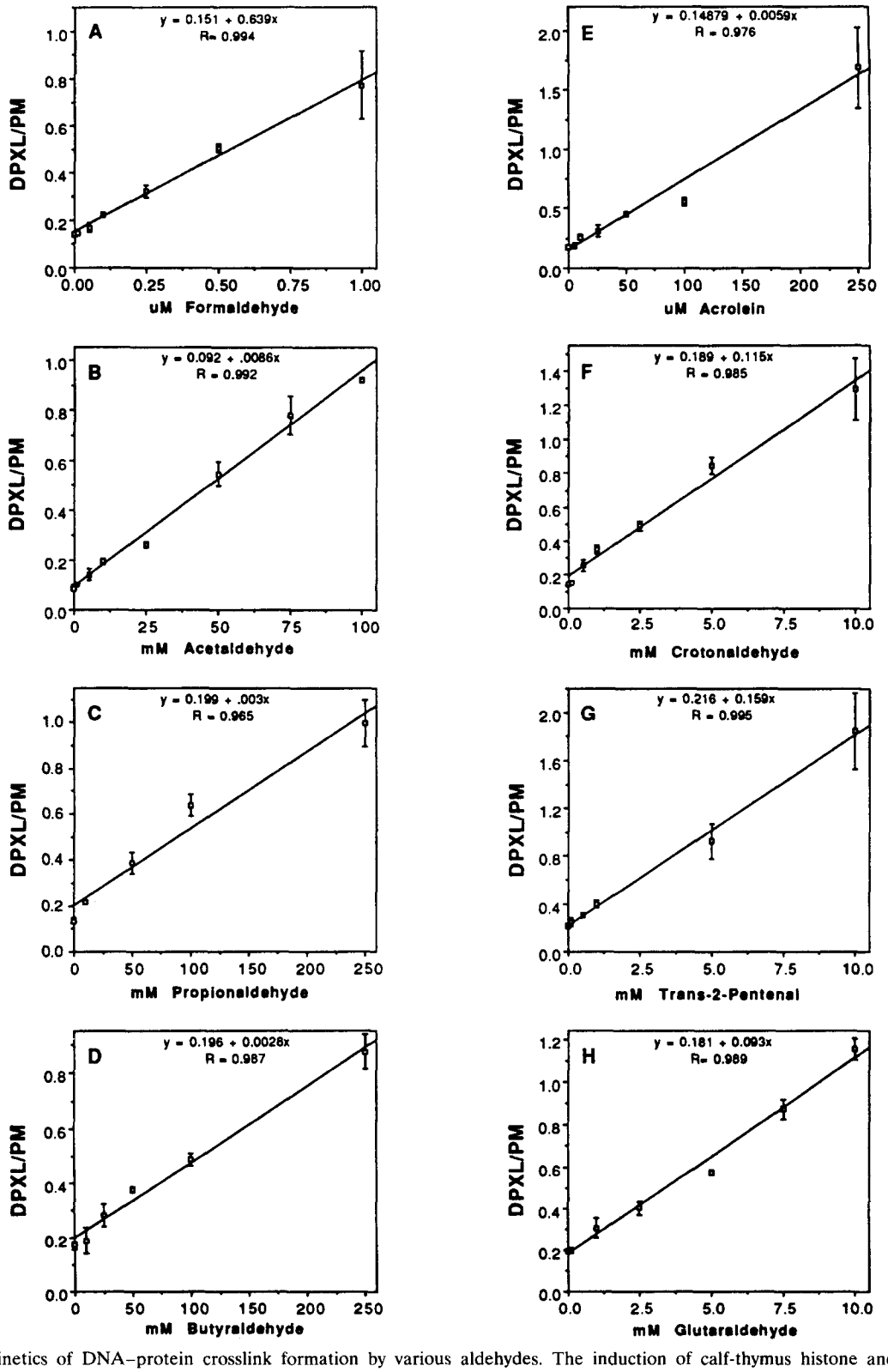

Fig. 1. Kinetics of DNA–protein crosslink formation by various aldehydes. The induction of calf-thymus histone and pUC13 plasmid-crosslink formation was studied in vitro using an SDS/KCl precipitation of complexes followed by filter-binding (Trask et al., 1984). The rate equations are shown in the top center of each graph. All data presented are the mean ± S.D. of triplicate assays.

cells and their nuclear compartment, as well as possible differences in the repair of crosslinks formed by each compound, would make interpretation of the data in chemical terms very difficult. With the use of a modified procedure (Trask et al., 1984) based on the precipitation of protein and covalently bound DNA, we were able to measure the formation of DNA–histone crosslinks in vitro. In this way, the differences in the efficiency of crosslink formation itself could be compared between various compounds, with minimal interference from biological effects. The data presented in this study complements existing studies of crosslink formation by this class of compounds, and explains differences in efficiency of crosslink formation as a function of the crosslinking reaction itself.

In this study, formaldehyde was a very potent crosslinking agent, active in the low μM concentration range. The reaction mechanism for this agent is thought to involve initial addition of formaldehyde to a primary amine on either an amino acid residue or DNA base to yield a hydroxymethyl intermediate. In a second and subsequent step, the hydroxymethyl group condenses with a second primary amine to yield a methylene bridge (Fraenkel-Conrat and Olcott, 1948).

Compounds such as acetaldehyde, propionaldehyde and *trans*-2-pentenal, with a chain length of 2, 3 or 4 saturated carbons, respectively, proved less efficient at crosslinking histones to DNA than formaldehyde. In addition, these compounds were sequentially less reactive with increasing length. The presence of an unsaturated bond between α,β-carbons dramatically enhanced the ability of these compounds to cause formation of DPXLs over their saturated analogs. For example, acrolein and propionaldehyde differ chemically only by the presence or absence of an unsaturated bond, yet acrolein is 575 times more potent in crosslink formation than propionaldehyde. Likewise, crotonaldehyde is 25 times more potent than its saturated analog, butyraldehyde. We speculate that this increased reactivity of compounds containing an unsaturated bond may involve induced partial polarity of the molecule due to resonance around the double bond. Electron withdrawal from the oxygen of the aldehyde groups would make it more positively charged, and possibly more reactive.

Glutaraldehyde, which is a 5-carbon saturated dialdehyde, is a very potent crosslinking agent in the μM concentration range. If one applies the above assumptions that long-chain saturated aldehydes are not very potent crosslinking agents, glutaraldehyde is an exception. This is most likely due to the difunctional nature of this compound, but it is not known if the binding of the second moiety is accelerated by the binding of the first. This could explain its very potent crosslinking ability, but further work is necessary to determine the reactivity of these two functional aldehyde groups.

Acknowledgement

This work was funded in part by the Society of the Plastics Industry.

References

Auerbach, C., M. Moutschen-Dahmen and J. Moutschen (1977) Genetic and cytogentical effects of formaldehyde and related compounds, Mutation Res., 39, 317–362.

Casanova-Schmitz, M., and H. d'A. Heck (1983) Effects of formaldehyde exposure on the extractability of DNA from proteins in the rat nasal mucosa, Toxicol. Appl. Pharmacol., 70, 121–132.

Casanova, M., D.F. Deyo and H. d'A. Heck (1989) Covalent binding of inhaled formaldehyde to DNA in the nasal mucosa of Fischer 344 rats: Analysis of formaldehyde and DNA by high-performance liquid chromatography and provisional pharmacokinetic interpretation, Fund. Appl. Toxicol., 12, 397–417.

Chung, F.-L., and S.S. Hecht (1983) Formation of cyclic 1,N2-adducts by reaction of deoxyguanosine with α-acetoxy-*N*-nitropyrrolidine, 4-(carbethoxynitrosamino) butanal, or crotonaldehyde, Cancer Res., 43, 1230–1235.

Costa, M. (1991) DNA–protein complexes induced by chromate and other carcinogens, Environ. Health Perspect., 22, 45–52.

Craft, T.R., E. Bermudes and T.T. Skopek (1987) Formaldehyde mutagenesis and formation of DNA–protein crosslinks in human lymphoblasts in vitro, Mutation Res., 176, 147–155.

Ewig, R.A.G., and K.W. Kohn (1978) DNA–protein crosslinking and DNA interstrand cross-linking by haloethylnitrosoureas in L1210 cells, Cancer Res., 38, 3197–3203.

Feldman, M.Y. (1973) Reactions of nucleic acids and nucleoproteins with formaldehyde, Prog. Nucleic Acid Res. Mol. Biol., 13, 1–49.

Fraenkel-Conrat, H., and H.S. Olcott (1948) The reaction of

formaldehyde with proteins, V. Crosslinking between amino and primary amide or guanidyl groups, J. Am. Chem. Soc., 70, 2673–2684.

Goldmacher, V.S., and W.G. Thilly (1983) Formaldehyde is mutagenic for cultured human cells, Mutation Res., 116, 417–422.

Gordon, H.H. (1972) Hyperhiderosis: treatment with glutaraldehyde, Cutis, 9, 375–378.

Heck, H. d'A., M. Casanova, C.-W. Lam and J.A. Swenberg (1986) The formation of DNA–protein crosslinks by aldehydes present in tobacco smoke, in: Banbury Report 23, Mechanisms in Tobacco Carcinogenesis, Cold Spring Harbor Laboratory.

Kerns, W.D., L.K. Pavkov, D.J. Donofrio, E.J. Gralla and J.A. Swenberg (1983) Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure, Cancer Res., 43, 4382–4392.

Lam, C.-W., M. Casanova and H. d'A. Heck (1986) Decreased extractability of DNA from proteins in the rat nasal mucosa after acetaldehyde exposure, Fund. Appl. Toxicol., 6, 541–550.

Lambert, B., Y. Chen, S.-M. He and M. Sten (1985) DNA cross-links in human leucocytes treated with vinyl acetate and acetaldehyde in vitro, Mutation Res., 146, 301–303.

Majchrowicz E. (1975) Metabolic correlates of ethanol, acetaldehyde, acetate and methanol in human and animals, in: E. Majchrowicz (Ed.), Biochemical Pharmacology of Ethanol, Plenum, New York, pp. 111–140.

Miller, C.A., M.D. Cohen and M. Costa (1990) Comparison of DNA–protein crosslinks (DPCs) formed by chromium compounds with those induced by *cis*-diamminedichloroplatinum (II) (cis-Pt) and formaldehyde (CH_2O), Toxicologist, 10, 316.

Milner, N.A., J.W. McDowell, G.W. Willcockson, N.I. Bruckner, R.L. Stark and E.J. Whitmore (1977) Antimicrobial and other properties of a new stabilized alkaline glutaraldehyde disinfectant/sterilizer, Am. J. Hosp. Pharmacol., 34, 376–382.

Natarajan, A.T., F. Darroudi, C.J.M. Bussman and A.C. van Kesteren-van Leeuwen (1983) Evaluation of the mutagenicity of formaldehyde in mammalian cytogenetic assays in vivo and in vitro, Mutation Res., 122, 355–360.

Nethercott, J.R., D.L. Holness and E. Page (1988) Occupational contact dermatitis due to glutaraldehyde in health care workers, Contact Dermatitis, 18, 193–196.

Rosenkranz, H.S. (1977) Mutagenicity of halogenated alkanes and their derivatives, Environ. Health Perspect., 21, 79–84.

Sasaki, Y., and R. Endo (1978) Mutagenicity of aldehydes in Salmonella, Mutation Res., 54, 251–252.

Sellakumar, A.R., C.A. Snyder, J.J. Solomon and R.E. Albert (1985) Carcinogenicity of formaldehyde and hydrogen chloride in rats, Toxicol. Appl. Pharmacol., 81, 401–406.

Simon, P., J.G. Filser and H.W. Bolt (1985) Metabolism and pharmacokinetics of vinyl acetate, Arch. Toxicol., 57, 191–195.

Solomon, M.J., P. Larsen and A. Varshavsky (1988) Mapping protein–DNA interactions in vivo with formaldehyde: evidence that histone H4 is retained on a highly transcribed gene, Cell, 53, 937–947.

St. Clair, M.B., E. Bermudez, E.A. Gross, B.E. Butterworth and L. Recio (1991) Evaluation of the genotoxic potential of glutaraldehyde, Environ. Mol. Mutagen., 18, 113–119.

Trask, D.K., J.A. DiDonato and M.T. Muller (1984) Rapid detection and isolation of covalent DNA/protein complexes: application to topoisomerase I and II, EMBO J., 3, 671–767.

Communicated by Th.R. Skopek