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The Dissociation Constant of the Hydroxysulphonate of 3,4-Dideoxy-4-Sulphohexosulose in the Sulphite-Inhibited Maillard Reaction of Glucose and Glycine

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ABSTRACT

The dissociation constant of the hydroxysulphonate of 3,4-dideoxy-4-sulphohexosulose (DSH) was measured by iodimetric determination of free and reversibly bound sulphur (IV) oxospecies (S(IV)) in mixtures of DSH and S(IV). The reaction medium was chosen to simulate the conditions used in the model Maillard system of McWeeny et al. *(1969) and contained DSH* ($4{\cdot}3 \times 10^{-3}$ M)*, glycine* ($0{\cdot}5$ M) *and S(IV)* ($0{\cdot}0035 - 0{\cdot}035$ M) *at pH 5·5 and 55°C. Under these conditions, DSH forms a monohydroxysulphonate with a dissociation constant of* $4{\cdot}4 \times 10^{-3}$ M, *adding credence to the suggestion that DSH is a major contributor to the increase, with time, in reversible binding of S(IV) in the system glucose–glycine–S(IV).*

INTRODUCTION

The inhibition of Maillard browning by sulphur (IV) oxospecies (S(IV)) leads to reversible and irreversible combination of S(IV) (McWeeny *et al.*, 1969). In the case of the system glucose–glycine–S(IV), a product of irreversible combination of S(IV) is 3,4-dideoxy-4-sulphohexosulose (DSH). A kinetic model of the reversible and irreversible binding of S(IV) suggests that, under the conditions used by McWeeny *et al.* (initial concentrations: [glucose] = 1 M; [glycine] = 0·5 M; [S(IV)] = 0·039 M; pH 5·5 and 55 °C), reversible binding of S(IV) is the result of two reactions—the formation of the hydroxysulphonates of glucose and of DSH (Wedzicha, 1984). In order for this conclusion to be true, the dissociation constant of the hydroxysulphonate of DSH is required to be 5×10^{-3} M, a value predicted from the kinetic data of McWeeny *et al.* (1969) since direct measurements of the dissociation constant were not available at the time when the model was derived. Here we report the measurement of the equilibrium constant in question under the conditions of the model experiments of McWeeny *et al.*

EXPERIMENTAL

Preparation of 3,4-dideoxy-4-sulphohexosulose

The preparation of DSH was carried out by a modification of the method of Ingles (1962). Glucose (18 g) and sodium sulphite (12·6 g) in water (50 ml) were heated at 100 °C for 5 h. The mixture was diluted to 250 ml and passed through a column of Dowex 50W-X8 cation exchange resin in the H^+ form. The acidic effluent was steam distilled until no more sulphur dioxide was present in the distillate. The acidic components of the residue, after steam distillation, were adsorbed on to a column of Dowex 1-X8 anion exchange resin in the Cl^- form and neutral components removed by washing with water. The adsorbed acids were displaced with 0·1 M sulphuric acid and the column effluent fractionated (100 fractions). A sample of each fraction was mixed with a solution of 2,4-dinitrophenylhydrazine hydrochloride (2 g) in perchloric acid (100 ml, 30 %). Any precipitate which formed was filtered and washed with decreasing concentrations of perchloric acid and, finally, water, before it was dissolved in a small volume of pyridine. The resulting solution was subjected to thin layer chromatography on silica gel type G with butanol: 0·880 ammonia (4:1) as solvent. Fractions showing a single purple spot at R_f 0·42 due to the bis(2,4-dinitrophenylhydrazone) of DSH (Wedzicha & McWeeny, 1975) were bulked; the sulphuric acid was neutralised by the addition of barium carbonate and filtered to give the barium salt of DSH in the filtrate. This solution was immediately passed through a column of Dowex 50W-X8 cation exchange resin in the H^+ form to give a solution of the sulphonic acid which was concentrated to a viscous syrup under reduced pressure at 40 °C. The product was stored at −40 °C and its purity checked by thin layer chromatography of the bis(2,4-dinitrophenylhydrazone), as described above, before use.

Measurement of dissociation constant

It was not possible to obtain DSH in an anhydrous state and therefore the method of Burroughs & Sparks (1973) for the determination of hydroxysulphonate dissociation constants, where the concentration of the carbonyl component is not known, was adopted. If the apparent equilibrium constant for the dissociation of hydroxysulphonate:

$$\mathrm{DSH.S(IV)} \rightleftharpoons \mathrm{DSH} + \mathrm{S(IV)}$$

is given by:

$$K = \frac{S(X - x)}{x}$$

where: S is the molar concentration of free S(IV) in any form; X is the total molar concentration of the carbonyl compound (free and bound) and x is the molar concentration of hydroxysulphonate, then the equation can be rearranged to give:

$$x = X - K\frac{x}{S} \qquad (1)$$

Therefore, a graph of x as a function of x/S should be linear, with a slope of K and intercept X.

For measurement of the equilibrium constant, a series of reaction mixtures containing constant amounts of DSH (approximately 5×10^{-3} M) and glycine (0·5 M) and variable amounts of S(IV) (0·0035–0·035 M) were prepared. The pH of each mixture was adjusted with sodium hydroxide to 5·5 before the solutions were made up to their final volumes. The mixtures were placed in a waterbath at 55 °C for 24 h for equilibration. The concentration of free S(IV) was determined on aliquots (20 ml) of the reaction mixtures by adding hydrochloric acid (2 ml, 25 %) and titrating with 0·01 M iodine solution to a starch end-point. The solutions were then made mildly alkaline by saturating with solid sodium hydrogen carbonate and reversibly bound S(IV), thus liberated, was titrated with 0·01 M iodine solution as before.

RESULTS AND DISCUSSION

The method of preparing DSH adopted here involves the removal of free and reversibly bound S(IV) from reaction mixtures by steam distillation prior to chromatographic separation of reaction products. This

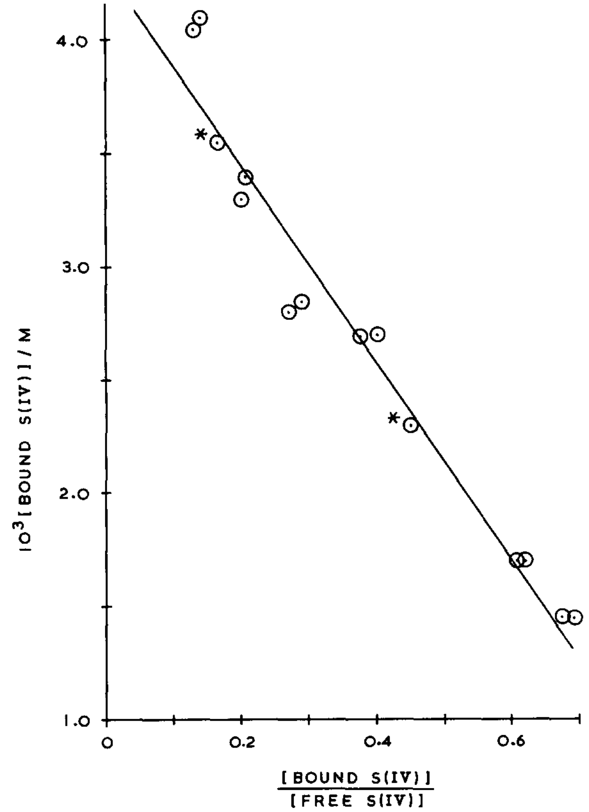

Fig. 1. Graphical determination of the apparent dissociation constant of the hydroxysulphonate of 3,4-dideoxy-4-sulphohexosulose in the presence of glycine (0·5 M) at pH 5·5 and 55 °C. * Denotes two data points. superimposed.

operation is found to have the advantage that, when mixtures are subsequently applied to an anion exchange resin, only the sulphonates which remain after steam distillation are adsorbed on to the resin. In contrast, anion exchange separations carried out on mixtures containing an excess of S(IV) lead to the elution of carbonyl components which are adsorbed as hydroxysulphonates and which contaminate the DSH. When converted to the 2,4-dinitrophenylhydrazone derivative and subsequently analysed by thin layer chromatography, the DSH sample was found to be homogeneous.

The conditions for the reaction between DSH and S(IV) were chosen to correspond as closely as possible to those used by McWeeny *et al.* (1969) for a model of sulphite-inhibited Maillard (glucose–glycine) browning. Glucose was not included in the system as it is considered that the only consequence of its presence would be to contribute to the reversible binding of S(IV) and thus complicate the results. On the other hand, the inclusion of glycine was considered to be essential since interaction between the carbonyl groups of DSH and glycine could alter the apparent stability of the hydroxysulphonate of DSH. Also, Ingles (1967) has reported that glyoxal and diacetyl may form addition compounds involving both amine and S(IV) and the possibility of this type of interaction modifying the equilibrium constant for the dicarbonyl–S(IV) interaction should not be discounted.

Preliminary experiments showed that an equilibration time of 24 h was sufficient for the reaction between DSH and S(IV). Experimental data, for the amounts of free and reversibly bound S(IV), in linearised form according to eqn. (1) are shown in Fig. 1 and the regression line gives a value of $4{\cdot}4 \times 10^{-3}$ M for the dissociation constant of the hydroxysulphonate of DSH at a total DSH concentration of $4{\cdot}3 \times 10^{-3}$ M. The latter is reasonable since the amount of DSH weighed out as a syrup for the preparation of solutions corresponded to a concentration of 5×10^{-3} M. The essentially linear plot shown in Fig. 1 is consistent with the prediction made earlier (Wedzicha, 1984) that DSH forms a monohydroxysulphonate. The value of the dissociation constant is encouragingly close to that predicted (5×10^{-3} M) from kinetic data, adding credence to the suggestion that DSH is a major contributor to the increase, with time, in reversible binding of S(IV) in the system glucose–glycine–S(IV).

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