Sodium metabisulfite as a contact allergen – an example of a rare chemical mechanism for protein modification

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Summary

Background. Skin-sensitizing chemicals that cause allergic contact dermatitis do so by reacting with self-proteins such that the modified structure becomes antigenic. The reaction chemistry involved is well characterized, but there are exceptions, such as the occasional allergen sodium metabisulfite.

Objectives. To identify the potential *in cutaneo* reaction chemistry of sodium metabisulfite.

Methods. The established protein reaction chemistry associated with aqueous sulfite chemistry was explored in the context of the protein modification stage in allergic contact dermatitis.

Results. A probable mechanism for the *in cutaneo* modification of proteins by sodium metabisulfite involves the sulfite di-anion, acting as a nucleophile towards electrophilic centres in proteins, which is a rare mechanism, as most known skin-sensitizing chemicals behave as electrophiles.

Conclusions. Sodium metabisulfite is an unusual but not infrequent contact allergen whose chemistry suggests a previously unrecognized protein modification mechanism involving nucleophilic attack by sulfite di-anions on target electrophilic centres in skin proteins. The chemical properties required for sensitization by nucleophilic attack on skin proteins are quite restrictive, so the domain of nucleophilic sensitizers is expected to be small. Thiourea derivatives are among the sensitizers likely to act by this mechanism.

Key words: allergic contact dermatitis; reaction mechanism; sodium metabisulfite.

Sodium metabisulfite has been reported as a contact allergen and as a cause of allergic contact dermatitis for many years (e.g. 1–9). Clinically, the skin reaction of a positive diagnostic patch test is clear and often felt to be relevant to the patients' eczema (1, 2). However, the chemical does not immediately fit into the well-described types commonly associated with skin-sensitizing properties (10–14). In the present work, we have explored the probable chemical mechanism by which sodium metabisulfite could behave as an allergen and considered what consequences this might have regarding related substances that could sensitize via the same or similar mechanisms.

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Chemical rationale

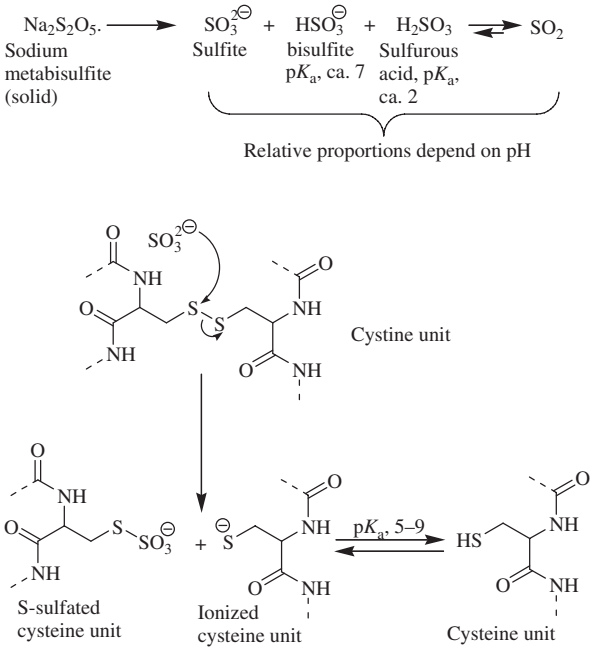
There is extensive evidence, dating back many decades, that skin sensitization to low molecular weight chemicals involves (either directly or after metabolic or abiotic

transformation) covalent reactions with skin proteins. Although there are still significant uncertainties, in particular regarding the identity and *in cutaneo* location of the skin proteins involved, substantial progress has been made in understanding the reaction chemistry underlying skin sensitization. Chemistry-based interpretation of quite large datasets of skin sensitization test results, covering the range of potency from non-sensitizing to extreme, supports the argument that most, if not all, skin sensitizers (or their derivatives after metabolic or abiotic activation) are electrophilic, and able to react with nucleophilic groups on proteins, such as cysteine units (reacting through the ionized SH group) or lysine units (reacting through the non-ionized NH_2 group). This does not mean that all electrophilic chemicals are sensitizers – some are insufficiently reactive or inadequate in other physicochemical properties, particularly hydrophobicity, to sensitize (14). Regarding non-electrophilic skin sensitizers, there is strong evidence, although so far not conclusive proof, that the potency of some sensitizers results not from electrophilicity but from their ability to react with proteins via free radical reactions. Examples are organic hydroperoxides, and certain aromatic amines (15–17).

Sodium metabisulfite appears to provide a rare example of a third chemical mechanism of sensitization. In water, it exists as a mixture of sulfite di-anions, bisulfite anions, sulfurous acid, and sulfur dioxide, in proportions depending on the pH (Scheme 1). The pK_a of bisulfite is 7.2 (18), so, at physiological pH, a significant proportion will exist as the sulfite di-anion. This equilibrium, shown in Scheme 1, is probably the basis of the clinical finding that the majority of patients with contact allergy to sodium metabisulfite react positively when patch-tested with sodium sulfite (19).

The sulfite di-anion is a reactive borderline soft nucleophile, and we consider that this is the basis of the sensitization potency of sodium metabisulfite. The concept of hardness or softness as applied to nucleophiles and electrophiles is a complicated topic in physical organic chemistry. The concept relates to the electronic polarizability at the reaction centre; the more polarizable, the softer. Other things being equal, hard nucleophiles react preferentially with hard electrophiles, and soft nucleophiles react preferentially with soft electrophiles. A sulfur atom is a relatively soft reaction centre, both as a nucleophile, such as a thiolate ion or a bisulfite ion, and as an electrophile when it is part of a leaving group.

For a nucleophile to sensitize, it must be able to react covalently with an electrophilic group in skin protein. The only significantly electrophilic groups in proteins are



Scheme 1. Reaction of metabisulfite with cystine S–S linkages.

the disulfide linkages of cystine units. These can act as soft electrophiles, as shown in Scheme 1. The nucleophile attacks one of the sulfur atoms, and the S–S bond breaks heterolytically, with the thiolate anion of the cysteine unit acting as a leaving group. The effectiveness of the thiolate anion as a leaving group depends inversely on the pK_a of the corresponding thiol. The SH groups of cysteine units in proteins have pK_a values ranging from ~5 to ~9, depending on the nature of the neighbouring amino acid units in the secondary and tertiary structure (20). Consequently, some cystine-based disulfide linkages in proteins are likely to be much more reactive than others towards nucleophiles.

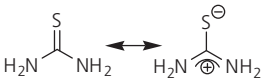
The reaction chemistry shown in Scheme 1 is well established (21) and is, at least in part, the basis of the preservative properties of sodium metabisulfite.

Other nucleophilic skin sensitizers?

For a nucleophile to act as a skin sensitizer, it needs to be able to react with S–S linkages of cystine units, in a reaction analogous to that shown in Scheme 1 for sulfite. To do this, it needs to be a soft nucleophile, or at least borderline, and to be sufficiently reactive. Table 1 shows a selection of nucleophiles, taken from a compilation of Swain–Scott *n*-values (quantifying nucleophilicity) (22) and a listing of hard, borderline and soft nucleophiles (23),

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Table 1. Nucleophiles that are likely to be able to react with cystine S–S linkages

Nucleophile	Swain-Scott *n**	Hard-soft classification
Aniline†	5.70	Borderline
Sulfite, $SO_3^{2\ominus}$	8.53	Borderline
Thiosulfate, $^{\ominus}S\text{-}SO_3^{\ominus}$	8.95	Soft
Thiourea‡ 	7.27	Soft
Cyanide, $^{\ominus}CN$	6.70	Soft
Thiocyanate, $^{\ominus}SCN$	6.70	Soft
Thiophenolate, $PhS^{\ominus}$	9.92	Soft
Triphenyl phosphine, Ph_3P:	8.69	Soft
Iodide, $I^{\ominus}$	7.42	Soft

*Based on reaction kinetics with methyl iodide as the reference electrophile, in methanol, with methanol assigned an *n*-value of 0. The original Swain–Scott study used water both as the solvent and as the reference nucleophile (with *n* defined as zero), and methyl bromide as the reference electrophile (*s* defined as 1). Subsequently, the methanol-based scale was developed – one of the driving forces was probably the fact that most organic chemicals tend to have low water solubility, and most organic chemistry was carried out in organic solvents rather than in water. As the *n*-values and *s*-values for the water system are strongly correlated with the corresponding *n*-values and *s*-values for the methanol system, the choice of system does not affect the argument. More *n*-values and *s*-values have been measured for the methanol system than for the water system, so the methanol values are more usually quoted in chemistry textbooks. The *in cutaneo* reactions discussed here might occur in either an aqueous medium or in a more 'organic solvent' medium, such as in a membrane.

†Aniline is included for comparison. Although it is borderline soft, like sulfite, its nucleophilicity is substantially lower, so it is much less likely to react.

‡Thiourea homologues with alkyl substituents on the nitrogen atoms should be similarly soft and have similar *n*-values.

which could meet this criterion for nucleophilic sensitization.

As a broad generalization, we can see from Table 1 that sulfur-based nucleophiles with a negative charge on the sulfur atom, carbon-based nucleophiles and phosphorus-based nucleophiles are likely to be able to react with S–S linkages of cystine units. Similarly, iodide ion is likely to be reactive. However, soft nucleophilicity towards S–S linkages may not be the only determinant of sensitization potency. If the reaction with S–S units is rapidly reversible, as is usually likely to be the case, then the sensitization potency will be influenced by the extent to which the equilibrium favours the reaction product, and whether the reversibly formed reaction product can react further to give a stable reaction product. Scheme 2 shows some examples of reported secondary reactions, as well as some possible reactions that seem plausible but for which we know of no evidence.

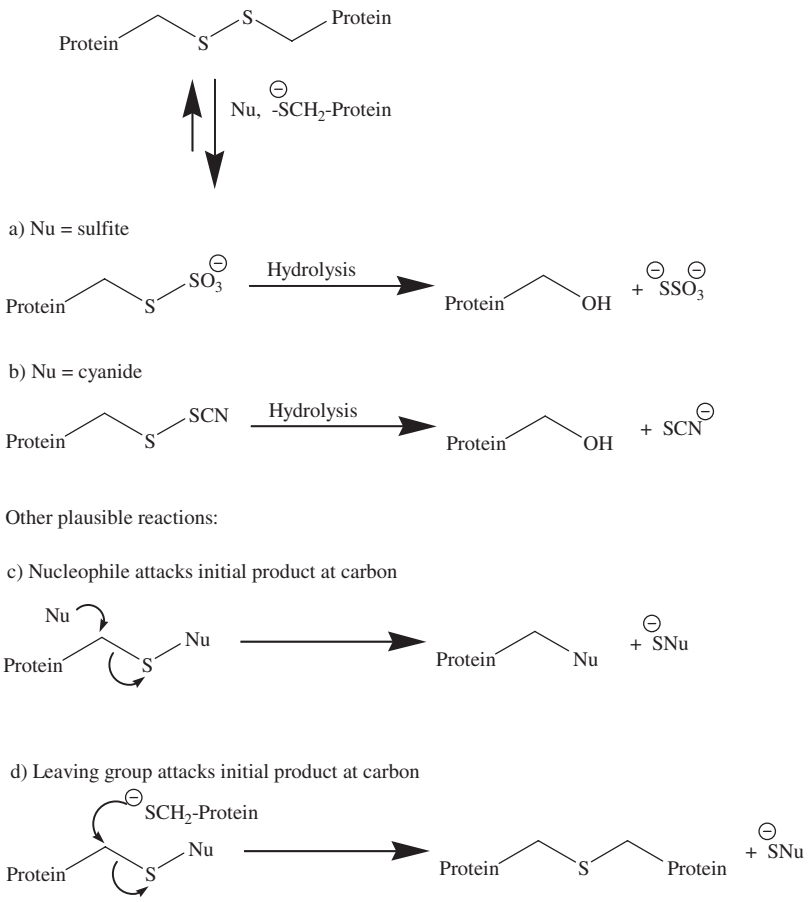
From the clinical perspective, many of the substances/species in Table 1 have been associated with skin sensitization. Aniline has been reported as a cause of allergic contact dermatitis (24, 25), although a free radical protein-binding mechanism seems to be a plausible alternative to the nucleophilic mechanism; thioureas are well recognized as being responsible for allergic contact dermatitis (26, 27); and also, case reports of skin allergy to sodium thiosulfate (28) and to rubidium iodide (29) exist. Furthermore, both aniline and diphenylthiourea are positive in predictive assays for skin sensitization (30, 31).

Biological mechanism

Two pathways have been distinguished in allergic contact dermatitis: Th1 and Th2, exemplified by 2,4-dinitrochlorobenzene (DNCB) and trimellitic anhydride (TMA) (32). Although better known as a respiratory allergen (32), TMA is a recognized contact allergen in humans (33–35). Evidence that this may be associated with the *in cutaneo* location of the carrier protein has been presented (36). Generalizing these conclusions, the DNCB type of sensitizer reacts with thiol nucleophiles, which are more likely to be cytoplasmic (they would be oxidized to S–S linkages outside the cells). In contrast, the TMA type will preferentially react with harder nucleophiles, mainly lysine, that are more likely to be present in membranes and/or outside cells (this does not, of course, preclude them being present inside cells as well). For the nucleophilic sensitizers discussed in this article, the targets are S–S links. These can probably be encountered in membranes and outside the cells. If the choice between Th1 and Th2 pathways is more about location of protein binding than about the type of functional groups that react, then nucleophilic sensitizers may well preferentially trigger the TMA-type pathway.

Conclusions

The contact allergenicity of sodium metabisulfite is most simply rationalized in terms of a previously unrecognized protein-binding mechanism, nucleophilic attack by the



Scheme 2. Further reactions of initial reaction products. Reactions (a) and (b) are discussed in reference 21. These hydrolysis reactions are observed *in vitro* with model peptides only under alkaline conditions, so their occurrence *in cutaneo* is hypothetical – for it to occur would probably require general base catalysis in the epidermal environment. For reaction (c), the attacking nucleophile could be a nucleophilic group, such as a lysine or cysteine unit, on the original protein, in which case the original disulfide bridge would be lost and a new bridge formed. Reaction (d) is a special case of reaction (c), resulting in the disulfide bridge being replaced by a sulfide bridge.

allergen on electrophilic S–S linkages in skin proteins. The chemical properties required for sensitization by nucleophilic attack on skin proteins are quite restrictive, so the domain of nucleophilic sensitizers is expected to be small. Thiourea derivatives are among other sensitizers that are likely to act by this mechanism.

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