Biochimica et Biophysica Acta, 979 (1989) 1–6
Elsevier

BBA 74298

Mechanism of spectrin degradation induced by phenylhydrazine in intact human erythrocytes

Arduino Arduini [1], Stefano Storto [1], Maurizio Belfiglio [1], Rosa Scurti [1], Giovanna Mancinelli [1] and Giorgio Federici [2]

[1] *Istituto di Scienze Biochimiche, Facoltà di Medicina, Università degli Studi G. D'Annunzio, Chieti and* [2] *Dipartimento di Biologia, Università di Tor Vergata, Roma (Italy)*

(Received 22 August 1988)

Key words: Erythrocyte; Phenylhydrazine; Spectrin; Protein degradation; Proteolysis; (Human)

The exposure of human erythrocytes to phenylhydrazine results in the degradation of both monomers of spectrin, a major cytoskeleton membrane protein. The degradative process, characterized by a loss of spectrin without the appearance of high-molecular-weight products, either under reducing conditions or not, is almost complete in 10 min when a 5% erythrocyte suspension is treated with 1 mM phenylhydrazine. Under these conditions, we found a loss of 62.3 and 48.5% for the α and β monomer, respectively. A similar degradative extent was obtained when the membrane ghost plus cellular free extracts, were dialyzed, and the membrane ghost plus hemoglobin was exposed to 1 mM phenylhydrazine for 10 min. The presence of different proteinase inhibitors and effectors, such as EDTA, diethylenetriaminepentaacetic acid, EGTA, leupeptin, aprotinin, phenylmethylsulfonyl fluoride, pepstatin, Ca^{2+} and ATP plus Mg^{2+}, in the membrane ghost plus cellular free extract system (undialyzed) did not affect the degree of the spectrin-degradative process induced by phenylhydrazine. In addition, a purified spectrin tetramer preparation exposed to 1 mM phenylhydrazine in the presence of hemoglobin was degraded to an extent comparable to that with intact cells. Our data suggest that the initial degradative step of spectrin induced by phenylhydrazine in intact erythrocytes may be ascribed more to a direct oxidative breakdown, probably involving main-chain cleavage and side-chain cleavage processes, than to an eventual proteolytic system.

Introduction

In the last few years, the red blood cell has been the subject of a considerable amount of study based upon the biological effects of oxidative stress [1–12]. The functional and structural properties of red cell membrane, in particular, have been investigated in great detail in red cells exposed to oxidants [3–12]. We have recently demonstrated that phenylhydrazine, a compound that undergoes coupled oxidation with oxyhemoglobin, induced spectrin degradation in intact red cells [8]. The degradation process was not followed by the appearance of any high-molecular-weight compound, either under reducing or non-reducing conditions, as judged by SDS-PAGE. However, at that time, we did not know the intimate mechanism responsible for the phenylhydrazine-induced red cell membrane spectrin degradation. We are now able to provide convincing evidence for the most probable action mechanism of phenylhydrazine: namely, a free-radical-mediated protein fragmentation * seems to be the basis of phenylhydrazine-induced spectrin degradation.

Materials and Methods

Chemicals. All salts and buffers were reagent quality RPE (ACS) and obtained from Carlo Erba (Milan). Phenylhydrazine, EDTA, EGTA, diethyldithiocarba-

Abbreviations: DTPA, diethylenetriaminepentaacetic acid; PMSF, phenylmethylsulfonyl fluoride; DPPD, *N,N'*-diphenyl-1,4-phenylenediamine; EGTA, ethyleneglycol bis(β-aminoethyl ether)*N,N,N',N'*-tetraacetic acid; KRG buffer, Krebs-Ringer glycylglycine buffer.

Correspondence: A. Arduini, Istituto di Scienze Biochimiche, Facoltà di Medicina, Università degli Studi G. D'Annunzio Chieti, 66100 Chieti, Italy.

* It has been proposed, recently, that the term protein fragmentation should signify the direct breakdown of protein mediated by free radicals, while the term "protein degradation" should signify the peptide-bond cleavage mediated by proteolytic enzymes. Since the aim of our work is only to discriminate between these two events, at least in the very early stages, throughout the Results and Discussion we will use the terms degradation, breakdown and fragmentation interchangably, in reference to the observed phenomenon of phenylhydrazine-induced breakdown.

mate, DTPA, β-mercaptoethanol, catalase (EC 1.11.1.6) from bovine liver (thymol-free), superoxide dismutase (EC 1.15.1.1) from bovine erythrocytes, mannitol, thiourea and glycylgline were purchased from Sigma Chemicals (U.S.A.). PMSF, aprotinin, leupeptin and pepstatin were purchased from Boehringer Mannheim (Milan). Desferoxamine B methanesulfonate (Desferal) was from Ciba-Geigy (Milan) and DPPD was from Aldrich Chemicals (U.S.A.). All electrophoretic reagents were obtained from Bio-Rad (Milan).

Preparation of washed isolated erythrocytes and ghost. Venous human blood was collected daily from healthy volunteers into heparinized test-tubes. Leukocytes and platelets were removed essentially by the procedure described by Beutler et al. [13], with a column containing a mixture of microcrystalline cellulose and α-cellulose (1 : 3, w/w). Neither leukocytes nor platelets were detected by counting filtered blood cells according to standard manual techniques. An additional three-step washing procedure, using 4 vol. of cold 0.9% NaCl containing 5 mM glucose, was performed to remove any plasmatic contaminant from the filtered cells. Membrane ghosts were prepared from washed cells which were lysed in 30 vol. of cold lysing buffer (5 mM NaH_2PO_4 (pH 8.0)/0.1 mM PMSF). The lysed cells were then centrifuged for 10 min at $37000 \times g$ at 4°C and the resulting pelletted ghost was washed repeatedly with lysing buffer until virtually all of the residual hemoglobin was removed. Throughout this work, membrane ghosts refers to those membranes which do not contain hemoglobin. The lysing buffer was used within 5 min in the solubilization of PMSF, in view of the short half-life of this compound in aqueous solution. Membrane ghosts obtained were washed again with cold hypotonic buffer (5 mM NaH_2PO_4 (pH 7.4)) and utilized immediately for the experiments.

Preparation of hemolysate, dialysed hemolysate and hemoglobin. Washed isolated erythrocytes were suspended in 4 vol. of cold hypotonic buffer and stirred for 60 min in order to completely lyse the cells. Hemolysate was collected after removal of membrane fractions by centrifugation for 30 min at $37000 \times g$. To prepare the dialysate, hemolysate was dialysed extensively against cold hypotonic buffer (membrane M_r cutoff 12000). Hemoglobin was purified by column chromatography of the hemolysate on DEAE-Sephadex, as described by Winterbourn [14]. Purified hemoglobin was subsequently dialysed against cold hypotonic buffer. Hemolysate, dialysed hemolysate and purified hemoglobin were used immediately after preparation. All of the operations were carried out at 4°C, checking step by step for the oxidative status of hemoglobin [14]. In all preparations, hemoglobin showed the spectral properties of oxyhemoglobin, and the methemoglobin content was found to be less than 3%. The total hemoglobin concentration was determined as cyanmethemoglobin by using Drabkin's reagent [15].

Spectrin preparation. Spectrin was extracted according to the procedure of Bennett [16] by incubation of white ghosts at 37°C for 30 min in a low-ionic-strength medium. Spectrin-depleted ghosts were then removed by centrifugation at $200000 \times g$ for 15 min and 20 mM KCl was added to the supernatant which was next warmed to 30°C for 3 h. Spectrin tetramer was then fractionated by density-gradient centrifugation on linear 5–20% sucrose gradient, dialysed against cold hypotonic buffer, and used within 3 days of preparation. The final concentration of the stock spectrin solution was calculated using an E 1%, 280 nm of 10 cm^{-1}.

Incubation conditions. For experiments with intact cells the washed isolated erythrocytes were resuspended in a Krebs-Ringer glycylglycine (KRG) buffer (120 mM NaCl/47 mM KCl/2.5 mM $CaCl_2$/1.2 mM KH_2PO_4/1.2 mM $MgSO_4$/50 mM glycylglycine (pH 7.4)) to a final hematocrit of 5% (about 280 μM of total hemoglobin). For the experiments with membrane ghosts the hypotonic buffer was used, and the final volume of the reaction mixture was adjusted to obtain a 5% red cell equivalent membrane ghost suspension. The final hemoglobin concentration was 280 μM. For experiments with purified spectrin the final concentrations of hemoglobin and spectrin were 280 μM and 80 nM, respectively, the latter corresponding to that of a 5% erythrocyte suspension, taking into account a spectrin tetramer content for each erythrocyte of 10^5 copies [17]. The hypotonic buffer was used in this case as well. In these experiments phenylhydrazine was always added last, after other additions, to a final concentration of 1 mM, unless otherwise stated. Antioxidants, proteinase inhibitors and proteinase effectors were added at final concentrations as stated in the figure legends. All incubations were conducted at 37°C in a Rotabath shaking water bath.

SDS-PAGE analysis. Washed ghosts were solubilized to a final concentration of 1 mg/ml in SDS sample buffer (1% sodium dodecyl sulfate/10% glycerol/63 mM Tris-HCl (pH 6.5)), with or without 1% β-mercaptoethanol, and were warmed to 95°C for 5 min. Equal amounts of solubilized membrane protein (100 μg) were loaded onto 7% polyacrylamide gels prepared according to Laemmli [18]. The gel system was calibrated for molecular-weight determination by measuring the migration of standard proteins (ranging from 29 kDa, for carbonic anhydrase, to 205 kDa, for myosin). Gels were fixed and stained by either Coomassie R-250 or silver procedures. The dye-protein complexes were labeled according to the nomenclature of Furthmayr and Marchesi [19]. The dye-protein complex intensities were quantified and the molecular weight determined on the Coomassie- or silver-stained gel using a Hoefer

scanner (GS-300) in the transmittance mode, interfaced with an IBM XT personal computer via a Data Translation A/D card utilizing the GS-360 Data System software (Hoefer).

The degradation percentage of the two spectrin monomers was calculated from the integration of the corresponding peak areas using the following relationship:

$$100 \times (\text{area}_{\text{control}} - \text{area}_{\text{treated sample}})/\text{area}_{\text{control}}$$

Results and Discussion

We have recently demonstrated that intact human erythrocytes exposed to phenylhydrazine showed a marked decrease of stain intensity of Coomassie-spectrin complexes as judged by SDS-PAGE technique without the appearance of a high-molecular-weight compound [8]. At that time, we speculated that a proteolytic system was, in some respect, involved in the degradation of spectrin induced by phenylhydrazine. In this context, several papers have appeared in the literature concerning the possibility that endogenous proteinases may recognize and degrade oxidatively modified proteins and/or their fragments [20–38]. Even phenylhydrazine seems to be responsible for an oxidative modification of hemoglobin, which makes it susceptible to degradation by a proteolytic system present in reticulocytes, erythrocytes and fibroblasts, though these authors did not describe the molecular modification involved in the oxidant-treated hemoglobin [20,31]. However, free radicals aside from their various damaging effects on proteins, are also able to fragment proteins, a process which could be potentially confused with a proteolytic action [34,39–44]. Thus, we decided to follow-up both of these possibilities, the proteolytic and the direct free-radical attack, in order to understand the mechanism of spectrin degradation induced by phenylhydrazine. It should be also noted that in the current paper the authors are not interested in the ultimate destiny of protein fragments derived from any initial cleavage process, though we have already demonstrated the existence of immunoreactive fragments toward spectrin in phenylhydrazine-treated erythrocytes [8].

Our previous data on spectrin degradation were related to experiments conducted with intact erythrocytes exposed to phenylhydrazine for 1 h, at 37 °C. The most efficient phenylhydrazine concentration, in terms of spectrin degradation without the appearance of a high-molecular-weight compound, was found to be 1 mM. Fig. 1 shows a time-dependent study of 1 mM phenylhydrazine-exposed erythrocytes, in which it is possible to observe, within the first few minutes of incubation, a marked degradation of both α and β monomers. At 10 min a plateau value of 62.3 and 48.5% for the α and β monomers of spectrin, respectively, was reached that was about the same as the percentage of degradation after 1 h of incubation. During this time-course study, the α monomer seemed to be more susceptible than the β to the degradative effect of phenylhydrazine. In addition, throughout the experiment, no high-molecular-weight compound could be detected either under reducing conditions or not.

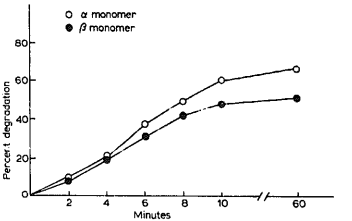

Fig. 1. A 5% red cell suspension in a final vol. of 1 ml of KRG buffer was incubated in the presence of 1 mM phenylhydrazine. At the indicated times the samples were diluted with 30 volumes of cold lysing buffer and immediately centrifuged to remove phenylhydrazine. The obtained pellets were dissolved in SDS sample buffer and analyzed by SDS-PAGE. The values are the average of three experiments. The variation between one experimental value to another was not more than 5%.

In Table I it is clearly evident that the percentage of degradation of the two spectrin monomers in the membrane ghost plus hemolysate system exposed to 1 mM phenylhydrazine is comparable to those with the intact cells in the presence of the same phenylhydrazine con-

TABLE I

Phenylhydrazine-induced spectrin degradation in both intact erythrocytes and membrane ghosts plus hemolysate

A 5% red cell equivalent membrane ghost suspension plus hemolysate in a final volume of 1 ml of KRG buffer and 1 ml of hypotonic buffer, respectively, as incubated in the presence of 1 mM phenylhydrazine for 10 min. At the end of the incubation, both the intact cell suspension and the membrane ghost plus hemolysate system were processed as reported in Fig. 1. Samples were subsequently analyzed by SDS-PAGE. The values are and average of three experiments. The variation between experimental values was not more than 5%. MG, membrane ghost; PH, phenylhydrazine; CH, hemolysate;

	Spectrin degradation (%)	
	α monomer	β monomer
Cells	0	0
Cells + PH	62.3	48.5
MG	0	0
MG + PH	1.3	1.7
MG + CH	1.2	2.3
MG + CH + PH	58.5	46.5

TABLE II

Effects of proteinase inhibitors and effectors on the phenylhydrazine-mediated spectrin degradation in the membrane ghost plus hemolysate system

A 5% red cell equivalent membrane ghost suspension in a final vol. of 1 ml of hypotonic buffer was mixed with hemolysate. One of the following proteinase inhibitors and effectors were added prior to 1 mM phenylhydrazine: 1 mM EDTA, 1 mM DTPA, 1 mM EGTA, 0.12 mg/ml leupeptin, 0.6 mg/ml aprotinin, 1 mM PMSF, 0.12 mg/ml pepstatin, 5 mM ATP plus 5 mM $MgCl_2$, and 1 mM $CaCl_2$. The samples were incubated for 10 min and processed as in Fig. 2. The values are an average of three experiments. The variation between experimental values was not more than 5%.

	Spectrin degradation [a] (%)	
	α monomer	β monomer
No addition	100	100
EDTA	101	100
DTPA	99	100
EGTA	98	99
Leupeptin	102	100
Aprotinin	105	101
Pepstatin	98	97
PMSF	102	99
PMSF	102	99
ATP + Mg^{2+}	100	102
Ca^{2+}	98	99

[a] Spectrin degradation was measured taking the degradation induced by phenylhydrazine in the membrane ghost plus hemolysate system without any additions as 100%.

centration. Neither membrane ghosts plus hemoglobin nor membrane ghost plus phenylhydrazine systems induce spectrin breakdown. In other words, only the concomitant presence of the two erythrocyte compartments, the membrane and soluble fraction, plus the oxidant results in spectrin degradation. In another set of experiments, we tested several compounds considered to be proteinase inhibitors or effectors, at least with respect to those proteinases identified in mature erythrocytes and reticulocytes [21,31–33,37,38]. The membrane ghosts plus hemolysate system exposed to phenylhydrazine was used. EDTA, DTPA, EGTA, leupeptin, aprotinin, PMSF, pepstatin, Ca^{2+} and ATP plus Mg^{2+} did not modify the extent of spectrin breakdown when exposed to the oxidant (Table II). It is important to note that the oxidation of phenylhydrazine initiated by hemoglobin is not affected by the presence of chelating agents [45]. All of these experiments seem to suggest that the bulk of the initial fragmentation process of membrane-ghost spectrin should be attributed more to a direct phenylhydrazine oxidative attack, rather than to a proteinase involvement (ATP- and Ca^{2+}-dependent proteinases, serine and metalloproteinases).

A further confirmation of the major role played by the direct phenylhydrazine oxidative action on the initial step of spectrin breakdown comes from an experiment in which we compared the degradative efficiency of the oxidant in three different preparations of the soluble extracts: hemolysate, dialysed hemolysate, and pure hemoglobin preparation. As shown in Table III, the hemoglobin preparation is the most efficient in phenylhydrazine-induced degradation of membrane ghost spectrin. The α monomer, further, was always more susceptible to the degradative action than the β monomer in both the hemolysate and dialysed hemolysate. Even the ubiquitin conjugation process, as a tag for the recognition of potential substrates by proteinase [22,46], may be excluded in our systems since the dialysed hemolysate extract was extensively dialysed against a M_r cutoff 12000 membrane. Yet, in an attempt to produce the possible substrate, membrane ghosts plus hemoglobin were treated with 1 mM phenylhydrazine over several time intervals (2, 4, 6 and 10 min), and then washed extensively. The exposure of these membrane ghosts to hemolysate did not yield any additional breakdown of spectrin with respect to a same membrane preparation not exposed to hemolysate. Hemolysate and dialysed hemolysate contain two enzymes capable of counteracting the oxidative damage of some intermediate of phenylhydrazine oxidation, namely, superoxide dismutase and catalase. The presence of these enzymes and other antioxidants could explain the differences in membrane-ghost spectrin fragmentation observed between the hemolysates (hemolysate and dialysed hemolysate) and pure hemoglobin. Thus, in an attempt to identify the reactive species involved in the spectrin degradation induced by phenylhydrazine in a membrane ghost plus hemoglobin system we found that the most efficient protective agent was superoxide dismutase (Table IV) which, by removing one of the mediators of phenylhydrazine oxidation [45,47], may justify the differences observed above. Ethanol and Desferal showed only a slight degree of protection, while mannitol, thiourea, DPPD and catalase did not.

TABLE III

Phenylhydrazine-induced spectrin degradation in membrane ghosts

A 5% red cell equivalent membrane ghost suspension in a final vol. of 1 ml of hypotonic buffer was mixed with either hemolysate, dialysed hemolysate or hemoglobin and incubated for 10 min in the presence of 1 mM phenylhydrazine. Incubations were terminated as described in Fig. 1 and samples were analyzed by SDS-PAGE. The values are the average of three experiments. The variation between one experimental value and another was not more than 5%. Spectrin degradation in MG, MG + PH, MG + CH, MG + CHD, and MG + HB was less than 3% for both monomers. CHD, dialysed hemolysate; HB, hemoglobin.

	Spectrin degradation (%)	
	α monomer	β monomer
MG + CH + PH	58.5	46.5
MG + CHD + PH	61.5	45.1
MG + HB + PH	68.5	54.3

TABLE IV

Effect of some scavangers on the phenylhydrazine-mediated spectrin degradation in the membrane ghost plus hemoglobin system

A 5% red cell equivalent membrane ghost suspension in a final vol. of 1 ml of hypotonic buffer was mixed with purified hemoglobin. One of the following antioxidants were added prior to 1 mM phenylhydrazine: 200 μM desferal, 100 mM mannitol, 20 mM thiourea, 0.5% (v/v) ethanol, 40 μM DPPD, 1200 U/ml catalase and 78 μg superoxide dismutase. The samples were incubated for 10 min and processed as in Fig. 2. The values are an average of three experiments. The variation between experimental values was not more than 5%.

Additions	Inhibition of spectrin degradation (%)	
	α monomer	β monomer
Desferal	7.1	9.8
Mannitol	3.1	1.5
Thiourea	2.7	1.8
DPPD	1.8	0.7
Ethanol	9.6	14.9
Catalase	0.5	0.8
Catalase (boiled)	1.2	1.3
Superoxide dismutase	21.1	28.3
Superoxide dismutase (boiled)	2.5	1.7

Since it has been described that some proteinases, such as the Ca^{2+}-dependent proteinase Calpain I and the three acidic proteinases, may be located in membrane ghosts [48,49], the above results could be affected by the action of one of these proteinase and/or other unknown proteinases, which are insensitive to the inhibitors we used and which during the oxidative stress might have degraded membrane ghost spectrin. In this respect, we decided to perform an experiment in which purified spectrin tetramer was exposed to phenylhydrazine in the presence or absence of purified hemoglobin. In order to obtain data easily comparable with those obtained in both the intact cells and membrane ghosts plus hemolysate system, the molar ratio and the final concentration of hemoglobin and spectrin were identical to those in the intact erythrocytes.

The first important consideration from the observation of Table V is that hemoglobin by itself, in the presence of phenylhydrazine, is able to induce a spectrin degradation close to the values obtained with the intact cell experiments, 65.3 and 62.3% for the α monomer, and 51.2 and 48.5% for the β monomer, respectively. In addition, the presence of hemoglobin is an absolute requirement to promote the spectrin breakdown mediated by the oxidant. The exposure of spectrin to either phenylhydrazine or hemoglobin alone did not cause any significative degradation of the cytoskeletal protein. It is noteworthy that hemoglobin is a classic initiator of phenylhydrazine oxidation, from which a series to reactive intermediates, comprised of reduced oxygen species, may be the mediator(s) of the spectrin degradation [2]. It should also be noted that in all the systems investigated the concentration of phenylhydrazine used was the maximal possible without the appearance of either a high-molecular-weight compound stabilized by intermolecular covalent bonds other than S–S bonds or a real S–S interchain high-molecular-weight compound (see also Ref. 8). In addition, the exposure of membrane ghosts containing a high-molecular-weight compound, produced from the oxidative action of a phenylhydrazine concentration more than 1 mM, to hemolysate did not affect the content of either the high-molecular-weight compound or the remaining membrane ghost proteins (data not shown).

Our data suggest that phenylhydrazine-induced spectrin degradation in red blood cells is initiated by the reactive intermediates produced during the course of the phenylhydrazine oxidation mediated by hemoglobin, most likely excluding the involvement of a proteolytic pathway devoted to the recognition of oxidatively modified proteins. In this context, it has been clearly demonstrated that oxygen free radicals can fragment or aggregate several globular proteins [34]. The same author has also reported that oxidative modification, involving alterations of the secondary an tertiary structure of bovine serum albumin and its fragmentation but not its aggregation products, increased its proteolytic susceptibility toward several proteinases, among which a novel form was identified in the cytosol of mature red blood cells [35–37]. However, they could not discriminate the extent of the proteolytic event between fragmentation and conformational alteration of bovine serum albumin exposed to oxygen radicals.

Currently, we are evaluating whether a proteolytic system is actually involved in an eventual second degradative step dedicated to the removal of the spectrin degradation products generated from the phenylhydrazine-induced oxidative stress [8].

TABLE V

Purified-spectrin degradation induced by phenylhydrazine

Purified spectrin tetramer was mixed with hemoglobin and incubated for 10 min with 1 mM phenylhydrazine. The reaction was terminated by diluting samples with 50 vol. of cold hypotonic buffer. Samples were then dialysed against the same hypotonic buffer and concentrated before solubilization in SDS sample buffer to a final concen. of 1 mg/ml and analyzed by SDS PAGE. The incubation of spectrin in the absence of hemoglobin under the same conditions resulted in no degradation. The values are the average of three experiments. The variation between one experimental value and another was not more than 5%.

	Spectrin degradation (%)	
	α monomer	β monomer
Spectrin + PH	1.5	2.3
Spectrin + HB	2.4	1.9
Spectrin + HB + PH	65.3	51.2

References

1 Chiu, D., Lubin, B. and Shohet, S.B. (1982) in Free Radicals in Biology (Pryor, W.A., ed.), Vol. 5, pp. 115–160, Academic Press, New York.
2 Stern, A. (1985) in Oxidative Stress (Sies, H., ed.), pp. 331–345, Academic Press, London.
3 Girotti, A.W. and Thomas, J.P. (1984) J. Biol. Chem. 259, 1744–1752.
4 Sullivan, S.G. and Stern, A. (1984) Biochim. Biophys. Acta 774, 215–220.
5 Girotti, A.W., Thomas, J.P. and Jordan, J.E. (1986) Arch. Biochem. Biophys. 251, 639–653.
6 Heller, K.B., Jahn, B. and Deuticke, B. (1987) Biochim. Biophys. Acta 901, 67–77.
7 Schwartz, R.S., Chiu, D. and Lubin, B. (1984) in Erythrocyte Membranes 3: Recent Clinical and Experimental Advances (Kruckenberg, W.C., Eaton, J.W., Aster, J. and Brewer, G.J., eds.), pp. 89–122, Alan R. Liss, New York.
8 Arduini, A. and Stern, A. (1985) Biochem. Pharmacol. 34, 4283–4289.
9 Arduini, A., Sullivan, S.G. and Stern, A. (1985) in Cellular and Molecular Aspects of Aging. The Red Cell As a Model (Eaton, J.W., Konzen, D.K. and White, J.G., eds.), pp. 123–131, Alan R. Liss, New York.
10 Arduini, A., Chen, Z. and Stern A. (1986) Biochim. Biophys. Acta 862, 65–71.
11 Deuticke, B., Heller, K.B. and Haest, C.W.M. (1987) Biochim. Biophys. Acta 899, 113–124.
12 Rosen, G.M., Barber, M.J. and Rauckman, E.J. (1983) J. Biol. Chem. 258, 2225–2228.
13 Beutler, E., West, C. and Blume, K.G. (1976) J. Lab. Clin. Med. 88, 328–333.
14 Winterbourn, C.C. (1986) in Handbook of Methods for Oxygen Radical Research (Greenwald, R., ed.), pp. 137–141, CRC Press, Boca Raton.
15 Drabkin, D.L. and Austin, J.H. (1932) J. Biol. Chem. 98, 719–723.
16 Bennett, V. (1983) Methods Enzymol. 906, 313–324.
17 Bennett, V. (1985) Annu. Rev. Biochem. 54, 273–304.
18 Laemmli, U.K. (1970) Nature 227, 680–685.
19 Furthmayr, H. and Marchesi, V.T. (1976) Biochemistry 15, 1137–1144.
20 Hendil, K.B. (1980) J. Cell. Physiol. 105, 449–460.
21 Dubiel, W., Muller, M. and Rappoport, S.M. (1981) Biochem. Int. 3, 165–171.
22 Chin, D., Kuehl, I. and Rechsteiner, M. (1982) Proc. Natl. Acad. Sci. USA 79, 5857–5861.
23 Fucci, L., Oliver, C.N., Coon, M.J. and Stadtman, E.R. (1983) Proc. Natl. Acad. Sci. USA 80, 1521–1525.
24 Levine, R.L. (1983) J. Biol. Chem. 258, 11823–11827.
25 Levine, R.L. (1983) J. Biol. Chem. 258, 11828–11833.
26 Matoo, A.K., Hoffman-Falk, H., Marder, J.B. and Edelman, M. (1984) Proc. Natl. Acad. Sci. USA 81, 1380–1386.
27 Nakamura, K. and Stadtman, E.R. (1984) Proc. Natl. Acad. Sci. USA 81, 2011–2015.
28 Rivett, A.J. (1985) J. Biol. Chem. 260, 300–305.
29 Stadtman, E.R. and Wittenberg, M.E. (1985) Arch. Biochem. Biophys. 239, 379–387.
30 Wolf, S.P., Garner, A. and Dean, R.T. (1986) Trends Biochem. Sci. 11, 27–31.
31 Fagan, J.M., Waxman, L. and Goldberg, A.L. (1986) J. Biol. Chem. 261, 5705–5713.
32 Davies, K.J.A. and Goldberg, A.L. (1987) J. Biol. Chem. 262, 8220–8226.
33 Davies, K.J.A. and Goldberg, A.L. (1987) J. Biol. Chem. 262, 8227–8234.
34 Davies, K.J.A. (1987) J. Biol. Chem. 262, 9895–9901.
35 Davies, K.J.A., Delsignore, M.E. and Lin, S.W. (1987) J. Biol. Chem. 262, 9902–9907.
36 Davies, K.J.A. and Delsignore, M.E. (1987) J. Biol. Chem. 262, 9908–9913.
37 Davies, K.J.A., Lin, S.W. and Pacifici, R.E. (1987) J. Biol. Chem. 262, 9914–9920.
38 Davies, K.J.A. (1988) in Cellular Antioxidant Defence Mechanisms (Chow, C.K., ed.), Vol. 2, pp. 25–67, CRC Press, Boca Raton, FL.
39 Garrison, W.M., Jayko, M.E. and Bennett, W. (1962) Radiat. Res. 16, 483–502.
40 Dean, R.T., Roberts, C.R. and Forni, L.G. (1984) Biosci. Rep. 4, 1017–1026.
41 Schuessler, H. and Schilling, K. (1984) Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 45, 267–281.
42 Chung, M.H., Kesner, L. and Chan, P.C. (1984) Agents Actions 15, 328–335.
43 Wolff, S.P. and Dean, R.T. (1986) Biochem. J. 234, 399–403.
44 Hunt, J., Simpson, J.A. and Dean, R.T. (1988) Biochem. J. 250, 87–93.
45 Misra, H.P. and Fridovich, I. (1976) Biochemistry 15, 681–687.
46 Hershko, A. and Ciechanover, A. (1982) Annu. Rev. Biochem. 51, 335–364.
47 Goldberg, B., Stern, A. and Peisach, J. (1976) J. Biol. Chem. 251, 3045–3051.
48 Pontremoli, S., Salamino, F., Sparatore, B., Melloni, E., Morelli, A., Benatti, U. and De Flora, A. (1979) Biochem. J. 181, 559–568.
49 Hatanaka, M., Yoshimura, N., Murakami, T., Kannagi, R. and Murachi, T. (1984) Biochemistry 23, 3272–3276.