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Kinetics of the Reaction of 3-Deoxyhexosulose with Thiols

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ABSTRACT

Mercaptoethanol (ME) and glutathione (GSH) react with 3-deoxyhexosulose (DH) with 2:1 stoichiometry, the initial reaction being first order with respect to DH but zero order with respect to H^+ and thiol. The reaction is accelerated by glycine and two parallel rate-determining processes, one dependent on glycine concentration, are proposed. The rate constants are compared with those for the reaction of DH with sulphite species (S(IV)), which shows similar kinetic behaviour; however, the rate constant of the DH–thiol reaction is c. *2–3 times the value of that for the DH–S(IV) reaction but the effect of glycine is smaller by a factor of* c. *3. Possible differences in mechanism are considered.*

The reaction of DH with cysteine shows complex behaviour. An initial rapid formation of a 1:1 adduct is followed by liberation of thiol. A mechanism involving the formation of a thiazolidine compound by addition of cysteine to a carbonyl group, followed by Strecker degradation, is suggested.

INTRODUCTION

The amine-assisted dehydration of aldoses, which constitutes an essential step in the Maillard reaction, leads to the formation of 3-deoxyaldosuloses as important intermediates in colour formation. In the case of a typical Maillard reaction, that between glucose and glycine, the corresponding intermediate is 3-deoxyhexosulose (DH) which further loses the elements of water to give 3,4-dideoxyhexosulos-3-ene (DDH), one of the most reactive intermediates in browning (McWeeny *et al.*, 1974).

The α,β-unsaturated carbonyl structure of DDH renders the molecule susceptible to attack by nucleophiles in position 4 (Wedzicha, 1984) and the

ability of sulphite species (S(IV)) to inhibit the Maillard reaction is attributed to the 3,4-addition of $H^+SO_3^{2-}$ to the double bond. This reaction causes irreversible binding of S(IV). A characteristic of the inhibition of browning by this additive is that it delays the onset of colour formation, which subsequently proceeds at a rate almost independent of how much inhibitor had been added. It is found (Song & Chichester, 1967) that simple thiols such as mercaptoethanol exert a similar effect on colour formation and it is, therefore, tempting to suggest that these, too, add to DDH; the thiolate anion is of similar nucleophilicity to the sulphite ion (Davies, 1968). It is well known that when the Maillard reaction is carried out with cysteine as the amino-compound, characteristic meaty flavours are generated (Marbrock *et at.*, 1969). The extent to which any reaction with 3-deoxyosuloses or the corresponding 3,4-dideoxyosulos-3-enes contributes to such flavour formation is not known.

The reaction between 3-deoxyhexosulose and sulphite ion is of first order with respect to DH but of zero order with respect to the nucleophile (Wedzicha & Kaban, 1986). This is interpreted as a rate-determining transformation of DH, possibly to DDH followed by a mopping up of this intermediate by the nucleophile. The reaction is catalysed by glycine but inhibited by sulphite species. The catalysis is possibly the result of some amine-assisted dehydration of DH and kinetic data suggest two parallel processes, one of which involves the amino acid in the rate-determining step. The first order rate constant k is related to glycine concentration by:

$$k = 5{\cdot}32 \times 10^{-3}(1 + 4{\cdot}45[\text{glycine}])\ \text{h}^{-1}$$

at pH 5·5, 55°C (recalculated from Wedzicha & Kaban, 1986). The inhibition by S(IV) is due to the reversible formation of a hydroxysulphonate of DH which was assumed to be unreactive towards the nucleophile.

The object of the present investigation was to study the kinetics of the reaction between DH and thiols in order to compare the mechanism with that which is known with reasonable certainty for the DH–S(IV) reaction. The thiols chosen were mercaptoethanol (ME) as a simple water-soluble thiol, cysteine as an important thiol-containing constituent of foods and capable of generating useful flavours in Maillard systems and glutathione (GSH) as an example of a simple peptide with a thiol group.

MATERIALS AND METHODS

Wherever possible, reagents were AnalaR grade and were obtained from BDH Chemicals Ltd., Poole, Pyruvaldehyde (PA) and acetaldehyde were obtained from Aldrich, Gillingham.

The preparation of DH was based on the method of Madson & Feather (1981) as used by Wedzicha & Kaban (1986) and the homogeneity of the product established as described previously. Solutions of DH were standardised by conversion to the metasaccharinic acid according to the method of Anet (1961). The reaction between DH and thiols (ME, cysteine and GSH) was followed by measuring the concentration of thiol spectrophotometrically. To calibrate the spectrophotometer, a solution (*c.* 0·05M) of thiol was standardised with iodine and used to prepare a set of standards by mixing with a solution of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) at pH 8·0, using the same reagent and procedure as described previously for the determination of S(IV) (Wedzicha & Kaban, 1986).

Reaction mixtures contained DH (5–50 mM), thiol (5–50 mM) and glycine (0–1·0M) and, unless otherwise stated, were prepared in a pH 5·5 acetate buffer ($[CH_3COOH] + [CH_3COO^-] = 50$ mM) containing EDTA (0·5 mM) to prevent autoxidation of thiol. Reaction mixtures containing sodium cyanoborohydride (50–75 mM) were prepared in a pH 6·9 phosphate buffer ($[KH_2PO_4] = [Na_2HPO_4] = 25$ mM). Reactant solutions were prepared immediately before use and mixed at room temperature. Reaction mixtures (*c.* 2 ml) were dispensed into screw-cap vials (2 ml capacity) and placed in a water bath at $55{\cdot}0 \pm 0{\cdot}1$°C. Vials were withdrawn at timed intervals and the contents analysed for residual thiol-content.

When reactions were followed at 10°C, buffered reactants were allowed to attain thermal equilibrium, mixed rapidly and aliquots withdrawn at timed intervals. These were immediately diluted with water at 10°C and analysed for thiol-content as rapidly as possible.

To measure free thiol-content, an aliquot (10 ml) of reaction mixture was made acid with HCl (1 ml, 4M) and titrated with iodine solution (10 mM). Total thiol-content was measured by saturating an aliquot (10 ml) of reaction mixture with solid $NaHCO_3$ and titrating the mixture with iodine solution (10 mM).

Preliminary analysis of DH-cysteine reaction products was carried out by tlc on silica gel G with butanol:acetic acid:water (4:1:1) as solvent. Thiols were revealed as yellow spots by spraying plates with a solution of DTNB in ethanol.

RESULTS

Preliminary experiments

The procedure for the analysis of thiols using 5,5′dithiobis(2-nitrobenzoic acid) is a well established method (Ellman, 1959) and is based on

the cleavage of the disulphide bond in the reagent to form 5-mercapto-2-nitrobenzoic acid which, at pH 8, is present as the highly absorbing thiolate anion. The resonance stabilisation of this species causes the equilibrium between the reagent + thiol and the reaction product to be pushed well to the side of the product giving rise to a quantitative analytical reaction. The analysis for thiols was found to obey Beer's law at the concentrations used. Simple thiols are known to react reversibly with aldehydes to form hemimercaptals (*cf.* the formation of hydroxysulphonates with hydrogen sulphite ion). When a reaction mixture initially containing DH (5·2 mM), ME (19·5 mM) and glycine (0·5M) was allowed to react for 21 h, the resulting concentration of free thiol was 8·4 mM by iodine titration. The end point was stable, indicating that the reversibly formed adducts were sufficiently stable to allow the free thiol-content to be obtained. Total thiol-content, obtained iodimetrically after raising the pH of the mixture, was 16·9 mM, whilst the corresponding result by the spectrophotometric method was 16·6 mM. For a similar reaction mixture containing GSH, the concentrations were, respectively, 8·8 mM, 15·8 mM and 15·6 mM, after 24 h reaction. These results show that a significant proportion of the thiol exists reversibly bound and that the spectrophotometric method of analysis measures the total (free + reversibly bound) thiol. This observation reflects the situation previously reported for the DH-S(IV) reaction (Wedzicha & Kaban, 1986). An important aspect of the results is that the concentration of reversibly bound thiol (8·5 and 7·0 mM for the ME and GSH reactions respectively) exceeds the initial concentration of DH (5·2 mM). In the case of acetaldehyde–ME or PA–ME mixtures, the end point in the titration with iodine was unstable when free thiol was being measured, and the adducts so formed were clearly less stable than those formed during the course of the DH–thiol reaction. The spectrophotometric method of analysis gave the same total thiol-content as found iodimetrically.

Preliminary kinetic experiments showed that reproducible results could only be obtained if reaction mixtures contained a small concentration (0·1–1·0 mM) of EDTA. The apparent rate of loss of thiol was greatly accelerated by the addition of $MnCl_2$ (10 μM) suggesting that a metal-catalysed autoxidation of thiol had been arrested by the addition of EDTA. Subsequently, all reaction mixtures contained 0·5 mM EDTA. The reproducibility of thiol concentrations in replicate kinetic runs was better than $\pm 2\%$.

Reactions of mercaptoethanol and glutathione

The initial reaction

Initial rates were measured as the slope of concentration–time curves at zero time. When the concentrations of DH and ME or GSH were varied in the

range 10–50 mM with [glycine] = zero or 0·5M the reaction was found to be of first order with respect to DH and of zero order with respect to thiol. Variation of pH in the range 3·85–6·00 by changing the composition of the acetate buffer with [DH] = [ME] = 25 mM and [glycine] = 0·5M, gave an initial rate of $(1{\cdot}06 \pm 0{\cdot}06) \times 10^{-3}$ M h^{-1} (mean of five measurements ± standard deviation) and the reaction is, therefore, of zero order with respect to H^+. The first order rate constants for the initial reactions of ME and GSH in the presence of 0·5M glycine are, respectively, $0{\cdot}044 \pm 0{\cdot}003\ h^{-1}$ (mean of 15 measurements ± standard deviation) and $0{\cdot}032 \pm 0{\cdot}002\ h^{-1}$ (mean of six measurements ± standard deviation). When the reaction was carried out in D_2O as the solvent, rate constants were 0·045 and $0{\cdot}033\ h^{-1}$, respectively, indicating absence of any kinetic solvent-isotope effect. The effect of glycine concentration on the reaction of ME is illustrated in Fig. 1. This implies that the first order rate constant, k, varies with concentration according to:

$$k = k_1(1 + k_2[\text{glycine}])\ h^{-1}$$

where the values of k_1 and k_2 for the reactions of ME and GSH are as shown

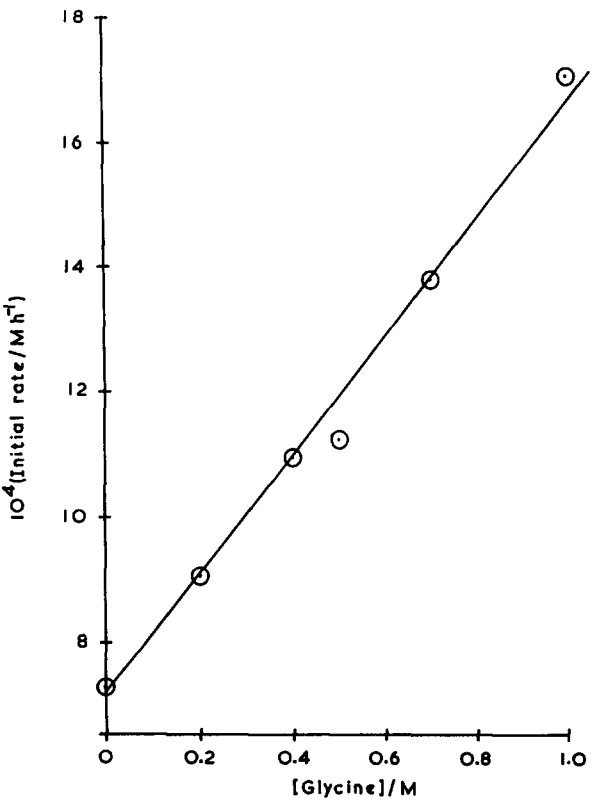


Fig. 1. Effect of glycine concentration on the initial rate of loss of thiol in the reaction between DH and ME. Initial reaction conditions: [DH] = [ME] = 25 mM; 50 mM acetate buffer, pH 5·5; 55°C.

TABLE 1

Values of Rate Constants k_1 and k_2 in the Equation for the Pseudo-First Order Rate Constant k of the Reaction between 3-Deoxyhexosulose and Mercaptoethanol or Glutathione at 55°C. The Relationship between Rate Constants is, $k = k_1(1 + k_2[\text{glycine}])$

	k_1/h^{-1}	k_2/M^{-1}
Mercaptoethanol	0·029	1·31
Glutathione	0·020	1·35

in Table 1. The reaction of both thiols with DH appears to proceed by two paths, one of which involves glycine. The kinetic order with respect to the DH, thiol and H^+ is the same whether or not glycine is present and the complete rate equation is, therefore, given by:

$$-d[SH]/dt = k'[DH](1 + k_2[\text{glycine}])$$

In the presence of sodium cyanoborohydride (50 mM) the initial rate of the DH–ME reaction ([DH] = 25 mM, [ME] = 25 mM, [glycine] = 0·5M) is reduced to approximately one twentieth of its value without the reagent.

The overall reaction

There is kinetic evidence to suggest that the mechanism of reactions of DH with S(IV), at long reaction times, is different from that of the initial reaction (Wedzicha & Kaban, 1986). A typical first-order plot ($\ln(c-c_\infty)$ versus t, where c and c_∞ are, respectively, thiol concentrations at times t and infinity) for a reaction with initial concentrations [DH] = 15·3 mM, [ME] = 49·9 mM, [glycine] = 0·5 M and c_∞ = 19·8 mM is shown in Fig. 2. Thus, first order behaviour is followed for at least 90% of the reaction and the value of rate constant is 0·0106 h^{-1}. This result is seen to be approximately one-quarter of the value of the first order rate constant obtained from initial rates under the same conditions.

The stoichiometry of the reaction was measured by finding the value of c_∞ in runs where an excess of thiol was present, and using the amount of thiol reacted and the initial concentration of DH, in the calculation. The stoichiometries (DH:thiol) of the reactions with ME and GSH were, respectively, 1:1·97 and 1:2·03, indicating that the overall reaction involves two molecules of thiol with one molecule of DH. It has not been possible to measure with sufficient precision the time-dependent concentration of DH to establish whether this stoichiometry applies to the initial reaction.

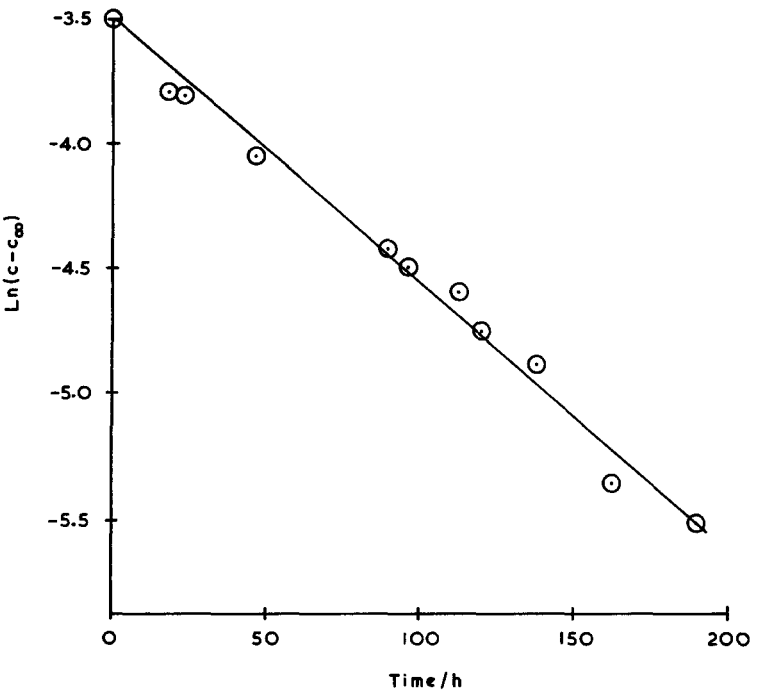


Fig. 2. First order plot for the reaction of DH with ME where c is the concentration of ME at time t, and $c_\infty = 19{\cdot}8$ mM, measured as the asymptote to concentration–time curves. Initial conditions: [DH] = 15·3 mM; [ME] = 49·9 mM; [glycine] = 0·5M; 50 mM acetate buffer, pH 5·5; 55°C.

Reaction of 3-deoxyhexosulose with cysteine

The concentration–time characteristics of the reaction of DH with cysteine are shown in Fig. 3. There appears to be a rapid initial binding of cysteine followed by a slower release of thiol. When analysed by tlc on silica, reaction mixtures which contained the liberated thiol showed a component which ran at R_f 0·12 and which gave a positive reaction with DTNB reagent. Cysteine ran at R_f 0·27. The extent of the initial change depends on DH concentration. The concentrations of bound cysteine $[\text{cysteine}]_{bd}$, after 1 h, at different initial DH concentrations $[DH]_0$, taken from Fig. 3, are as shown in Table 2. A significant formation of reaction intermediate is taking place on a short time scale. It is likely that the true stoichiometry is closer to 1:1 because the relatively fast initial reaction of the *formation* of new thiol will cause an underestimate of the bound cysteine concentration. At the lowest initial concentration of DH (9·7 mM), the rate of formation of thiol product is sufficiently slow to reliably extrapolate the concentration-time data to zero time, to give a better estimate of the initial concentration of

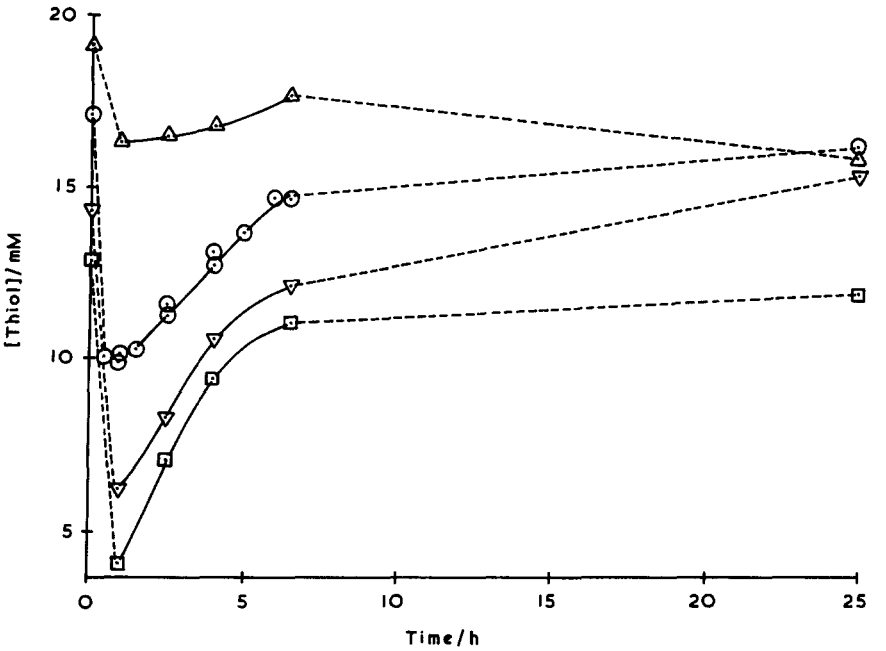


Fig. 3. Concentration–time data for the reaction of DH with cysteine. Initial conditions: [cysteine] = 24·9 mM; [glycine] = 0·5 M; 50 mM acetate buffer, pH 5·5; 55°C. Initial DH concentration was varied as follows: △ 9·7 mM; ⊙ 24·6 mM; ▽ 38·8 mM; ⊡ 48·5 mM.

bound cysteine as 9·3 mM. Thus, the stoichiometry of the adduct formed initially is (DH:cysteine) 1:0·96. It was noticed that mixtures of DH with cysteine develop cooked-meat aromas within the time scale of the reactions investigated here.

The concentration–time characteristics of the DH–cysteine reaction in the presence of sodium cyanoborohydride ([cysteine] = [DH] = 25 mM, $[NaBH_3CN]$ = 75 mM) are illustrated in Fig. 4. This shows that the initial loss of thiol (obtained by extrapolation of concentration–time data to zero

TABLE 2

Concentration of Cysteine bound to DH after 1 h and the Stoichiometry of the Reaction. ($[DH]_0$ is the initial DH concentration and $[cysteine]_{bd}$ the concentration of cysteine bound. Reaction conditions: [cysteine] = 24·9 mM; [glycine] = 0·5 M: 50 mM acetate buffer, pH 5·5; 55°C)

$[DH]_0$ (mM)	9·7	24·6	38·8	48·5
$[cysteine]_{bd}$ (mM)	8·5	14·9	18·7	20·8
$[cysteine]_{bd}/[DH]_0$	0·88	0·61	0·48	0·43

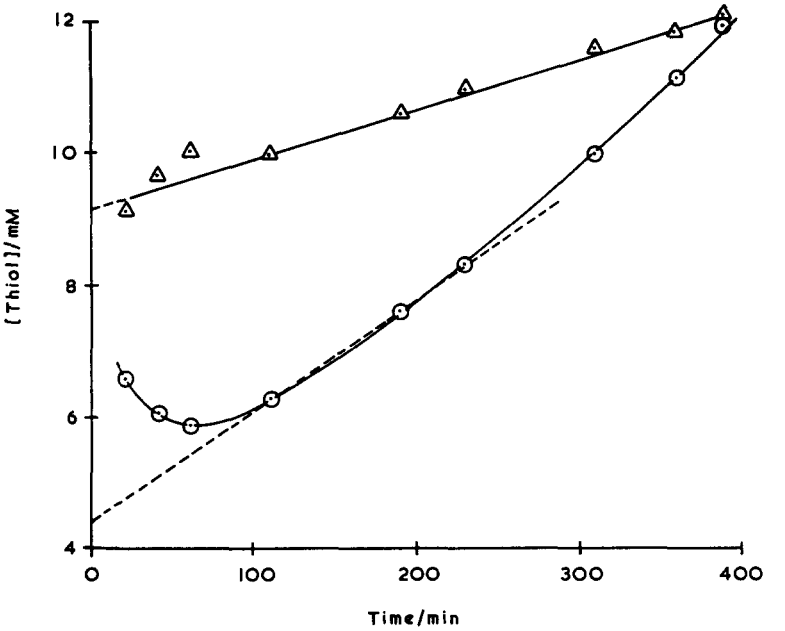


Fig. 4. Effect of sodium cyanoborohydride on the concentration–time behaviour of the reaction between cysteine and DH. Reaction conditions: [cysteine] = [DH] = 25 mM; 50 mM phosphate buffer, pH 6·9; 55°C; ⊙ no $NaBH_3CN$; △ [$NaBH_3CN$] = 75 mM.

time) is reduced by some 25% on addition of reagent whilst the ratio of initial rates of subsequent formation of new thiol (rate without cyanoborohydride/rate with cyanoborohydride) is 2·2.

It was possible to reduce the rate of the initial stage of the reaction to a measurable value by reducing the temperature to 10°C. Under such conditions and sampling reaction mixtures at 3 min intervals, the initial rates obtained as a function of cysteine concentration when [DH] = 24·4 mM in the absence of glycine are shown in Table 3. There appears to be no kinetic effect of the amino acid on its rate of reaction on the short time scale.

In order to investigate if the observed reactivity of cysteine was a feature peculiar to DH or simply a feature of an α-dicarbonyl compound, the

TABLE 3

Effects of Cysteine Concentration on the Initial Rate of Reaction of Cysteine with DH. (Reaction conditions: [DH] = 24·4 mM; 50 mM acetate buffer, pH 5·5; 10°C)

[cysteine]/mM	10	15	25	40	50
10^4 rate/M min^{-1}	3·90	3·94	3·79	4·08	3·46

reactions of acetaldehyde and PA with cysteine were followed as described for DH. Pyruvaldehyde reacts in the same way as DH whilst with acetaldehyde, a stable reaction product is rapidly formed. Preliminary experiments on the kinetics of the PA–cysteine reaction at 10°C suggest that its rate and final thiol concentration after 1 h depend on PA concentration as was observed with DH.

DISCUSSION

Reaction with mercaptoethanol and glutathione

The rate law for the DH–ME and DH–GSH reactions is the same as that for the DH–S(IV) reaction (Wedzicha & Kaban, 1986) including the fact that glycine promotes the loss of nucleophile. An important difference is that the initial stages of the reactions reported here are not inhibited by the thiols; thus, no evidence of hemimercaptal formation between DH and ME or GSH can be found despite significant reversible binding of mercaptoethanol in the later stages of the reaction. Either the time for initial rate measurements is too short for hemimercaptals to be formed or the adducts are highly dissociated. Aldehyde–thiol adducts are generally less stable than the corresponding hydroxysulphonates; the equilibrium constant for the formation of hemimercaptal between isobutyraldehyde and methoxymercaptoethanol is $11{\cdot}8\,\text{M}^{-1}$ (Lienhard & Jencks, 1966) compared with $4{\cdot}4 \times 10^4\,\text{M}^{-1}$ (Green & Hine, 1974) for the formation of the corresponding hydroxysulphonate. The observation that the concentration of reversibly bound ME, in the later stages of reaction, exceeded the initial concentration of DH implies (i) the concentration of carbonyl groups may increase as the reaction proceeds, (ii) each carbonyl group may react with more than one thiol group to form the more stable mercaptals (Wander & Horton, 1976) rather than hemimercaptals or (iii) DH forms a diadduct as a result of it having two carbonyl groups. The available data do not allow these possibilities to be distinguished from one another but it is clear that the analytical procedure used for measuring residual thiol in kinetic studies accounts for all thiol that can be released and measured iodimetrically after raising the pH of reaction mixtures.

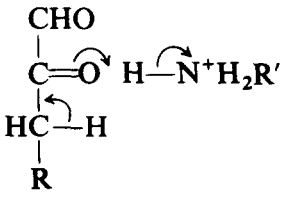
A further dissimilarity between the reactions of DH with thiols and with S(IV) is that the overall stoichiometry of the former is 1:2 whereas that of the latter is 1:1. Adherence to first order behaviour over 90% of the reaction implies that there is no substantial change in mechanism during the course of the measurements. If it is assumed that 1:2 stoichiometry applies to the processes involved in the initial stages of the DH–thiol reaction, the

relationship between the initial rates of loss of thiol, DH and S(IV) (in the DH–S(IV) reaction) is:

$$-\mathrm{d[SH]}/\mathrm{d}t = -2\,\mathrm{d[DH]}/\mathrm{d}t = -2\,\mathrm{d[S(IV)]}/\mathrm{d}t$$

Thus, the first order rate constant for the loss of DH in the DH–thiol reaction is 0·022 and 0·016 h^{-1} when the thiols are ME and GSH, respectively, with [glycine] = 0·5M. It is misleading to compare these values with a reported value of first order rate constant of 0·0164 h^{-1} for the DH–S(IV) reaction under the same conditions (Wedzicha & Kaban, 1986) because their similarity is fortuitous. It is the result of different weightings of the glycine-dependent and glycine-independent mechanisms in the two reactions. The rate constant of the glycine-independent step in the DH–thiol reaction is approximately 2–3 times greater than the value of that for the DH–S(IV) reaction but the effect of glycine is smaller by a factor of approximately 3.

The mechanism of the DH–thiol reaction involves a spontaneous, pH-independent, rate-determining change of DH to an activated complex which reacts in a fast step with 1 or 2 molecules of thiol. The rate is independent of pH whether or not glycine is present and the absence of a kinetically significant protonation step is confirmed by the lack of any solvent isotope effect. As pH is varied in the range 3·85–6·00, the proportion of carboxylate ion (pK_a 2·35, Weast, 1981) increases from *c.* 97% of the glycine present to *c.* 99·9%; the amino group (pK_a 9·78) will be almost completely protonated. The species whose concentrations do not change significantly with pH are, therefore, $-COO^-$ and $-NH_3^+$. A possible spontaneous change which DH undergoes and which could be conceived as independent of pH is its dehydration to DDH. This has been suggested as the pathway for activation of the DH–S(IV) reaction (Wedzicha & Kaban, 1986); if a common pathway exists, the faster rate of the DH–thiol reaction implies that the activated complex which reacts with thiols could be formed at an earlier stage. Thus, an example of a plausible spontaneous change could be enolisation of DH as a prerequisite to reaction. This is likely to be acid-base catalysed and the protonated amino group could act as an appropriate proton-donor:



$R = (CHOH)_2CH_2OH$

A proton transfer of this type would, however, be subject to a kinetic solvent-isotope effect, though its magnitude cannot be estimated reliably; it is not possible to say whether a measurable effect is likely. Alternatively, the

important intermediate could be a Schiff's base as suggested previously for the DH–S(IV) reaction. Data on the effect of pH on the rates and equilibria of Schiff's base formation with DH are required, but it is interesting to see that the rates of formation of oximes (reaction of carbonyl groups with NH_2OH) are relatively insensitive to pH at pH 4 (Jencks, 1959). The reaction is, however, acid-base catalysed but it is not possible to extrapolate published data on simple aldehydes and ketones to DH.

Sodium cyanoborohydride reduces carbonyl groups and Schiff's bases; the reduction of the former is favoured at low pH (pH 3–4) whilst the reduction of Schiff's bases is rapid at around neutral pH (Borch *et al.*, 1971). Thus, the reagent may be used to selectively reduce Schiff's bases in the presence of aldehydes and ketones and was used here to provide evidence for the involvement of these reaction intermediates. Once the Schiff's bases are reduced, both the carbonyl group and the amino compound are made unavailable, irreversibly. The rate of the DH–ME reaction in the presence of glycine is reduced markedly by the addition of sodium cyanoborohydride and we infer that a Schiff's base between DH and glycine is formed. This could be the kinetically significant species but it is not possible to distinguish this involvement from a situation in which the reduction of Schiff's base merely serves to remove DH from the system and thereby prevent reaction with ME. When the concentration of glycine is 0·5M, the rate of the glycine-catalysed reaction is two thirds of that of the uncatalysed reaction. The fact that sodium cyanoborohydride reduces the rate of the reaction by a factor of approximately 20 is indicative that it is not only the glycine-catalysed reaction which is inhibited and we therefore favour the explanation in terms of removal of DH. However, the data provide unequivocal evidence for the formation of a Schiff's base which may be postulated within the mechanism of the glycine-dependent reaction.

The rate determining step could be either formation of the Schiff's base, or a rearrangement of the Schiff's base to the activated complex. Thus, the glycine-dependent reaction could proceed as follows:

$$\text{DH} + \text{glycine} \overset{\text{slow}}{\rightleftharpoons} \text{Schiff base} \xrightarrow[\text{thiol}]{\text{fast}} \text{Product}$$

or,

$$\text{DH} + \text{glycine} \overset{\text{fast}}{\rightleftharpoons} \text{Schiff base} \overset{\text{slow}}{\longrightarrow} I \xrightarrow[\text{thiol}]{\text{fast}} \text{Product}$$

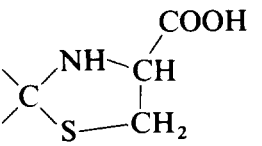
where I is a reaction intermediate.

The fact that the presence of simple thiols causes inhibition of browning of DH–glycine mixtures suggests that the reaction prevents formation of α,β-unsaturated carbonylic intermediates such as DDH. A mechanism which involves subsequent addition of thiol to DDH, or a precursor of it as DH is converted to DDH, is attractive but there is no simple way in which a

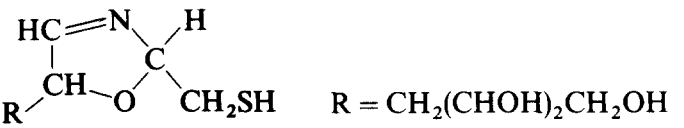
second molecule of thiol can be accommodated in the product. The mechanism can only be further elucidated after the structure of the reaction product has been established. The overall reaction of ME and GSH at [glycine] = 0·5M proceeds more slowly than does the initial reaction.

Reaction with cysteine

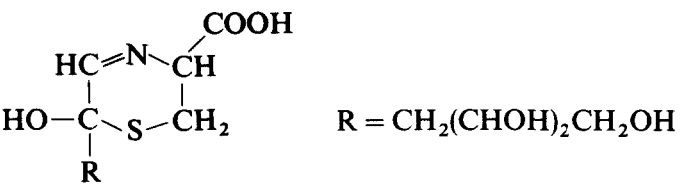
The adduct formed between carbonyl groups and cysteine is not decomposed during the spectrophotometric analysis for thiols. The product, which is formed with 1:1 stoichiometry, is likely to be the thiazolidine (Schubert, 1936),



and it is known that amino-thiols react rapidly with aldehydes to form such adducts above pH 4 (Ratner & Clarke, 1937). The reaction of ninhydrin with cysteine also leads to the formation of a thiazolidine (Prota & Ponsiglione, 1973) indicating that compounds with α-dicarbonyl groups could well react as if there was only one carbonyl group present. An alternative structure for the initial addition product is based on that suggested by Ho & Hartman (1982) for the reaction of 2,3-butanedione with cysteine, and is as follows:

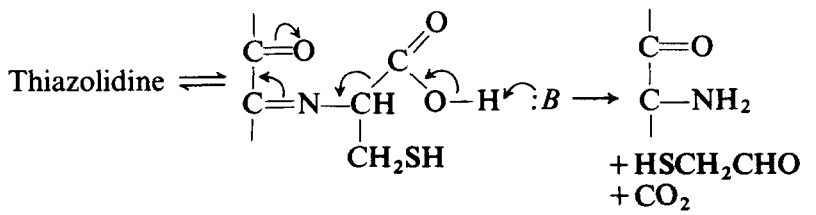


This was envisaged because 2,4,5-trimethyloxazole and 4,5-dimethyloxazole were isolated from 2,3-butanedione-cysteine reaction mixtures, and the structure offers an opportunity for forming the correct ring. The essential step is base-catalysed decarboxylation of the cysteine bound to one of the carbonyl groups, as in Strecker degradation, followed by attack at the neighbouring carbonyl group by the carbanion so formed at what was the α-position of the cysteine molecule. However, in the present experiments, the adduct formed does not have an available thiol group which would tend to suggest that the oxazole structure is not present. A third possibility is the following six-membered thiazine structure:



which is formed by addition of the thiol to the second carbonyl group, after the conventional Schiff's base had been formed with the first. There is, however, no reason to prefer such a structure to the thiazolidine compound in the case of α-dicarbonyl compounds. Jocelyn (1972) considers that hemimercaptals do not form when the thiols have an amino group in the β-position. A thiazine structure was originally proposed for the product of reaction between ninhydrin and cysteine but subsequently evidence was presented to show that the assignment had been incorrect (Prota & Ponsiglione, 1973). Also, the fact that sodium cyanoborohydride is not a strong inhibitor of any subsequent reaction of this intermediate is suggestive of the possibility that the intermediate does not contain a C=N bond.

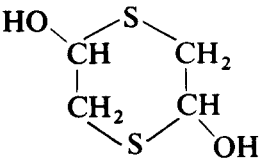
It is suggested here that thiazolidines were formed rapidly when acetaldehyde, PA and DH were allowed to react with cysteine. The mechanism of the reaction involves either initial formation of a Schiff's base followed by attack at the C=N bond by a thiolate anion, or the addition of an amino group to an initially formed hemimercaptal. Any Schiff's base which is formed probably exists after the rate-determining step as shown for the cysteine–formaldehyde reaction (Kallen, 1971) and is short lived; there will be little opportunity for it to be reduced by sodium cyanoborohydride and the reagent therefore has only a small effect on the rate and extent of the initial reaction. Kinetic data for the reaction with DH show that the rate-determining step is independent of the thiol and it is possible that some relatively slow rearrangement of DH, e.g. involving keto-enol forms, is necessary before it becomes susceptible to attack by cysteine. Only in the cases of the reactions involving PA and DH was there any further change. This could be the result of the presence of the α-dicarbonyl or activated-methylene groups. An attractive possibility is that the thiazolidine could be in equilibrium with the Schiff's base which could subsequently undergo a Strecker reaction as follows:



where *B* is a base acting as a catalyst. If the Schiff's base had a significant lifetime, reduction by sodium cyanoborohydride would be possible; this reduction would also cause release of measurable thiol groups. Since ring opening of the thiazolidine would precede Strecker degradation, the rate of release of thiol groups in the presence of sodium cyanoborohydride should be at least as fast as in the absence of reducing agent. It was found that the

rate of formation of thiol groups was approximately halved on adding sodium cyanoborohydride; this could be due to a lower concentration of intermediate formed in the initial reaction or as yet unidentified reactions.

Mercaptoacetaldehyde is only known in solution and readily dimerises to a 2,5-dihydroxy-1,4-dithiane (Thiel *et al.*, 1958; Takahashi *et al.*, 1982); that is,



This is a hemimercaptal and may be sufficiently labile to decompose under the conditions for spectrophotometric analysis of thiols, thereby accounting for the slow increase in apparent thiol-content. Alternatively, the six-membered ring could render the dithiane stable, but the mercapto-acetaldehyde may not cyclise immediately and one is, therefore, observing the formation of the monomer. Kobayashi & Fujimaki (1965) report the formation of mercaptoacetaldehyde when ninhydrin reacts with cysteine (steam distillation conditions, 1 h) and state, without supporting evidence, that DH and dehydroascorbic acid react similarly. The occurrence of the Strecker reaction proposed above is, therefore, likely, but we are continuing with the synthesis and evaluation of the reactivity of mercaptoacetaldehyde with respect to its ability to react with the DTNB reagent. The fact that a reaction capable of leading to meaty flavours has been observed at relatively low temperatures is very significant and the DH–cysteine reaction may be a source of relevant intermediates.

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REFERENCES

Anet, E. F. L. J. (1961). Degradation of carbohydrates II. The action of acid and alkali on 3-deoxyhexosones. *Aust. J. Chem.*, **14**, 295–301.

Borch, R. F., Bernstein, M. D. & Durst, H. D. (1971). The cyanohydridoborate anion as a selective reducing agent. *J. Amer. Chem. Soc.*, **93**, 2897–904.

Davies, R. E. (1968). Mechanisms of sulphur reactions. In *Inorganic Sulphur Chemistry*. ed. G. Nickless, Elsevier Scientific Publishing Co., Amsterdam.

Ellman, G. L. (1959). Tissue sulphydryl groups. *Arch. Biochem. Biophys.*, **82**, 70–77.

Green, L. R. & Hine, J. (1974). The pH-independent equilibrium constants and rate

constants for formation of the bisulphite addition compound of isobutyraldehyde in water. *J. Org. Chem.*, **39**, 3896–901.

Ho, C. T. & Hartman, G. J. (1982). Formation of oxazolines and oxazoles in Strecker degradation of DL-alanine and L-cysteine with 2,3-butanedione. *J. Agric. Food Chem.*, **30**, 793–94.

Jencks, W. P. (1959). Studies on the mechanism of oxime and semicarbazone formation. *J. Amer. Chem. Soc.*, **81**, 475–81.

Jocelyn, P. C. (1972). *Biochemistry of the SH Group.* Academic Press, London. Pp. 63–93.

Kallen, R. G. (1971). The mechanism of reactions involving Schiff base intermediates. Thiazolidine formation from L-cysteine and formaldehyde. *J. Amer. Chem. Soc.*, **93**, 6236–48.

Kobayashi, N. & Fujimaki, M. (1965). On the formation of mercaptoacetaldehyde, hydrogen sulphide and acetaldehyde on boiling cysteine with carbonyl compounds. *Agric. Biol. Chem.*, **29**, 698–99.

Lienhard, G. E. & Jencks, W. P. (1966). Thiol addition to the carbonyl group. Equilibria and kinetics. *J. Amer. Chem. Soc.*, **88**, 3982–95.

Madson, M. A. & Feather, M. S. (1981). An improved preparation of 3-deoxy-D-*erythro*-hexosulose *via* the bis(benzoylhydrazone) and some related constitutional studies. *Carbohydr. Res.*, **94**, 183–91.

Marbrock, A. F., Jarboe, J. K. & O'Connor, E. M. (1969). Water-soluble flavour precursors of beef. *J. Agric. Food Chem.*, **17**, 5–14.

McWeeny, D. J., Knowles, M. E. & Hearne, J. F. (1974). The chemistry of non-enzymic browning in foods and its control by sulphites. *J. Sci. Food Agric.*, **25**, 735–46.

Prota, G. & Ponsiglione, E. (1973). On the reaction of ninhydrin with cysteine and its analogues: a revision. *Tetrahedron*, **29**, 4271–4.

Ratner, S. & Clarke, H. T. (1937). The action of formaldehyde upon cysteine. *J. Amer. Chem. Soc.*, **59**, 200–206.

Schubert, M. P. (1936). Compounds of thiol acids with aldehydes. *J. Biol. Chem.*, **114**, 341–50.

Song, P. & Chichester, C. O. (1967). Kinetic behaviour and mechanism of inhibition in the Maillard reaction. III. Kinetic behaviour of the inhibition in the reaction between D-glucose and glycine. *J. Food Sci.*, **32**, 98–106.

Takahashi, H., Miwa, K., Igarashi, T. & Kobayashi, Y. (1982). Conformational studies of mercaptoacetaldehyde and mercaptoacetone by ^{1}H-NMR, Raman and infrared spectroscopy. *Spectrochim. Acta, Ser. A*, **38**, 759–65.

Thiel, M., Asinger, F. & Schmiedel, K. (1958). Über die gemeinsame Einwirkung von elementarum Schwefel und gasförmigem Ammoniak auf Ketone VIII. Die kondensation von α-Mercaptoaldehyden mit Oxo-verbindungen und gasförmigem Ammoniak zu Thiazolinen. *Justus Liebigs Ann. Chem.*, **611**, 121–7.

Wander, J. D. & Horton, D. (1976). Dithioacetals of sugars. *Adv. Carb. Chem. Biochem.*, **32**, 15–123.

Weast, R. C. (1981). *Handbook of Chemistry and Physics*, 62nd edn. CRC Press, Inc., Boca Raton, p. D-139.

Wedzicha, B. L. (1984). *Chemistry of Sulphur Dioxide in Foods.* Elsevier Applied Science Publishers, London. Pp. 51–53.

Wedzicha, B. L. & Kaban, J. (1986). Kinetics of the reaction between 3-deoxyhexosulose and sulphur(IV) oxospecies in the presence of glycine. *Food Chem.*, **22**, 209–23.