Metachromatic interactions of dimethylmethylene blue with some polyanionic polysaccharides†

Ashoke Kumar Bhattacharyya* & Nirmal Chandra Chakravorty
Department of Chemistry, Maharaja Bir Bikram College, Agartala 799 004 (Tripura)
Received 7 February 1989; revised and accepted 15 May 1989

1,9-Dimethylmethylene blue (DMMB) exhibits stronger metachromatic ability than methylene blue (MB) and toluidine blue (TB). Unlike MB and TB, DMMB undergoes metachromatic spectral shift in presence of Bael (Aegle marmelosi) exudate gum polysaccharide (BP), a polyanion with relatively low charge density (0.19 per monosaccharide unit), and Bael (fruit) gum polysaccharide, a polysaccharide (AMA) of still lower charge density (0.074). Dextran sulphate (DS), a sulphated polysaccharide with very high charge density (2.0), induces very sharp and stable metachromasia in DMMB in its dilute aqueous solution (conc. $\approx 10^{-5} M$) at a low polymer to dye ratio (P/D) (0.75). Increased stability of metachromatic compound of DMMB-DS system is reflected in its disruption to the extent of only $\approx 50$ per cent by high ethanol concentration ($\sim 60\%$). High urea concentration ($8 M$) does not lead to the half-plateau value. The corresponding metachromatic compounds of DS with TB and MB get destroyed at lower ethanol or urea concentrations.

The structure of gummy material engulfing the seeds of Bael (Aegle marmelosi) fruit has been reported, and its polyelectrolytic, chromotropic and anticoagulant behaviour described. It is a branched chain polysaccharide (AMA) containing galactose, rhamnose, arabinose and glucuronic acid in the molar ratio of $\sim 19:2:3:2$. Bael exudate gum polysaccharide (BP) has been characterised and shown to contain D-galactose, L-rhamnose, L-arabinose and D-glucuronic acid in the molar ratio of $\sim 9:3:1:3$.

1,9-Dimethylmethylene blue (DMMB) is a cationic dye of thiazine group with enriched hydrophobic elements. In this paper we report our observations on the metachromatic interactions of DMMB with some polyanions widely differing in charge densities; the stabilities of the metachromatic compounds toward disruptive factors like ethanol, urea, etc. have also been studied.

Materials and Methods

The isolation of AMA has been described earlier. The exudate gum obtained from the trunk of Bael tree was isolated by repeated precipitation of an aqueous solution of the gum with ethanol. The product so obtained was designated as BP.

Dextran sulphate (DS), chondroitin sulphate (CS) and 1,9-dimethylmethylene blue (DMMB) were products of M/s Serva Feinbiochemica (Heidelberg) and were used as such. Methylene blue (MB) and toluidine blue (TB) were E. Merck products and were purified as reported. Aqueous solutions of the dyes were used. Stock solutions of the dyes and other experimental solutions were kept in dark when not in use.

Absorbances were measured with a Bausch and Lomb visible spectrophotometer or a Toshniwal digital spectrophotometer.

Results and Discussion

Metachromatic dyes are hydrophobic in nature, having a large hydrophobic aromatic portion and a relatively small cationic charge. Proper aggregation of dye cations is responsible for exhibition of metachromasia. The dye-dye interaction is facilitated by dye-chromatic electrostatic bonding. DMMB belongs to thiazine group with the chromophoric group $\overset{-N}{\text{R}} \overset{\text{S}}{\text{R}}$. Other dyes of this group are thionine, azure, $\text{A}$, $\text{B}$, $\text{C}$, MB and TB. Introduction of two methyl groups in MB at 1 and 9 positions increases its hydrophobic character, which is reflected in greater aggregating tendency of DMMB. Enhanced aggregating tendency of DMMB is evident from the appearance of a sharp dimer band ($\beta$-band) around 590 nm (Fig. 1) in its very dilute aqueous solution ($\sim 10^{-5} M$). MB and TB show no distinct dimer bands at this concentration. Moreover, on doubling
the dye concentration \(2.0 \times 10^{-5} \) M, \(\beta\)-band around 590 nm tends to dominate the monomer band (\(\alpha\)-band) around 650 nm (figure not shown). The methyl groups linked with the ring C-atoms contribute to the hydrophobicity of the dye molecule, whereas methyl groups linked with the amino N-atoms constitute a part of the hydrophilic cationic charge. In DMMB, two methyl groups are linked with ring C-atoms and four methyl groups are linked with amino N-atoms in comparison with 4 amino N-linked methyl groups and none with ring C in MB, and two amino N-linked methyl groups and one ring C-linked methyl group in TB.

Greater metachromatic ability of DMMB than that of MB or TB is reflected in its exhibition of metachromasia with BP, an acid polysaccharide of equivalent weight 854 (Fig. 1D), and AMA, another acid polysaccharide having still lower charge density (Eq. wt. 255) (Fig. 1E).

Fig. 1D shows increased absorbance around \(\lambda_{\text{max}}\) of the dye, DMMB. This effect is more pronounced for DMMB-AMA system (curve-1E). This reflects weaker chromotropic ability of BP and AMA. Absorbances around the \(\lambda_{\text{max}}\) of the dye, however, get slightly reduced with increase in P/D ratio (curves not shown). Both BP and AMA fail to induce metachromasia in MB and TB. DS—a sulphated polysaccharide of very high charge density (Eq. wt. 167 as the Na-salt)—induces very sharp and stable metachromasia with DMMB, TB and MB (Figs 1, 2 and 3). The magnitudes of blue shift (Table 1) with MB, TB and DMMB in presence of DS are a bit more than their usual average values. For the DMMB—polyanions systems studied here, the magnitude of the blue shift is in the order: DS > CS > BP > AMA.

The increasing order of blue shift is in agreement with the charge densities of the polyanions as well as with the nature of the anionic groups. DS is a sulphated polysaccharide having charge density 2.0 per monosaccharide unit. CS is characterised by a disaccharide repeating unit having one –COOH group and one –OSO\(_3\)H group (charge density 1.0 per monosaccharide unit). BP and AMA are acid polysaccharides having only –COOH group with charge densities 0.19 and 0.074, respectively. In fact, induction of metachromasia by polyanions of the type BP and AMA with such low charge densities has been rarely reported.

Stability of metachromatic compounds depends upon the nature of both the dye and the polyanion. Relative stabilities of metachromatic compounds are evaluated on the basis of the concentration of ethanol/urea needed to cause disruption of metachromasia. Fig. 4 shows the influence of urea on the metachromatic interaction of DMMB with DS (curve-4D), CS (curve-4C), and BP (curve-4B) at P/D 3.0. Fig. 5 shows the effect of ethanol on the stability of metachromatic compounds of DMMB with DS, CS and BP.

Stabilities of metachromatic compounds of a single dye with different chromotropes are different (Figs 4 and 5). Same is the case with the metachromatic compounds of different dyes with the single chromotrope (Figs 5 and 6).

The extent of the destruction of metachromasia by ethanol/urea is obviously a function of ethanol/urea concentration, which in turn grades the stability of metachromatic compounds. Decrease in stability of metachromatic compounds is accompanied by increasing absorbance of the solution of dye-chromotrope system at the mon-
Fig. 2—Absorption spectra of $1.0 \times 10^{-5} \ M$ toluidine blue in water (A), in presence of DS at P/D 1.5 (B), 3.0 (C) and 6.0 (D)

Fig. 3—Absorption spectra of $1.0 \times 10^{-5} \ M$ methylene blue in water (A), in presence of DS at P/D 1.5 (B), 3.0 (C) and 6.0 (D)
BHATTACHARYYA et al.: METACHROMATIC INTERACTIONS OF DMMB WITH POLYSACCHARIDES

Fig. 4—Absorbance at the α-band of $1.0 \times 10^{-5} M$ (DMMB) vs molarity of urea in water (A), in presence of BP (B), CS (C) and DS (D) at $P/D = 3.0$. Curves E, F, G show the corresponding absorbances at the μ-bands of the dye vs molarity of urea.

OMER BAND (α-band) and the decreasing absorbance at metachromatic band (μ-band). A sharper measure of stability of the metachromatic compounds is the concentration of ethanol/urea at which the α-band absorbance curve attains its half-plateau value (Table 2). The half-plateau value is taken half-way between the upper plateau value approached or reached for each family and the lower level of absorbance approached at low ethanol/urea concentration in presence of strongest chromotrope.

Simultaneous increase in dye and chromotrope concentrations, so as to maintain the same P/D, is accompanied by the weakening of the effect of ethanol in disrupting metachromasia. So, higher ethanol concentration is required for destroying the metachromatic compound.

With a given chromotrope, the metachromatic abilities of the dyes belonging to thiazine group follow the order: DMMB > TB > MB, and with a given dye of this group the chromotropic abilities of different polyanions vary in the order: DS > CS > BP > AMA.

In the DMMB-DS system (DMMB: $1.0 \times 10^{-5} M$; DS: $3.0 \times 10^{-5} M$; $P/D = 3.0$) an ethanol concentration of $\sim 10 M$ (59.9%) is capable of disrupting metachromasia to the extent of about 50%. Same is the case with urea. $8 M$ urea does not even correspond to half-plateau value. On extrapolating the α-band absorbance curve, it is observed that intersection of half-plateau line with the α-band absorbance curve corresponds to $\sim 10 M$ urea.

With increased concentration of DMMB and DS (DMMB: $2.0 \times 10^{-5} M$; DS: $6.0 \times 10^{-5} M$; $P/D = 3.0$), higher ethanol concentration ($\sim 70\%$) is required to destroy the metachromatic compound to the extent of 50 per cent.

Acknowledgement

The authors are thankful to the DST, Tripura for financial assistance.

<table>
<thead>
<tr>
<th>Table 2—Half-plateau values (Figs. 4-6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>System</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>DMMB-BP</td>
</tr>
<tr>
<td>DMMB-CS</td>
</tr>
<tr>
<td>DMMB-DS</td>
</tr>
<tr>
<td>TB-DS</td>
</tr>
<tr>
<td>MB-DS</td>
</tr>
</tbody>
</table>
Fig. 5 — Absorbance at the \( \alpha \)-band of \( 1.0 \times 10^{-5} \, M \) (DMMB) vs per cent ethanol in water (A), in presence of BP (B), CS (C) and DS (D) at P/D 3.0 (\( \alpha \)-bands not shown)

Fig. 6 — Absorbance at the \( \alpha \)-band of \( 1.0 \times 10^{-5} \, M \) (MB) vs per cent ethanol in water (A), in presence of DS at P/D 3.0. (B), (C) and (D) are the corresponding curves for the dye TB
References
4 Toepfer K, Histochemic, 21 (1970) 64.