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Modification of the glyoxalase system during the functional activation of human neutrophils

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The glyoxalase system catalyses the metabolism of methylglyoxal to D-lactic acid, via the intermediate *S*-D-lactoylglutathione. It is present in human neutrophils and undergoes a significant modification during functional activation – induction of chemotaxis, phagocytosis and degranulation. During the activation of neutrophils with serum-opsonised zymosan and the tumour-promoting phorbol diester 12-*O*-tetradecanoylphorbol 13-acetate, the activity of glyoxalase I increases and the activity of glyoxalase II decreases by 20–40% of their activities in resting cells, in the initial 10 min of the activation period. Determination of the Michaelis constant, K_m, and the apparent maximum velocity, V_{max}, for these enzymatic reactions indicates that the change in activity is due to a non-competitive activation and inhibition of glyoxalase I and glyoxalase II, respectively. This is consistent with a modification of the glyoxalase enzyme protein during the activation response. This modification occurs under aerobic and anaerobic incubation conditions. The concentration of *S*-D-lactoylglutathione increases approx. 100% of the resting cell concentration during the initial 10 min of the activation period. The presence of *S*-D-lactoylglutathione in neutrophils may be related to its ability to stimulate microtubule assembly.

Introduction

The glyoxalase system catalyses the metabolism of methylglyoxal to D-lactic acid, via the putative intermediate *S*-D-lactoylglutathione [1]. It comprises two enzymes and a catalytic amount of reduced glutathione. Glyoxalase I (EC 4.4.1.5.) catalyses the formation of *S*-D-lactoylglutathione from the non-enzymatically formed hemimercaptal adduct of methylglyoxal with reduced glutathione [2].

$$CH_3COCHO + GSH \rightleftharpoons CH_3COCH(OH)\text{–}SG$$

$$\xrightarrow{\text{glyoxalase I}} CH_3CH(OH)CO\text{–}SG$$

S-D-lactoylglutathione

Glyoxalase II (EC 3.1.2.6) catalyses the hydrolysis of *S*-D-lactoylglutathione to D-lactic acid and reduced glutathione [3].

$$CH_3CH(OH)CO\text{–}SG + H_2O$$

$$\xrightarrow{\text{glyoxalase II}} CH_3CH(OH)CO_2H + GSH;$$

D-lactic acid

The glyoxalase system occurs widespread throughout biological life [4].

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; DMSO, dimethylsulphoxide

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The glyoxalase system occurs widespread throughout biological life [4].

The apparent ubiquity of the glyoxalase system throughout biological life may suggest a fundamental role for this system in metabolism. In contrast, the major product of the glyoxalase system, D-lactic acid, is not utilised in mammalian metabolism and is excreted [5]. Gillespie [6] has demonstrated that the intermediate *S*-D-lactoylglutathione stimulates microtubule assembly and also stimulates the release of histamine from human leukocytes [7] – a secretory process known to be mediated by the assembly of cytoplasmic microtubules [8].

Given a constant supply of methylglyoxal, modification of glyoxalase activities would be expected to change the cellular concentrations of *S*-D-lactoylglutathione and thereby regulate microtubule assembly. One such system where this regulation of microtubule assembly is expected to be operative is the functional activation (chemotaxis, phagocytosis and degranulation) of neutrophils [9].

In this report, we describe the changes in the glyoxalase system in human neutrophils during functional activation with the tumour-promoting phorbol diester, 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and serum-opsonised zymosan. Glyoxalase I activity increases, glyoxalase II activity decreases and the concentration of *S*-D-lactoylglutathione increases early in the activation response. The glyoxalase system appears competent to receive and act on functional signals to stimulate microtubule assembly in human neutrophils.

Materials and Methods

Human neutrophils. Neutrophils, more than 95%, were prepared by dextran sedimentation and centrifugation on Ficoll-Hypaque gradients [10] using citrate anti-coagulated venous blood obtained from healthy adult donors. After hypoosmotic lysis of contaminating erythrocytes, the cells were suspended in incubation medium containing 110 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$ and 25 mM Hepes (pH 7.4), with 5 mM glucose.

Incubations. Freshly prepared human neutrophils (10^7 cells/ml) were incubated at 37 °C in a shaking water bath with and without stimulant: 0.3 and 3.0 mg/ml zymosan, opsonised with human serum [11]; 5, 10, 50 and 100 ng/ml TPA. Samples were withdrawn after 0, 2, 5 and 10 min and injected in 9 vol. of ice-cold incubation buffer. Cells were sedimented by centrifugation and treated as described below for assay of glyoxalase activities and glutathione metabolites.

Incubations under anaerobiosis were performed using stoppered tubes under a nitrogen atmosphere. Prior to the incubation, the buffer was degassed with nitrogen for 2 h. After addition of the cells, the suspension was incubated on ice for 30 min under a nitrogen gas flow to remove residual oxygen introduced by the addition of the neutrophils. Incubation mixtures were then warmed to 37 °C, and treated with stimulant as the incubation period was initiated.

Assay of glyoxalase activity. The cell pellet from each sample (10^7 cells) was suspended in cold sonication buffer (10 mM Hepes, pH 7.0, with 0.02% Triton X-100) and ruptured by sonication (100 W, 20 s). The membrane fragments were sedimented by centrifugation ($50\,000 \times g$ for 30 min). The supernatant was assayed for activities of glyoxalase I and glyoxalase II. The membrane pellet was re-suspended in buffer and assayed for NADPH oxidase activity – see below. The activity of glyoxalase I (*S*-lactoylglutathione methylglyoxal-lyase, (isomerising); EC 4.4.1.5) in the cell extract supernatant was assayed by measuring the initial rate of formation of *S*-D-lactoylglutathione from hemimercaptal, followed spectrophotometrically by the increase in absorbance at 240 nm, $\Delta\epsilon_{240} = 2.86\ mM^{-1} \cdot cm^{-1}$ at pH 6.6 [12]. The hemimercaptal was prepared by preincubating methylglyoxal and reduced glutathione in 100 mM sodium phosphate (pH 6.6 at 37 °C) for 30 min; the reference sample, omitting methylglyoxal, was similarly incubated. Nominal hemimercaptal concentrations were calculated using the equilbrium constant $K_d = 3.0$ mM [11] for the equilibrium:

$$MeCOCH(OH)\text{-}SG \rightleftharpoons MeCOCHO + GSH$$

The initial hemimercaptal concentrations were 1.33 mM and also 0.33, 0.19 and 0.13 mM where the Michaelis constant, K_m, and the apparent limiting velocity, V_{max}, were determined by the double-reciprocal method of Lineweaver and Burk.

After preparation of hemimercaptal and reference solutions, the assay was initiated by adding equal aliquots of cell extract to assay and reference mixtures and observing the rate of change in absorbance at 240 nm. The rates for the blanks were determined for each hemimercaptal concentration by the addition of sonication buffer only. These blank rates were substracted from all assays with the corresponding equivalent substrate concentration. Glyoxalase I activities are given in units per 10^6 cells (and/or per mg protein) where one unit of glyoxalase I activity is the amount of enzyme required to catalyse the formation of 1 μmol *S*-D-lactoylglutathione per min under assay conditions. The percentage change ($\Delta\%$) in glyoxalase I activity (a_{GI}) is calculated from the equation

$$\Delta\%_{GI} = \frac{a_{GI(activated)} - a_{GI(resting)}}{a_{GI(resting)}} \times 100$$

The activity of glyoxalase II (*S*-hydroxyacyl glutathione hydrolase (EC 3.1.2.6) in the cell extract was assayed by measuring the initial rate of decrease of *S*-D-lactoylglutathione concentration, followed spectrophotometrically by the decrease in absorbance at 240 nm. $\Delta\epsilon_{240} = 3.10\ mM^{-1} \cdot cm^{-1}$ at pH 7.4 [12] in 50 mM Tris-HCl and 37°C. The initial substrate concentrations were 0.300 mM, and also 0.050, 0.033 and 0.025 mM where K_m and V_{max} were determined. Blank rates of hydrolysis of *S*-D-lactoylglutathione, using sonication buffer instead of cell extract, were determined and subtracted from the respective assay measurement. Glyoxalase II activity is given in units per 10^6 cells where 1 unit is that amount of glyoxalase II required to catalyse the hydrolysis of one μmol of *S*-D-lactoylglutathione per min, under assay conditions. The percentage change ($\Delta\%$) is calculated from the following equation

$$\Delta\%_{GII} = \frac{a_{GII(activated)} - a_{GII(resting)}}{a_{GII(resting)}} \times 100$$

Assay of NADPH oxidase activity. The activity of neutrophilic NADPH oxidase was assayed by a modification of the method of Bellavite et al. [13]. The membrane pellet from the preparation of the cell extract for glyoxalase activity measurement was resuspended in 50 mM Hepes (pH 7.0) with 1 mM diethylenetriaminepentaacetic acid, 2 mM NaN_3, 1 mM $MgSO_4$, 0.15 mM NADPH, 0.05% sodium deoxycholate and 80 μM ferricytochrome *c*. NADPH oxidase activity was assayed by measuring the initial rate of superoxide formation from the membrane homogenate in the presence of NADPH and ferricytochrome *c*. Superoxide formation was determined by measuring superoxide dismutase-inhibitable reduction of ferricytochrome *c* [14], followed spectrophotometrically at 550 nm, $\Delta\epsilon_{550} = 21\ mM^{-1} \cdot cm^{-1}$ [15].

Assay of S-D-lactoylglutathione and reduced glutathione. Incubation samples (10^7 cells), quenched in 9 vol. ice-cold incubation buffer, were immediately centrifuged ($2000 \times g$, 10 min) and the resultant pellet was treated with 0.25 ml ice-cold 10% trichloroacetic acid in 0.02% hydrochloric acid and mixed thoroughly. 0.25 ml 0.25% Triton X-100 was added to the cell extract. The extract was mixed again, then held on ice for 20 min. The extract was then centrifuged ($2000 \times g$, 10 min). The supernatant was removed and the pellet extracted with 1 more vol. (0.25 ml) of trichloroacetic acid. The combined supernatants wer extracted with 5×1 vol. of water-saturated diethyl ether. The resultant aqueous layer was assayed for reduced glutathione and *S*-D-lactoylglutathione by a modification of the method for reduced glutathione described in Ref. 16. Reduced glutathione is assayed spectrophotometrically at 340 nm as the thioether *S*-2,4-dinitrophenylglutathione, formed with 1-chloro-2,4-dinitrobenzene in a reaction catalysed by glutathione *S*-transferase (EC 2.5.1.18). When there is no further change in absorbance, glyoxalase II is added and *S*-D-lactoylglutathione may be determined from the increase in absorbance at 240 nm due to conjugation of reduced glutathione released by the enzymatic reaction of glyoxalase II. Assays were performed in 50 mM Tris-HCl buffer (pH 7.4), incubating samples for 1 h initially with 10 U/ml glutathione *S*-transferase, then for 1 h with 1.0 U/ml glyoxalase II. The increase in absorbance at 340 nm was recorded for each stage. The assay was calibrated and controlled by extracting and assaying solutions of known concentration of *S*-D-lactoylglutathione and reduced glutathione and blanks (incubation buffer), respectively.

Materials. *S*-D-Lactoylglutathione was prepared and purified by the method of Uotila [17]. The purity was more than 99% and samples were devoid of reduced and oxidised glutathione.

Glutathione *S*-transferase from equine liver, was supplied by Sigma Chemical Co. Ltd., Poole, Dorset, U.K. This enzyme has an activity of 65 U/mg protein where 1 unit is defined as the amount of enzyme required to catalyse the conjugation of 1 μmol of 1-chloro-2,4-dinitrobenzene per min at pH 6.5 and 25° C. Glyoxalase II, from bovine liver, was purchased from Sigma and had an activity of 10 units per mg protein.

The phorbol diester, 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and zymosan A were purchased from Sigma. TPA was dissolved in DMSO; less than 0.1% (v/v) DMSO was added to the incubation medium in activation experiments.

Results

The glyoxalase system in resting human neutrophils

In the cytosolic fraction of human neutrophils there is enzymatic activity of glyoxalase I and glyoxalase II. Glyoxalase activities in the neutrophil cytosol extracts from six healthy human donors were examined. The mean glyoxalase activities ± standard error of the mean were: glyoxalase I, 9.11 ± 0.77 mU/10^6 cells or 10.26 ± 0.57 mU/mg protein; glyoxalase II, 0.94 ± 0.20 mU/10^6 cells or 1.06 ± 0.13 mU/mg protein. The data suggest that the activity of glyoxalase I is approx. 10-times that of glyoxalase II under our assay conditions.

The Michaelis constants, K_m, for the glyoxalase enzymes with the physiological substrates, hemimercaptal and *S*-D-lactoylglutathione, were determined from double reciprocal plots–typical data are presented in Fig. 1. The mean values for K_m are: glyoxalase I, 378 ± 8 μM; glyoxalase II, 80 ± 8 μM. The data show that glyoxalase II has a much higher affinity for *S*-D-lactoylglutathione than does glyoxalase I for hemimercaptal.

The concentration of reduced glutathione in human neutrophils has been determined [18,19,20]: Estimates are in the range 0.6–1.5 nmol/10^6 cells. Measurements made during this study gave a mean neutrophilic reduced glutathione concentration of 0.91 ($n = 5$, S.E. = 0.03) nmol/10^6 cells. This is

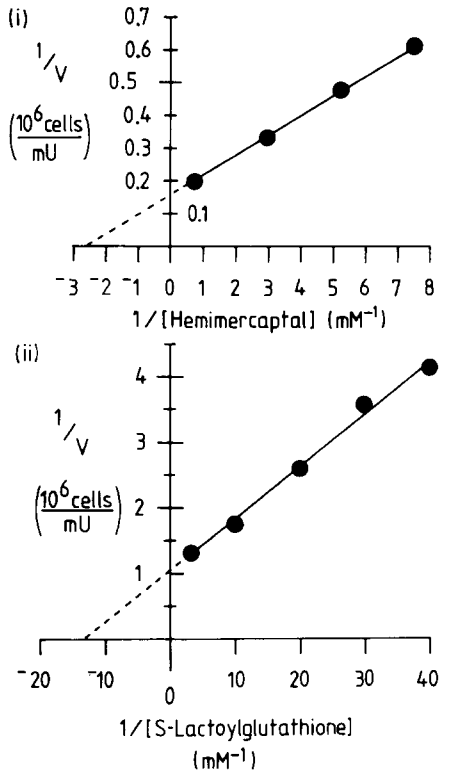

Fig. 1. Lineweaver-Burk analysis of substrate concentration-reaction velocity data for (i) glyoxalase I (ii) glyoxalase II in resting human neutrophils. Data are the means of three determinations using the homogenate from freshly isolated human neutrophils. Errors (S.E.) lie within the data point symbols given.

easily accomodated in the reported range of estimates of reduced glutathione concentration in the neutrophil. This is equivalent to a concentration of approx. 3.37 mM reduced glutathione in the neutrophil cytosol – assuming that the mean volume of a human neutrophil is 0.27 pl [21].

The mean concentration of *S*-D-lactoylglutathione in the resting neutrophil was found to be 0.26 nmol/10^6 cells (S.E. = 0.03, $n = 6$), equivalent to a cytosol concentration of 0.96 mM in the resting human neutrophil. In resting neutrophils, the concentration of *S*-D-lactoylglutathione is, therefore, approx. 30% of the reduced glutathione concentration.

The glyoxalase system in functionally activated human neutrophils

Human neutrophils may be functionally acti-

vated by serum-opsonised zymosan and by the tumour-promoting phorbol diester, 12-*O*-tetradecanoylphorbol 13-acetate. When neutrophils (10^7 cells/ml) are incubated with opsonised zymosan (3 mg/ml) and TPA (100 ng/ml) the activity of glyoxalase I increases and the activity of glyoxalase II decreases from the resting cell activities (see Fig. 2). The changes in glyoxalase activities occur rapidly upon treatment with the stimulant. With zymosan, the neutrophil glyoxalase activities progressively diverge from their resting cell activities throughout the initial 10-min period of observation. In contrast to this, with TPA there is a maximal inhibition of glyoxalase II activity after 5 min. After 10 min, the glyoxalase II activity is at the same level as unstimulated cells.

The relationship of the change in glyoxalase activities in activated neutrophils to the kinetic properties of the enzymes, K_m and V_{max}, was investigated by constructing double reciprocal substrate-reaction velocity plots for glyoxalase I and glyoxalase II in resting and activated cells. Data presented are for the glyoxalase activities for resting cells, for zymosan treated cells at $t = 10$ min and for TPA-treated cells at $t = 5$ min, to illustrate the Lineweaver-burk analysis at the point of maximum effect (see Figs. 3 (glyoxalase I) and 4 (glyoxalase II)). Inspection of the linear regression analysis indicates that the K_m of both enzymes is constant during the activation period; the change in enzyme activity is due to a change in V_{max}. This is consistent with a non-competitive activation of glyoxalase I (or removal of non-competitive inhibition) and non-competitive inhibition of glyoxalase II.

The effect of oxygen, and oxygen metabolites of the respiratory bursts, on neutrophilic glyoxalase activities was investigated by performing activation experiments in anaerobic buffer. The changes in the activities of neutrophilic glyoxalases after incubation for 5 min with 3 mg/ml zymosan and 100 ng/ml TPA, in anaerobiosis and aerobiosis, are given in Table I. The changes in the glyoxalase activities during functional activation with and without oxygen are approximately the same. This demonstrates that the modification of

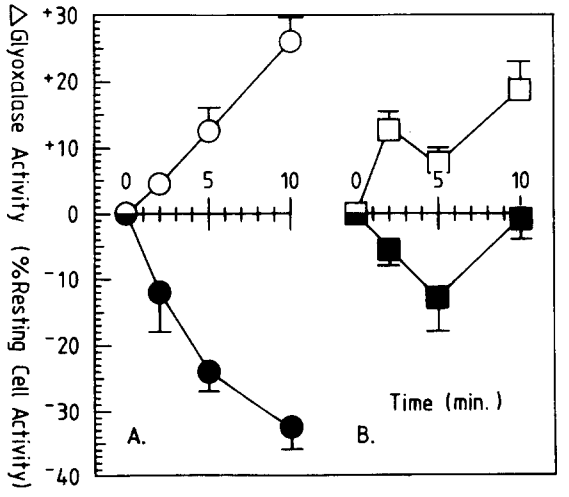

Fig. 2. Changes in the activities of neutrophilic glyoxalase I and glyoxalase II induced by incubation with opsonised zymosan and TPA. Incubation contained 10^7 neutrophils/ml with (A) 3.0 mg/ml opsonised zymosan and (B) 100 ng/ml TPA in incubation buffer (pH 7.4 and 37° C). ○, □, glyoxalase I; ●, ■, glyoxalase II. Data are means ± S.E. of four experiments.

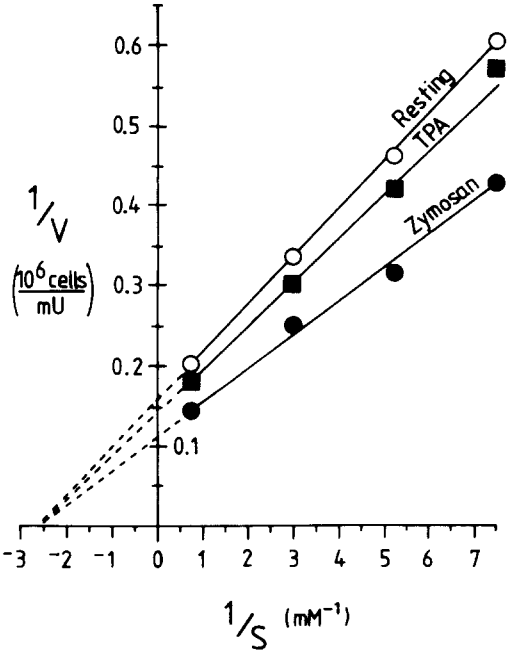

Fig. 3. Lineweaver-Burk analysis for the hemimercaptal concentration dependence of the velocity of glyoxalase I-catalysed reaction in resting and functionally activated human neutrophils. ○, resting cell data; ●, data from zymosan-treated neutrophils after 10 min incubation; ■, data from TPA-treated neutrophils after 10 min incubation. Data are the mean ± S.E. of three determinations.

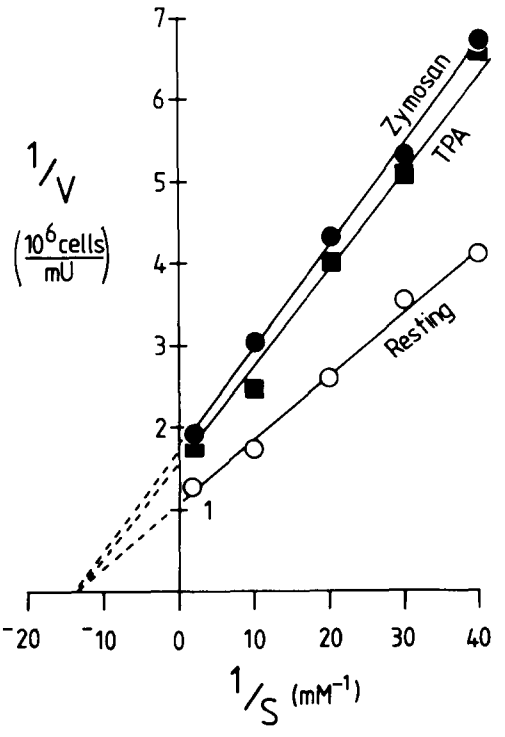

Fig. 4. Lineweaver-Burk analysis for the *S*-D-lactoylglutathione concentration dependence of the velocity of the glyoxalase II-catalysed reaction in resting and functionally activated human neutrophils. ○, resting cell data; ●, data from zymosan-treated neutrophils after 10 min incubation; ■, data from TPA-treated neutrophils after 5 min incubation. Data are means ± S.E. of three determinations.

TABLE I

CHANGES IN NEUTROPHIL GLYOXALASE ACTIVITIES: LACK OF DEPENDENCE ON OXYGEN

Neutrophils (10^7/ml) were incubated for 5 min with and without stimulant at 37 °C, under aerobic (normal) conditions and in anaerobiosis (nitrogen degassing and under nitrogen atmosphere). The neutrophils were isolated and sonicated after incubation and analysed for glyoxalase I and glyoxalase II activities. Data are means ± S.E. of four estimations.

Stimulant		Change in glyoxalase activity (% resting cell activity)	
		anaerobiosis	aerobiosis
+ Opsonised zymosan (3.0 mg/ml)	glyoxalase I	+16±1	+12±2
	glyoxalase II	−34±4	−31±4
+ TPA (100 ng/ml)	glyoxalase I	+9±2	+7±4
	glyoxalase II	−28±1	−38±3

glyoxalase activities is independent of oxygen metabolites (H_2O_2 and O_2^-).

The observed changes in glyoxalase activities in neutrophil activation are dependent on the presence of the stimulant. The effect of stimulant concentration on this response was studied by measuring the glyoxalase II activity after a fixed time period (5 min) with different concentrations of TPA and opsonised zymosan (see Fig. 5). The response appears to increase with stimulant concentration to a maximum, limiting response. For a cell concentration of 10^7 cells/ml, after 5 min incubation there is still a considerable change in glyoxalase activity (more than 25% resting cell activities) with stimulant concentrations of 0.3 mg/ml zymosan and 10 ng/ml TPA.

When human neutrophils were treated with opsonised zymosan and TPA, concurrent with the observed changes in the activities of the glyoxalases, there is an activation of the superoxide-generating NADPH oxidase and a marked decrease in the cellular concentration of reduced glutathione (see Figs. 6 and 7).

The activation of the NADPH oxidase (Fig. 6) shows the characteristic rapid activation of the enzyme with TPA and the slower activation with zymosan – reflecting the phagocytic (zymosan) and non-phagocytic (TPA) characteristics of the activation processes.

The concentration of reduced glutathione in zymosan-treated and TPA-treated cells falls rapidly in the initial 10 min of the activation

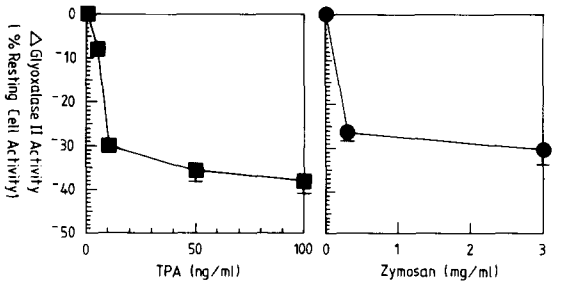

Fig. 5. The effect of a stimulant concentration on the activity of neutrophilic glyoxalases. Incubations contained: 10^7 neutrophils/ml with 100, 50, 10 and 5 ng/ml TPA and 3.0 and 0.3 mg/ml opsonised zymosan. Data are the means ± S.E. of four experiments taking the decrease in the activity of glyoxalase II after 5 min incubation time as being indicative of the magnitude of the response.

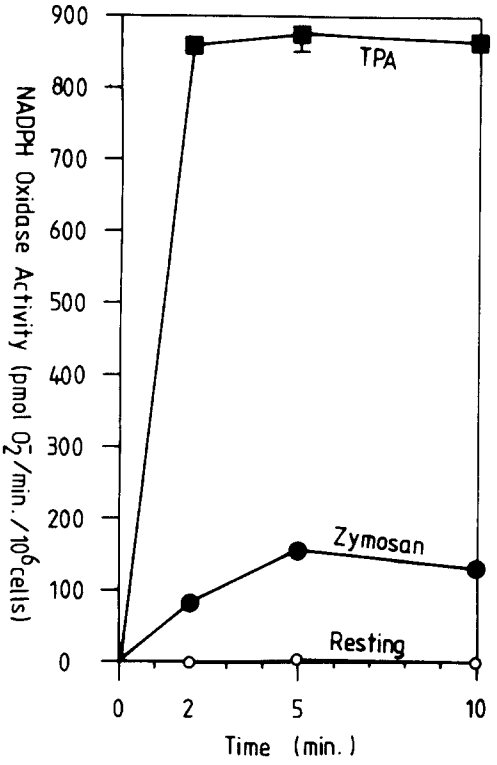

Fig. 6. The activation of the neutrophilic NADPH oxidase by zymosan and TPA. Incubations contained: 10^7 neutrophils/ml incubation buffer at pH 7.4 and 37 °C with: resting, (○) no further additions; zymosan, (●) +3.0 mg/ml opsonised zymosan; and TPA, (■) +100 ng/ml TPA. Data are means ± S.E. of four determinations.

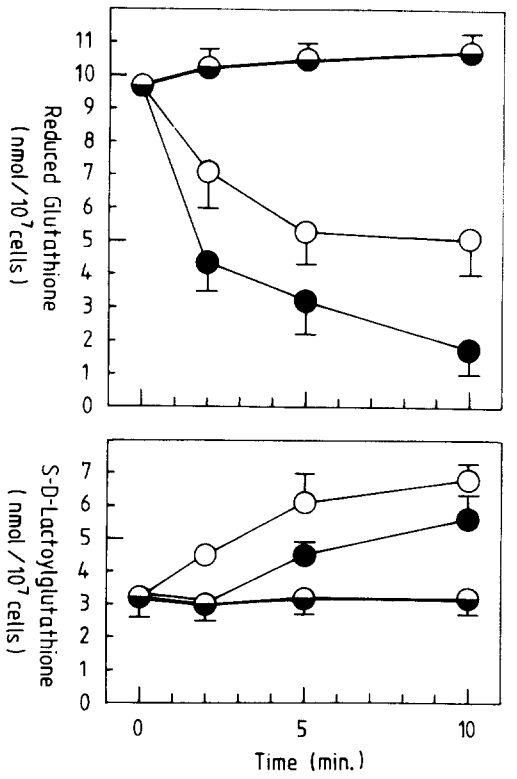

Fig. 7. Changes in neutrophilic reduced glutathione and *S*-D-lactoylglutathione during functional activation. Incubations contained 10^7 neutrophils/ml incubation buffer at pH 7.4 and 37 °C with: control (◒) no further additions; (○) +3.0 mg/ml opsonised zymosan; and (●) +100 ng/ml TPA. Incubations were sampled at the times indicated and assayed for reduced glutathione and *S*-D-lactoylglutathione content. Data are means ± S.E. from four experiments.

period (Fig. 8). In contrast, the concentration of *S*-D-lactoylglutathione in these cells rises. In TPA-activated cells, the increase in the concentration of *S*-D-lactoylglutathione occurs later than in the zymosan-activated cells.

Discussion

The neutrophilic glyoxalase system and microtubule assembly: resting cells

The activity of glyoxalase I and glyoxalase II in human neutrophils was determined in six independent experiments. The rate-limiting step of the neutrophils glyoxalase system can be determined by comparing the activities of the glyoxalase enzymes at physiological concentrations of a glyoxalase substrate. The mean resting cell concentration of *S*-D-lactoylglutathione was 0.96 mM. The double-reciprocal plot for glyoxalase II (Fig. 1, ii), this corresponds to a reaction velocity of 0.73 mU/10^6 cells. For a hemimercaptal concentration of 0.96 mM, reading from the double-reciprocal plot for glyoxalase I (Fig. 1, i), the corresponding reaction velocity is 4.5 mU/10^6 cells. The activity of glyoxalase II is approx. 16% of the activity of glyoxalase I at a normal substrate concentration of glyoxalase II. The glyoxalase II-catalysed reaction is clearly the rate-limiting step and glyoxalase II is the pacemaker enzyme of the neutrophilic glyoxalase pathway.

There is one previous report of the activity of the glyoxalase system in human neutrophils [22]. The 'glyoxalase activity' of human neutrophils determined by McKinney was calculated from

rates of lactic acid formation with addition of methylglyoxal and is similar to the glyoxalase II activity reported here. (Mean 'glyoxalase' activity was 0.82 mU/10^6 cells, determined over a 5 h period with an initial methylglyoxal concentration of 5.6 mM with no exogenous reduced glutathione added, and with a red blood cell contamination of 20%).

The concentration of reduced glutathione in resting neutrophils reported here corroborates well with previous reports [18,19,20]. An interesting feature of the resting cell glyoxalase system is the concentration of *S*-D-lactoylglutathione, in concentration terms, the second most important glutathione metabolite in the resting human neutrophil (cf. Ref. 19).

The neutrophilic glyoxalase system: functionally activated cells

In both the zymosan and TPA-stimulated response, the activity of glyoxalase I is increased and the activity of glyoxalase II is decreased by non-competitive modifications of the enzymes. These modifications are independent of oxygen. Consequently, the cytosolic concentration of *S*-D-lactoylglutathione increases by approx. 100% of resting cell levels. The cytosolic concentration of reduced glutathione falls: a major component of this decrease with zymosan activation, and a minor component with TPA activation, represents the demand for reduced glutathione in *S*-D-lactoylglutathione synthesis.

Since the glyoxalase II-catalysed reaction is the rate-limiting step in resting cells, it is not immediately clear why the activity of glyoxalase I is also modified to produce an increase in cellular *S*-D-lactoylglutathione concentration. In resting cells, the activity of glyoxalase II is approx. 16% of the activity of glyoxalase I. However, in activated neutrophils the concentration of reduced glutathione may fall by 50–90% of the resting cell concentrations. This would effectively decrease the rate of the glyoxalase I-catalysed reaction to approx. the rate of the glyoxalase I-catalysed reaction. The activity of glyoxalase I is increased to maintain the glyoxalase II-catalysed reaction as the rate-limiting step, even at low cellular concentrations of reduced glutathione.

The response of the neutrophil glutathione metabolism to functional stimulation has been previously investigated. Some investigators report a severe fall (more than 90%) in the concentrations of reduced glutathione upon activation with zymosan [20], others report a modest fall (39%) in levels of reduced glutathione [18]; this study gave a decrease in glutathione levels between these two extremes. Previous investigations of neutrophil glutathione metabolism have been oblivious to the presence of *S*-D-lactoylglutathione. However, Voetman et al. [18] reported that only 40% of the fall in reduced glutathione levels upon activation was recovered as oxidised glutathione. Similarly, inspection of the results of Burchill et al. [19] indicates that in an experiment where the fall in reduced glutathione during activation was 28% of resting cell values, the recovery of glutathione metabolites (oxidised glutathione and protein glutathione adducts were also measured) decreased as the activation progressed; after 9.5 min, the recovery was only approx. 81%. This indicates that of the 28% fail in reduced glutathione levels, 19% of glutathione metabolites was not recovered as reduced glutathione, oxidised glutathione or protein-glutathione adducts. It is clear that the detailed studies of these two groups have produced data which leave a significant component of glutathione metabolism unaccounted. This is consistent with our estimation of *S*-D-lactoylglutathione levels in human neutrophils.

Glyoxalases, S-D-lactoylglutathione and the involvement of glutathione in the control of microtubule assembly in neutrophils

The role of glutathione in microtubule assembly in the neutrophil was initially studied by observing the effects of oxidising agents of reduced glutathione (e.g. *tert*-butyl hydroperoxide and diamide) on the induction of microtubule assembly by binding of the plant lectin concanavalin A to the plasma membrane [23]. Severe depletion of reduced glutathione levels in neutrophils inhibited microtubule assembly and produced the movement of concanavalin A into surface 'caps'. When the hexosemono-phopshate shunt had been activated and reduced glutathione levels had recovered from the oxidative insult, microtubule assembly returned to control rates. Neutrophils from patients with chronic granulomatous disease

give a similar effect indicating that there is no direct relationship between NADPH oxidase activity and control of microtubule assembly in the neutrophil [24]. Gillespie [25] has previously reported that the activity of glyoxalase I increases and the activity of glyoxalase II decreases in neutrophils stimulated with TPA. This study corroborates this report and shows how these changes in glyoxalase activities are related to the enzymatic kinetic parameters, K_m and V_{max}, and illustrates their influence of the cellular concentration of *S*-D-lactoylglutathione.

We have also demonstrated the presence of *S*-D-lactoylglutathione in resting human neutrophils and the increase in the concentration of *S*-D-lactoylglutathione during functional activation. Gillespie has demonstrated that *S*-D-lactoylglutathione potentiates and glyoxalase II inhibits microtubule assembly in vitro [6]. The mechanism of in vivo microtubule assembly is still not fully understood. It is thought to be controlled by the phosphorylation of microtubule-associated proteins [26,27]. Other chemical modifications of tubulin are thought to be responsible for the stabilisation of assembled microtubules [28]. During the functional activation of neutrophils with opsonised zymosan there is a small increase (0–25%) in the mean number of microtubules but a substantial (approx. 100%) increase in the mean microtubular length [29,30]. During this period, the mean concentration of *S*-D-lactoylglutathione increases by approx. 100%. There is an approximate correlation between the increase in the concentration of *S*-D-lactoylglutathione and the mean microtubular length. *S*-D-Lactoylglutathione may serve to regulate the length of cytoplasmic microtubules during the functional activation of neutrophils. The modification of glyoxalase activities in the neutrophil, through its influence on the cellular concentration of *S*-D-lactoylglutathione may be a mechanism by which functional signals are transmitted to the microtubular components of the cytoskeleton. If the glyoxalase system fulfils this role in metabolism, its widespread distribution in tissue would be immediately reconciled with its function.

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