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The Oxidation of Reduced Nicotinamide Nucleotides by Hydrogen Peroxide in the Presence of Lactoperoxidase and Thiocyanate, Iodide or Bromide

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Lactoperoxidase (EC 1.11.1.7) catalysed the oxidation of NADH by hydrogen peroxide in the presence of either thiocyanate, iodide or bromide. In the presence of thiocyanate, net oxidation of thiocyanate occurred simultaneously with the oxidation of NADH, but in the presence of iodide or bromide, only the oxidation of NADH occurred to a significant extent. In the presence of thiocyanate or bromide, NADH was oxidized to NAD^+ but in the presence of iodide, an oxidation product with spectral and chemical properties distinct from NAD^+ was formed. Thiocyanate, iodide and bromide appeared to function in the oxidation of NADH by themselves being oxidized to products which in turn oxidized NADH, rather than by activating the enzyme. Iodine, which oxidized NADH non-enzymically, appeared to be an intermediate in the oxidation of NADH in the presence of iodide. NADPH was oxidized similarly under the same conditions. An assessment was made of the rates of these oxidation reactions, together with the rates of other lactoperoxidase-catalysed reactions, at physiological concentrations of thiocyanate, iodide and bromide. The results indicated that in milk and saliva the oxidation of thiocyanate to a bacterial inhibitor was likely to predominate over the oxidation of NADH.

It is well established that certain peroxidases (e.g. horseradish peroxidase and uterine peroxidase) can catalyse the oxidation of NADH by hydrogen peroxide provided that a suitable cofactor (e.g., certain phenols) is present (Akazawa & Conn, 1958; Hollander & Stephens, 1959). Oram & Reiter (1966*b*) have reported that LP* catalyses the oxidation of NADH by hydrogen peroxide in the presence of thiocyanate. This paper reports further studies on this reaction together with studies on analogous oxidation reactions involving iodide and bromide.

MATERIALS AND METHODS

Enzymes and chemicals. Lactoperoxidase and solutions of thiocyanate and hydrogen peroxide were prepared as described previously (Hogg & Jago, 1970). NADH, NAD^+, NADPH and NADase (NAD glycohydrolase, EC 3.2.2.5) from *Neurospora* and yeast alcohol dehydrogenase (EC 1.1.1.1) were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.

Bacteria. Streptococcus cremoris 972 was maintained and grown as described previously (Hogg & Jago, 1970).

Estimation of nicotinamide nucleotide oxidation. The oxidation of NADH and NADPH was followed by the decrease in E_{340} by using a Zeiss spectrophotometer, model PMQII. For kinetic studies the spectrophotometer was equipped with a thermostatically controlled cell holder and a Rikadenki recorder, model B14, to measure the initial rates of reaction.

Spectral determination. Spectra were obtained by using a Cary Recording Spectrophotometer, model 14.

Formation of NAD^+–cyanide complex. Reaction mixtures were prepared containing 0.133mM-KSCN, -KI or -KBr, 0.133mM-NADH, H_2O_2 (0.33mM for SCN^-, 0.133mM for I^-, 0.067mM for Br^-) and 0.23μM-LP in 8.3mM-sodium phosphate buffer, pH7.0. To 2.0ml of reaction mixture was added 2.0ml of M-KCN and the spectrum was recorded after the reaction.

NADase treatment of oxidation products. To 2.0ml of the above reaction mixtures was added 0.1ml of NADase solution (1.8mg of protein/ml). The solutions were incubated at 37°C for 30min, and the presence of NAD^+ was tested for by the addition of 2.0ml of M-KCN as described above.

Other methods. The amperometric method for studying inhibition of oxygen uptake by bacteria, the polarographic method for detecting the antibacterial oxidation product of SCN^- and the method for estimating cyanide, thiocyanate and ammonia after acidification of the reaction mixture have been described elsewhere (Hogg & Jago, 1970).

* Abbreviation: LP, lactoperoxidase (EC 1.11.1.7).

RESULTS

Anion specificity of the reaction. The oxidation of NADH by hydrogen peroxide in the presence of LP and thiocyanate was confirmed. Further, it was found that iodide or bromide, but not chloride, could replace thiocyanate in this reaction. In the absence of these anions, no oxidation of NADH was observed.

Stoicheiometry of NADH oxidation. The stoicheiometry of the reaction with respect to hydrogen peroxide is illustrated in Fig. 1. In the presence of thiocyanate, the addition of hydrogen peroxide in small amounts initially caused only a slight change in E_{340}. As more hydrogen peroxide was added, the extinction change became more marked and was proportional to the amount of hydrogen peroxide added until most of the NADH had been oxidized. The molar ratio, NADH oxidized/hydrogen peroxide added, was 0.19 at this point. In the presence of iodide or bromide, the E_{340} decreased sharply with the first addition of hydrogen peroxide and continued to fall steadily as further hydrogen peroxide was added. The ratio, NADH oxidized/hydrogen peroxide added, was 0.96 for iodide and 1.01 for bromide.

In the reaction involving thiocyanate, the stoicheiometry of the oxidation was dependent on the amount of thiocyanate present (Fig. 2). The amount of hydrogen peroxide required to produce the maximum decrease in E_{340} became less as the amount of thiocyanate was decreased, that is, the ratio, NADH oxidized/hydrogen peroxide added, increased with a decrease in the amount of thiocyanate. In the presence of iodide or bromide, however, this ratio was independent of the amount of iodide or bromide present. With all these anions, the stoicheiometry was independent of the LP concentration.

A study of the acid hydrolysis products during the oxidation of NADH in the presence of thiocyanate indicated that a reaction occurred that was basically similar to that found previously to occur in the absence of NADH (Hogg & Jago, 1970). A gradual utilization of thiocyanate was observed together with a gradual accumulation of ammonia and a peak of cyanide production at a hydrogen peroxide/thiocyanate ratio of 1. However, whereas the reaction in the absence of NADH reached completion at a hydrogen peroxide/thiocyanate ratio of 4, the reaction in the presence of NADH reached completion at a hydrogen peroxide/thiocyanate ratio of between 5 and 6.

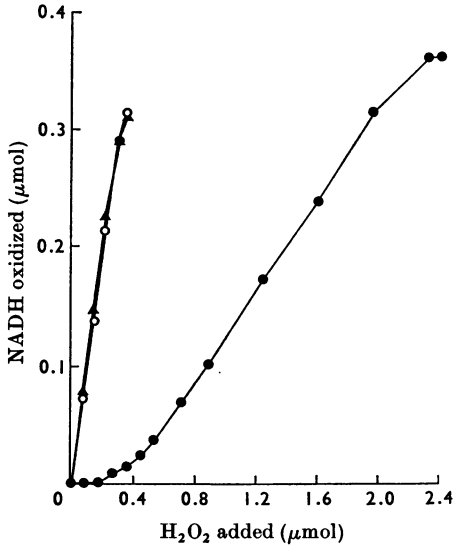

Fig. 1. Stoicheiometry of the oxidation of NADH by H_2O_2 in the presence of LP and thiocyanate, iodide or bromide. The reaction mixture (initial volume 3.0ml) contained NADH (0.4μmol), KSCN (●), KI (○) or KBr (▲) (0.4μmol) and LP (69nmol) in 25mM-sodium phosphate buffer, pH7.0, at room temperature. Small increments of 18mM-H_2O_2 were added and the E_{340} was measured after the reaction following each addition had gone to completion. E_{340} values were corrected for volume changes due to the addition of H_2O_2.

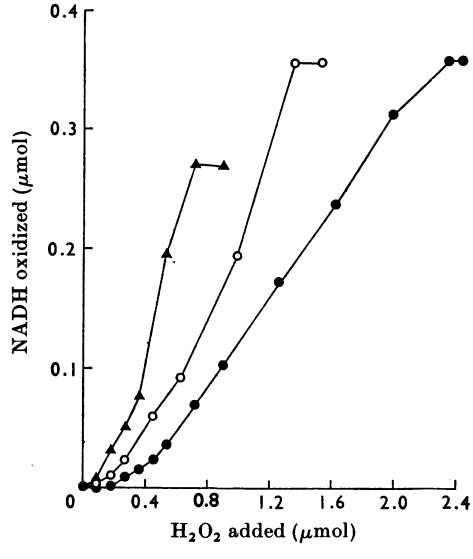

Fig. 2. Effect of SCN^- concentration on the stoicheiometry of the oxidation of NADH by H_2O_2 in the presence of LP and thiocyanate. The experimental details are given in Fig. 1 except that the amount of SCN^- was varied. ●, 0.4μmol of SCN^-; ○, 0.2μmol of SCN^-; ▲, 0.08μmol of SCN^-.

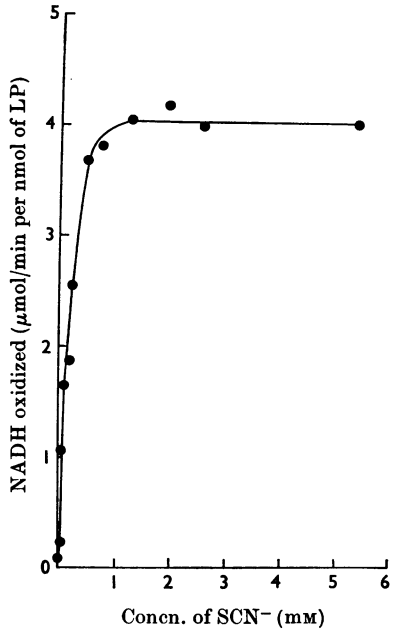

Fig. 3. Effect of thiocyanate concentration on the rate of oxidation of NADH by H_2O_2 in the presence of LP. The reaction mixture contained SCN^- as indicated, 0.60mM-H_2O_2, 0.13μM-LP and 0.133mM-NADH in 25mM-sodium phosphate buffer, pH7.0, at 30°C. The reaction was followed by the decrease in E_{340}.

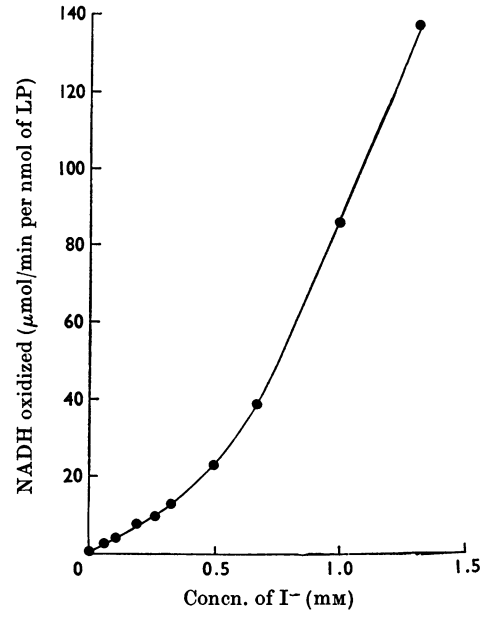

Fig. 4. Effect of iodide concentration on the rate of oxidation of NADH by H_2O_2 in the presence of LP. The reaction mixture contained I^- as indicated, 0.60mM-H_2O_2, 13.8nM-LP and 0.133mM-NADH in 25mM-sodium phosphate buffer, pH7.0, at 30°C. The reaction was followed by the decrease in E_{340}.

Kinetics of NADH oxidation. The reactions involving the oxidation of NADH were found to differ markedly in order with respect to thiocyanate, iodide or bromide. Thiocyanate displayed saturation kinetics, the oxidation approaching its maximum velocity at a thiocyanate concentration of 1mM (Fig. 3). With iodide or bromide, the reaction with respect to halide was of an order greater than 1 (Figs. 4 and 5). Iodide was much more reactive than bromide, and much higher concentrations of bromide than iodide were required to obtain the same rate of reaction.

All these reactions were first order with respect to LP. The rates of the reactions were independent of the concentration of NADH. The kinetics with respect to hydrogen peroxide were complicated by the marked inhibition of LP by high concentrations of hydrogen peroxide. In each case the maximum rate of reaction was observed at a hydrogen peroxide concentration of 0.1mM.

Formation of the antibacterial oxidation product of thiocyanate during the oxidation of NADH in the presence of thiocyanate. A solution containing NADH, thiocyanate and hydrogen peroxide in

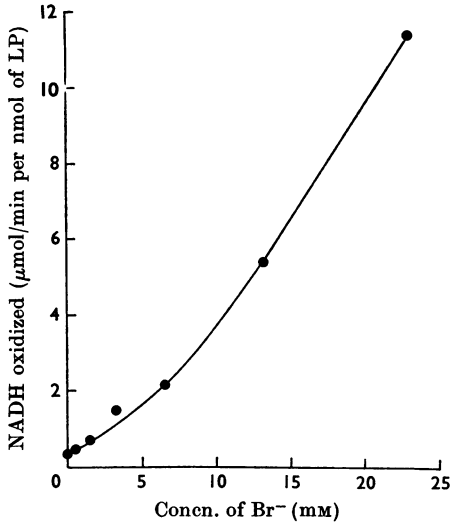

Fig. 5. Effect of bromide concentration on the rate of oxidation of NADH by H_2O_2 in the presence of LP. The reaction mixture contained Br^- as indicated, 0.60mM-H_2O_2, 0.138μM-LP and 0.133mM-NADH in 25mM-sodium phosphate buffer, pH7.0, at 30°C. The reaction was followed by the decrease in E_{340}.

equimolar amounts and LP inhibited oxygen uptake by *S. cremoris* 972, indicating that the inhibitor formed during the LP-catalysed oxidation of thiocyanate by hydrogen peroxide (Hogg & Jago, 1970) was formed also in the presence of NADH. This solution displayed the normal polarographic wave for the inhibitor (Hogg & Jago, 1970), confirming its existence in the system.

Properties of the oxidation products of NADH. (*a*) Ultraviolet spectra. The oxidation of NADH in the presence of thiocyanate or bromide was accompanied by a decrease in E_{340} and a slight increase in E_{260} (about 20%) as is normally observed during the oxidation of NADH to NAD^+. In the presence of iodide however, the increase in E_{260} was much greater (about 70%) and the wavelength maximum of the peak shifted to 267nm.

(*b*) Reaction with alcohol dehydrogenase. When the product arising from the oxidation of NADH in the presence of thiocyanate or bromide was treated with yeast alcohol dehydrogenase and ethanol, it was partially reduced again to NADH (Table 1). However, the product resulting from the oxidation of NADH in the presence of iodide showed no reaction. NAD^+ in the presence of iodide was readily reduced by this system, indicating that the absence of a reaction was not a result of inhibition of alcohol dehydrogenase by iodide.

(*c*) Spectra with cyanide. Nicotinamide derivatives containing a quaternary nitrogen atom and an intact amide group in the pyridine ring react with cyanide in alkaline solution to form a complex with a characteristic extinction peak around 325nm (Colowick, Kaplan & Ciotti, 1951). This reaction occurred with the NADH oxidation product formed in the presence of thiocyanate or bromide but not with the product from iodide oxidation.

(*d*) Treatment with NADase. When the products arising from the oxidation of NADH in the presence of thiocyanate or bromide were treated with NADase, then tested for complex-formation with cyanide, the spectra showed a decrease in extinction at 325nm compared with the controls. The spectrum of the product obtained when this experiment was performed with iodide, however, showed no change as a result of NADase treatment, indicating the absence of NAD^+.

Effect of oxidizing thiocyanate, iodide or bromide before addition of NADH. In the previous experiments, hydrogen peroxide was always the last component to be added to the reaction mixture, so that NADH was present throughout the oxidation. However, if NADH was not added to a mixture of LP, thiocyanate and hydrogen peroxide until several minutes after the addition of hydrogen peroxide, no oxidation of NADH occurred. As shown in Table 2, the amount of NADH oxidized decreased rapidly with increasing time-lag between the additions of hydrogen peroxide and NADH. The disappearance of NADH-oxidizing activity was less rapid at higher hydrogen peroxide concentrations, suggesting that the loss of activity was due to hydrogen peroxide depletion.

When iodide was oxidized by LP and hydrogen peroxide in the absence of NADH, the resulting solution still oxidized NADH rapidly, even after the LP had been removed by Millipore filtration. This solution was brown and showed the extinction maxima for tri-iodide at 288 and 350nm. A solution of iodine in potassium iodide prepared chemically at the same concentration oxidized NADH in the absence of LP and hydrogen peroxide, suggesting the involvement of iodine as an intermediate in the reaction. When a solution of bromide, hydrogen peroxide and LP was passed through a Millipore membrane filter (type VM) to remove the LP, the filtrate did not oxidize NADH at a significant rate. However, a solution of bromine at an equivalent concentration oxidized NADH in the absence of LP and hydrogen peroxide.

Oxidation of NADPH. The above reactions were not specific for NADH, as, under the same reaction conditions, NADPH was also oxidized by hydrogen peroxide in the presence of LP and thiocyanate or iodide to yield products which displayed respectively the spectrum for $NADP^+$ or an atypical spectrum similar to that observed on the corresponding oxidation of NADH with iodide.

Table 1. *Alcohol dehydrogenase-catalysed reaction between ethanol and the products arising from the oxidation of NADH by hydrogen peroxide in the presence of LP and thiocyanate, iodide or bromide*

The reaction mixtures in 3.0ml of 0.83mM-sodium phosphate, pH7.0, at 30°C contained the components indicated at the following concentrations: KSCN, 0.133mM; KBr, 0.133mM; KI, 0.133mM; NADH, 0.133mM; NAD^+, 0.133mM; LP, 0.23μM; H_2O_2 (added last), as indicated. A portion (1.9ml) of the reaction mixture was added to 1.0ml of 1.25M-ethanol in 0.25M-tris, pH10.1, and the E_{340} was recorded. Yeast alcohol dehydrogenase was added (0.1ml, final concentration 0.37mg of protein/ml) and the E_{340} at the end of the reaction was measured. ADH, yeast alcohol dehydrogenase.

Reaction mixture	E_{340} −ADH	E_{340} +ADH
NAD^+	0.072	0.655
NADH + SCN^- + H_2O_2(0.333mM) + LP	0.392	0.589
NADH + Br^- + H_2O_2(0.067mM) + LP	0.481	0.560
NADH + I^- + H_2O_2(0.133mM) + LP	0.115	0.126
NAD^+ + I^-	0.076	0.669
Control	0.054	0.056

Table 2. *Effect of the time-lag between the additions of hydrogen peroxide and NADH on the extent of NADH oxidation by hydrogen peroxide in the presence of LP and thiocyanate*

To 2.5ml of the reaction mixture containing 0.4μmol of KSCN and 0.138nmol of LP in 0.05M-sodium phosphate buffer, pH 7.0, was added 0.1ml of H_2O_2 at the concentrations indicated. After the time-interval indicated, 0.4μmol of NADH in 0.4ml of solution was added. At the completion of the reaction, the decrease in E_{340} was measured.

H_2O_2 concn. (mM)	Time between additions of H_2O_2 and NADH (s)	NADH oxidized (nmol)
0.27	0	41.7
0.27	10	15.3
0.27	20	2.4
0.27	120	0
0.60	0	90.0
0.60	10	86.4
0.60	75	30.6
0.60	90	11.4
0.60	120	0

DISCUSSION

Although the LP-catalysed oxidation of NADH by hydrogen peroxide can be brought about in the presence of either thiocyanate, iodide or bromide, the reaction shows marked differences according to which of these three anions is present.

The first difference is with respect to the stoicheiometry of the reaction. In the presence of iodide or bromide, approximately 1μmol of NADH was oxidized/μmol of hydrogen peroxide added. This would be expected for the net reaction:

$$NADH + H^+ + H_2O_2 \rightarrow NAD^+ + 2H_2O$$

As the stoicheiometric relationship between NADH and H_2O_2 was independent of the amount of iodide or bromide present, it would appear that no net oxidation of iodide or bromide was occurring.

In contrast, the oxidation of NADH in the presence of thiocyanate was accompanied by net oxidation of thiocyanate itself. During the initial stages of the addition of hydrogen peroxide, oxidation of thiocyanate to the bacterial inhibitor discussed in a previous paper (Hogg & Jago, 1970) occurred as indicated polarographically and by inhibition of oxygen uptake by bacterial cells, but there was very little oxidation of NADH. On the addition of further hydrogen peroxide, oxidation of both the inhibitor and NADH occurred concurrently. The stoicheiometry for the complete oxidation was in approximate agreement with the reactions:

$$SCN^- + 4H_2O_2 \rightarrow SO_4^{2-} + HOCN + 3H_2O + H^+$$
$$NADH + H^+ + H_2O_2 \rightarrow NAD^+ + 2H_2O$$

The second difference is with respect to the kinetics of the reaction. Oxidation of NADH in the presence of thiocyanate displayed saturation kinetics with respect to thiocyanate, but oxidation of NADH in the presence of iodide or bromide was of an order greater than 1 with respect to the halide. The reason for this difference is not apparent.

The third difference is with respect to the products of the reactions. In the presence of thiocyanate or bromide, the major product from oxidation of NADH by LP and hydrogen peroxide showed clearly the characteristics of NAD^+. In the presence of iodide, however, the product showed none of these characteristics.

It is unlikely that this product is an iodinated form of NAD as iodide was not required in stoicheiometric amounts. The formation of a complex of NAD^+ with cyanide requires both the quaternary pyridine nitrogen and an intact amide group in the pyridine ring (Colowick *et al.* 1951), and the failure to form such a complex suggests that at least one of these functional groups is absent. This could result from a cleavage reaction at one of these points which requires the presence of iodide but does not involve its net utilization. This reaction is specific for the reduced nicotinamide nucleotide as it did not occur with NAD^+ (D. McC. Hogg & G. R. Jago, unpublished work). Whatever reaction is involved must account for the observed change in the extinction maximum of the adenine ring at 260nm.

Possibly related to this reaction is the oxidation of NADH by myeloperoxidase and hydrogen peroxide in the presence of chloride. K. Agner unpublished work, cited by Paul, 1963) reported that, in this reaction also, NADH was converted not, into NAD^+, but irreversibly into several unidentified products.

Thiocyanate, iodide or bromide could function in NADH oxidation either by being in turn oxidized by hydrogen peroxide and then reduced by NADH, or by functioning as essential activators, enabling

LP to oxidize NADH directly. Evidence for the first of these mechanisms is indicated by the observation that iodide was oxidized to iodine (or tri-iodide) in the absence of NADH and that iodine oxidized NADH in the absence of LP and hydrogen peroxide. No such stable intermediate which would oxidize NADH non-enzymically was obtained with bromide or thiocyanate. While the oxidation of thiocyanate to the bacterial inhibitor occurred concurrently with NADH oxidation in the presence of thiocyanate, these two reactions need not be closely related. Further evidence that NADH did not react directly with LP and hydrogen peroxide was indicated by the observation that, in all cases, the rate of oxidation was independent of NADH concentration.

It has been suggested that this type of mechanism for the oxidation of NADH is important in tissues in which the mitochondrial oxidation of NADH is limited by the rate of hydrogen transfer across the mitochondrial membrane. de Duve & Baudhuin (1966) considered that such a mechanism was physiologically important in peroxisomes of liver, kidney and certain plant tissues, particularly in relation to gluconeogenesis. In these particles, catalase was postulated to function as a peroxidase utilizing hydrogen peroxide to oxidize various substrates. The products of such oxidations in turn oxidize NADH under the action of specific enzymes in the peroxisomes. However, no evidence has yet been obtained that LP functions in a similar manner *in vivo*. Nevertheless, there is an indication that the physiological importance of LP is associated with the metabolism of iodide or thiocyanate.

LP is found in the mammary, salivary, lacrimal and Harderian glands of mammals and in their respective secretions, milk, saliva and tears. It has been found also that mammary and salivary glands accumulate iodide (Fletcher, Honour & Rowlands, 1956; Freinkel & Ingbar, 1956; Logothetopoulos & Myant, 1956; Wolff & Maurey, 1961) and salivary glands accumulate thiocyanate (Logothetopoulos & Myant, 1956; Fletcher *et al.* 1956) and bromide (Ullberg *et al.* 1964). Apart from the oxidation of NADH, there are several other reactions catalysed by LP which utilize these anions. These are the oxidation of thiocyanate to a bacterial inhibitor (Oram & Reiter, 1966*a*,*b*; Hogg & Jago, 1970), the oxidation of iodide to iodine (or tri-iodide) (Alexander, 1962) and the iodination and bromination of tyrosine (Alexander, 1961; Klebanoff, Yip & Kessler, 1962). The oxidation of thiocyanate to the bacterial inhibitor has been suggested as a physiologically important function of LP in combatting bacterial growth in the region of glands producing LP (Klebanoff & Luebke, 1965; Morrison & Allen, 1966; Slowey, Eidelman & Klebanoff, 1968). The iodination of tyrosine in milk proteins occurs *in vivo* (Potter, Tong & Chaikoff, 1959), and has been shown to be catalysed by LP (McQuillan, Morton, Stanley & Trikojus, 1954). However, the physiological significance of this is not clear. A more likely role for the iodination of tyrosine by LP was suggested by Klebanoff (1967) who found that the iodination of bacterial proteins by LP or myeloperoxidase caused inhibition of bacterial growth and loss of viability. Bacterial inhibition by LP in the presence of iodide and hydrogen peroxide has been demonstrated also by Zeldow (1963). This mechanism of bacterial inhibition was clearly distinct from the one involving thiocyanate, as it was necessary to perform the oxidation of iodide by LP and hydrogen peroxide in the presence of the bacteria for inhibition to be obtained. In contrast, the oxidation of thiocyanate to the inhibitor can be performed before the addition of the bacteria, without affecting the inhibition (Hogg & Jago, 1970).

An indication of the relative physiological importance in milk and saliva of the nicotinamide nucleotide oxidations and other LP-catalysed reactions involving thiocyanate, iodide or bromide can be obtained by comparing the physiological concentrations of these anions with the reaction rates shown in Figs. 3, 4 and 5 and with the results obtained for the rates of oxidation of thiocyanate to the bacterial inhibitor and of the iodination of tyrosine (D. McC. Hogg & G. R. Jago, unpublished work). These are summarized in Table 3.

It is apparent that in milk and saliva the rate of oxidation of NADH in the presence of iodide or bromide would be insignificant compared with the rate of oxidation of NADH in the presence of thiocyanate. This is a consequence of the much higher concentration of thiocyanate, which is present at a value close to that required for the maximum rate of NADH oxidation. However, the rate of oxidation of thiocyanate to the bacterial inhibitor is even higher, suggesting that this reaction would be the one occurring to the greatest extent. This is confirmed by the observation that in the presence of LP, NADH and limiting amounts of hydrogen peroxide, thiocyanate is oxidized to the bacterial inhibitor with very little NADH oxidation occurring.

Because of the low concentration of iodide, the iodination of tyrosine in milk or saliva is unlikely to be significant. Klebanoff (1967) and Zeldow (1963) found that, in the presence of LP and hydrogen peroxide, iodide was inhibitory at concentrations as low as 50 and 14 μM respectively, but these concentrations are significantly higher than normal physiological values.

In milk and saliva, LP exists in a soluble form, but within the cells of salivary and mammary glands it is possible that the enzyme could be loosely

Table 3. *Rates of LP-catalysed reactions at physiological concentrations of thiocyanate, iodide and bromide*

The concentrations quoted for milk refer to cow's milk and those for saliva refer to human saliva. The rates of reaction for the oxidation of NADH were obtained from Figs. 3, 4 and 5 and for the other reactions from unpublished results of D. McC. Hogg & G. R. Jago.

	Milk		Saliva	
Reaction	Physiological concn. of SCN^-, I^- or Br^- (mM)	Rate of reaction*	Physiological concn. of SCN^-, I^- or Br^- (mM)	Rate of reaction*
Oxidation of NADH in the presence of:				
SCN^-	1.7×10^{-3}–3.6×10^{-1} [a]	0.02–3	1.7×10^{-1}–4.7 [d]	2–4
I^-	5×10^{-5}–1.3×10^{-3} [b]	0.002–0.06	2.8×10^{-4}–2×10^{-3} [e]	0.01–0.08
Br^-	2.0×10^{-3}–2.7×10^{-3} [c]	0.0004–0.0005	1.1×10^{-2}–8×10^{-2} [f]	0.002–0.016
Oxidation of SCN^- to the bacterial inhibitor	1.7×10^{-3}–3.6×10^{-1}	0.08–13	1.7×10^{-1}–4.7	7–40
Iodination of tyrosine	5×10^{-3}–1.3×10^{-3}	0.005–0.13	2.8×10^{-4}–2×10^{-3}	0.03–0.2

* μmol of substrate utilized/min per nmol of LP.

References: [a] Wokes, Wedgwood & Wyatt (1952); Boulange (1959); Virtanen & Gmelin (1960); Boulange, Han & Vert (1963); [b] Ling, Kon & Porter (1961); Stolc & Nemeth (1961); Wayne, Koutras & Alexander (1964); [c] Ling *et al.* (1961); [d] Zeldow (1963); Densen, Davidow, Bass & Jones (1967); [e] Burger, Hinton & Lough (1941); [f] Jenkins (1960).

bound to subcellular particles. This could influence its affinities for different substrates and the relative rates of the reactions it catalyses. Also, the concentrations of iodide, thiocyanate and bromide may differ within the cells from the concentrations found in their secretions. It is therefore conceivable that the LP-catalysed oxidation of NADH may be important within these glands.

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