Journal of Chromatography, 614 (1993) 245–251
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6746

C_{18} thin-layer chromatographic enhancement of the ^{32}P-postlabeling assay for aromatic or bulky carcinogen–DNA adducts: evaluation of adduct recoveries in comparison with nuclease P1 and butanol methods

M. Vijayaraj Reddy

Environmental and Health Sciences Laboratory, Mobil Oil Corporation, P.O. Box 1029, Princeton, NJ 08543-1029 (USA)

(First received October 20th, 1992; revised manuscript received January 8th, 1993)

ABSTRACT

The suitability of C_{18} reversed-phase thin-layer chromatography (TLC) for enrichment of adducts in the ^{32}P-postlabeling assay was investigated for structurally diverse classes of DNA adducts derived from benzo[*a*]pyrene, 2-acetylaminofluorene, benzoquinone, safrole, and mitomycin C. The TLC enrichment involved retention of adducts to the C_{18} phase followed by elution with organic solvent–water. Adduct patterns obtained by the C_{18} purification were qualitatively similar to those obtained by the nuclease P1 and butanol procedures, the two commonly used enrichment methods. Adduct recoveries by the C_{18} method varied for different adducts and were significantly lower than those obtained by the other two techniques.

INTRODUCTION

The ^{32}P-postlabeling method is a sensitive method for detecting and quantifying carcinogen–DNA adducts implicated in the initiation of chemical carcinogenesis [1,2]. Following the development of this method to normal bases and the modified base, m^5C in DNA [3], it was extended to DNA bases modified with structurally diverse mutagens/carcinogens [4–6]. Initial description of the assay comprised no enrichment of adducts and involved digestion of DNA to 3′-monophosphates of normal and adducted nucleotides, their labeling with [γ-^{32}P]ATP and detection after TLC. Subsequently, procedures were developed to enrich adducts or remove normal nucleotides prior to labeling to increase the assay's sensitivity. These include: purification of adducts by octadecylsilane (C_{18}) reversed-phase TLC [7], extraction of adducts with butanol [8], treatment of DNA digests [9,10] or DNA [11,12] with nuclease P1 [9,11,12] or nuclease S1 [10], and purification of adducts by C_{18} reversed-phase HPLC [13–18]. Nuclease P1 treatment and butanol extraction of DNA digests have been widely used for aromatic adducts. The nuclease P1 procedure, the simpler of the two, is as effective as the butanol method for most aromatic adducts from one or more aromatic ring carcinogens, but gives lower recovery of certain aromatic adducts [7,19–21]. On the other hand, the butanol technique shows little or no recovery of small aromatic adducts from styrene oxide and bulky adducts from diaziquone [22], mitomycin C (MMC; this paper), benzoquinone (BQ; this paper), and oxidative DNA damage [23].

Because of these differences, there is a desire for a more versatile method for enrichment of adducts, and C_{18} reversed-phase TLC can potentially be one such method, because the C_{18} phase

0378-4347/93/$06.00

retains both aromatic and bulky adducts as 3′,5′-bisphosphate nucleotide products [6,7,22,24–28]. The purpose of this investigation was to determine the suitability of the C_{18} TLC purification method for structurally diverse aromatic and bulky carcinogen–DNA adducts by comparing their recoveries with those obtained by the nuclease P1 and butanol methods. To date, the C_{18} TLC method has been applied to the purification of mycotoxin–DNA adducts [7].

EXPERIMENTAL

Materials

Glass-backed KC_{18} reversed-phase TLC plates (20 × 20 cm) were from Whatman (Hillsboro, OR, USA) and isopropanol (HPLC grade) from Fisher Scientific (Springfield, NJ, USA). Plastic-backed polyethyleimine (PEI)-cellulose plates (20 × 40 cm) were Macherey-Nagel brand from Alltech Assoc. (Deerfield, IL, USA). The sources of other materials for the postlabeling assay have been reported previously [5,7–9,24].

TLC tanks and saturation

TLC was performed either in small glass tanks (27 × 13 × 7 cm) or custom-made acrylic chambers (29 × 29 × 22 cm) [29]. Unless contraindicated, appropriate volume of solvent was added to tanks or chambers just before the start of TLC and their saturation was not performed.

Preparation of adducted carcinogen–DNAs

For comparative evaluation of adducts by different procedures, the following DNA samples were used: benzo[*a*]pyrene (BP)–DNA isolated by an abbreviated extraction method [26] from the lungs of female CF1 mice (about 26 g) at 24 h after four daily topical applications of 50 μg of BP in 50 μl of acetone, 2-acetylaminofluorene (AAF)–DNA extracted from treated rat liver [20], BQ–DNA prepared by *in vitro* reaction [12], safrole (SAF)–DNA from treated mouse liver [30], and MMC–DNA from female rat liver [26].

^{32}P-Postlabeling analysis of DNA adducts by C_{18} TLC enrichment

Digestion. BP–DNA (5 μg), AAF–DNA (2 μg), BQ–DNA (0.25 μg), SAF–DNA (1 μg), and MMC–DNA (3 μg) were mixed with prepurified calf thymus DNA to bring total quantity to 10 μg. Commercially obtained calf thymus DNA (Sigma, St. Louis, MO, USA) was purified by enzymatic digestions and solvent extractions [26]. DNA samples were then enzymatically digested to 3′-mononucleotides with micrococcal nuclease (0.5 U) and spleen phosphodiesterase (0.01 U) in 10 μl of 10 m*M* $CaCl_2$ and 20 m*M* sodium succinate, pH 6.0, at 37°C for 3 h [8].

Enrichment and ^{32}P-labeling. For C_{18} purification of adducts, the KC_{18} reversed-phase TLC plates (20 × 20 cm) were cut into smaller size (10 × 10 cm), and purified as follows. Each plate was developed overnight (about 15 h) in ascending fashion in a glass tank with 0.4 *M* ammonium formate, pH 6.2, onto a 15-cm Whatman 1 paper wick that had been attached with paper clips to the top of the plate. Following development, the wick was removed, and the plate was dried and developed in the direction of previous solvent flow with isopropanol–water (1:1, v/v), to the top and dried. A glass TLC tank containing 50 ml of 0.4 *M* ammonium formate, pH 6.2, and the top sealed with Saran Wrap and a lid was precooled overnight at 4°C. The above DNA digests were applied in small aliquots to the C_{18} plate (four samples per plate) at 2 cm from bottom edge and 2 cm apart. The wet plate was then developed in precooled ammonium formate at 4°C to 8 cm above the origin to remove normal nucleotides. The plates were dried under an air dryer for 5 min with cool air and for 10 min with warm air (about 40°C). To extract adducts, the C_{18} material from the origins (0.2 cm below, 2 cm above, 0.5 cm either side) was scraped with TLC scraper (Alltech Assoc.), transferred to 1.5-ml polypropylene microcentrifuge tubes, and extracted with 600 μl of isopropanol–water (1:1, v/v) at 37°C for 30 min with continuous stirring on a mechanical mixer (Vortex-2 Genie, Scientific Industries, Bohemia, NY, USA). After centrifugation on a table-top microcentrifuge at 13 000 *g* for 3 min, the

supernatant was collected, and the pellet was reextracted with 400 μl of cocktail and repelleted. The combined supernatant fractions were centrifuged again for 10 min to remove fine particles before evaporation in vacuo. The residue was dissolved in 14 μl of water, mixed with 2 μl of 1 *M* 2-(N-cyclohexylamino)ethanesulfonic acid (CHES)–NaOH, pH 9.6, and ^{32}P-labeled with a 4-μl solution containing each of the following: 2.0 μl of kinase buffer [200 m*M* N,N-bis(2-hydroxyethyl)glycine (Bicine)–NaOH, 100 m*M* dithiothreitol, 100 m*M* $MgCl_2$, 10 m*M* spermidine, pH 9.6], 0.76 μl (100 μCi) of carrier-free [γ-^{32}P]ATP (ICN Radiochemicals, Cat. No. 35020), 0.16 μl of polynucleotide kinase (4.8 U), and 1.08 μl of 10 m*M* Tris–HCl, pH 9.6. After incubation at 37°C for 30 min, 1 μl of apyrase (50 mU) was added, and the reaction mixture was further incubated at 37°C for 30 min. To ensure that normal nucleotides are removed and [γ-^{32}P]ATP is present in excess during labeling, a 2-μl aliquot of the labeled solution (before or after apyrase treatment) was diluted to 375 μl in 10 m*M* Tris–HCl, pH 9.6, and a 5-μl aliquot of the diluted solution was chromatographed by one-dimensional polyethyleneimine (PEI)–cellulose TLC [9] to resolve nucleotides.

Resolution of ^{32}P-labeled adducts. For mapping of adducted nucleotides of BP, AAF, and BQ, a 15-μl aliquot of the labeled solution was resolved by the previously described multi-directional PEI–cellulose TLC [5], with modifications [10]. Prior to use, the PEI–cellulose (20 × 40 cm) sheets were cut into 12.5 × 20 cm, developed with water in acrylic chambers for about 16 h with lid slightly opened for continuous development of the solvent once it reached the top, and air-dried. The sheet was marked with an origin at 7.5 cm from the bottom of the 12.5-cm edge and 2 cm from the left-hand side of the 20-cm edge and attached by stapling an 8 cm Whatman 3 paper wick [5,29]. The labeled digest was applied to the origin by clamping the pipet to a device and allowing the solution to flow from the pipet tip on to the cellulose layer by capillary action [29]. For initial purification, the wet sheet was then developed (D1 direction) in an acrylic chamber [29] with 100 ml of 1.7 *M* sodium phosphate, pH 6.0, until the solvent reached at least 70% of the wick which took 20–30 h. After development, the sheet was cut with a pair of scissors at 2.8 cm below the origin in D1 direction, and the wick containing bulk of radioactivity as $^{32}P_i$ was discarded. The other portion of the sheet containing adducts at or near the origin was washed in water stirred continuously [29] in a 38-l glass aquarium, dried, and developed two-dimensionally to resolve adducts. The first dimension along D3, opposite D1, was with 100 ml of solvent in an acrylic chamber. Different concentrations of D3 solvent were used for each compound and prepared by diluting stock solution with water. The composition of the stock D3 solution was 4.5 *M* lithium formate, 8.5 *M* urea, pH 3.5. D3 solvents corresponding to 95% (*i.e.* prepared by mixing 95 ml of stock solution and 5 ml of water), 80%, and 60% of the stock concentration were used for the separation of adducts from BP, AAF, and BQ, respectively. After D3 development, the wet sheet was cut 2–3 mm above the origin in D3 direction, and the portion containing the origin was discarded. The remaining portion of the sheet was washed in water to remove salts, dried, and developed in the second dimension, perpendicular to D3, in D4 solvent. Stock D4 solvent composition was 0.8 *M* sodium phosphate, 0.5 *M* Tris–HCl, 8.5 *M* urea, pH 8.0, and concentrations corresponding to 90%, 70%, and 50% of stock were employed for adducts from BP, AAF, and BQ, respectively. After D4 development, the sheet was washed in water, dried, and developed (along D4) in 1.7 *M* sodium phosphate, pH 6.0 (D5), on to a 3-cm Whatman 1 wick attached to the top of the sheet to reduce background radioactivity. The wick was removed and discarded, and the sheet was given a brief water wash, dried, and exposed to X-ray films to locate adducts.

For quantitation, adduct spots and appropriate background areas were cut out from the plastic sheet and counted without liquid scintillation (Cerenkov radiation). The relative adduct labeling (RAL) was calculated from adduct cpm according to: RAL = (adduct cpm after background correction)/(pmol of DNA labeled ×

specific activity of [γ-^{32}P]ATP in cpm/pmol). The specific activity of [γ-^{32}P]ATP was determined by labeling a known amount of dAp [9], followed by TLC using 1.8 *M* ammonium formate, pH 3.5 [31]. A value of *N* for (RAL × 10^9) corresponds to *N* number of adducts per 10^9 DNA nucleotides provided adduct recovery by enzymatic and chromatographic methods is quantitative.

SAF and MMC lesions were resolved by a combination of C_{18} TLC and PEI–cellulose TLC [24, 26]. Prewashed glass-backed C_{18} plates (10 × 10 cm) were marked with four origins and prescored with a carbide wheel cutter around the origins for later excision, and a 15-cm Whatman 1 wick was attached at the top with paper clips. Labeled digests (15 μl) were applied to the origins of the C_{18} plate. A glass tank with 60 ml of 0.4 *M* ammonium formate, pH 6.2, was precooled at 4°C overnight. For initial purification of adducts the plates were placed in the tank with paper wick projecting out of the tank, allowing the solvent to develop continuously on to the paper wick once the top was reached. After development, the plate was broken along a scored line to remove and discard the wick containing bulk of radioactivity. The plate was dried thoroughly as above and broken along several prescored lines so as to obtain origins (1.44 cm^2 chips) containing the retained adducts. For contact transfer of adducts from C_{18} layer to PEI–cellulose layer, each chip was attached with a clip to a PEI–cellulose acceptor sheet (10 × 10 cm), which was then developed in ascending fashion at 50°C in a preheated glass tank with *n*-propanol–water (1:1, v/v) containing 1% Nonidet P-40 [24]. After development, the chips were removed, and the PEI-cellulose sheets were washed in water, dried, and developed two-dimensionally to resolve adducts. Development in the first dimension (D3) was with 50% of stock D3 solvent of composition given above, and in the second dimension (D4) was with 45% of stock D4 solvent of composition 0.8 *M* lithium chloride, 0.5 *M* Tris–HCl, 8.5 *M* urea, pH 8.0; the chromatograms were cut 2–3 mm above the origin in D3 direction after D3 development, and the origin portion discarded. After D4 development, the plates were further developed along D4 in 1.7 *M* sodium phosphate, pH 6.0 (D5), to a 3-cm Whatman 1 paper wick, washed, dried, and autoradiographed as above.

^{32}P-Postlabeling analysis of DNA adducts after enrichment by butanol and nuclease P1

In the analysis of adducts by the butanol and nuclease P1 procedures, DNA samples were digested as above. To 10 μl of the digest was added a 4-μl solution containing 1.4 μl of nuclease P1 (5.6 μg, 5.6 U of 3′-nucleotidase), 0.7 μl of 2 m*M* $ZnCl_2$, 0.7 μl of 0.8 *M* sodium acetate, pH 5.0, and 1.2 μl of water and incubated at 37°C for 45 min [9]. Butanol extraction was performed as described before [8], and the dried samples were rehydrated in 16 μl of water. The butanol extracts and nuclease P1 digests were ^{32}P-labeled, treated with apyrase, and chromatographed as above.

RESULTS AND DISCUSSION

DNA samples modified with BP, AAF, BQ, SAF, and MMC produced the autoradiograms shown in Fig. 1 when analyzed by the ^{32}P-postlabeling assay with enhancements by C_{18} TLC, nuclease P1 (except AAF–DNA), and butanol (only AAF–DNA). Adduct patterns for each modified DNA were qualitatively similar by different procedures, but adduct levels varied. With low-modified MMC–DNA, spot 1 was barely seen, while the other two adducts were not detected by the C_{18} TLC enrichment. Some representative adducts from each modified DNA were quantitated, and the data are given in Table I. Adduct recoveries by the C_{18} enhancement varied for different adducts and were significantly lower than those obtained by the nuclease P1 digestion and butanol extraction. The major BP adduct (spot 1) showed 45% of the recovery obtained by the nuclease P1 method. For other adducts, this value was as follows: BQ adduct 1, 21%; SAF adduct 1, 4%; SAF adduct 2, 51%; and MMC adduct 1, 31%. The major AAF adduct (No. 1) was 8% of that obtained by the butanol method. The recovery of BQ and MMC adducts by the butanol method was little to none compared with the nuclease P1 digestion.

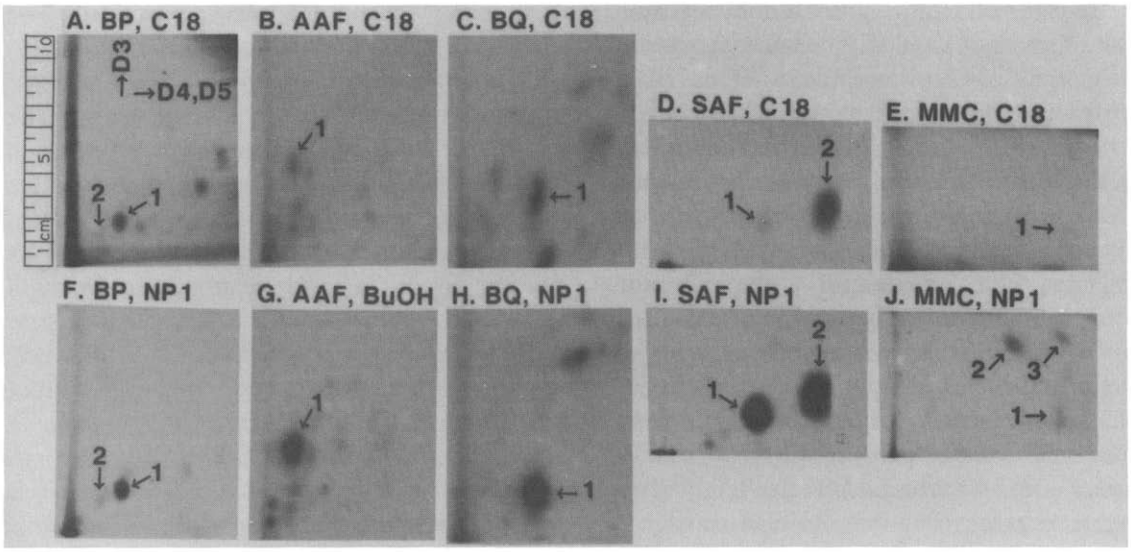

Fig. 1. Comparison of various DNA adduct patterns obtained by the ^{32}P-postlabeling assay after enhancement with C_{18} reversed-phase TLC, nuclease P1 (NP1), and butanol (BuOH). DNA samples (0.25–5 μg modified DNA plus calf thymus DNA to a total of 10 μg) adducted with the indicated carcinogens were digested to 3′-nucleotide adducts, which were then enriched by C_{18} TLC (A–E) or NP1 treatment (F, H–J) or butanol extraction (G), ^{32}P-labeled, and resolved by PEI–cellulose TLC. Orientation of D3, D4, and D5 developments are as indicated. The origins were removed after D3 development (see text). Autoradiographic conditions were at −80°C with Dupont Cronex-4 X-ray films and intensifying screen for 12 h (A, E, F, J), 3 h (B, C, G, H), and 1 h (D, I). Representative carcinogen-specific adducts have been identified by numbers.

TABLE I

COMPARISON OF DNA ADDUCT LEVELS MEASURED BY THE ^{32}P-POSTLABELING ASSAY WITH ENHANCEMENTS BY C_{18} REVERSED-PHASE TLC, NUCLEASE P1, AND BUTANOL

For each DNA, the RAL values represent mean ± S.D. of triplicate postlabeling analyses. The values are given to two significant numbers. See Fig. 1. for adduct number assignment.

DNA type	Enrichment method	RAL × 10^9	
		Spot 1	Spot 2
BP–DNA	C_{18}	28.0 ± 1.2	7.8 ± 1.6
	Nuclease P1	62.0 ± 3.0	11.5 ± 4.0
	P[a]	0.000	0.213
AAF–DNA	C_{18}	110.0 ± 54.0	
	Butanol	1300.0 ± 650.0	
	P	0.02	
BQ–DNA	C_{18}	6300.0 ± 1800.0	
	Nuclease P1	30 000.0 ± 2100.0	
	P	0.000	
	Butanol	19.0 ± 8.9	
SAF–DNA	C_{18}	930.0 ± 300.0	7600.0 ± 610.0
	Nuclease P1	21 000.0 ± 3300.0	15 000.0 ± 3600
	P	0.000	0.035
MMC–DNA	C_{18}	14.0 ± 1.3	
	Nuclease P1	45.0 ± 17.0	
	P	0.09	
	Butanol	0.0 ± 0.0	

[a] Significant difference between the C_{18} and P1 or butanol values was evaluated by unpaired Student's *t*-test using a computer program. *P* values of 0.05 or less were considered statistically significant.

The recovery of major AAF adduct was not improved (data not shown) when the above-described C_{18} TLC conditions were altered as follows: extraction of adducts at 25 or 50°C; extraction with *n*-propanol–2-butoxyethanol–water (1:1:2), development of the C_{18} plate in a higher salt concentration [22], which has been shown to increase the retention of diaziquone adducts; development of the C_{18} plate in 0.4 *M* ammonium formate, pH 6.2, containing 5 or 10 m*M* tetrabutylammonium chloride, an ion-pair reagent which aids in the retention of polar adducts to the C_{18} phase; and longer elution of the plate with solvent, *i.e.*, overnight for 16 h onto a Whatman paper wick [24], to accomplish better removal of normal nucleotides.

Analysis of an aliquot of labeled solution by one-dimensional PEI–cellulose TLC showed that the normal 3′-nucleotides were effectively removed by C_{18} TLC, and that [γ-^{32}P]ATP was left over after labeling reaction as evident by a strong [γ-^{32}P]ATP spot before apyrase reaction or a strong $^{32}P_i$ spot after apyrase (autoradiograms not shown). Among the three methods, the nuclease P1 digestion removed normal nucleotides slightly better than the C_{18} and butanol purifications, while the latter two showed comparable results.

The major adduct of BP (spot 1) has previously been characterized as the 3′,5′-bisphosphate of 10β-(deoxyguanosin-N^2-yl)-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene [5,32], AAF adduct 1 as the 3′,5′-bisphosphate of N-(deoxyguanosin-8-yl)-2-aminofluorene [5,19], BQ adduct as a deoxyguanosine derivative [33], probably (3′-hydroxy)benzetheno[N^1,N^2]deoxyguanosine 3′,5′-bisphosphate [34], SAF adduct 1 as the 3′,5′-bisphosphate of N^2-(*trans*-isosafrol-3′-yl)deoxyguanosine [24,25], SAF adduct 2 as the 3′,5′-bisphosphate of N^2-(safrol-1′-yl)deoxyguanosine [24,25], and MMC adduct 1 as a deoxyguanosine derivative [26].

Our results indicate that the C_{18} TLC enrichment gives reasonable recoveries of some adducts (*e.g.*, BP adduct 1 and SAF adduct 2) considering the extent of experimental manipulations, but much less of others (*e.g.*, adduct 1 of AAF, BQ, and SAF). Variable recoveries may result from different extents of retention of each adduct to the C_{18} phase, although attempts to improve retention of the major AAF adduct using higher salt [22] or an ion-pair agent failed. Taking increasing area of C_{18} plate might improve adduct recovery, as only a standard area of the plate (2 cm above the origin) was utilized for adduct extraction, and some poorly retained adducts might migrate above this area. This approach was not examined in this communication, but it has been employed for the recovery of ^{32}P-labeled adducts and polar I-compounds from a PEI–cellulose TLC sheet [35,36]. Variable recovery may also result from some soluble material which may be extracted from the C_{18} phase and may inhibit the labeling of each adduct to different degrees. SAF adduct 1 appeared to be better retained as the 3′,5′-bisphosphate derivative than as the 3′-monophosphate derivative, since the ratio of adduct 1 to adduct 2 is 0.1 by the C_{18} enrichment, whereas it is 1.4 by the nuclease P1 method which also included a C_{18} purification after labeling.

Among the three methods for enrichment, the nuclease P1 method is apparently much simpler than the butanol and C_{18} TLC procedures and would, therefore, be the method of choice when adduct recovery is satisfactory. However, the nuclease P1 method being not suitable for certain aromatic amine adducts was first recognized by Reddy and Randerath [9] and later confirmed by Gupta and Earley [19]. Although butanol extraction allowed recovery of most aromatic adducts including aromatic amine derivatives, it is not as effective for some small aromatic or bulky adducts as the P1 method is (see Introduction). We investigated the C_{18} TLC approach here hoping that it will cover a wider range of structurally diverse adducts than nuclease P1 and butanol techniques. Unfortunately, the results were contrary to the expectation for adducts studied from five different classes of carcinogens, suggesting that the C_{18} method as described is less useful than the other two methods.

In conclusion, the results indicate that the C_{18} TLC procedure is not suitable for enrichment of all aromatic or bulky adducted nucleotides.

ACKNOWLEDGEMENTS

This work was supported by a Joint-Industry Research Program sponsored by Amoco Corp., Ashland Oil Inc., The Dow Chemical Co., Mobil Oil Corp., Standard Oil Co., and Sun Co., Inc. I am grateful to Scott C. Schultz for his excellent technical assistance.

REFERENCES

1 E. C. Miller and J. A. Miller, *Cancer*, 47 (1981) 2327.
2 I. B. Weinstein, *J. Supramol. Struct. Cell. Biochem.*, 17 (1981) 180.
3 M. V. Reddy, R. C. Gupta and K. Randerath, *Anal. Biochem.*, 117 (1981) 271.
4 K. Randerath, M. V. Reddy and R. C. Gupta, *Proc. Natl. Acad. Sci. U.S.A.*, 78 (1981) 6126.
5 R. C. Gupta, M. V. Reddy and K. Randerath, *Carcinogenesis*, 3 (1982) 1081.
6 M. V. Reddy, R. C. Gupta, E. Randerath and K. Randerath, *Carcinogenesis*, 5 (1984) 231.
7 M. V. Reddy, T. R. Irvin and K. Randerath, *Mutat. Res.*, 152 (1985) 85.
8 R. C. Gupta, *Cancer Res.*, 45 (1985) 5656.
9 M. V. Reddy and K. Randerath, *Carcinogenesis*, 7 (1986) 1543.
10 M. V. Reddy, *Carcinogenesis*, 12 (1991) 1745.
11 K. Randerath, E. Randerath, T. F. Danna, K. L. van Golen and K. L. Putman, *Carcinogenesis*, 10 (1989) 1231.
12 M. V. Reddy, W. T. Bleicher, G. R. Blackburn and C. R. Mackerer, *Carcinogenesis*, 11 (1989) 1349.
13 B. P. Dunn and R. H. C. San, *Carcinogenesis*, 9 (1988) 1055.
14 N. J. Gorelick and G. N. Wogan, *Carcinogenesis*, 9 (1989) 1567.
15 W. P. Watson and A. E. Crane, *Mutagenesis*, 4 (1989) 75.
16 F.-L. Chung, R. Young and S. S. Hecht, *Carcinogenesis*, 10 (1989) 1291.
17 P. G. Shields, A. C. Povey, V. L. Wilson, A. Weston and C. C. Harris, *Cancer Res.*, 50 (1990) 6580.
18 R. Mustonen, A. Forsti, P. Hietanen and K. Hemminki, *Carcinogenesis*, 12 (1991) 1423.
19 R. C. Gupta and K. Earley, *Carcinogenesis*, 9 (1988) 1687.
20 M. V. Reddy, G. R. Blackburn, S. E. Irwin, C. Kommineni, C. R. Mackerer and M. A. Mehlman, *Environ. Health Perspect.*, 82 (1989) 239.
21 J. E. Gallagher, M. J. Kohan, M. H. George and J. Lewtas, *Carcinogenesis*, 12 (1991)1685.
22 R. C. Gupta, A. Garg, K. Earley, S. C. Agarwal, G. R. Lambert and S. Nesnow, *Cancer Res.*, 51 (1991) 5198.
23 P. L. Carmichael, M. N. She and D. H. Phillips, *Carcinogenesis*, 13 (1992) 1127.
24 K. Randerath, R. E. Haglund, D. H. Phillips and M. V. Reddy, *Carcinogenesis*, 5 (1984) 1613.
25 D. H. Phillips, M. V. Reddy and K. Randerath, *Carcinogenesis*, 5 (1984) 1623.
26 M. V. Reddy and K. Randerath, *Mutat. Res.*, 179 (1987) 75.
27 S.-F. Liu, S. M. Rappaport, J. Rasmussen and W. J. Bodell, *Carcinogenesis*, 9 (1988) 1401.
28 K. Yamashita, K. Wakabayashi, Y. Kitagawa, M. Nagao and T. Sugimura, *Carcinogenesis*, 9 (1988) 1905.
29 M. V. Reddy and G. R. Blackburn, *Carcinogenesis*, 11 (1990) 683.
30 M. V. Reddy and K. Randerath, *Mutat. Res.*, 241 (1990) 37.
31 M. V. Reddy, W. T. Bleicher and G. R. Blackburn, *Cancer Commun.*, 3 (1991) 109.
32 L.-J. W. Lu, R. M. Disher, M. V. Reddy and K. Randerath, *Cancer Res.*, 46 (1986) 3046.
33 M. V. Reddy, G. R. Blackburn, C. A. Schreiner, M. A. Mehlman and C. R. Mackerer, *Environ. Health Perspect.*, 82 (1989) 253.
34 L. Jowa, S. Winkle, G. F. Kalf, G. Witz and R. Snyder, in J. Kocsis, D. J. Jollow, C. M. Whitmer, J. O. Nelson and R. Snyder, (Editors), *Advances in Experimental Medicine and Biology. Biological Intermediates III*, Plenum Press, New York, 1986, p. 825.
35 K. Randerath, M. V. Reddy and R. M. Disher, *Carcinogenesis*, 7 (1986) 165.
36 G. Talaska, J. H. Roh and T. Getek, *J. Chromatogr.*, 580 (1992) 293.