Thermochromic behaviour of 6-bromoindigotin: key to understanding purple dyeing with banded dye-murex

I. Irving Ziderman, Yoshiko Sasaki, Masanori Sato and Ken Sasaki

Abstract In contrast to the purple dyes from all other species of shellfish, banded dye-murex (*Hexaplex trunculus*) typically yields a bluish tint, and is therefore identified as the hyacinthine purple of antiquity, biblical *tekhelet*. Chemical reactions of indoxyl and bromoindoxyl dye precursors from the hypobranchial glands compete to form a mixture of 6,6'-dibromoindigotin (DBI), 6-bromoindigotin (MBI) and indigotin. A reddish purple, which was obtained from banded dye-murex by some workers, has been found to readily change colour to blue on heating, a phenomenon attributed to a hypothetical tinctorial lability of MBI supposedly present in the dye. Wool dyed violet with synthetic MBI indeed turns blue on mild heating. Visible absorption spectroscopy shows a bathochromic shift after heating. Gas mass spectrometry (MS), single ion monitoring (SIM) and X-ray fluorescence (XRF) spectroscopy reveal no change in chemical composition, showing that no indigotin is formed by debromination of MBI. Pure MBI also undergoes this irreversible change in colour from violet to blue at 60 °C. This discontinuous thermochromic transition of MBI is the key to understanding the nature of hyacinthine purple and to explaining conflicting findings regarding the colour of the dye from banded dye-murex.

Introduction

Banded dye-murex (*Hexaplex trunculus*) was the major source for dyeing purple in the ancient Mediterranean world.[1] Purple from this shellfish is found typically to have a bluish tint, giving it a violet colour. It is therefore identified as 'hyacinthine purple' of antiquity, biblical *tekhelet*.[2] The other shellfish dye of antiquity, 'Tyrian purple' (biblical *argaman*), was made from two other species: spiny dye-murex (*Bolinus brandaris*) and red-mouthed rockshell (*Stramonita haemastoma*).[3] Chemically, this 6,6'-dibromoindigotin (DBI) dye is formed as an artefact by chemical reactions when natural bromoindoxyl dye precursors present in the hypobranchial glands of these species are exposed to sunlight and atmospheric oxygen. Characteristic features of these two murex purples of antiquity are listed for comparison in Table 1.

Hyacinthine purple is a mixture of DBI with indigotin and 6-bromoindigotin (MBI), the latter two accounting for its bluish tint. The three dyestuffs are formed in competing chemical coupling reactions of indoxyl and bromoindoxyl dye precursors extracted from the hypobranchial gland, on their exposure to atmospheric oxygen, as illustrated in Figure 1. Elsner discovered a sex-dependent distribution of the dyestuffs in banded dye-murex from the Bay of Haifa, Israel, the male snails giving preferentially bluish shades (mainly indigotin) and the females purples (DBI).[4] Accordingly, the glands of the males contain largely indoxyl dye precursors and the females bromoindoxyls; this means that the brominating enzyme, bromoperoxidase,[5] is often

Table 1 The two murex purples of antiquity.

Name in: *Modern English*	Hyacinthine purple	Tyrian purple
Sumerian (3000 BCE)	*SIG-ZA-GIN*	*SIG-ZA-GIN-DIR*
Nuzi (1500 BCE)	*ugni*	*kinaahhu*
Ugaritic (1000 BCE)	*iqnu*	*kinahhu*
Biblical Hebrew	*tekhelet*	*argaman*
Syriac	*tekelta*	*argewana*
Akkadian (1500–700 BCE)	*takiltu*	*argamannu*
Hellenistic Greek	*hyakinthos*	*porphyra*
Latin[6]	*Purpura hyacinthea, P. violacea, P. ianthina, P. hyacinthina, P. amethystina*	*P. blatta, P. oxyblatta, Tyria*
Tyndale's Bible (1529)	jacinth	purple
King James' Version (1611)	blue	purple
Jerusalem Bible, London (1966)	violet	purple
New English Bible, Oxford (1970)	violet	purple
Tint of purple	bluish	reddish
Colour of dye, in current English usage	violet	purple
Shellfish species source of the dye	banded dye-murex	all other porphyrogenic species
Natural precursors in shellfish's hypobranchial gland	bromoindoxyls+indoxyls	bromoindoxyls
Necessity for photolysis of C-2 substituent in precursors[7]	no	yes
Vatting required for dyeing[7]	yes	no
Chemical composition of dye	indigotin+DBI+MBI	DBI

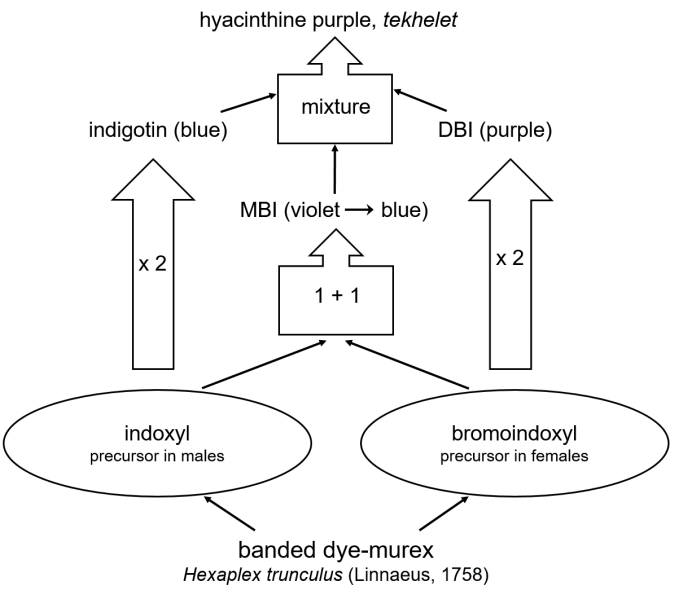

Figure 1 Diagram depicting the formation of DBI, MBI and indigotin from the precursors present in banded dye-murex.

deficient, or at least inactive, in these banded dye-murex males, uniquely among purple molluscs.

The dye obtained will contain the three dyestuffs in varying proportions, depending on the reaction conditions prevailing, such as temperature, pH, illumination, ionic strength and relative concentrations of precursors. The dye composition will also depend on the season of fishing and on the ratio of males to females in the shellfish catch used. But this factor is further complicated by protandrous hermaphroditism, the males undergoing sex reversal to functioning females.[8] The sex changes after one season, the proportion of indigotin gradually decreasing until only DBI remains in the dye from the females. When its penis is retained, such a female may be erroneously scored as male, even though it now contains predominantly bromoindoxyl dye precursors: Elsner therefore selected males according to the greenish colour of the gland and almost all gave bluish dyes.

Furthermore, the ratio of the amounts of dyestuffs actually dyed onto the textile, and the resulting dye colour, will be determined by additional factors, such as their affinity for the fibre substrate under the reaction conditions used, and, in vat dyeing, by their redox potentials and the illumination. Thereby, the dye composition obtained on the textile will become enriched in one of the three ingredients.

Thermochromic behaviour of MBI

An additional recently discovered complication affecting the colour obtained from banded dye-murex is the discontinuous thermochromic transition (colour change on heating) exhibited by MBI. In the hands of Lacaze-Duthiers, a reddish purple was sometimes obtained from banded dye-murex, which visually had the same colour as Tyrian purple.[9] He erroneously understood this observation to mean that it was the same dyestuff as obtained from spiny dye-murex, namely DBI. It has recently been found that wool dyed to such a reddish purple with the banded murex has the peculiar property

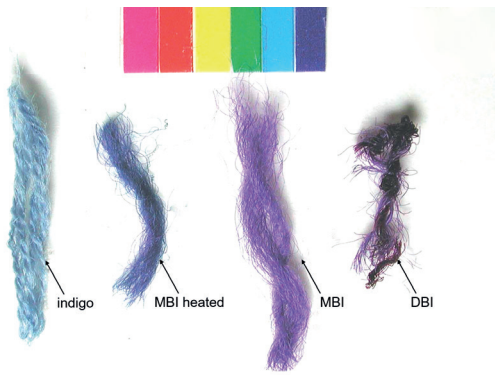

Figure 2 Photograph of the colour change on heating MBI dyed on wool, as compared to the colours of DBI and indigotin dyed on wool. Left to right: synthetic indigotin, heated MBI, unheated MBI, synthetic DBI.

of readily turning blue on heating.[10] Ziderman ascribed the change to a hypothetical tinctorial lability of MBI, which he presumed to be the effective dyestuff in the dyeing. The lability appeared to be due to debromination of MBI to indigotin. Previously detected only chromatographically without being isolated, synthetic MBI later became available for chemical research.[11] In a test of his proposition, Ziderman discovered that, on heating MBI dyeings on wool, the colour changes from violet to blue, just as is required by his hypothesis.[12]

In order to prove whether the blue product of heating MBI is merely another physical form of MBI formed by thermochromism or that it is indeed indigotin, instrumental techniques were employed including visible absorption spectroscopy, gas mass spectrometry (MS) and X–ray fluorescence (XRF) spectroscopy.

Experimental

MBI and the wool dyed with MBI were made and kindly supplied by Chris Cooksey. For the heating treatment, the dyed wool was placed in an oven at about 100 °C for a few minutes until it changed colour from violet to blue. Visible spectra were obtained with a Hitachi UV-Visible Spectrophotometer U–3500, with the integrating

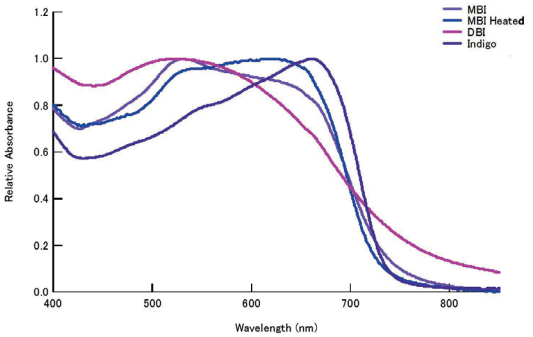

Figure 3 Visible spectra of wools dyed with DBI, indigotin and MBI, the latter before and after heating.

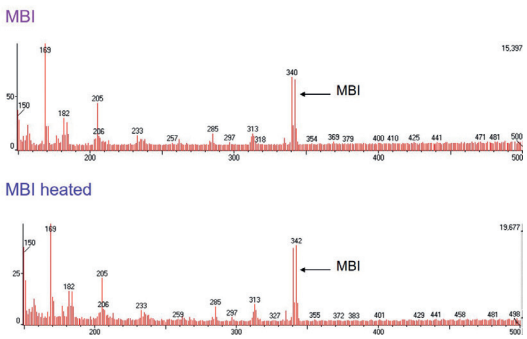

Figure 5 Gas MS of DMF extracts of wool dyed with MBI, before and after heating.

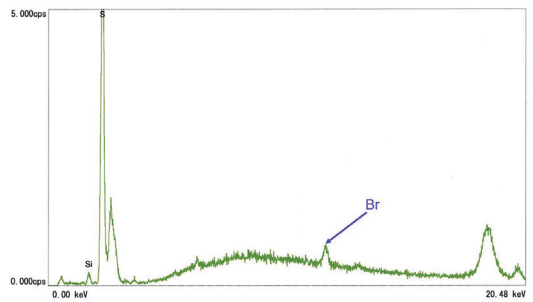

Figure 4 XRF spectra of wool dyed with MBI, after heating.

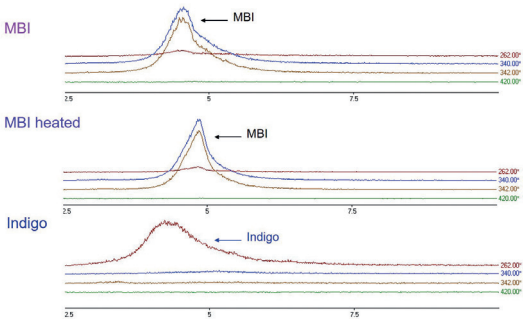

Figure 6 SIM traces (gas MS) of samples as in Figure 5, compared with indigotin.

sphere attachment for the visible reflectance absorption. The reflectance spectrum was automatically transformed into an absorption spectrum by the Kubelka-Munk equation. Each absorbance spectrum was normalised at maximum absorbance. X-ray fluorescence spectra were measured with a Horiba XGT2000W X-ray analytical microscope, at 50 kV, 1.0 mA, 300 sec, and diameter 100 μm.

Gas MS was performed using a Shimadzu QP-5000 gas chromatograph-mass spectrometer. Dyestuff extraction for gas MS was performed at room temperature in the dark with N, N'-dimethylformamide (DMF). For DBI, the extraction took several days, but for the other materials it was easy and soon gave the extract. The unfiltered sample was injected directly without using the chromatograph, with electron impact at 70 eV, and ion source temperature at 300 °C.[13]

Results

The colour deepening exhibited by MBI on being heated is depicted in Figure 2. In Figure 3, the absorption spectrum of wool dyed with MBI is shown to change after the thermal colour shift to blue. The absorption maximum shifts bathochromically from about 510 nm to about 640 nm, from the range for DBI to that for indigotin. XRF (Fig. 4) reveals the presence of bromine in the dyed wool after dyeing. However, it may be inorganic bromide that has been eliminated from the MBI on heating and not bromine in unchanged MBI.

Gas MS was used to prove that the bromine present is in MBI and that indigotin is not formed. In Figure 5, the extracts of dyed wool before and after the colour change are compared, both having peaks for MBI at *m/z* 340 and 342. This finding was corroborated by single

ion monitoring (SIM), which is highly sensitive (Fig. 6); blue MBI has the same peak as violet MBI, and no indigotin is present. It was found that, on heating to 60 °C, pure synthetic MBI undergoes the same irreversible change in colour to blue, as does the dyed wool. Accordingly, the colour alteration in dyed wool is not dependent on some interaction between wool and MBI, but is an inherent solid-state property of the compound.

Discussion

The thermochromic behaviour of MBI is the key to a renewed understanding of the chemical nature of hyacinthine purple and of conflicting findings that have been reported during the last two centuries regarding the colour of the dye made from banded dye-murex. Reddish-purple dyeings obtained with banded dye-murex can be essentially MBI, and not DBI, which, on the other hand, would always be the dyestuff in purples from spiny dye-murex and red-mouthed rockshell. This conclusion provides cogent substantiation of the uniqueness of the banded murex species as the source of hyacinthine purple dye, but not of Tyrian purple. It therefore corroborates Bizio's view, in his disputation on this issue with Lacaze-Duthiers.[14]

Characterisation of the mechanism of the thermal colour change exhibited by MBI may prove to be an intriguing undertaking. It should be possible to detect and quantify the thermal transformation by differential thermal analysis techniques. High power microscopy may detect a change in particle size.

Thermochromism must arise because of a change in the electronic structure of a material, but the electronic change can be driven by geometric changes of either the molecule or the solid lattice.[15] Thus a solid-state change in molecular organisation would presumably be the cause of the shift in spectral absorption. The colour shift may be due to a crystalline transition that could be ascertained by hot-stage polarised microscopy, X-ray powder refraction and crystallography.

Addendum

In a recent paper, Ramig and co-workers presented evidence confirming our hypothesis that the thermochromic transition of MBI dyeings is due to a decrease in MBI particle size.[16] They conclude that this transition with MBI dyeings is essentially independent of the nature of the dyed substrate, as is consistent with the thermochromism of bulk MBI, as reported in the present paper. Anomalously, however, they report that their bulk MBI itself did not exhibit the thermochromic shift. We suggest that this apparent inconsistency occurs because their MBI had been recrystallised directly to the thermodynamically stable state that would not undergo a thermochromic transition. This altered behaviour may be ascribed to the particular experimental conditions used for recrystallising their MBI samples, which they state differed from those used by Cooksey[17] for recrystallising the present bulk sample that exhibited the thermochromic transition. It would be pertinent to ascertain whether the analogous hypsochromic thermochromism they report for DBI dyeings is also exhibited by bulk DBI.

Editor's note

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Authors' addresses

- *Author for correspondence*: Irving Ziderman, Tekhelet Foundation, 1 Yefe Nof Street, Shoresh, 9086000 Israel (ziderman@smile.net.il)
- Yoshiko Sasaki, Museum and Archives, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan
- Masanori Sato, Nara National Research Institute for Cultural Properties Museum and Archives, , 2-9-1 Nijo-cho, Nara 630-8577, Japan
- Ken Sasaki, Department of Biomolecular Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585 Japan