Developmental regulation of rat lung Cu,Zn-superoxide dismutase

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In the present investigation we found that lung Cu,Zn-superoxide dismutase (SOD) activity (units/mg of DNA) increases steadily in the rat from birth to adulthood. The specific activity (units/ μ g of enzyme) of Cu,Zn-SOD was unchanged from birth to adulthood, excluding enzyme activation as a mechanism responsible for the increase in enzyme activity. Lung synthesis of Cu,Zn-SOD peaked at 1 day before birth and decreased thereafter to adult values. Calculations, based on rates of Cu,Zn-SOD synthesis and the tissue content of the enzyme, indicated that lung Cu,Zn-SOD activity increased during development owing to the rate of enzyme synthesis exceeding its rate of degradation by 5–10%. These calculations were supported by measurements of enzyme degradation in the neonatal (half-life, $t_{1/2}$ = 12 h) and adult lung ($t_{1/2}$ = > 100 h); the difference in half-life did not reflect the rates of overall protein degradation in the lung, since these rates were not different in lungs from neonatal and adult rats. We did not detect differences in the M_r or pI of Cu,Zn-SOD during development, but the susceptibility of the enzyme to inactivation by heat or copper chelation decreased with increasing age of the rats. We conclude that the progressive increase in activity of Cu,Zn-SOD is due to a rate of synthesis that exceeds degradation of the enzyme. The data also suggest that increased stabilization of enzyme conformation accounts for the greater half-life of the enzyme in lungs of adult compared with neonatal rats.

INTRODUCTION

The evolution of cells and tissues to their present level of complexity was probably facilitated by the increased efficiency brought to cellular energetics by the availability of molecular oxygen and the development of oxidative metabolism. However, incomplete reduction of oxygen leads to the production of superoxide (O_2^-) and other oxygen radicals that can cause considerable cellular damage by reacting with proteins, lipids and DNA (Clark & Lambertsen, 1971; Kellogg & Fridovich, 1977). As a result, enzymes such as catalase, glutathione peroxidase and glutathione reductase have assumed considerable importance in the maintenance of a favourable balance between deleterious by-products of oxygen metabolism and cellular antioxidants. Superoxide dismutase (SOD) is perhaps the most important antioxidant enzyme because of its ability to convert superoxide to the less reactive H_2O_2 , thereby diminishing the formation of the even more highly reactive hydroxyl radical ($OH^\cdot$) (Fridovich, 1972).

Bacteria and yeast grown anaerobically have very low SOD activity, but can respond to aerobic conditions with a 5–10-fold increase in SOD, presumably in response to greatly increased production of superoxide (Gregory & Fridovich, 1973; Gregory *et al.*, 1974). Similarly, exposure of neonatal animals from several species to hyperoxia results in an increase in lung SOD activity (Frank *et al.*, 1978a; Hoffman *et al.*, 1980). The capacity of neonatal animals to respond to hyperoxia with an increase in lung SOD activity appears crucial to their ability to survive prolonged hyperoxic exposure with minimal lung damage. In contrast with neonatal animals, adults of these species do not increase lung antioxidant

enzyme activity in response to hyperoxia, suffer severe O_2 -induced lung damage and die (Frank *et al.*, 1978a). Inhibition, by the copper chelator diethyl dithiocarbamate, of the usual rise in SOD that occurs in the lungs of neonatal animals exposed to hyperoxia, abrogates the neonatal animal's tolerance to O_2 and leads to fatal lung injury (Frank *et al.*, 1978b).

Cu,Zn-SOD comprises approx. 90% of the total SOD activity in adult rat lung. Its activity increases during the period shortly preceding birth, is maintained at a plateau for a few days after birth, and then gradually increases into adulthood (Tanswell & Freeman, 1984; Gerdin *et al.*, 1985). We now present evidence that: (1) the increase of Cu,Zn-SOD activity, which takes place from birth to adulthood, is brought about by a rate of Cu,Zn-SOD synthesis that slightly exceeds its rate of degradation; (2) the half-life of Cu,Zn-SOD increases from 12 h in the lungs of neonatal rats to > 100 h in the lungs of adult rats; (3) this change in degradation does not reflect differences in the overall rate of proteolysis between lungs of neonatal and adult rats, but (4) the prolongation of the half-life of the enzyme is associated with a diminution in its susceptibility to inactivation by heat or copper chelation.

EXPERIMENTAL PROCEDURES

Materials

Aquasol was obtained from New England Nuclear, and [2,6- 3H]phenylalanine was from ICN Pharmaceuticals. Triton X-100 and all chemicals used in polyacrylamide-gel electrophoresis, isoelectric focusing and immunoblotting were obtained from Bio-Rad. Soluene-

Abbreviation used: SOD, superoxide dismutase.

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350 was obtained from Packard Instruments. All other biochemicals were obtained from Sigma.

Methods

Animals. Sprague-Dawley rats (200–250 g) were obtained from Charles River Breeding Laboratories and maintained in the Animal Care Facility of the University of Miami. Timed pregnancies were produced by cohabitation of male and female rats overnight (12 h) and confirmed by the presence of sperm in the female's genital tract. The time of conception was estimated to be 02:00 h, the midpoint of the cohabitation period. The time of birth was determined by frequent observation of the gravid female during the day or was assumed to be 02:00 h if birth occurred during the night. Preterm fetuses were delivered by Caesarian section after anaesthetizing the dam with sodium pentobarbital.

Protein synthesis by the lung *in vivo*. We are unaware of reports of the rate of lung protein synthesis *in vivo* in newborn animals. Therefore, to provide a comparison for our studies of protein synthesis by lung slices, we measured the rate of lung protein synthesis *in vivo* in 6-day-old rats (Preedy *et al.*, 1983). To do this we injected a large dose of [3 H]phenylalanine (1.5 mmol/kg body wt., 50 mCi/mmol) intraperitoneally, and 4 or 14 min later the rats were anaesthetized and, within about 1 min, their lungs removed and frozen. We measured the specific radioactivity of phenylalanine released by alkaline treatment of aminoacyl-tRNA as previously described in detail (Fan *et al.*, 1982). The rate of lung protein synthesis was calculated from this specific radioactivity and from the amount of [3 H]phenylalanine incorporated into acid-precipitable lung protein (Hass *et al.*, 1982). The synthesis rate represents the mean value for six rats at each time point.

Protein synthesis *in vitro*. We killed rats by cutting the great vessels of the abdomen after the rats had been anaesthetized with sodium pentobarbital (~50 mg/kg). To remove blood from the pulmonary vasculature, the lungs were perfused with 0.154 M-NaCl/0.1 M-potassium phosphate, pH 7.4, although before 21 days of gestation the effectiveness of the perfusion was very inconsistent. The lungs were removed and sliced with a McIlwain tissue chopper set to make 1.0 mm slices. Slices from several rats were pooled to achieve approx. 0.5 g of lung tissue per incubation flask.

Each flask contained 10 ml of Krebs-Ringer bicarbonate buffer, with 5.5 mM-glucose, adult rat plasma concentrations of 19 amino acids (Morgan *et al.*, 1971) and 0.7 mM- 3 H-phenylalanine (45 Ci/mol). At this phenylalanine concentration, the specific radioactivity of tRNA-bound phenylalanine equals that of medium phenylalanine within 15 min of the start of the incubation, enabling us to use the medium specific-radioactivity value to calculate absolute rates of lung protein synthesis (Clerch *et al.*, 1987). The flasks were shaken at 120 oscillations/min at 37 °C for 2 h with a gas phase of O₂/CO₂ (19:1).

We should point out that the concentration of amino acids in the serum of newborn rats (Girad *et al.*, 1975) differs from the concentration in the serum of adult rats (Morgan *et al.*, 1971). However, it has been shown that the rate of protein synthesis by lung slices from neonatal

lungs is not different if the slices are incubated with neonatal rather than adult serum concentrations of amino acids (Clerch *et al.*, 1987).

Tissue preparation. After incubation, the lung tissue was rinsed twice in saline (0.9% NaCl), blotted and homogenized in 10 ml of 5 mM-potassium phosphate, pH 7.8, for 45 s with a Brinkman Instruments Polytron operated at the highest setting. A 1 ml portion of the homogenate was added to cold 20% (w/v) trichloroacetic acid for assay of DNA and extraction of radioactive lung protein (Schneider, 1957). The remainder of the homogenate was centrifuged at 100 000 *g* for 1 h (4 °C). The supernatant fraction was dialysed overnight in 4 litres of the homogenizing buffer, then centrifuged for 20 min at 20 000 *g* (4 °C).

Enzyme analysis. Lung Cu,Zn-SOD activity was determined in the dialysed clarified 100 000 *g* supernatant fraction using the xanthine oxidase–ferricytochrome *c* method that at pH 7.8 distinguishes Cu,Zn- and Mn-forms of the enzyme by differential sensitivity to 10 mM-NaCN (McCord & Fridovich, 1969). Values for enzyme activity and protein-synthesis rates are expressed per mg of DNA, the ratio of protein to DNA remaining virtually constant throughout postnatal lung development (Clerch *et al.*, 1987). The 100 000 *g* supernatant fraction was also used for immunoassay of Cu,Zn-SOD by nephelometry and for immunoprecipitation of Cu,Zn-SOD using monospecific goat antibodies prepared against purified rat liver Cu,Zn-SOD (Hass *et al.*, 1982).

Electrophoresis on 11%-(w/v)-polyacrylamide slab gels was by the method of Laemmli (1970), except that the electrode buffer was 0.1 M-Tris/0.03 M-glycine, pH 8.8. Blotting on to nitrocellulose membranes was by mass action, using 2–4 litres of 25 mM-potassium phosphate, pH 7.8. Cu,Zn-SOD was located by incubating the membranes with goat anti-(rat Cu,Zn-SOD) serum and then with Protein A–Gold, followed by silver staining using the Bio-Rad silver enhancement kit.

Fig. 1. Developmental time course of Cu,Zn-SOD activity and content

Lung 100 000 *g* supernatant fractions were assayed enzymically for enzyme activity (●) and immunologically for enzyme content (○).

Table 1. Specific activity of Cu,Zn-SOD during development

Cu,Zn-SOD activity and content both were determined in the same lung 100 000 g supernatant fraction from rats of various ages. The specific activity was calculated as the ratio of enzyme activity to enzyme content. Data are means $\pm$ S.E.M. of values from the number of rats indicated in parentheses.

Age (days)	Cu,Zn-SOD specific activity (units/ μ g)
20 (gestation)	9.9 $\pm$ 1.1 (9)
21 (gestation)	3.8 $\pm$ 0.7 (6)
1 (term)	10.2 $\pm$ 0.9 (11)
3	9.7 $\pm$ 0.9 (8)
8	9.3 $\pm$ 1.6 (5)
12	8.6 $\pm$ 1.1 (9)
25	8.1 $\pm$ 1.1 (6)
60 (adult)	8.2 $\pm$ 0.6 (7)

Isoelectric focusing. Isoelectric-focusing gels (7.98 % polyacrylamide) with a pH gradient from approx. 4.0 to 6.5 were prepared by adding ampholyte mixtures at the following concentrations: Biolytes 3–10, 0.5 % (v/v), Biolytes 4–6, 2.5 % (v/v), and Biolytes 5–7, 2.5 % (v/v). Lung samples were electrofocused for 16 h at 400 V and then for 1 h at 800 V, with 20 mM-sodium hydroxide as cathode solution and 20 mM-acetic acid as anode solution. Some gels were electroblotted at 500 mA for 8 h, and at 250 mA for 12 h in 0.7 % acetic acid. Cu,Zn-SOD was then revealed by immunolocalization as previously described above. Other gels were stained for Cu,Zn-SOD activity by the NitroBlue Tetrazolium method (Beauchamp & Fridovich, 1971).

Statistical analysis. Data are expressed as means $\pm$ S.E.M. Differences between group means were determined by analysis of variance (Duncan, 1955; Kramer, 1956). Protein half-lives were calculated from the group means by using least-squares analysis.

Fig. 2. Developmental time course of Cu,Zn-SOD and total protein synthesis by lung slices

Incorporation of [3 H]phenylalanine into Cu,Zn-SOD (●) was determined by immunoprecipitation. Incorporation into total protein (○) was determined by precipitation with trichloroacetic acid. * $P < 0.05$ versus values at other ages (analysis of variance).

RESULTS

Developmental time course of Cu,Zn-SOD activity

Lung Cu,Zn-SOD activity decreased from gestational day 20 to 21, then rose sharply during the rest of gestation, reaching a perinatal peak at postnatal day 3 (Fig. 1). After decreasing 20 % from day 3 to day 8, Cu,Zn-SOD activity resumed its rise, reaching an adult level 3 times that at birth. With the exception of gestational day 21, the amount of lung Cu,Zn-SOD protein detected by immunoassay closely paralleled the Cu,Zn-SOD enzymic activity, resulting in the specific activity remaining nearly constant throughout development (Table 1).

Table 2. Calculated rates of Cu,Zn-SOD turnover

Average rates of Cu,Zn-SOD turnover were determined by subtraction, using the measured increment in enzyme content and the average rate of enzyme synthesis for each age interval. Each synthesis rate (ng of SOD/h per mg of DNA) was calculated by dividing the age interval's average value for phenylalanine incorporation (pmol/h per mg of DNA) (Fig. 2) by 6 mol of phenylalanine/mol of enzyme and multiplying by the M_r , 32 000 (McCord & Fridovich, 1969). The synthesis rate (ng of SOD/h per mg of DNA) was then multiplied by the time interval (h) to obtain the total (ng/mg of DNA) enzyme synthesized. The difference of the total enzyme synthesized less the increment in enzyme content was divided by the time interval to obtain the rate of enzyme degradation (ng of SOD/h per mg of DNA). Abbreviation: g, gestational.

Age interval (days)	Increment in Cu,Zn-SOD content (ng of SOD/mg of DNA)	Cu,Zn-SOD synthesis rate (ng of SOD/h per mg of DNA)	Cu,Zn-SOD degradation rate (ng of SOD/h per mg of DNA)	Ratio (degradation/synthesis)	Fractional degradation rate (K_d) (%/h)	Half-life ($t_{1/2}$) (h)
21 g–1	300	154	148	0.96	11.3	6
1–3	500	105	95	0.92	6.6	11
3–8	–120	66	67	1.02	3.5	20
8–12	–40	50	51	1.02	2.8	25
12–60	3360	38	36	0.95	2.0	35
60–		31	28	0.92	0.5	139

Fig. 3. Degradation rates of Cu,Zn-SOD and total protein in lung slices

Lung slices from 1-day-old (●) or 60-day-old (○) rats were labelled by incubation in medium containing trace [^3H]phenylalanine for 1 h. The slices were rinsed with saline, blotted and divided between flasks. The first flask was frozen (-20°C) immediately. The remaining three flasks were incubated, for the time indicated, in non-radioactive medium containing 10 mM-phenylalanine, sufficient to prevent reincorporation of released [^3H]phenylalanine by the lung (Chiang *et al.*, 1979). After appropriate treatment, all samples were subsequently thawed and homogenized. (a) Radioactivity remaining in Cu,Zn-SOD was determined by immunoprecipitation. (b) Radioactivity remaining in total protein was determined by precipitation with trichloroacetic acid.

Protein and Cu,Zn-SOD synthesis and degradation

The rate of protein synthesis by lung slices was high during late gestation, fell at birth and, by the third postnatal day, reached and then maintained a level similar to that of adult rats (Fig. 2). Synthesis of Cu,Zn-SOD was low on gestational days 20 and 21, increased 4-fold on gestational day 22, fell rapidly over the next 72 h, and then gradually decreased to adult values (Fig. 2). The lung protein-synthesis rate *in vivo* (32.1 nmol of phenylalanine incorporated/h per mg of DNA, $n = 6$) measured in 6-day-old rats was $\sim 30\%$ higher than the rate *in vitro* (24.0 nmol of phenylalanine incorporated/h per mg of DNA). In addition, Cu,Zn-SOD synthesis *in vivo* (18.7 pmol of phenylalanine incorporated/h per mg

of DNA, $n = 5$) constituted 0.06% of total lung protein synthesis, compared with 0.04% in the experiments *in vitro* (see Fig. 2 for values *in vitro*).

Because the adult lung contained three times more Cu,Zn-SOD/mg of DNA than the neonatal lung, despite having a lower rate of Cu,Zn-SOD synthesis, it appeared that alterations in enzyme degradation might play an important role in determining the level of Cu,Zn-SOD activity throughout development. We therefore used our values for Cu,Zn-SOD synthetic rates and for Cu,Zn-SOD immunodetectable protein and calculated both the average rates of enzyme synthesis during the intervals between the developmental ages examined and the predicted average rates of enzyme degradation (Table 2). From the calculations it appeared that, throughout most of the period studied, Cu,Zn-SOD synthesis exceeds Cu,Zn-SOD degradation by 5–10%, and that the half-life ($t_{1/2}$) of Cu,Zn-SOD progressively increased throughout development.

These considerations led us to estimate the half-life of Cu,Zn-SOD. To do this we labelled Cu,Zn-SOD by incubating lung slices for 1 h with [^3H]phenylalanine, followed by incubation in non-radioactive medium containing 10 mM-phenylalanine (pulse-chase), and determined the radioactivity remaining in Cu,Zn-SOD after various intervals. We found that Cu,Zn-SOD in

Fig. 4. Electrophoresis and immunolocalization of Cu,Zn-SOD from lungs of rats of various ages

Lungs were perfused with saline and homogenized in 5 mM-potassium phosphate, pH 7.8, containing 1 μg of leupeptin/ml, 1 μg of pepstatin/ml, 10 mM-sodium bisulphite, 1 mM-benzamidine, 0.2 mM-phenylmethylsulphonyl fluoride and 100 μg of soybean trypsin inhibitor/ml. The homogenates were centrifuged at 100 000 g for 1 h and supernatant fractions isolated; a sample was subjected to electrophoresis. After electrophoresis the enzyme was localized by immunoblotting as described under 'Methods'. Lanes: 1, 21 days gestation; 2, 22 days gestation; 3, 1 day (postnatal); 4, 8 days; 5, 15 days; 6, 30 days; 7, 60 days. The migration of protein standards having known M_r values is indicated on the left of the gel.

Fig. 5. Isoelectric focusing and immunolocalization of Cu,Zn-SOD from lungs of rats of various ages

Lung supernatant fractions were prepared as described in the legend to Fig. 4. After isoelectric focusing the enzyme was localized by immunoblotting as described under 'Methods'. Lanes: 1, 21 days gestation; 2, 1 day (postnatal); 3, 8 days; 4, 60 days.

Fig. 6. Localization of Cu,Zn-SOD activity after isoelectric focusing

Lung supernatant fractions were prepared as described in the legend to Fig. 4. After isoelectric focusing the enzyme activity was localized by using the NitroBlue Tetrazolium stain (Beauchamp & Fridovich, 1971). Lanes: 1, 21 days gestation; 2, 1 day (postnatal); 3, 8 days; 4, 30 days; 5, 60 days.

1-day lung had a $t_{1/2}$ of 12 h, whereas the $t_{1/2}$ in adult lung was > 100 h (Fig. 3a). These differences were not attributable to altered rates of overall lung proteolysis, since the $t_{1/2}$ of general lung protein was nearly equal in the neonatal animal ($t_{1/2} = 11$ h) and the adult ($t_{1/2} = 9$ h) (Fig. 3b).

Developmental changes in stability of Cu,Zn-SOD

To investigate whether there were physical differences in rat lung Cu,Zn-SOD at different ages, lung 100 000 g supernatant fractions were first subjected to SDS/polyacrylamide-gel electrophoresis. The mobility of Cu,Zn-SOD, assessed by immunolocalization, failed to reveal detectable changes in the M_r of Cu,Zn-SOD (Fig. 4). Immunolocalization of Cu,Zn-SOD after isoelectric focusing showed the presence of two to three bands in material from lungs of rats at least 8 days of age, but only one band in lungs of younger rats (Fig. 5). However, localizing Cu,Zn-SOD by enzyme activity on similar gels

Fig. 7. Heat-inactivation of Cu,Zn-SOD from lungs of rats of various ages

Lung 100 000 g supernatant fractions prepared as described in the legend to Fig. 4 were incubated at 80 °C for the times indicated. Cu,Zn-SOD activity was determined at each time point and compared with that of the sample before heating.

Fig. 8. Inactivation of Cu,Zn-SOD by diethyl dithiocarbamate

Lung 100 000 g supernatant fractions prepared as described in the legend to Fig. 4 were incubated for 2 h with the indicated concentrations of diethyl dithiocarbamate. Cu,Zn-SOD activity was determined and compared with that of the sample before incubation.

showed five to six isoenzymes in rat lungs of each age examined, without clear differences in the relative intensity of the bands (Fig. 6).

Cu,Zn-SOD activity from lungs of different ages was found to be stable to heating at 70 °C for at least 1 h. At 80 °C, Cu,Zn-SOD from fetal and newborn lungs was rapidly and completely inactivated (not shown); thereafter enzyme stability increased with increasing age of the rat (Fig. 7). When extracts from 1-day and 60-day lungs were mixed, the heat-stability of Cu,Zn-SOD was intermediate (56%) between the two individual values, indicating the absence of diffusible factors capable of altering the enzyme's stability.

Lung Cu,Zn-SOD from 1-day-old rats was nearly

completely inhibited by 2 h incubation with 1 mM-diethyl dithiocarbamate (a copper chelator), whereas Cu,Zn-SOD from lungs of 30-day- and 60-day-old rats was considerably more resistant to inactivation (Fig. 8). Cu,Zn-SOD purified 50–80-fold from lungs of both 1-day-old and 60-day-old rats (Crapo & McCord, 1976) retained its full activity and normal electrophoretic migration on SDS/polyacrylamide gels despite 2 h incubation with 1 mg of trypsin/ml at 37 °C (results not shown).

DISCUSSION

Developmental time course of Cu,Zn-SOD

A late gestational increase in activity of the major antioxidant enzymes has been found in the lungs of all species studied (Frank, 1985). The signal for this increased activity, especially as it pertains to the SODs, is not known. Since, in general, there seems to be a balance between the substrate, superoxide, and the activity of the SODs (Gregory & Fridovich, 1973), one could argue that superoxide production increases with development, perhaps owing to improved vascularization, and hence oxygenation, of the lung, as occurs in late gestation (Burri, 1985). However, fibroblasts from fetal-rat lungs, maintained in culture under hypoxic conditions [O_2 partial pressure (pO_2) of 3.33 kPa (~ 25 torr)] similar to those present in the fetus, undergo a spontaneous increase in SOD activity (Randhawa *et al.*, 1987). This implies the increase in activity *in vivo* is programmed within at least some of the cells of the lung. This suggestion is supported by the observation that a corticosteroid (dexamethasone), which as a class accelerates differentiation of some types of lung cells (Kikkawa *et al.*, 1971; Burri, 1985), stimulates the developmental increase in SOD activity both *in vivo* and *in vitro* (Frank *et al.*, 1985; Randhawa *et al.*, 1986).

In comparison with the prenatal increase in lung antioxidant enzymes, the stimulus for an early postnatal increase in SOD activity is understandable, since the change from a fetal environment with a pO_2 of 3.33 kPa to a postnatal one with a pO_2 of 13.3 kPa probably increases the production of superoxide (Dejours, 1981). In addition, there is a postnatal increase in the volume fraction of mitochondria in the two lung cells in which this parameter has been measured (Massaro & Massaro, 1986; Massaro *et al.*, 1986), and, since mitochondria are a source of superoxide (Fisher *et al.*, 1984), there might be a further increase in superoxide production on these grounds alone, without a change in pO_2 . The basis for the continued (2-fold) increase in SOD activity, which occurs between age 12 days and adulthood (Fig. 2), is not clear. However, there is a comparable time course of increase in the fraction of lung cell volume occupied by mitochondria during this period. Hence, because mitochondria are a source of superoxide, and since there seems to be a balance between the substrate and the enzymes that dismutate it, the increase in SOD activity may be in response to a greater production of its substrate, superoxide.

Mechanisms for the developmental changes in Cu,Zn-SOD activity

Since we had difficulty perfusing fetal lungs free of blood, it is possible that blood contamination influenced the results of our studies on fetal lungs. This probably

did not affect our measurement of Cu,Zn-SOD activity, however, since erythrocyte contribution to SOD activity in lung during the perinatal period is only 1–3 % (Tanswell & Freeman, 1984). The constancy, except for the value on gestational day 21, of the specific activity (units/ μ g) of Cu,Zn-SOD (Table 2), excludes the possibility that enzyme activation contributes to the increase in lung Cu,Zn-SOD activity.

The measurement of the specific radioactivity of phenylalanine in the medium, which has been shown to equal that of phenylalanine released from phenylalanyl-tRNA of lung slices under the conditions of our experiments (Clerch *et al.*, 1987) has allowed us to measure the absolute rate of synthesis of lung proteins including Cu,Zn-SOD. We think the measurements in neonatal lungs have physiological relevance, since the rates in slices are 60–70 % of the rates *in vivo* we observed in neonatal rats. In addition, the rate of protein synthesis we found in slices made from lungs of adult rats is virtually identical with that reported in the intact perfused and ventilated adult rat lung (Watkins & Rannels, 1980).

Our measurements of the rates of synthesis of Cu,Zn-SOD and of the Cu,Zn-SOD immunodetectable protein allowed us to calculate that the progressive rise in Cu,Zn-SOD activity was brought about because, over most of the period studied, Cu,Zn-SOD synthesis exceeds its degradation by 5–10 %. Our measurements of the degradation of the enzyme do not allow us to generate an absolute rate of degradation, but in a general way they support our calculations in that we found at least a 10-fold prolongation of the half-life of the enzyme in the adult compared with the neonatal animal. This large prolongation of the half-life of Cu,Zn-SOD occurred without the development of a difference in the overall rate of lung proteolysis between neonates and adults.

Developmental changes in stability of Cu,Zn-SOD

Since the half-life of general lung proteins did not change between the neonatal period and adulthood, we sought a change in the enzyme itself that might decrease its susceptibility to proteolysis. The M_r of Cu,Zn-SOD does not differ at the various ages, but the enzyme's resistance to inactivation by heat and copper chelation does correlate with the calculated half-lives, suggesting the possibility that low- M_r ligands or non-covalent binding proteins may be involved in limiting the enzyme's degradation, perhaps by causing conformational changes in the enzyme's tertiary structure. Although isoelectric focusing fails to demonstrate the appearance of new Cu,Zn-SOD forms, it cannot exclude developmental changes in the relative proportion of the various forms that may also differ in their enzyme stability. The biphasic behaviour of the enzyme during inactivation by heat and copper chelation does suggest the presence of a mixture of enzyme molecules, some of which are more stable than others. The marked difference in enzyme half-life that occurs as the animal ages makes it an excellent molecule to test the recent hypothesis that the N-terminal amino acid determines the susceptibility of a protein to degradation (Bachmair *et al.*, 1986). Decreased enzyme $t_{1/2}$ with aging has been described previously for liver ferritin in rats of age 0.5–1.5 years (Ove *et al.*, 1972), but to our knowledge no reports investigating protein half-lives during early postnatal development have been published.

In studies of protein properties during long-term aging (i.e. 0.5–2.5 years), M_r is generally unchanged, whereas susceptibility to heat inactivation frequently increases (Rothstein, 1977). Several years ago, Reiss & Gershon (1976) reported that rat liver Cu, Zn-SOD M_r , antigenic and kinetic properties were unaltered between 0.5 and 2.3 years of age, whereas its susceptibility to heat inactivation increased, suggesting the accumulation of abnormal or modified enzyme molecules (Reiss & Gershon, 1976). In our study of the period from birth to early adulthood (60 days), there was an apparent, though not statistically significant, trend toward decreased enzyme specific activity (units/ μ g) with age, suggesting the possible accumulation of inactive enzyme molecules, but the increasing stability of the enzyme to heat and copper chelation with age appears to contradict this possibility and provides additional evidence of developmental alterations in enzyme stability.

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