ACKNOWLEDGMENTS

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The Reaction of β-Lactoglobulin Sulfenyl Iodide with Several Antithyroid Agents*

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Although β-lactoglobulin sulfenyl iodide is relatively stable toward reaction with cysteine or mercaptoethanol, it reacts very rapidly with other reagents such as thiourea, thiouracil, and related compounds which are known to interfere with the *in vivo* iodination of tyrosine in thyroglobulin. Similarly, I^- and SCN^-, which are known to exert characteristic effects upon thyroid metabolism, also react with the protein sulfenyl iodide, but these reactions are characterized by less specificity as evidenced by the approximate equilibrium constants. The reaction of thiouracil or thiourea with the sulfenyl iodide leads to the formation of the respective mixed disulfide with lactoglobulin The similarity of this product to the intermediate proposed by Maloof and Soodak (*Pharmacol. Rev. 15*, 43, 1963) in the thyroid-catalyzed desulfuration of thiourea lends support to the hypothesis that a sulfenyl iodide may function as a reactive intermediate in thyroidal-iodine metabolism.

The reactions of iodine with proteins may be grouped into essentially two classes; substitution reactions involving tyrosine and histidine residues in the peptide chain, and oxidative reactions involving cysteine and, to some extent, tryptophan (Putnam, 1953; Anson, 1941). The substitution reactions are favored in solutions of alkaline *p*H, but occur to a negligible extent at *p*H values below 7, particularly at temperatures near 0° (Putnam, 1953; Cunningham and Nuenke, 1959, 1960a). Under these conditions the predominant reaction of iodine with most proteins appears to involve oxidation of free-sulfhydryl groups of cysteine residues to the level of disulfide. However, in the case of several proteins, notably ovalbumin and β-lactoglobulin, we have been able to show (Cunningham and Nuenke, 1960a, 1961) that reaction with the sulfhydryl groups proceeds only to the level of sulfenyl iodide

$$P\text{—}SH + 2I^- \rightleftarrows P\text{—}SI + H^+ + I^- \quad (1)$$

These protein sulfenyl iodides may be quite stable depending upon the conditions of formation, but react readily with a variety of reagents including simple mercaptans to give the corresponding mixed disulfides.

$$P\text{—}SI + RSH \rightleftarrows P\text{—}SS\text{—}R + H^+ + I^- \quad (2)$$

In previous communications (Cunningham and Nuenke, 1959, 1960a,b) we have described the preparation of protein sulfenyl iodides and reported on some application of these reactions to the study of protein structure. Several aspects of the chemistry of the protein sulfenyl iodide group were suggestive of the hypothesis that it might play a role in iodide metabolism *in vivo*. Of greatest significance, perhaps, was the observation, which has been reported briefly previously (Cunningham and Nuenke, 1960b), that thiourea and thiouracil were the most reactive substances known to participate in reaction (2) with protein sulfenyl iodide.

$$P\text{—}SI + H_2N\text{—}\overset{\overset{S}{\|}}{C}\text{—}NH_2 \rightleftarrows P\text{—}S\text{—}S\text{—}\overset{\overset{NH}{\|}}{C}\text{—}NH_2 + H^+ + I^- \quad (3)$$

The report by Maloof and Soodak (1960, 1963) that a disulfide bond was formed between cellular protein and thiourea and thouracil in the course of metabolism of these substances by the thyroid further strengthened our interest in a possible physiological role for sulfenyl iodide. We have now undertaken a more comprehensive examination of the reactions of the sulfenyl iodide

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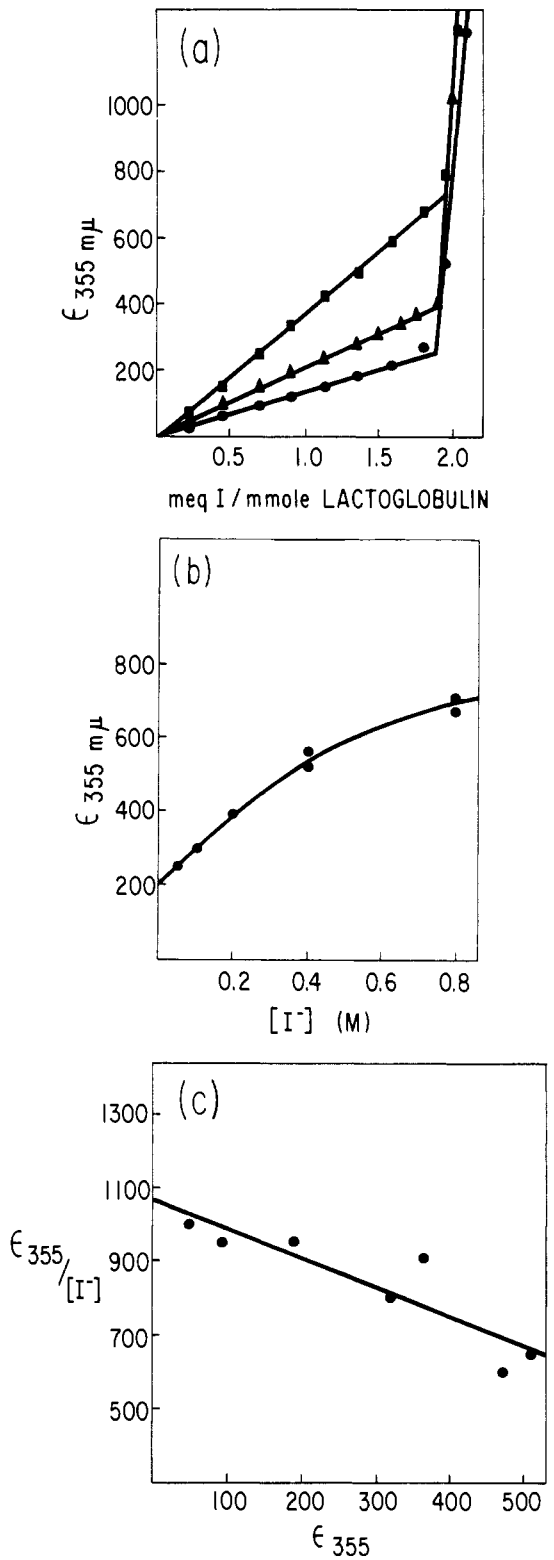


FIG. 1.—Titration, extinction-coefficient, and equilibrium-constant data for β-lactoglobulin. (a) Titration of β-lactoglobulin with I_3^- at *p*H 6.1 and 5° in the presence of different concentrations of I^-. (●), 0.05 M KI; (▲), 0.2 M KI; (■), 0.8 M KI. [β-Lactoglobulin] = 21.4 × 10^{-7} mole/3 ml. (b) Variation of the extinction coefficient of β-lactoglobulin sulfenyl iodide with the concentration of I^-. (c) Evaluation of the equilibrium constant for the binding of I^- by β-lactoglobulin sulfenyl iodide and of the extinction coefficient at infinite I^- concentrations.

derivative of β-lactoglobulin with a variety of reagents (including iodide, thiocyanate, thiouracil, and goitrin) which have been implicated in special roles in the biochemistry and pharmacology of the thyroid. The β-lactoglobulin sulfenyl iodide was chosen as the model for these studies since this protein contains only one sulfhydryl group per monomer unit (mw 18,500) (Townend and Timasheff, 1957). Results similar to those reported here have also been obtained with ovalbumin; but as the latter protein contains 3 sulfenyl iodide residues of differing reactivity per mole (mw 45,000), the interpretation of the results is less straightforward (Cunningham and Nuenke, 1959, 1960a, 1961).

EXPERIMENTAL PROCEDURES

Spectrophotometric Titrations.—The titration of β-lactoglobulin with I_3^- was followed by measuring the residual optical density at 355 mμ after the addition of increasing amounts of a standard solution of 0.2 N iodine in 0.05 M potassium iodide to a solution of β-lactoglobulin in 0.125 M sodium phosphate buffer, *p*H 6.1. Potassium iodide was also present in all titrations, but the concentration was varied (see Fig. 1). The end point was detected by the sharp increase in residual absorbance at 355 mμ which occurs when excess I_3^- remains in the solution. All solutions were kept at 5° throughout all experiments. A Beckman Model DU spectrophotometer with double thermospacers permitted adequate temperature control during these measurements. Similar titrations have been described in detail in earlier publications (Cunningham and Nuenke, 1959, 1961).

Spectrophotometric Reaction-Rate Studies.—Standard solutions of β-lactoglobulin sulfenyl iodide in 0.2 M potassium iodide–0.125 M sodium phosphate buffer, *p*H 6.1, were prepared at 5° by titration as described with the exception that the titration was halted when approximately 90–95% of the protein had been converted to sulfenyl iodide, so that no free I_3^- would be present to interfere in subsequent studies. The β-lactoglobulin sulfenyl solution (1.2 ml) was placed in a cuvet in the spectrophotometer and aliquots of 10–50 μl of the desired reagent were added. The cuvet was maintained at 5°, and the rate of loss of sulfenyl iodide was followed by determining the rate of disappearance of the absorbance at 355 mμ (Cunningham and Nuenke, 1959, 1960b).

Measurement of the Rate of Hydrogen-Ion Liberation.—The formation of β-lactoglobulin sulfenyl iodide and its subsequent reaction with different reagents was also followed with the aid of the *p*H-stat assembly of Radiometer, Inc., Copenhagen, as described in detail in a previous publication (Cunningham and Nuenke, 1961).

MATERIALS

β-Lactoglobulin was obtained from the Nutritional Biochemicals Corp. Protein concentrations were determined with the use of the relation: mg of protein ml^{-1} = 1.11 × OD at 280 mμ (Cunningham and Nuenke, 1959). Stock solutions of β-lactoglobulin were prepared in 0.05 M NaCl to facilitate solution. For calculation purposes, a monomer weight of 18,500 and the presence of one reactive sulfhydryl group per monomer were assumed for β-lactoglobulin. The concentrations of iodine solutions in KI were routinely determined (Cunningham and Nuenke, 1959) by dilution in 0.2 M KI, and measurement of the absorption at 355 mμ, with the aid of the relationship: meq I ml^{-1} = 7.77 × 10^{-5} × OD. All other compounds employed were commercial products of reagent grade or the equivalent.

[2-^{14}C]Thiouracil was obtained from the New England Nuclear Corp. Crystalline goitrin (5-vinyl-2-thiooxazolidone) was a gift from Dr. H. J. Eichel, Lloyd

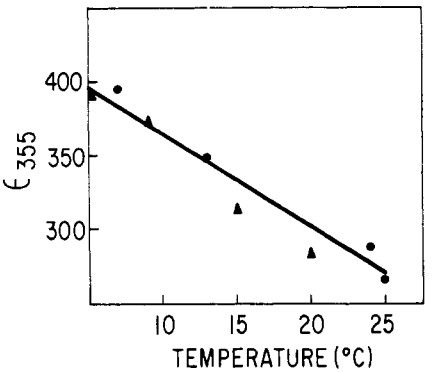


FIG. 2.—The variation of the extinction coefficient of β-lactoglobulin with temperature. (▲), temperature increasing from 6° to 25°; (●), temperature decreasing from 25° to 6°. [β-Lactoglobulin sulfenyl iodide] = 8.1×10^{-4} M, *p*H 6.1, 0.2 M KI.

Brothers, Inc., and a sample of U-9189 (1,1,3-tricyano-2-amino-1-propene) was generously provided by Dr. F. S. Eberts, Jr., of the Upjohn Co.

RESULTS

Effect of Iodide Concentration on the Titration of β-Lactoglobulin with I_3^-.—In previous studies, it was demonstrated that, at 5° in phosphate buffer near *p*H 6.1 and in the presence of 0.2 M KI, about 1.9 equivalents of iodine are consumed per mole of β-lactoglobulin before an excess of I_3^- is detected. The stoichiometry, as well as studies of hydrogen-ion liberation and mixed-disulfide formation (Cunningham and Nuenke, 1959, 1960a, 1961), afforded the conclusion that a stable β-lactoglobulin sulfenyl iodide is created by this reaction. In Figure 1a, the titration under these conditions is included with others identical except for variations in the concentration of KI present in the titration vessels. It may be seen that the stoichiometry of the reaction is not affected by a difference in KI concentration, but the magnitude of the residual absorbance at 355 mμ of the resulting sulfenyl iodide solution does change. Figure 1b summarizes this variation, calculated as the molar extinction coefficient, ϵ_{355}, of the β-lactoglobulin sulfenyl iodide (evaluated from the optical densities at the end points) with changes in the concentration of iodide ion. Despite difficulties in working with solutions of iodine at low iodide concentrations, it is possible to provide a tentative analysis of these data in terms of an equilibrium between excess iodide and the protein sulfenyl iodide (reaction 5). This in turn suggests that the following equations (reactions 4 and 5) may represent the formation of protein sulfenyl iodide in our system more adequately than does reaction (1).

$$P\text{—}SH + I_3^- \rightleftharpoons P\text{—}SI_2^- + H^+ + I^- \qquad (4)$$

$$P\text{—}SI_2^- \overset{K_d}{\rightleftharpoons} P\text{—}SI + I^- \qquad (5)$$

The extinction coefficient of the P—SI form was estimated from the intercept at zero iodide concentration of the curve in Figure 1b to be 206 ± 20. Subtraction of the contribution of this form from the optical density at the several different iodide concentrations permits the construction of a plot of ϵ_{355}/I^- versus ϵ_{355} (Fig. 1c) and thus allows the estimation of ϵ_{355} for the $P\text{—}SI_2^-$ form, 1560 ± 300, and of K_d, 0.78 ± 0.30 M.

An apparent alternative explanation for the increased optical density at 355 mμ near the end points of titration at increasingly higher concentrations of I^- (Fig. 1a), as the result of the presence of increased amounts of I_3^- resulting from the reversibility of reaction (4), is eliminated by the failure of the optical density at 355 mμ to be even partially discharged upon the subsequent addition of mercaptoethanol (see Fig. 3) at the very rapid rate characteristic of the reaction I_3^- with simple thiols. The equilibrium point of reaction (4) therefore appears to be far in the direction of the sulfenyl iodide. This is further substantiated by the sharp end points observed in titrations at all iodide concentrations (Fig. 1a).

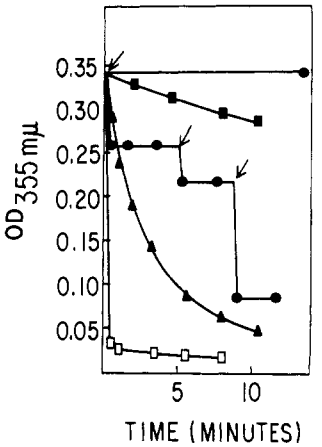


FIG. 3.—The disappearance of absorption due to β-lactoglobulin sulfenyl iodide (OD_{355}) as a result of its reaction with various compounds. (●), SCN^-, 49 × 10^{-4} M at first addition, 98 × 10^{-4} M total concentration after second addition, 588 × 10^{-4} M total concentration after third addition; (■), mercaptoethanol, 20.8 × 10^{-4} M; (▲), thiouracil, 10.4 × 10^{-4} M; (□), thiourea, 27.0 × 10^{-4} M, *p*H 6.1, 0.2 M KI, 5°.

Effect of Temperature on the Molar Extinction Coefficient of β-Lactoglobulin Sulfenyl Iodide.—A complex equilibrium involving the sulfenyl iodide function is also indicated by studies of its temperature stability and is reflected in reversible changes in optical density resulting from warming and recooling solutions of β-lactoglobulin sulfenyl iodide. This behavior is illustrated in Figure 2. The decrease in optical density upon warming is consistent with the dissociation of I^- postulated in reactions (4) and (5). It should also be noted that the sulfenyl iodide is remarkably stable even at room temperature. The optical density at 5° of samples which had been kept at room temperature, 22–25°, for 1 hour was within 5% of those of control samples which were maintained constantly at 5°.

Reaction of Thiocyanate with β-Lactoglobulin Sulfenyl Iodide.—Figure 3 illustrates the results obtained when several sequential additions of SCN^- are made to a solution of lactoglobulin sulfenyl iodide. The rate of disappearance of part of the sulfenyl iodide absorption may be seen to be essentially instantaneous. The reaction appears to be a freely reversible equilibrium, since subsequent changes in the concentration of either I^- or SCN^- produce increases and decreases, respectively, in the optical density at 355 mμ (Cunningham and Nuenke, 1960b). Hydrogen ions are neither evolved nor taken up during this reaction. We have interpreted these results as reflecting chiefly the equilibrium

$$P\text{—}SI + SCN^- \overset{K_b}{\rightleftharpoons} P\text{—}SSCN + I^- \qquad (6)$$

The present lack of values for the extinction coefficients of the molecular species $[PS(SCN)(I)]^-$ and P—SSCN leads to considerable uncertainty in the analysis of these equilibria. However, as reported previously, the equilibrium constant for reaction (6),

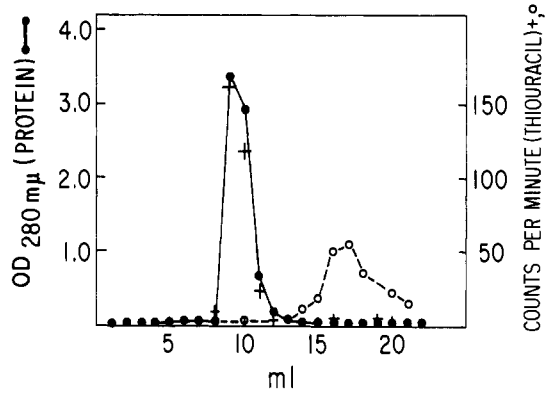


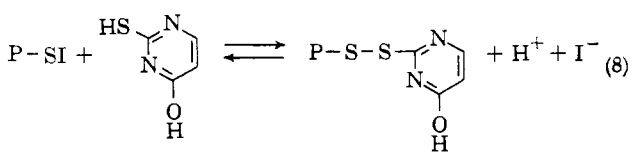
FIG. 4.—Fractionation of the reaction mixture on Sephadex G-25 following the addition of 5 μmoles of [2-^{14}C]-thiouracil to 8.1 μmoles of β-lactoglobulin sulfenyl iodide at *p*H 6.1, 0.2 M KI, at 5°. (●), absorption at 280 mμ; (+), [^{14}C]thiouracil after reaction with protein sulfenyl iodide; (○), [^{14}C]thiouracil in the absence of protein sulfenyl iodide.

K_b = ([P—SSCN][I^-])/([P—SI][SCN^-]), may be calculated approximately from data similar to that of Figure 3 to be 10.5 ± 2.0, since the points of equilibrium of reactions (4) and (5) are well in the direction of P—SI.

Reaction of Mercaptans and Related Compounds with β-Lactoglobulin Sulfenyl Iodide.—The reaction of simple mercaptans, and other compounds with the sulfenyl iodide, may also be followed by measuring the rate of disappearance of the optical density at 355 mμ resulting from the reaction

$$[P—SI_2]^- + R—SH \rightleftharpoons P—S—SR + H^+ + 2I^- \quad (7)$$

and reaction (2). Figure 3 also includes a comparison of the rate of reaction of mercaptoethanol with that of thiourea and thiouracil. It has previously been shown (Cunningham and Nuenke, 1959, 1961) that the reaction of mercaptoethanol with β-lactoglobulin sulfenyl iodide is accompanied by the liberation of 1 mole of hydrogen ion per mole of sulfenyl iodide, in conformity with equations (2) or (7). The disappearance of sulfenyl iodide color owing to reaction with thiourea or thiouracil, illustrated in Figure 3, is accompanied in each case by the liberation of a single mole of H^+ as illustrated in simplified form by equations (3) and (8).



This reaction has been substantiated by the use of [2-^{14}C]thiouracil as shown in Figure 4. A solution containing β-lactoglobulin sulfenyl iodide was prepared in the customary way and then 10 μl of a solution containing 5 μmoles of [2-^{14}C]thiouracil, specific activity 0.1 mc/mmole, was added. When reaction was complete as judged by the change in optical density at 355 mμ, an aliquot of the solution was subjected to gel filtration on Sephadex G-25. The same buffer used in the reaction was also used for chromatography. Since an excess of protein sulfenyl iodide was present, all the ^{14}C emerged with the protein peak after reaction with the [^{14}C]thiouracil. It may therefore be concluded that the stable disulfide shown in equation (8) has been formed. The positions of the protein and of free [^{14}C]-thiouracil were determined under identical conditions in separate experiments.

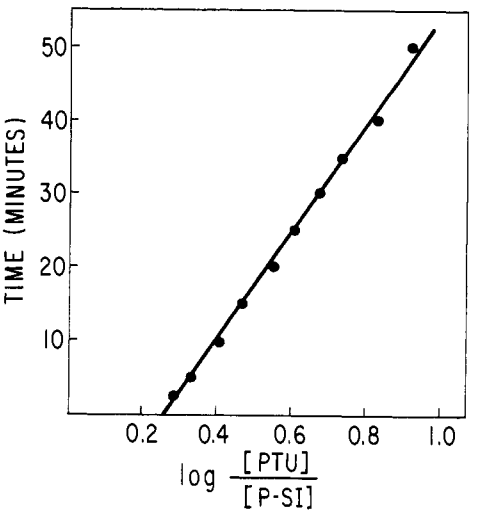


FIG. 5.—The reaction of β-lactoglobulin sulfenyl iodide with propylthiouracil. [β-Lactoglobulin sulfenyl iodide (P-SI)] = 6.1 × 10^{-4} M; [propylthiouracil (PTU)], 10 × 10^{-4} M; *p*H 6.1, 0.2 M KI, 5°.

TABLE I
A COMPARISON OF RATE CONSTANTS FOR THE REACTION OF SEVERAL REAGENTS WITH β-LACTOGLOBULIN SULFENYL IODIDE[a]

Compound Tested	Concentration Tested, M × 10^4	k_2 (liter mole^{-1} min^{-1})
1-Methyl-2-mercaptoimidazole	2.1; 10.4	>3000
Goitrin[b]	10.4	>1100
Thiourea	27.3	830 ± 110
Thiouracil	10.4; 24.3	270 ± 30
Propylthiouracil	10.4	78 ± 8
Mercaptoethanol	487; 208	8.0 ± 4
CN^-	433; 43.3	3.5 ± 1
SO_3^-	43.3; 533	3.7 ± 1
Cysteine	249	1.7 ± 1.2
Alanine	200	<0.1
H_2NOH	43.3	83 ± 20
Imidazole	1300	<0.1
U 9189[c]	10.4	<0.1

[a] The concentration of the sulfenyl iodide was between 6.1 and 8.7 × 10^{-4} M in all these experiments. The reaction was carried out at 5° in the *p*H 6.1 0.2 M KI buffer used for all routine titrations. [b] 5-Vinyl-2-thiooxazolidone. [c] 1,1,3-Tricyano-2-amino-1-propene.

Kinetics of the Reaction of the Sulfenyl Iodide.—From the stoichiometry of the equations which have been discussed, the rate of disappearance of sulfenyl iodide during reaction with mercaptoethanol or any of the other compounds might be expected to obey second-order kinetics.

$$-\left[\frac{d(P—SI)}{dt}\right] = k_2[P—SI][R—SH] \quad (9)$$

In Figure 5 the reaction of lactoglobulin with propylthiouracil is seen to be described rather well as a second-order reaction, with a rate constant, k_2, of 78 ± 2 liters mole^{-1} min.$^{-1}$ Most reactions, however, were not carried out to completion and rate constants were approximated from the initial slope for purposes of comparison. Table I lists the compounds we have tested, together with the second-order constants evaluated in this fashion. Constants given which exceed 500–1000

liters mole^{-1} min^{-1} represent minimal estimates because of technical limitations of our system. In such cases, complete reaction occurred within 0.5 to 1 minute under conditions where protein sulfenyl iodide and the compound being studied were present in essentially equimolar quantities at concentrations near 10^{-3} M.

Discussion

The specificity and stoichiometry of the reaction of iodine with the single sulfhydryl group of β-lactoglobulin (mw 18,500) has been described in previous communications and is further clarified by the results presented here. Thus it seems probable that two forms of the sulfenyl iodide product exist in equilibrium in solutions where iodide ion is present in excess. This equilibrium is reminiscent of the iodide-iodine-triiodide equilibrium, and implies that the reaction of iodine with the sulfhydryl group may be viewed as two related processes.

$$\begin{array}{ccc} P\text{—}SH + I_3^- & \rightleftharpoons & [P\text{—}SI_2]^- + I^- + H^+ \\ \downarrow\uparrow & & \uparrow\downarrow K_d \\ P\text{—}SH + I_2 + I^- & \rightleftharpoons & P\text{—}SI + I^- + H^+ \end{array} \qquad (10)$$

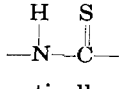
The quantitative effect of iodide concentration upon the optical density of solutions of the β-lactoglobulin sulfenyl iodide can be interpreted on the basis of this relationship, as has been described, so that molar extinction coefficients ϵ_{355} for the P—SI form and the P—SI_2^- form can be obtained. The equilibrium constant K_d for the dissociation of iodide from P—SI_2^- was determined to be about 0.8 M. Thus the great majority of the protein is in the P—SI form, even under our usual conditions where 0.2 M KI is present. In a typical physiological solution it is apparent that little, if any, P—SI_2^- would be present. It should be emphasized that while this interpretation is consistent with the observed equilibria, a direct confirmation of the structure of the P—SI_2^- form would clearly be desirable.

The reaction of the β-lactoglobulin sulfenyl iodide with simple mercaptans such as mercaptoethanol, cysteine, and cysteamine to yield the respective mixed disulfides of lactoglobulin has been described in previous publications. A related class of reactions appears to be those in which cyanide or sulfite react with the sulfenyl iodide, although these reactions have not yet been characterized in detail.

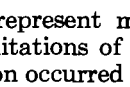
$$P\text{—}SI + CN^- \rightleftharpoons P\text{—}SCN + I^- \qquad (11)$$

The reaction of the sulfenyl iodide with thiocyanate ion (equation 6) appears to be in a class by itself. It is more rapid than any other that we have observed and its easily demonstrated reversibility is unique. Further examination will be necessary to elucidate fully the nature of this reaction. Nitrate ions were found to have no observable effect on the stability of the sulfenyl iodide.

Of the various reagents we have examined, those which react most rapidly with sulfenyl iodide at near equimolar concentrations are thiourea, thiouracil, goitrin, and several closely related compounds. All contain the structure



which is at least theoretically capable of existing in the alternative sulfhydryl form,

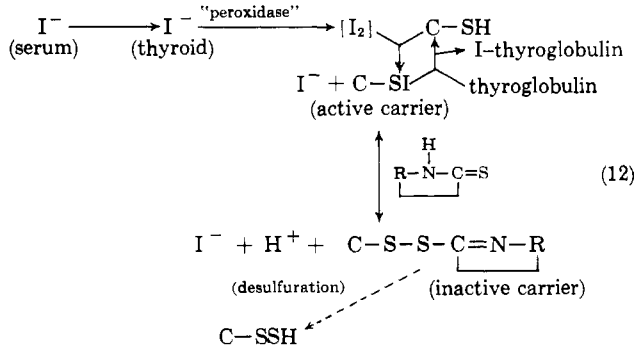


It is not known which form preferentially attacks the sulfenyl iodide, though, in view of the high pK_a values of the potential sulfhydryl groups of these compounds, pK_a >8, when compared to the pH at which these reactions are carried out, pH 6.1, it seems probable that the thione, not the thiol, is the reactive species. The overall reaction (reactions 3 and 8) is, however, apparently the same as that for simple mercaptans (reactions 2 and 7). In the case of thiouracil the existence of a stable protein-thiouracil compound, presumably the mixed disulfide, after reaction with the protein sulfenyl iodide, has been demonstrated by the stoichiometry with respect to sulfenyl iodide and hydrogen ion, and by chromatography on Sephadex G-25.

The fact that the compounds which were most reactive toward β-lactoglobulin sulfenyl iodide were all substances known to be potent inhibitors of tyrosine iodination in the thyroid first led us to suggest several years ago that a protein sulfenyl iodide intermediate might be formed in the course of iodine metabolism in the thyroid. Before and during the period of our studies, Maloof and Soodak (1957, 1960) and Maloof and Spector (1958) reported extremely interesting observations on the metabolism of thiourea and thiouracil by the thyroid, which greatly enhanced our interest in a possible metabolic role for sulfenyl iodide. They found that a sulfur-sulfur bond is formed between a protein of the cytoplasmic particulate fraction and thiourea in an early stage in the metabolism of this drug. The overall course of the reaction involves desulfuration of thiourea, leaving the sulfur bound to the protein, and the remainder of the molecule, as urea, free in the solution. This reaction specifically requires thiocyanate ion, but is inhibited by iodide, sulfite, cyanide, and various thiols. It is interesting to consider that all these substances react also with the β-lactoglobulin sulfenyl iodide (Table I) and that their activity in the thyroid-desulfurase system might be interpreted by suggesting that the desulfuration process begins with reaction of a sulfenyl iodide derivative with thiourea. The basis for the special character of thiocyanate both in activating the desulfuration reaction and in its interaction with β-lactoglobulin sulfenyl iodide remains obscure.

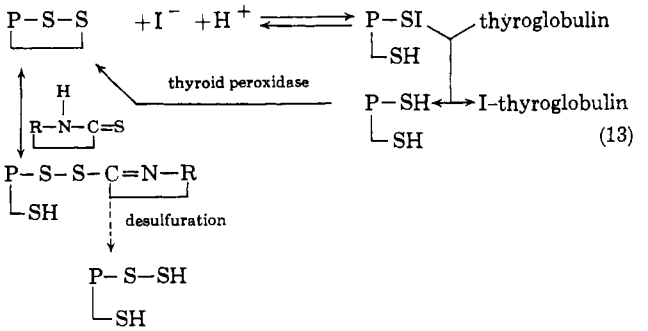
In recent years several reports have appeared describing preparations of particulate systems and enzymes from the thyroid which catalyze *in vitro* the incorporation of iodide into iodotyrosine (Tong *et al.*, 1957; Alexander, 1959; De Groot and Davis, 1962). It is apparently not clear whether iodination occurs as a result of the generation of iodine from iodide in a reaction catalyzed by a peroxidase, or by another route, but the available data indicate that the system catalyzing the synthesis of iodotyrosine is located in a microsomal-mitochondrial fraction very closely related to, if not identical with, that containing the thiourea-desulfurase system. Furthermore, many of the same compounds which react with β-lactoglobulin sulfenyl iodide and which inhibit the desulfurase system have also been reported to be inhibitors of the thyroid-iodination systems of Tong *et al.* (1957) and of De Groot and Davis (1962). It is important, also, to emphasize that the compounds which react most rapidly with β-lactoglobulin sulfenyl iodide, including thiocyanate ion, have as a common structural feature the sequence N—C—S and that the electronic configuration of this structure has long been associated with effectiveness in inhibiting iodination reactions in the thyroid (Astwood, 1943; Rimington, 1961). These results lend

support to the view that a single fundamental enzymatic property of thyroid tissue is being explored in all these studies. Many of these findings may be tentatively correlated by a sequence of reactions in which a central role in the mechanism of iodination reactions in the thyroid is assigned to a sulfenyl iodide derivative with chemical properties similar to those of the β-lactoglobulin sulfenyl iodide as well as additional hypothetical enzymatic properties.



In this scheme, I^- accumulated within the thyroid is oxidized to I_2 by the thyroid peroxidase or a similar enzyme (Alexander, 1959; De Groot and Davis, 1962; Tong *et al.*, 1957). The iodine, however, instantaneously combines with the reactive sulfhydryl group of the carrier, which may be a part of the peroxidase or a separate enzyme or coenzyme. The resulting sulfenyl iodide intermediate serves to stabilize I^+, lowering its reactivity toward the sulfhydryl groups of other enzymes of the thyroid and glutathione, thereby preventing the toxic effects which otherwise might be expected to result from the generation of free molecular iodine. It also serves to preserve I^+ from dissipation back to I^- by these same reactions. The finding that the sulfenyl iodide of β-lactoglobulin reacts with cysteine at less than 0.001 the rate of the reaction of free iodine with cysteine supports this view. Compounds such as thiouracil and thiourea, however, which have very high rates of reaction with sulfenyl iodide that approach those of the corresponding reactions with molecular iodine, would be expected to interfere with normal function of the C—SI carrier by reacting with it, as indicated, to liberate I^- and to form an inactive mixed disulfide. This mixed disulfide may then undergo the desulfuration sequence found by Maloof and Soodak (1957, 1960), or at very high iodide concentration it is probable that some reversal of inhibition would occur. An alternative scheme which has several attractive aspects has been briefly presented by Maloof and Soodak (1963). This sequence employs their finding that a reactive disulfide in the thyroid is a functional carrier in the desulfuration reaction (Maloof and Soodak, 1960, 1963), and therefore, presumably, in the iodination process as well.

In this sequence of reactions it was suggested that the thyroid peroxidase functions by oxidizing the sulfhydryl groups of a carrier protein to a reactive disulfide. Com-



bination of the reactive disulfide with iodide generates a sulfenyl iodide which functions here, as in reaction (12), as the thyroglobulin-iodinating agent. In contrast with reaction (12), however, the antithyroid action of thiourea and thiouracil was explained as a competition of these substances with iodide for the reactive disulfide.

Clearly these two mechanisms are not mutually exclusive. The common central feature is the function of a protein sulfenyl iodide as a key intermediate in thyroidal-iodine metabolism, while the major differences lie in the role of the thyroid peroxidase and in the nature of the intermediate which reacts with the antithyroid compounds. Further metabolic investigation of the thyroid should provide definitive information about which features of these proposals may be relevant to the problem of the physiological chemistry of iodine.

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