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MYELOPEROXIDASE INACTIVATION IN THE COURSE OF CATALYSIS OF CHLORINATION OF TAURINE

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Summary

Myeloperoxidase (donor: hydrogen-peroxide oxidoreductase, EC 1.11.1.7) was isolated from leukocytes of patients with chronic granulocyte leukemia. In the presence of H_2O_2 and Cl^- at pH 4.0—6.6 the myeloperoxidase catalyses chlorination of taurine to monochloramine taurine and simultaneously undergoes inactivation. The myeloperoxidase inactivation rate depends on the concentration of H_2O_2 and Cl^-: both the initial rate of chlorination and myeloperoxidase inactivation rate increase with increasing concentration of H_2O_2. However, an increase in concentration of Cl^- results in a decrease in enzyme inactivation. At a given H_2O_2 concentration, myeloperoxidase inactivation is a first order reaction, which implied that the enzyme may react with a substrate a limited number of times.

Introduction

Myeloperoxidase catalyses the oxidation of aminoacids, peptides and halogen ions [1—5]. At pH values below 6.5 myeloperoxidase can catalyse the oxidation of Cl^- with the production of a chlorinating agent [4—6]. This paper presents some data concerning the interactions of myeloperoxidase, Cl^- and H_2O_2 during the enzymatic chlorination of taurine.

Materials and Methods

The granulocytes were collected from blood of patients with chronic granulocyte leukemia as described elsewhere [7].

The myeloperoxidase was isolated from granulocytes using the following procedure:

1. Granulocyte pellets were suspended in 20—30 vols. of 0.05 M sodium

phosphate buffer, pH 7.4, and homogenized for 3 min in a Waring Blendor.

2. Solid NaCl was added to the homogenate to obtain a 6% (w/v) solution. The solution was allowed to stand at 4° C for several hours, during which there is a marked increase of viscosity. A 2% solution of protamine sulphate in 0.05 M phosphate buffer pH 7.4 was added until a thread-like sediment formation stopped. Usually, for each 10 ml of leukemic granulocyte mass, about 150 ml of protamine sulphate solution were required. However, a greenish-gray colour of the sediment indicated a coprecipitation of myeloperoxidase and need of the further protamine sulphate treatment. The mixture was allowed to stand overnight and then centrifuged for 15 min at 3000 × *g* yielding a clear yellow-green supernatant and a white pellet. The sediment was discarded. The supernatant was dialyzed for 36 h against several changes of 0.1 M acetate buffer pH 5.4. The sediment which was formed during dialysis was removed by centrifugation.

3. The clear supernatant was treated overnight with CM-cellulose (Whatman CM C-52, preswollen) equilibrated with the 0.1 M acetate buffer, pH 5.4. The mixture was transferred to a G-1 (Shotten-Baumann) glass filter funnel and the CM-cellulose was filtered off under hydrostatic pressure. The filtrate which had no peroxidase activity was discarded. The rest of the filtrate was separated from the cellulose by suction under diminished pressure and then a small volume of 1.2 M sodium acetate pH 7.8 (about 3 ml for each ml of the wet cellulose mass) was added to the cellulose. A brownish-dark green solution of crude myeloperoxidase was eluted from the cellulose. The procedure was repeated several times unitl the cellulose was no longer green. Myeloperoxidase solutions were pooled for further purification.

4. 20 ml of the myeloperoxidase preparation were applied to a Sephadex G-150 superfine column (4.7 × 65.0 cm, about 600 ml bed volume) equilibrated with 0.6 M sodium acetate pH 7.8. 4-ml fractions were collected. Myeloperoxidase emerged from the column with the first protein peak followed by several other protein fractions. This resulted in a peroxidase preparation with a ratio of light absorbance at 430 nm to that of 280 nm (R_Z coefficient) of about 0.60—0.66.

5. The partially purified myeloperoxidase was dialyzed against 0.14 M sodium acetate in 0.01 M sodium phosphate pH 7.0 and absorbed on a CM Sephadex C-50 column (0.8 × 15.0 cm) equilibrated with 0.1 M acetate/0.01 M phosphate, pH 7.0. The enzyme was eluted using a linear gradient of acetate obtained by the gradual mixing of 250 ml of the starting buffer with 250 ml of 1.2 M acetate in 0.01 M phosphate, pH 7.8. Myeloperoxidase emerged from the column as a single sharp peak when acetate concentration was about 0.5 M.

The preparation had a light absorbance 430/280 ratio equal to 0.75—0.79 and the spectral characteristics of myeloperoxidase.

Finally, the myeloperoxidase was dialyzed against several changes of distilled water and stored in 0.5-ml vials at —18° C. The enzyme (1.6 mg of protein per ml) did not show any detectable change in activity when stored frozen for several months and was stable standing at 4° C in a refrigerator for several days.

Myeloperoxidase was assayed with guaiacol as a substrate as previously described [9].

Myeloperoxidase chlorinating activity was estimated on the basis of the rate

of chlorination of taurine at 25° C [4]. The reaction mixture consisted of 0.2 M NaCl, 6.7 mM of taurine and 1.7 or 3.3 mM of H_2O_2 in 0.1 M sodium acetate buffer pH 5.4. The amount of myeloperoxidase used varied from 2.7 to 10 μg per ml (which is approx 18—67 nM) in the reaction mixture. The reaction was initiated by the addition of 0.05 ml of 0.1 M H_2O_2. The change in optical density at 255 nm, which is a measure of the production of chloramine taurine was measured using Pye Unicam, SP 800 Spectrophotometer with thermostatically temperature controlled cell.

Results

Fig. 1 shows the production of chloramine taurine as a function of the concentration of H_2O_2. With high myeloperoxidase concentrations, the yield of chloramine taurine is stiochiometrically equal to the amount of hydrogen peroxide used. However, with lower concentrations the yield is far below theoretical.

An analysis of the reaction rate with lower concentrations of myeloperoxidase shows that, as the reaction proceeds, the rate decreases and finally stops. Upon addition of more myeloperoxidase, however, the reaction will again proceed (Fig. 2). The total yield of chloramine taurine produced prior to inactivation is proportional to the initial concentration of myeloperoxidase (Fig. 3). Thus it would appear that in the course of the chlorination of taurine there is an inactivation of myeloperoxidase.

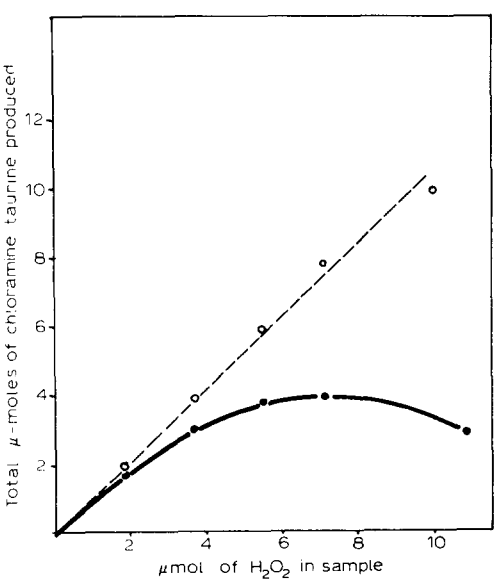

Fig. 1. Chloramine taurine produced as a function of H_2O_2 concentrations: (o- - - - -o) using 0.22 μM myeloperoxidase and (●———●) 18 nM myeloperoxidase. The reaction mixture contained 6.6 mM taurine in 0.2 M NaCl and 0.1 M acetate buffer, pH 5.4. Amounts of H_2O_2 are indicated on the graph.

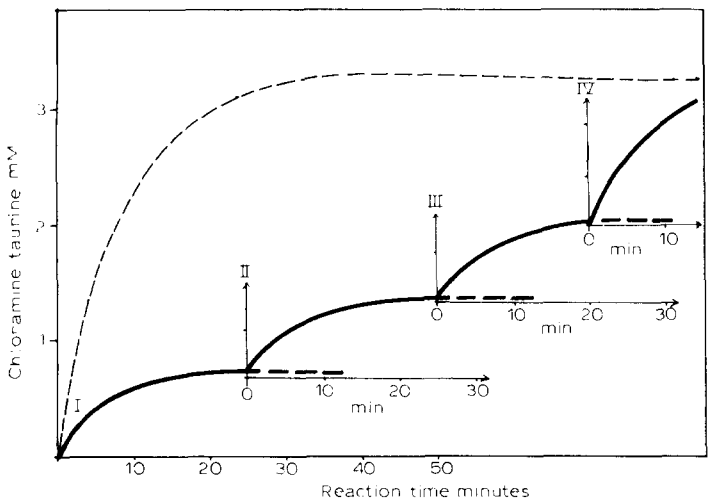

Fig. 2. The effect of the employed concentration of myeloperoxidase on total yield of chloramine taurine; ------ represents the increase of chloramine taurine when 100 μg myeloperoxidase (to obtain concentration of 0.22 μM) was used. ———, chloramine taurine synthesis with additions of : I, 10 μg; II, 10 μg; III, 10 μg; IV, 30 μg of myeloperoxidase to the sample. The additions were to the same reaction mixrure at 30-min intervals. The reaction mixture was as for Fig. 1, except for H_2O_2 concentration which was 3.4 mM.

Kinetics of taurine chlorination with simultaneous inactivation of myeloperoxidase

From the experiments shown in Figs, 1 and 2, one can presume that whilst mediating chlorination of taurine myeloperpxidase becomes inactivated. Thus,

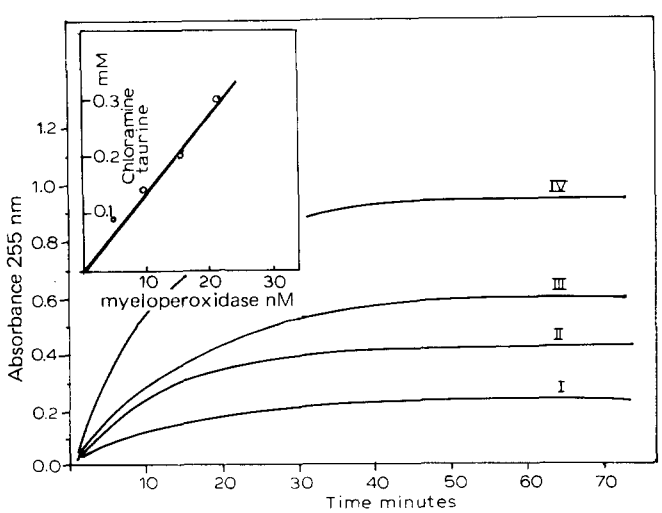

Fig. 3. Chloramine taurine produced prior inactivation of myeloperoxidase. Curves I—IV present chloramine taurine accumulation vs. time patterns for 6, 10, 16 and 24 nM myeloperoxidase respectively. The insert shows final concentrations of chloramine taurine produced as a function of the concentrations of myeloperoxidase employed. The reaction mixture was as for Figs. 1 and 2.

rate of the reaction of chlorination of taurine:

$$\text{Taurine} + Cl^- + H_2O_2 \xrightarrow[H^+]{\text{Myeloperoxidase}} \text{Taurine-Cl} + 2\,H_2O \quad (1)$$

will at any time depend on advance of the simultaneous reaction:

$$\text{Active myeloperoxidase} \rightarrow \text{Inactive myeloperoxidase} \quad (2)$$

Assuming that myeloperoxidase inactivation (reaction 2) is first order, one can write:

$$-\frac{dE}{dt} = k_{\text{inact.}} E \quad (3)$$

Where E is the concentration of myeloperoxidase and $K_{\text{inact.}}$ is the reaction 2 rate constant.

Integrating Eqn. 3 from time 0 to t:

$$-\int_{E_0}^{E_t} \frac{dE}{E} = k_{\text{inact.}} \int_0^t dt,$$

$$\text{or, } E_t = E_0\, e^{-k_{\text{inact.}} t} \quad (4)$$

One can thus obtain myeloperoxidase concentration at a given moment t.

If rate of the reaction 1 (chlorination of taurine) meets the usual condition, that $V = dP/dt = kE$, where P is concentration of the reaction product, and k is reaction 2 rate constant, one may calculate the rate of the reaction 1 at any moment t by substituting E in the above equation by E_t from the Eqn. 4:

$$V_t = \frac{dP}{dt} = kE_t \quad (5)$$

and:

$$P_t = kE_0 \int_0^t e^{-k_{\text{inact.}} t}\, dt \quad (6)$$

thus,

$$P_t = \frac{k}{k_{\text{inact.}}} E_0 (1 - e^{-k_{\text{inact.}} t}) \quad (7)$$

Eqn. 7 written for $t = \infty$ (after the myeloperoxidase is inactivated will be:

$$P = \frac{k}{k_{\text{inact.}}} E_0. \quad (8)$$

This is in full agreement with the experimental results (Fig. 3).

Equation III written for $t = 0$ is:

$$V_0 = kE_0 \quad (9)$$

where V_0 is an initial rate of the reaction of chlorination of taurine. Therefore differentiating Eqn. 7 and substituting kE_0 by V_0 we obtain:

$$V_t = \frac{dP}{dt} = V_0 e^{-k_{inact.}t}$$

or,

$$\ln V_t = \ln V_0 - k_{inact.}t \quad (10)$$

Assuming that 10 s increase in concentration of chloramine tauringe (P), is at a given moment t from the beginning of the reaction, virtually equal to V_t value, one can plot the data presented in Fig. 3 according to Eqn. 10. The results of this conversion is a straight line expressing natural logarithmus of V_t ploted versus reaction time t. In the experimental conditions used in this study a plot of ln V_t vs. t maintains linearity for time intervals ranging from the beginning of the reaction to 120 s.

Eqn. 10 allows determination of V_0 by extrapolating. In V_t to $t = 0$. Use of the above procedure for determination of V_0 values for 2, 3, 4 and 6 nM myeloperoxidase gives a perfect straight line relationship as stated in Eqn. 10. Therefore, the data present in Fig. 3 and obtained from calculations based on Eqn. 10 support the assumed model of the reaction kinetics of myeloperoxidase-catalyzed chlorination of taurine.

Factors influencing myeloperoxidase inactivation rate

The reaction model discussed above was valid if concentrations of the substrates changed negligibly from the initial values. However a change of the

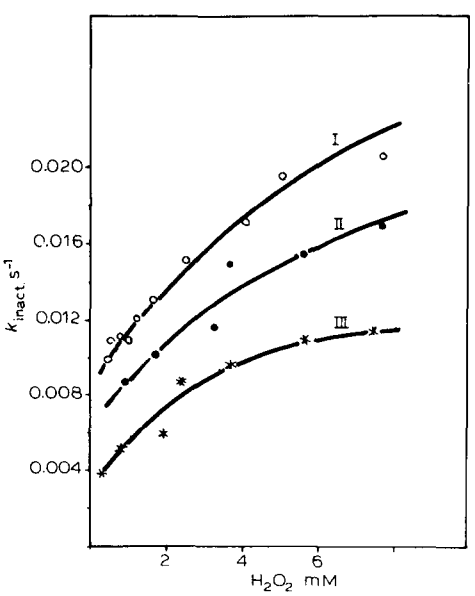

Fig. 4. The effect of H_2O_2 and Cl^- concentrations on myeloperoxidase $k_{inact.}$ value: Curves I, (o———o), II (•———•) and III (*———*) represent $k_{inact.}$ values for Cl^- concentrations of 0.20, 0.33, and 0.60 M respectively. The reaction mixture was 6.7 mM taurine in 0.1 M acetate buffer, pH 5.4, myeloperoxidase was 18 nM. Concentrations of Cl^- and H_2O_2 as indicated on the graphs.

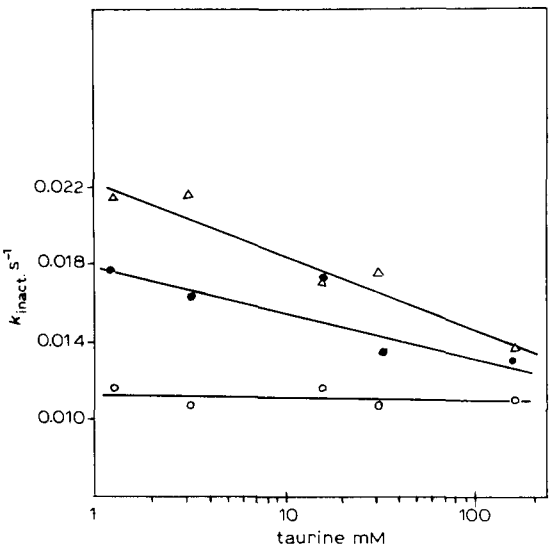

Fig. 5. The effect of concentration of taurine on myeloperoxidase $k_{inact.}$ value: at H_2O_2 concentration 0.08 mM, (○—, 1.7 mM (●) and 6.8 mM (△) respectively. The reaction mixture was as for Fig. 4 except Cl^- concentration was 0.2 M and taurine concentration as indicated on the graph.

initial values of concentration of the substrates may influence the myeloperoxidase inactivation rate. Eqn. 10 provides a possibility for studying the kinetic constant values for given concentrations of H_2O_2, Cl^- and taurine. The results of this study are shown in Figs. 4 and 5. It was found, that myeloperoxidase inativation rate depends on the concentration of H_2O_2 and Cl^-, both the initial rate of chlorination and myeloperoxidase inactivation rate increase with increasing concentration of H_2O_2. However, as increase in concentration of Cl^- results a decrease in enzyme inactivation.

An influence of increasing concentrations of taurine on myeloperoxidase inactivation rate is shown in Fig. 5. It was found that increasing concentration of taurine does result in decreased enzyme inactivation. A protective effect of taurine seems to be primarily dependent on the taurine: H_2O_2 ratio.

The pre-exsisting chloramine taurine in the reaction medium up to a concentration of 0.18 mM had no effect of myeloperoxidase inactivation rate.

The pH optimum for myeloperioxidase chlorination of taurine is between 5.0 and 5.5. In this pH range and below there is comparatively little enzyme inactivation. Above pH 5.6 there is decrease in the rate of chlorination and increase in the rate of enzyme inactivation.

On the basis of an initial concentration of myeloperoxidase and concentration of chloramide taurine produced prior to inactivation of myeloperoxidase one can calculate an average number of times the enzyme molecule will react with substrates prior to inactivation. This amounts about $8 \cdot 10^4$ at pH 5.4 and decreases by factor of 2.6 if pH changes to 6.0.

Treatment of the myeloperoxidase with OCl^- did not resulted in immediate breakdown of the enzyme. Three consecutive additions of equimolar amounts of OCl^- gave a spectral shift suggesting the formation of myeloperoxidase Compound II (Fig. 6). The Soret band then slowly decreases in absorption. Reduc-

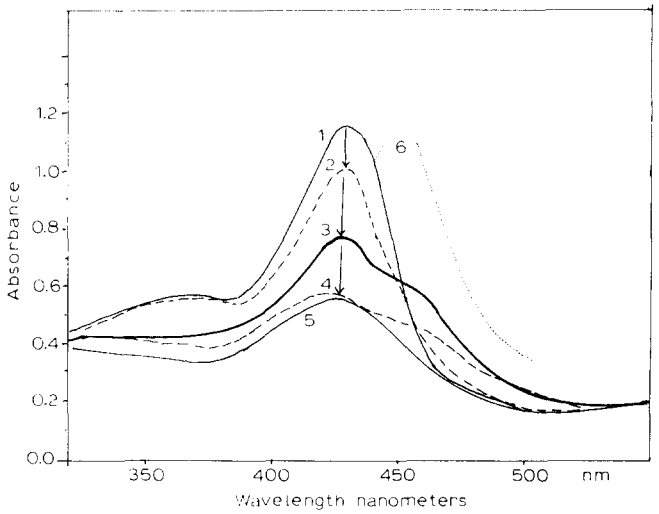

Fig. 6. Change of spectrum of myeloperoxidase upon treatment with OCl^-: 1, spectrum of myeloperoxidase standard solution (120 μM) in 0.05 M phosphate buffer pH 5.4. 2, 3 and 4, spectra of the same myeloperoxidase solution upon addition of one, twice and three times the stoichiometric amounts of OCl^- respectively. 5, the same solution after addition of ascorbate. 6, light absorption peak of 120 μM myeloperoxidase solution after treatment with H_2O_2 at pH 5.4.

tion of the enzyme with ascrobic acid did not restore the Soret band (Fig. 6). Thus, one can presume the slow breakdown of the heme grup in the enzyme molecule.

Discussion

The difficulties in obtaining polymorphonuclear leukocytes limit availability of myeloperoxidase. Although other sources of myeloperoxidase such as puss, peritoneal exudates and leukemic cells may be more readily available, there are technical difficulties in obtaining the enzyme from these sources. The cells thus obtained are non-homogenous and the extracts from these cells are highly viscous if treated with high concentration of ammonium sulphate or sodium chloride. This hindered chromatographic separations and salt precipitation procedures for purification.

The procedure described presents techniques for the elimination of the viscosity of salt extracts obtained from human leukemic cells (also human puss cells). Thus it allows one to isolate myeloperoxidase from more available materials then the normal polymorphonuclear leukocytes.

The inactivation of myeloperoxidase in the presence of Cl^- and H_2O_2 complicates procedures for the elimination of myeloperoxidase chlorination activity [16]. The exponential character of a function of accumulation of the reaction product vs. the reaction time flow occasions substantial difficulty in any attempt at graphical estimation of the initial rate [V_0] value. Because in practice a certain time ($t_0 + \epsilon$) has to pass (after reagents have been mixed) before a spectrophotometric measurement begins (usually 7—10 s), the direct graphical approximation of V_0 would be drawing of the slope of an experimentally unavailable section of the curve. The model of the myeloperoxidase inactivation

process proposed in this study presents, on the other hand, the possibility of releable calculation of the V_0 value as a function of the experimentally obtained P/t values. Thus the presented procedure gives an opportunity to estimate of chlorinating activity of myeloperoxidase.

Myeloperoxidase is inactivated during the oxidation of the Cl^- ion, although similar inactivation is not observed when guaiacol or tyrosine are substrates. This may suggest that the products formed by the Cl^- oxidation system specifically inactivate the enzyme. The product of the myeloperoxidase oxidation of Cl^- ion is hypochlorous acid [5—7,9—11]. If this is the case, the reaction of hypochlorous acid with myeloperoxidase may cause the inactivation of the enzyme. Treatment of the myeloperoxidase with an equimilar concentration of OCl^- gives a spectral shift suggesting the formation of Compound II (Fig. 6). A slow decrease in absorption in the Soret band may suggest the breakdown of the heme group of the enzyme molecule. Primary formation of myeloperoxidase Compound II in the presence of OCl^- may suggest that the heme prosthetic group is destroyed by some secondary product rather than $HOCl^-$ itself. The formation of a singlet oxygen in the myeloperoxidase-halide-H_2O_2 systems was recently reported [10,11]. Thus single oxygen may possibly be the enzyme-inactivating factor. Some protection of the enzyme by high concentration of Cl^- seems to support this idea. Since taurine is a hypochlorous acid scavanger and would therefore prevent some secondary reaction, protection of myeloperoxidase by the excess of taurine is understandable.

Myeloperoxidase inactivation in the presence of halides and H_2O_2 has been previously observed [2,6,12]. Similar inactivation with a limited turnover number was also found for chloroperoxidase [13].

Since myeloperoxidase halogenation is of great importance in the bacteria-killing systems of polymorphonuclear leukocytes [1,3,10], one may speculate that H_2O_2- and pH-dependent inactivation of myeloperoxidase are involved in natural regulatory mechanisms which could thus limit the effects of myeloperoxidase inside the phagocytic vesicle.

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References

1 Agner, K. (1947) Nature 271, 159—160
2 Zgliczyński, J.M., Stelmaszyńska, T., Ostrowski, W., Naskalski, J. and Sznajd, J. (1968) Eur. J. Biochem. 4, 504—547
3 Kelbanoff, S.J. (1975) Semin. Hematol. 12, 117—142
4 Stelmaszyńska, T. and Zgliczyński, J.M. (1974) Eur. J. Biochem. 45, 305—312
5 Harrison, J.E. and Schultz, J. (1976) J. Biol. Chem. 251, 1371—1374
6 Zgliczyński, J.M., Selvaraj, B., Paul, B., Poskitt, P., Luis, F. and Sbarra, A. (1977) Proc. Soc. Exp. Biol. Med. 154, 418—423
7 Zgliczyński, J.M., Stelmaszyńska, T., Domański, J. and Ostrowski, W. (1971) Biochim. Biophys. Acta 235, 419—424
8 Sznajd, J. and Naskalski, J.W. (1973) Biochim. Biophys. Acta 302, 282—292

9 Stelmaszyńska, T. and Zgliczyński, J.M. (1971) Eur. J. Biochem. 19, 56—63
10 Allen, R.C. (1975) Biochem. Biophys. Res. Commun. 63, 675—683
11 Allen, R.C. (1975) Biochem. Biophys. Res. Commun. 63, 684—691
12 Odajima, T. and Yamazaki, I. (1972) Biochim. Biophys. Acta 284, 368—374
13 Hager, L.P., Doubek, D.L., Silverstein, R.M., Lee, T.T., Thomass, J.A., Hargis, J.H. and Martin, J.C. (1973) in Oxidases and Related Redox Systems, Vol. 1. pp. 311—332, University Park Press Baltimore