The reaction of wool keratin with tetrazonium cations

Savel Ifrim

14 Str N Gane, Iasi 6600, Romania

A novel dyeing method is described in which wool can be treated with salts formed by tetrazotising benzidine, *o*-dianisidine and 4,4′-diaminostilbene. Evidence is provided that the salts react with aromatic nuclei present in certain amino acids that form part of the keratinous molecular structure of wool, to form *in situ* azo dyes. Details of the process and a number of potential practical advantages are discussed.

INTRODUCTION

Wool keratin is a biological macromolecule whose structural complexity is related to the range of different amino acids that can be present in the polypeptide chains, and also to the spatial orientation of these chains. The multitude of free functional groups available in wool makes the fibre amenable to reaction with a variety of organic compounds; one example is the formation of a covalent bond between wool and reactive dye molecules [1]. The free amino groups present in wool are particularly important in such reactions [2,3].

Many organic substances can react with keratin, and hence there are several possibilities in the way that the structure of keratin can be modified. For example, it can appear to react intramolecularly under certain conditions, with the cystine bond position changing or with lanthionine being formed [4]. It has been shown previously that keratin can react with several different organic compounds, including organohalogens, aldehydes and benzoquinone [5–8].

The present study examined the interaction of tetrazonium salts with wool, which induced a coloured appearance in the wool.

THEORETICAL BACKGROUND

Between the side chains of wool there are also various aromatic groups, such as phenyl groups bound to phenylalanine. It is known that aromatic nuclei frequently participate in electrophilic substitution reactions, even when the nuclei are part of a larger compound such as keratin. Thus the Pauly reaction, often used to identify wool fibres, is based on the ability of the aromatic ring in tyrosine to couple with a diazonium salt. In this reaction an azo dye is formed within the fibre and the wool appears coloured [9]. But tyrosine is found only in the cortical layer and it is absent in the cuticle, which acts to protect the rest of the fibre. The presence of an undamaged cuticle prevents the penetration of reactive chemical species into the fibre interior, towards the cortical layer.

However, the cuticle need not be degraded in order for chemically reactive species to penetrate into the cortex. It is sufficient for the wool fibre to undergo a swelling process, during which the scales that form the cuticular layer move away from the cortex, allowing the reactive species access to the latter.

It is known that swelling of wool fibre can take place by simple immersion of the wool in water, because the keratin has a great number of free hydrophilic groups, which can retain the water molecules by forming hydrogen bonds with them [10]. Swelling of wool can also be readily produced in an aqueous solution of sodium carbonate, the alkaline medium providing the conditions necessary for the aromatic nuclei to couple with a diazonium salt.

Quantitatively, wool consists of 3.4–4.7% phenylalanine, at least 6% tyrosine and 1.7–2.2% tryptophan [11]. Hence the proportion of wool made up of amino acids that have aromatic nuclei in their molecules can exceed 12%. This percentage is sufficiently high to permit keratin to act as a coupling component.

In an earlier paper we indicated that phenylalanine, tyrosine, tryptophan and hystidine present in wool keratin can participate in coupling reactions with diazonium and tetrazonium salts to form water-soluble azo dyes [12]. In this article further investigations are described, using the wool as coupling component in the presence of tetrazonium cations.

EXPERIMENTAL

Wool was submitted to a swelling process in which 5 g samples were immersed in a 300 ml solution of 1% sodium carbonate, for 60 min at room temperature and pH 11. These relatively mild conditions allowed the wool fibre to swell without any degradation taking place.

Separately, 2 g samples of benzidine were tetrazotised by adding them to a small amount of concentrated hydrochloric acid and cooling to 5°C. To this was added 2 g sodium nitrite, pre-dissolved in a small quantity of water at the same temperature, with stirring. The solution of benzidine tetrazonium salt, which was slightly acidic in character, was then added to the wool sample, again with stirring, in the sodium carbonate solution. Initially no coloration was observed, but after a short while the wool

began to assume at first a yellow and then in a yellow-orange shade. Allowing the mixture to stand for more than 60 min yielded a striking maroon coloration in the wool.

A similar procedure was adopted for preparing tetrazonium salts of *o*-dianisidine and 4,4′-diaminostilbene, the final colours obtained with these two compounds being pink and light orange respectively.

Because the azo group is not resistant to the reducing action of sodium metabisulphite, it was decided to compare the tensile properties of tetrazotised wool with those of untreated samples. Specifically the resistance to tearing and extension were measured, using a Schoper dynamometer, taking the average of 100 determinations for each sample. The change in tensile properties (Table 1) provided evidence for the formation of crosslinks, as discussed below.

Fibre swelling tests were also carried out. Both treated and untreated wool samples were added to a sodium carbonate solution at pH 10 for 12 h. Any change in fibre diameter was assessed using an optical microscope. The average of several determinations is presented in Table 2.

Table 1 Tensile properties of tetrazotised wool fibres

Tetrazotisation treatment	Resistance to tearing (gf per fibre)	Extension (%)
Untreated	7.72	38.35
Benzidine	14.40	27.45
o-Dianisidine	14.10	27.70
4,4′-Diaminostilbene	14.19	28.20

Table 2 Comparative diameters of tetrazotised wool fibres after a swelling treatment

Tetrazotisation treatment	Diameter (μm)	Colour
Control	25.5	
Benzidine	21.6	Maroon
o-Dianisidine	21.1	Pink
4,4′-diaminostilbene	18.6	Light orange

RESULTS AND DISCUSSION

Swelling of wool fibres took place readily in an aqueous medium because the keratin contains many free hydrophilic groups in the polypeptide chains. These can retain water molecules by forming hydrogen bonds [12], or by dipole interactions. Tryptophan and tyrosine concentrations are at their greatest in the cortex [13,14], so the proportion of amino acids present that were able to couple with the tetrazonium cations was sufficiently high to allow the wool to behave as a coupling component. Following this treatment the wool appeared coloured, demonstrating that the tetrazonium salts were bound at the free aromatic nuclei, forming chromophore structures that generated the colour.

The longer the contact time between the wool fibre and tetrazonium salts was prolonged, the more intense the coloration obtained. This suggests that a slow diffusion process occurred during which the tetrazonium cations entered the cortical layer, where electrophilic substitution reactions could take place. This was essentially a form of azoic dyeing process in which the coupling component is actually part of the polypeptide chains of wool.

The nature of the keratin reaction with the tetrazonium cations may be variable. For example, the tetrazotised benzidine used in this work could couple at one end of the molecule with the tyrozine nuclei on one polypeptide chain while the other end of the molecule could be coupled to the aromatic nucleus of phenylalanine or tryptophan of an adjacent polypeptide chain (Figure 1). Taking into account the four amino acids that have aromatic nuclei in their structures and so can participate to the coupling reaction, there are up to 24 different types of crosslink that could be involved.

Comparing the tensile properties of the treated and untreated fibres, the resistance to tearing was greater and the extension much lower with the treated wool fibre. It is known that the resistance to tearing of wool depends on the existence of crosslinks; the more numerous the cross-links the greater the resistance. Conversely extensive crosslinking anchors the polypeptide, thus limiting fibre extension.

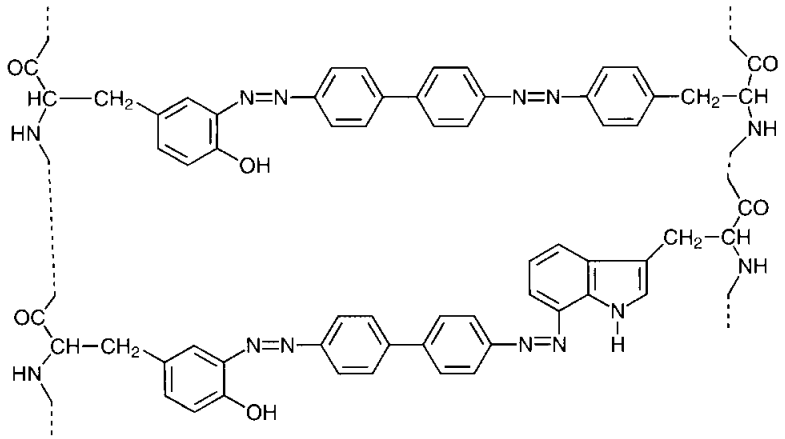

Figure 1 Possible crosslinks formed by reaction of tetrazotised benzidine with wool keratin

It has been demonstrated that the formation of crosslinks impairs wool fibre swelling [15,16]. In the present work it has been shown that, under subsequent identical swelling treatments, the diameters of the treated samples were less than those of the untreated ones. This demonstrates the existence of extensive crosslinking involving wool keratin and tetrazonium salts.

In addition this novel dyeing method offers a number of other potential advantages. Energy consumption is minimised because the treatment bath does not require heating, as dyeing takes place at 5°C. The dyeings produced tend to be fast to external agencies as the dye formed is covalently bound within the chemical structure of the keratin. However, the coloration produced disappears on treatment with reducing agents, because the azo chromophore is not resistant to the action of these agents.

A further advantage is that no special equipment is needed to carry out the dyeing.

CONCLUSION

The reaction of keratin using salts formed by tetrazotisation of benzidine, *o*-dianisidine and 4,4'-diaminostilbene has been studied. The nature of the reaction is explained by the existence in the keratin macromolecule of many amino acids side chains having aromatic nuclei. These nuclei can participate in electrophilic substitution reactions, even when they form part of the polypeptide chains in wool. Tetrazonium cations couple with the aromatic species to form new crosslinks, which are in effect *in situ* azo dyes that confer colour to the wool.

The fastness of these colorants is good, because of the covalent bond formed between the keratin and the tetrazonium cations, which penetrate into the interior of the swollen fibre. The high proportion of amino acids present in wool that have aromatic nuclei explains why wool behaves as a coupling component.

The modified tensile properties of the treated wool provides evidence for the proposed crosslinking mechanism. The improved tensile properties and the simplicity of the dyeing method employed means that this new process could offer significant practical possibilities for dyeing wool economically at low temperatures.

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