11. Lehmann, P. F., Hsiao, C. B. and Salkin, I. F., *J. Clin. Microbiol.*, 1989, **27**, 400–404.
12. Stevens, D. A., Odds, F. C. and Scherel, S., *Rev. Infect. Dis.*, 1990, **12**, 256–266.
13. Lodder, J. and Kreger, Rij van, N. J. W., in *The Yeasts the Taxonomical Study*, North Holland, Amsterdam, 1967.
14. Sambrook, J., Fritsch, E. F. and Maniatis, T., in *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989.
15. Burnie, J. P., Mathews, R. and Lee, W., *Epidem. Infect.*, 1987, **99**, 201–211.
16. Vaundry, W. L., Tierner, A. J., Wenman, W. M., *J. Infect. Dis.*, 1988, **158**, 1375–1379.
17. Moro, M. L., Maffeei, C., Manso, E., Morace, G., Polonelli, L. and Biavasco, F., *Infect. Control. Hosp. Epidemiol.*, 1990, **11**, 27–35.
18. Hasenclever, H. T. and Mitchell, W. O., *J. Bacteriol.*, 1961, **82**, 570–572.
19. Odds, F. C. and Abbott, A. B., *Sabouraudia*, 1980, **18**, 301–317.
20. Scherer, S. and Stevens, D. A., *J. Clin. Microbiol.*, 1987, **25**, 675–679.

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Antitumour agent coralyne: A guanine-cytosine specific DNA-binding alkaloid

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The base and sequence specificity of coralyne–DNA interaction were investigated by spectrophotometric, spectrofluorimetric, circular dichroic, thermal melting and viscometric studies. The strong binding of this alkaloid to various natural and synthetic DNAs was characterized by remarkably large changes of hypochromism and bathochromism in the absorption bands, quenching of steady state fluorescence intensity, decrease in quantum yield, perturbations in circular dichroic spectrum, stabilization of DNA against thermal denaturation and increase in contour length of sonicated rod-like duplex DNA. Binding of coralyne to various DNAs was dependent upon the base pair composition and sequence of DNA, being larger for GC-rich DNA and alternating GC polymer as observed from the above techniques. These studies demonstrate the GC base pair specificity in the intercalative DNA binding of coralyne.

THE molecular mechanism of the sequence-specific recognition of DNA by small molecules has received considerable attention in the last several years. A detailed understanding of the various factors that govern the binding of small molecules to specific nucleotide sequences in DNA is essential for the rational design of new anticancer drugs for gene targeting[1,2]. Extensive studies are therefore currently directed to delineate the molecular mechanism, site and regiospecificity by which these molecules bind to DNA targets[3,4]. In this context, our laboratory has been studying the binding aspects of natural alkaloids to different DNA polymers[5–11]. Alkaloids are known to have important role in medicinal chemistry due to their extensive biological activity. Especially noteworthy is the isoquinoline group of alkaloids for their wide distribution in several botanical families with myriad therapeutic applications[12,13]. The most important members of the protoberberine group are berberine and its synthetic analogue coralyne. Both of them are reported to possess promising antitumour activities, with the latter having very low cytotoxicity[14–16]. Coralyne has attracted recent attention for its ability to inhibit the topoisomerase I function[17]. The topoisomerase I poisoning of several coralyne derivatives has been reported very recently where mixed modes of DNA interaction have been envisaged depending on the state of saturation in the ring system for stabilizing the enzyme–DNA–drug ternary complex[18]. Coralyne has been earlier reported to form a complex with duplex DNA by mechanism of intercalation[19,20], but the details of the nature and base specificity in its interaction still remains obscure. In order to understand the molecular nature of its specificity towards sequence of base pairs, we have carried out a series of physico-chemical measurements on the interaction of coralyne with several natural and synthetic DNAs of varying base composition and sequences.

Coralyne chloride (Figure 1) was obtained from Aldrich Chemical Co., St. Louis, MO, USA and its purity was checked by thin-layer chromatography and melting point determinations. We have estimated a molar extinction coefficient (ε) of 17,500 M^{-1} cm^{-1} at 424 nm for determining the concentration of coralyne in our solution conditions. The four natural DNAs, namely *Clostridium perfringens* (CP), calf thymus (CT), *Escherichia coli*

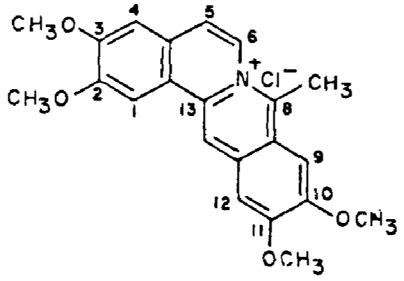

Figure 1. Chemical structure of coralyne chloride.

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(EC) strain B, *Micrococcus lysodeikticus* (ML) were from Sigma, USA and had respectively 30, 42, 50 and 72 mole% of guanine-cytosine content. The synthetic polymers poly(dG-dC)·poly(dG-dC) [hereafter poly[d(G-C)], poly(dA-dT)·poly(dA-dT) [hereafter poly[d(A-T)], poly(dG)·poly(dC) and poly(dA)·poly(dT) were obtained either from Sigma or Pharmacia, USA. The natural DNAs were sonicated to ~ 280 ± 60 base pairs in length while the average size of synthetic DNAs was ~ 270 ± 50 base pairs in length as revealed from viscometric studies. BPES buffer of pH 7.00 ± 0.01 having 0.75 mM of Na_2HPO_4, 0.25 mM NaH_2PO_4, 0.125 mM EDTA and 8.0 mM NaCl containing 30% (v/v) ethanol was used for spectrophotometric titration experiments. All other studies were performed in BPES buffer containing 8% (v/v) ethanol. The physico-chemical properties of duplex DNA remained unchanged under these conditions as revealed from UV and circular dichroic spectral characteristics. Ethanol was used as a co-solvent as coralyne undergoes aggregation in aqueous condition[21].

The absorption spectra of the alkaloid mixed with or without DNA were obtained using Shimadzu UV 260 spectrophotometer (Shimadzu Corporation, Japan) in quartz cells of 1 cm path length at 25°C and the melting profiles were recorded on the same spectrophotometer using the temperature programmer (KPC 5) and a temperature controller (SPR 5). Spectrophotometric titration data were used to construct Scatchard plots which in turn were fitted to a theoretical curve, which was drawn according to the excluded site model of Crothers[22] developed by McGhee and von Hippel[23] for a nonlinear non-cooperative ligand binding system.

$$r/C_f = K(1 - nr)[(1 - nr)/(1 - (n - 1)r)]^{n-1}, \qquad (1)$$

where K is the binding constant to an isolated DNA-binding site and n is the exclusion parameter in nucleotides. Steady-state fluorescence spectra were recorded in fluorescence free quartz cells of 1 cm path length on a Hitachi model F-4010 unit (Hitachi Ltd, Japan) at 25°C equipped with a temperature controller model Eyela Uni Cool UC-55 (Tokyo Rikakikai, Japan). Circular dichroism (CD) measurements were carried out on a Jasco J-720 spectropolarimeter (Jasco Corporation, Tokyo, Japan) at 25°C equipped with a Jasco temperature controller, model PTC 343, Compaq Prolinea 4/33 computer, Hewlett Packard Desk Jet 505J printer and Sekonic/SPL-430A X–Y plotter.

Linear duplex native and synthetic DNAs were sonicated in a Labsonic 2000 sonicator (B. Braun, Swiss). Viscometric measurements were performed using a Cannon Manning type 75 semimicro viscometer, mounted vertically in a constant temperature bath (Cannon Instruments Co., USA) at 25 ± 0.05°C. Flow times of DNA alone and DNA alkaloid complexes were measured by an electronic stopwatch (Fisher Scientific, USA) with an accuracy of 0.01 s. The increase in helix length of sheared DNA was calculated from the experimental data, which were transformed directly from flow times to values by the following expression as described earlier[24],

$$L/L_0 = \{(t_2 - t_0)/(t_1 - t_0)\}^{1/3} = 1 + \beta r, \qquad (2)$$

where L is the contour length in presence of the alkaloid, L_0 is the contour length of free DNA, t_2 is the flow time for the complex, t_1 is the flow time for pure DNA, t_0 is the flow time for buffer for a given volume in the viscometer and β is the slope of the plot of L/L_0 versus r.

The interaction of coralyne with various natural and synthetic DNAs was monitored using absorption spectroscopy in the 370–500 nm range, where DNA does not have any absorption. In Figure 2, the changes in the absorption spectrum of coralyne on progressively increasing the concentration of ML DNA are shown. The spectrum marked '1' represents the absorption spectrum of free coralyne in the absence of DNA, which undergoes hypochromic and bathochromic shift in presence of increasing concentration of DNA. Two isobestic points, one at 372 and another at 432 nm observed in

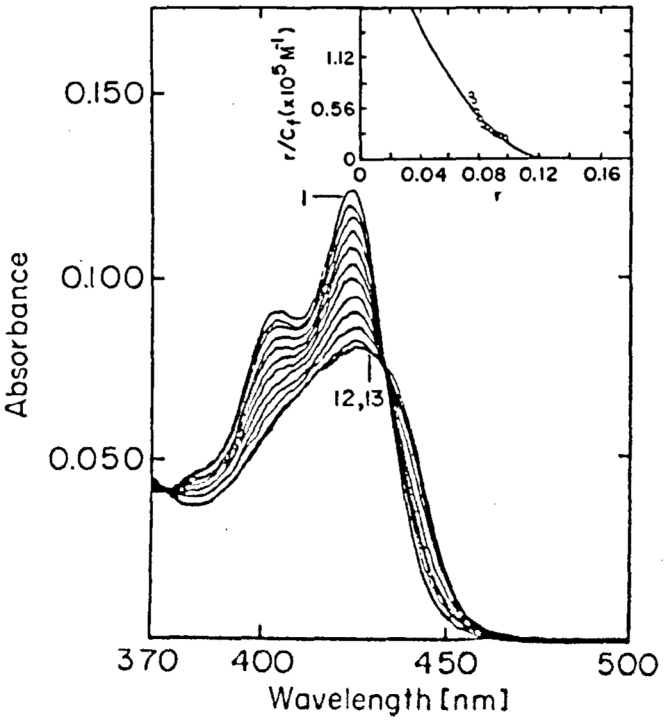

Figure 2. Absorption spectra of coralyne (7.09 μM, curve, 1) treated with ML DNA of 4.34, 8.68, 15.18, 21.66, 30.28, 38.88, 49.59, 60.27, 75.15, 85.73, 96.28 and 104.69 μM concentrations (curves 2–13) respectively. *Inset*: Scatchard plot for the binding process. The solid curve indicates the best fit theoretical line drawn by the method of McGhee and von Hippel[23] using the SCATFLOT program[25].

the series of spectra confirms equilibrium between free and the DNA bound alkaloid molecules. Similar spectral features were observed in all natural DNAs and synthetic polynucleotides, but the extent of changes varied. The maximum changes were seen in the GC rich ML DNA and the two GC polymers. The decrease in absorbance at 424 nm was used to express the data in the form of Scatchard plots. Such a plot in the case of ML DNA is shown as inset in Figure 2. A curvature was observed for Scatchard plots in all DNAs and polynucleotides studied which clearly indicated the involvement of more than one mode of binding in the association of coralyne with duplex DNAs. The isotherms were therefore analysed by the equation of McGhee and von Hippel (*vide supra*) for non-cooperative binding process. The solid line in the inset of Figure 2 indicates the best fit of the equation to the experimental data using the SCATPLOT program[25] that generates the binding parameters, namely the binding constant K and the exclusion parameter n. The values of these parameters obtained for coralyne binding to various natural and synthetic DNAs are presented in Table 1. It can be seen that the values of the binding constant K obtained were in the range of $0.38–9.80 \times 10^5\ M^{-1}$ which are generally observed for intercalators[1,8–11]. More importantly, in the series, the K values were higher for the GC-rich ML DNA and the GC polymers. This is again complemented by lower number of excluded sites in these cases. Taken together this implies a higher binding affinity for coralyne to the alternating GC polymer.

Coralyne has a remarkably strong fluorescence emission peak around 470 nm when excited at 424 nm. In presence of DNA a quenching of this fluorescence intensity was observed. The effect of various natural and synthetic DNAs on the fluorescence of coralyne was studied. The data were quantified in terms of Stern-Volmer quenching constant (K_{SV})[26] and relative quantum yields (ϕ/ϕ_o) (ref. 6) at saturation of each polymer and these are also collated in Table 1. The values of K_{SV} and ϕ/ϕ_o at the first instant suggest a strong interaction of coralyne to DNAs and polynucleotides. Furthermore, the K_{SV} values are higher for the GC-rich ML DNA and the GC polymers while the relative quantum yield values are lower in these cases, again suggesting the alternating GC specificity of coralyne–DNA binding.

The thermal melting (T_m) experiments of various coralyne–DNA/polynucleotide complexes reveal that the alkaloid can effectively stabilize DNA against thermal strand separation. A thermal stabilization (ΔT_m) of 8–9°C in the case of three natural DNAs and ~ 7.0°C in the case of the alternating AT polymer was seen. The homopolymer of AT showed only ~ 2.0°C stabilization (Table 1). T_m experiments with the GC polymers could not be performed as they melted at temperature > 90°C under the conditions of our experiment. Generally all the coralyne–DNA complexes studied melted co-operatively and the profiles were reversible within experimental errors. The alkaloid solution itself was found to be thermally stable in control experiments performed up to 90°C.

Coralyne is an optically inactive molecule and therefore does not have any CD spectrum. The B-form DNAs on the other hand, have conservative CD spectra in the

Table 1. Various parameters of the interaction of coralyne with different natural and synthetic DNAs[a]

Polymer	GC (mol%)	K ($\times 10^5 M^{-1}$)	n	ΔTm[b] (°C)	K_{SV}[c] ($\times 10^3\ M^{-1}$)	ϕ/ϕ_{wl}	β	ΔL (nm)
CP DNA	30	0.38 ± 0.02	14.0 ± 0.30	8.00	0.78	0.30	1.07 ± 0.03	0.18 ± 0.01
CT DNA	42	1.00 ± 0.05	9.0 ± 0.25	8.50	1.32	0.20	1.25 ± 0.03	0.21 ± 0.01
EC DNA	50	1.55 ± 0.08	8.5 ± 0.20	n.d.	n.d.	n.d.	1.56 ± 0.02	0.26 ± 0.01
ML DNA	72	2.87 ± 0.20	7.0 ± 0.21	9.00	2.46	0.24	2.13 ± 0.04	0.36 ± 0.02
Poly[d(G-C)]	100	9.80 ± 0.50	3.9 ± 0.11	n.d.	8.14	0.12	2.48 ± 0.04	0.42 ± 0.02
Poly(dG).poly(dC)	100	7.60 ± 0.30	4.5 ± 0.14	n.d.	n.d.	n.d.	n.d.	n.d.
Poly[d(A-T)]	0	6.42 ± 0.40	6.6 ± 0.17	7.00	2.00	0.55	2.20 ± 0.05	0.37 ± 0.02
Poly(dA).poly(dT)	0	1.00 ± 0.06	10.3 ± 0.20	2.00	n.d.	n.d.	n.d.	n.d.

[a]Four determinations each.

[b]Thermal melting profiles were measured at D/P (alkaloid/DNA nucleotide phosphate molar ratio) of 0.1 for each complex. In our buffer condition the melting temperatures of CP, CT, ML, poly[d(A-T)] and poly(dA).poly(dT) were 49.0, 54.5, 69.5, 32.0 and 41.5°C respectively.

[c]K_{SV} is then Stern–Volmer quenching constant determined from the equation $I_o/I = 1 + K_{SV}$ [DNA], where I_o and I are the fluorescence intensities in the absence and presence of DNA respectively[26]. Data were obtained at saturation P/D (DNA nucleotide phosphate/alkaloid molar ratio) for each complex.

[d]Steady state fluorescence quantum yield (ϕ) was determined from the equation, $\phi_s = (F_s \varepsilon_q C_q / F_q \varepsilon_s C_s) 0.55$ as described earlier[6], where s and q denote sample and quinine sulphate in 0.1 N H_2SO_4 respectively. F denotes the integral area of the fluorescence excitation at the same wavelength. ε is at the wavelength of excitation and C represents the corresponding concentration. ϕ/ϕ_o denotes the relative quantum yield. Data were determined at saturation P/D value for each complex.

n.d.: not determined.

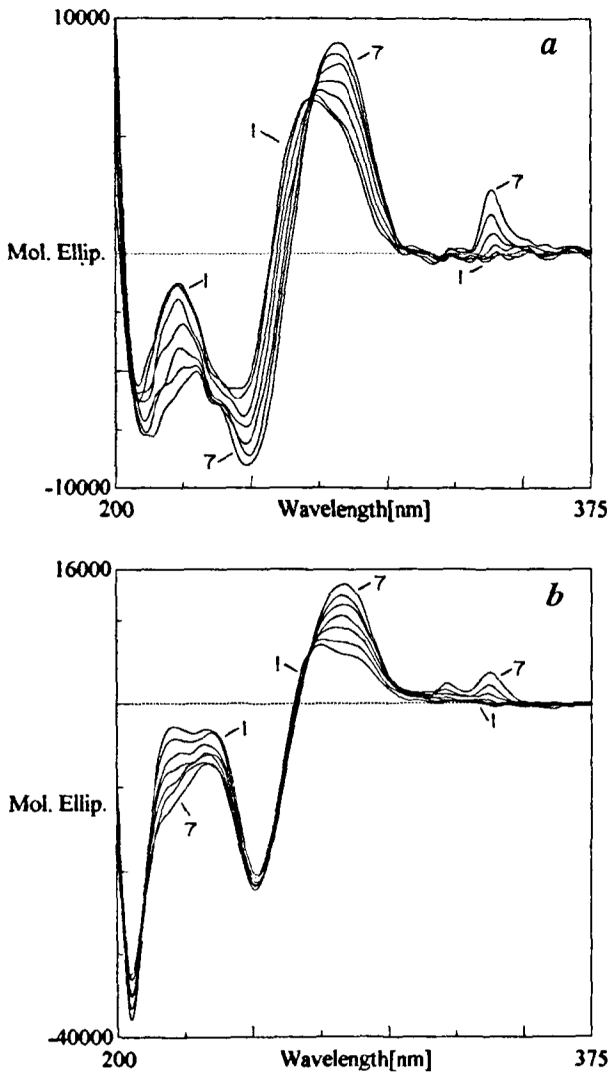

Figure 3. Circular dichroic spectral changes of ML DNA and poly[d(G-C)] on interaction with coralyne. *a*, Curve 1 denotes 85.21 μM of ML DNA treated with 0.97, 2.91, 4.84, 6.77, 8.69 and 10.61 μM of coralyne (curves 2–7) respectively. *b*, Curve 1 denotes 67.46 μM of poly[d(G-C)] treated with 2.08, 5.19, 7.26, 9.32, 11.38, and 14.45 μM of coralyne (curves 2–7) respectively. The expressed molar ellipticity is based on the DNA concentration.

200–300 nm region. In presence of coralyne, the CD spectra of all the DNAs and polymers were perturbed, but to different extents. There was an increase in the ellipticity of the long wavelength positive band with increasing concentration of the alkaloid. The changes were more pronounced in the GC-rich ML DNA and the alternating GC polymer. In Figure 3 the CD spectral changes observed in ML DNA and poly[d(G-C)] are depicted. Such changes in the intrinsic CD generally represent conformational changes consequent to strong binding of small molecules between the base pairs of duplex DNA[7,8]. Further evidence to this argument was obtained from the fact that the bound coralyne molecules acquire optical activity manifested by the generation of a positive extrinsic CD peak around its absorption maximum in the visible absorption region (~ 350 nm). Such non-conservative CD bands induced in symmetrical drug molecules have been characterized due to the electronic coupling between the intercalated drug and the nucleic acid base pairs[7,27,28]. This extrinsic CD band was seen more pronounced in the GC-rich ML DNA and the two GC polymers in comparison to other DNAs and polymers studied, once again implying a better intercalation geometry for coralyne in the alternating GC sequences.

Viscometric studies were performed as a test for conclusive evidence for both intercalation and the sequence specificity observed in spectroscopic experiments. Viscometric techniques are well established for investigating the helix length extension associated with intercalation[1,5,9,24]. Coralyne increased the viscosity of all the natural DNAs and synthetic polymers studied. The values of the helix length enhancement per intercalation site (ΔL) and β obtained for all the DNA duplexes studied are given in Table 1. The values for the ML DNA and the GC polymers fall very well within the range of values reported for classical intercalators like ethidium[1,2], sanguinarine[5,24] and aristololactam-β-D-glucoside[9] unequivocally establishing preferential intercalation of coralyne to the GC sequences.

The results presented here from a combination of spectroscopic and viscometric studies demonstrate that the antitumour agent coralyne binds to DNA, exhibiting a strong GC sequence specificity. The primary evidence to this has been obtained from the binding constants obtained from spectrophotometric titration data of various DNAs and polynucleotides. The binding affinity (K) is higher with the GC-rich DNA and the GC polymers. The quantitative data from fluorescence in terms of K_{sv} and (ϕ/ϕ_o) further testify the strong GC preference of the binding process. The circular dichroic changes in DNAs together with the induced CD signals generated for the bound coralyne molecules clearly underscores the intercalative binding mode and the associated GC specificity in coralyne–DNA interaction. The most authentic test for intercalation and the GC-specific binding of coralyne is obtained from the viscometric experiments where the helix length enhancement accounts for a classical intercalation model similar to that seen for ethidium[1] and sanguinarine[5]. To conclude, our results confirm the intercalative DNA binding mode of coralyne proposed earlier and furthermore establish the base pair specificity involved in the DNA binding of this important antitumour agent that would help in a better correlation of its strong inhibitory effects of topoisomerase I function and the antitumour activity.

1. Waring, M. J., *Annu. Rev. Biochem.*, 1981, **50**, 159–192.
2. Dervan, P. B., *Science*, 1986, **232**, 464–471.
3. Neidle, S. and Waring, M. J. (ed.), *Molecular Aspects of Anti-Cancer Drug Action*, CRC Press, Boca Raton, USA, 1993, vol. 1.

4. Pilch, D. S., Kirolos, M. A., Liu, X., Plum, G. E. and Breslauer, K. J., *Biochemistry*, 1995, **34**, 9962–9976.
5. Maiti, M., Nandi, R. and Chaudhuri, K., *FEBS Lett.*, 1982, **142**, 280–284.
6. Maiti, M., Nandi, R. and Chaudhuri, K., *Photochem. Photobiol.*, 1983, **38**, 245–249.
7. Debnath, D., Suresh Kumar, G. and Maiti, M., *J. Biomol. Struct. Dyn.*, 1991, **9**, 61–79.
8. Suresh Kumar, G., Debnath, D. and Maiti, M., *Anti-Cancer Drug Res.*, 1992, **7**, 305–314.
9. Nandi, R., Chakraborty, S. and Maiti, M., *Biochemistry*, 1991, **30**, 3715–3720.
10. Sen, A., Ray, A. and Maiti, M., *Biophys. Chem.*, 1996, **59**, 155–170.
11. Ray, A. and Maiti, M., *Biochemistry*, 1996, **35**, 7394–7402.
12. Suffness, M. and Cordell, A. G., *The Alkaloids* (ed. Brossi, A.), Academic Press, New York, 1985, vol. 25, pp. 1–355.
13. Bhakuni, D. S. and Jain, S., *The Alkaloids* (ed. Brossi, A.), Academic Press, New York, 1986, vol. 28, pp. 95–181.
14. Zee-Cheng, K. Y., Paull, K. D. and Cheng, C. C., *J. Med. Chem.*, 1974, **17**, 347–351.
15. Creasey, W. A., *Biochem. Pharmacol.*, 1979, **28**, 1081–1084.
16. Kuo, C. L., Chow, C. C. and Yung, B. Y. M., *Cancer Lett.*, 1955, **93**, 193–197.
17. Wang, L-K., Rogers, B. D. and Hecht, S. M., *Chem. Res. Toxicol.*, 1996, **9**, 75–83.
18. Pilch, D. S., Yu, C., Makhey, D., LaVoie, E. J., Srinivasan, A. R., Olson, W. K., Sauers, R. R., Breslauer, K. J., Geacintov, N. E. and Liu, L. F., *Biochemistry*, 1997, **36**, 12542–12553.
19. Gough, A. N., Jones, R. L. and Wilson, W. D., *J. Med. Chem.*, 1979, **22**, 1551–1554.
20. Wilson, W. D., Gough, A. N., Doyle, J. J.and Davidson, M. W., *J. Med. Chem.*., 1976, **19**, 1261–1263.
21. Pal, S., Suresh Kumar, G., Debnath, D. and Maiti, M. (unpublished report).
22. Crothers, D. M., *Biopolymers*, 1968, **6**, 575–584.
23. McGhee, J. D. and von Hippel, P. H., *J. Mol. Biol.*, 1974, **86**, 469–489.
24. Nandi, R. and Maiti, M., *Biochem. Pharmacol.*, 1985, **34**, 321–324.
25. Ray, A., Maiti, M. and Nandy, A., *Comput. Biol. Med.*, 1996, **26**, 497–503.
26. Sen, A. and Maiti, M., *Biochem. Pharmacol.*, 1994, **48**, 2097–2102.
27. Kamia, M., *Chem. Phys.*, 1980, **52**, 337–351.
28. Lyng, R., Hard, T. and Norden, B., *Biopolymers*, 1987, **26**, 1327–1346.

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Influence of fermented whey drink microflora on digestion of lactose

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Utilization of *chhana* whey in the form of a fermented beverage (wheyghurt drink) cultured with *Lactobacillus delbrueckii* subsp. *bulgaricus* W and *Streptococcus thermophilus* H, and its effect on the digestion of residual lactose in the gastrointestinal tract to alleviate lactose intolerance was investigated. Albino rats were fed with a preliminary diet of sucrose, lactose or sucrose with 20 ml wheyghurt drink for 7 days, followed by a test diet of galactose and glucose or lactose, fresh or pasteurized wheyghurt drink for another day. Consumption of sucrose and 20 ml wheyghurt drink as preliminary diet and wheyghurt drink containing viable culture microflora as test diet resulted in better digestion of lactose with maximum increase in serum galactose level (0.38 mg%/mg consumed × body wt/100 after 1 h of consumption) and highest lactase activity (8.20 µmol/g/h at pH 7.0). Gastrointestinal survival of culture organism was demonstrated *in vivo* up to 3 h after feeding, and, thus, improvement in lactose metabolism by consumption of whey drink with viable wheyghurt microflora is suggested in relieving symptoms of lactose intolerance associated with the consumption of milk-based food products.

In recent years much interest has evolved around the influence of lactose intolerance upon the acceptability of dairy products. Consumption of milk products by persons who lack the ability to digest lactose (lactose malabsorbers) often results in development of symptoms such as cramps, flatulence, and diarrhoea[1,2]. This problem which varies with race and geographic location, appears to be common in a high proportion of the worlds population[3,4]. Many researchers have recommended reduction of lactose in the diet of intolerant individuals either by modifying the milk product by enzymatic hydrolysis[5] or by substitution of fermented dairy products for fresh dairy products[6]. Although about 80% of the whey solids is lactose, Gallagher *et al.*[7] have shown that diets of fermented dairy products were effective in relieving symptoms of lactose intolerance in lactose-intolerant individuals who previously were fed unfermented dairy products. A survey of 15 microorganisms revealed that among others, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (yoghurt microflora) were able to hydrolyse 50–60% of lactose in acidified cheese whey within 5 h at 50°C (ref. 8). Kilara and Shahani[9] reported that yoghurt contains substantial lactase bound in the cells of the microbial culture