Cellular adaptation to down-regulated iron transport into lymphoid leukaemic cells: effects on the expression of the gene for ribonucleotide reductase

Christopher R. CHITAMBAR*[1], Janine P. WERELEY*, Thomas HEIMAN†, William E. ANTHOLINE‡ and William J. O'BRIEN†

*Division of Hematology/Oncology, Department of Medicine, Medical College of Wisconsin, 9200 West Wisconsin Ave, WI 53226, Milwaukee, U.S.A., †Department of Ophthalmology, Medical College of Wisconsin, 9200 West Wisconsin Ave, WI 53226, Milwaukee, U.S.A., and ‡Biophysics Research Institute, Medical College of Wisconsin, 9200 West Wisconsin Ave, WI 53226, Milwaukee, U.S.A.

Ribonucleotide reductase is an iron-containing enzyme that is essential for DNA synthesis. Whereas previous studies have used various iron chelators to examine the relationship between cellular iron metabolism and ribonucleotide reductase activity in cells, they have not elucidated the relationship between iron transport into cells and the expression of the gene for ribonucleotide reductase. To investigate this, we examined ribonucleotide reductase mRNA, protein and enzyme activity in a novel line of CCRF-CEM cells (DFe-T cells) that display an approx. 60 % decrease in their uptake of iron compared with the parental wild-type cell line. We found that DFe-T cells displayed an approx. 40 % decrease in ribonucleotide reductase specific enzyme activity relative to wild-type cells without a change in their proliferation. Kinetic analysis of CDP reductase activity revealed an approx. 60 % decrease in V_{max} in DFe-T cells without a change in K_m. Despite the decrease in enzyme activity, the mRNA and protein for the R1 and R2 subunits of ribonucleotide reductase in DFe-T cells were similar to those of wild-type cells. ESR spectroscopy studies revealed that DFe-T cells had a 22 % decrease in the tyrosyl free radical of the R2 subunit, suggesting that a larger amount of R2 protein was present as functionally inactive apo-R2 in these cells. Our studies indicate that ribonucleotide reductase activity in CCRF-CEM cells can be down-regulated by more than 50 % in response to down-regulated iron transport without an adverse effect on cell proliferation. Furthermore, our studies suggest a regulatory link between ribonucleotide reductase activity and iron transport into these cells.

Key words: cell proliferation, DNA synthesis, gallium, iron metabolism, tyrosyl radical.

INTRODUCTION

It is known that iron is essential for cell viability and proliferation [1]. Iron is a critical component of various proteins of the citric acid cycle and the mitochondrial electron transport chain [2] and is required for certain steps in the G_1 phase of the cell cycle [3,4]. However, its role in DNA synthesis relates to the activity of ribonucleotide reductase, an iron-containing enzyme responsible for the synthesis of deoxyribonucleotides [5–8]. Mammalian ribonucleotide reductase consists of two heterodimeric subunits termed R1 and R2, both of which are essential for enzyme activity and are encoded by different genes [9,10]. The R1 subunit first appears as cells enter G_1 from G_0 and remains constant throughout the cell cycle, whereas the R2 subunit gains prominence during late G_1 and early S phase [11–14]. The R1 subunit has substrate-binding and effector-binding sites [5–7], whereas the R2 subunit contains a binuclear non-haem iron centre and a tyrosyl free radical that produces a characteristic signal on ESR spectroscopy [15,16]. Both the iron centre and the tyrosyl radical of the R2 subunit are essential for the activity of ribonucleotide reductase.

Previous studies examining the role of iron in ribonucleotide reductase activity in cells have relied primarily on the use of various iron chelators to alter cellular iron status [17–20]. However, a potential limitation to that investigative approach is that iron chelators can also bind other metals and might therefore perturb cellular processes unrelated to iron. Moreover, chelators fail to distinguish between iron present in transport and intracellular compartments, because both might be indiscriminately affected by iron chelation. Therefore earlier studies have not specifically determined the effects of altered membrane iron transport as such on ribonucleotide reductase.

As part of our studies of the interaction of gallium with biological systems, we have developed a line of gallium-resistant human leukaemic CCRF-CEM cells that display a stable decrease in their transferrin-dependent and transferrin-independent uptake of iron [21]. These cells, designated DFe-T (decreased Fe transport) cells, have provided us with a model system to examine how the selective down-regulation of membrane iron transport affects the expression of the gene for ribonucleotide reductase. Our studies suggest that in intact cells, ribonucleotide reductase enzymic activity, but not the expression of its R1 and R2 subunits, is linked to iron transport.

MATERIALS AND METHODS

Materials

Aprotinin, PMSF, AMP-*p*-nitrophenyl phosphate, magnesium acetate, snake-venom phosphodiesterase I (from *Crotalus adamanteus*), dithiothreitol and nitrilotriacetic acid (NTA) were obtained from Sigma Chemical Company (St. Louis, MO,

Abbreviations used: DFe-T cells, decreased iron-transport cells; NTA, nitrilotriacetic acid.

[1] To whom correspondence should be addressed (e-mail chitambr@mcw.edu).

© 2000 Biochemical Society

U.S.A.). $^{59}FeCl_3$ and [^{32}P]dCTP were purchased from Amersham (Arlington Heights, IL, U.S.A.). ^{59}Fe-NTA was prepared as described previously [22]. [^{14}C]CDP (400 mCi/mmol) was obtained from Moravik Biochemicals (Brea, CA, U.S.A.).

Cells

Human T lymphoblastic leukaemic CCRF-CEM cells (wild-type cells) were obtained from American Type Culture Collection (Manassas, VA, U.S.A.) and were grown in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum in an air/CO_2 (47:3) atmosphere. The amount of iron in serum-supplemented medium is approx. 145 ng/ml (bound to bovine transferrin). A gallium-resistant CCRF-CEM cell line was developed from the parent line through a process of continuous exposure of cells to gallium nitrate over several months. These cells (DFe-T cells) displayed a decrease in iron uptake and were propagated in medium without gallium nitrate. Cell growth rates in culture were determined by counting cells with a haemocytometer.

cDNA probes and antibodies

The cDNA species for the R1 and R2 subunits of human ribonucleotide reductase cloned into the vector pCRII (Invitrogen, Carlsbad, CA, U.S.A.) were kindly provided by Dr. Yun Yen (City of Hope National Medical Center, Duarte, CA, U.S.A.), and have been described previously [23]. The R1 and R2 cDNA inserts were excised from the plasmids by using *Sac*1 and *Bam*HI respectively and were ^{32}P-labelled by a random primer method with a RadPrime DNA Labelling System from Gibco BRL (Gaithersburg, MD, U.S.A.). Mouse monoclonal antibody AD203 against human R1 was obtained from Accurate Chemical and Scientific Corp. (Westbury, NY, U.S.A.). Rabbit antiserum against human R2 was generously provided by Dr. Timothy Kinsella (Case Western Reserve University, Cleveland, OH, U.S.A.) and has been described previously [24].

Uptake of ^{59}Fe by cells

Iron uptake by wild-type and DFe-T cells was measured by using ^{59}Fe-NTA, a soluble iron complex, as a source of iron. Cells were plated in 1 ml multiwell plates (2×10^5 cells in 1 ml of medium per well) containing ^{59}Fe-NTA (4.2 ng of Fe; 20000 c.p.m./ml) and were incubated for 24–72 h in a CO_2 incubator. At specific time points, the cell number was determined and the cells were removed from the wells and washed twice by centrifugation with ice-cold PBS [10 mM K_2HPO_4/KH_2PO_4/150 mM NaCl (pH 7.4)]. ^{59}Fe radioactivity in the cell pellet was determined with a Wallac Compugamma gamma counter (Wallac, Gathersburg, MD, U.S.A.) and the amount of radioactivity in the cell pellet was expressed as c.p.m. per 10^6 cells.

RNA isolation and Northern blotting

Total cellular RNA was isolated from cells by using RNAzol (Tel-Test, Friendswood, TX, U.S.A.) in accordance with the manufacturer's recommendations. The integrity of the RNA was verified by agarose-gel electrophoresis. RNA (20 μg) from each preparation was subjected to electrophoresis on a 1% (w/v) agarose gel containing 2.2 M formaldehyde. The gel was stained with ethidium bromide to monitor for equal loading of RNA on the gel. RNA was transferred from the gel to Nytran membranes (Schleicher & Schuell, Keene, NH, U.S.A.) by capillary blotting. R1 and R2 mRNA were detected by sequential hybridization of the membranes with the corresponding ^{32}P-labelled cDNA probes (1.2×10^6 c.p.m./ml) by using QuikHyb Hybridization Solution (Stratagene, La Jolla, CA, U.S.A.) in accordance with the manufacturer's recommendations. Autoradiography of the membranes was performed by exposing the membranes to XAR-5 film (Eastman Kodak, Rochester, NY, U.S.A.) at -70 °C for 24–48 h. For quantification of band intensities, the ethidium-bromide-stained gel (to monitor RNA loading) and the autoradiograph were scanned with an AMBIS Optical Imaging System (AMBIS, San Diego, CA, U.S.A.).

Western blotting

R1 and R2 proteins in wild-type and DFe-T cells were detected by Western blotting with an ECL™ Western blotting detection system (Amersham, Arlington Heights, IL, U.S.A.). Cells (10^7) were harvested after 24–72 h of growth in fresh medium, washed twice by centrifugation with ice-cold 10 mM Tris/HCl (pH 7.4)/150 mM NaCl buffer and lysed in 200 μl of 50 mM Tris/HCl (pH 7.4)/150 mM NaCl buffer containing 1% (v/v) Triton X-100, 1% (w/v) sodium deoxycholate, 100 μg/ml PMSF, 75 μg/ml aprotinin and 1 mM sodium orthovanadate. Cellular debris was removed by brief centrifugation and the supernatant was saved for the measurement of protein content by bicinchoninic acid assay (Pierce, Rockford, IL, U.S.A.) and for the analysis of R1 and R2 protein levels by Western blotting. SDS/PAGE of the samples was performed as described [25]. Proteins were transferred from the gel to a nitrocellulose membrane as described [26], by using a Transblot system (Bio-Rad, Richmond, CA, U.S.A.). Membranes were first incubated for 1 h at room temperature in blocking buffer [PBS with 0.1% (v/v) Tween-20 containing 10% (w/v) non-fat dried milk], followed by sequential washes in PBS/Tween. Membranes were then incubated for 1 h at room temperature in PBS/Tween containing the primary antibody [either R1 (1:50 dilution) or R2 (1:10000) dilution]. Next the membranes were washed with PBS/Tween and incubated for 1 h at room temperature in the same buffer containing the secondary antibody [sheep anti-mouse Ig for R1 (1:3000 dilution), and donkey anti-rabbit Ig for R2 (1:10000 dilution)] conjugated with horseradish peroxidase. Membranes were then immersed in enhanced chemiluminescence detection solution and exposed to XAR-5 film for autoradiography.

CDP reductase assay

To prepare cell extracts for the CDP assay, cells (approx. 5×10^8) were harvested after 36 h of growth in fresh medium, washed by centrifugation with PBS and resuspended in homogenization buffer (100 mM Hepes, pH 7.4, containing 2 mM dithiothreitol and 0.2 mg/ml aprotinin). Cells were homogenized with a Dounce homogenizer equipped with a motor-driven pestle (20 strokes) and the lysate obtained was centrifuged at 10000 ***g*** at 4 °C for 20 min. The supernatant was removed and centrifuged at 100000 ***g*** at 4 °C for 90 min. The supernatant from this step was removed and proteins in it were precipitated by the addition of solid $(NH_4)_2SO_4$ (final 60%-satd. solution). After centrifugation of the sample (40000 ***g*** for 30 min), the pellet was dissolved in homogenization buffer and the sample was desalted by passing it through a small Sephadex G25 column. The eluted fractions with the highest protein concentration were pooled and stored at -80 °C. CDP reductase activity in the cell extract was assayed by using a modification of a method described previously [27,28]. The assay mixture (total volume 150 μl) contained 1.0 μmol of sodium phosphate buffer, pH 7.0, 0.60 μmol of magnesium acetate, 0.90 μmol of dithiothreitol, 0.15 μmol of AMP-*p*-nitrophenyl phosphate, 75–250 μg of cytoplasmic protein and 0.75–4.5 nmol of [U-^{14}C]CDP (specific radioactivity 20.4 μCi/mmol). (The amount of protein in the assay was varied

© 2000 Biochemical Society

from 75 to 250 μg to confirm that CDP reductase activity increased linearly over this range of protein concentration. Once this had been established, CDP reductase assays were performed with 200 μg of protein from wild-type and DFe-T cells.) After incubation of the mixture at 37 °C for 30 min, the reaction was stopped by heating it in a boiling-water bath for 4 min. The samples were cooled and incubated for 2 h with snake-venom phosphodiesterase mixture consisting of 1.2 μmol of Tris/HCl, pH 8.7, 3.0 μmol of $MgCl_2$, 20 nmol of dCMP and 1 mg/ml snake-venom phosphodiesterase. After the incubation with phosphodiesterase mixture, the sample was applied to a Dowex/borate column to separate cytidine from deoxycytidine [27,28]. Enzyme kinetic data were analysed with a computer program of Cleland [29].

ESR spectroscopy studies

Studies of the tyrosyl free radical of the R2 subunit were performed on intact cells as described previously [30]. Cells were harvested after 36 h of growth in culture and washed twice with ice-cold PBS. X-band ESR spectra were obtained with a standard Century series Varian E-100 spectrometer operating at X-band (9–9.5 GHz) and using 100 kHz field modulation. Direct ESR measurements were performed on frozen samples of cells (5×10^8 cells, 0.5 ml packed volume) in quartz finger Dewar vessels at −196 °C [30]. ESR spectra were recorded at least four times and were averaged by computer.

RESULTS

Cell growth

Wild-type and DFe-T cells were plated at 2×10^5 cells/ml; cell number was determined after specific times in culture. After 24, 48 and 72 h in culture, cell numbers were $(0.45 \pm 0.03) \times 10^6$, $(1.0 \pm 0.1) \times 10^6$ and $(1.9 \pm 0.1) \times 10^6$ cells/ml for wild-type cells, and $(0.4 \pm 0.03) \times 10^6$, $(1.1 \pm 0.1) \times 10^6$ and $(1.9 \pm 0.1) \times 10^6$ cells/ml for DFe-T cells respectively (means ± S.E.M. for three experiments). Hence the growth rate of DFe-T cells was similar to that of the parental wild-type cell line.

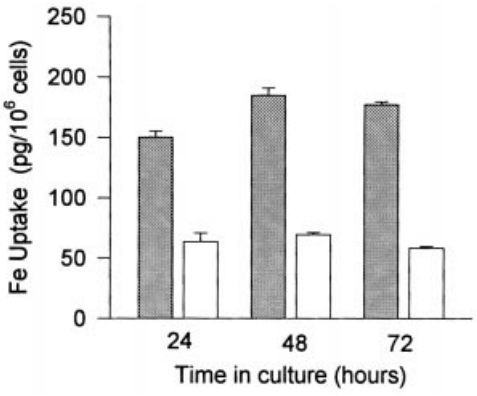

Figure 1 Uptake of ^{59}Fe by cells

DFe-T cells (open columns) and wild-type cells (grey columns) were incubated with ^{59}Fe-NTA and ^{59}Fe uptake was determined at specific time points as described in the Materials and methods section. Results are means ± S.E.M. for a representative experiment performed in triplicate.

Iron uptake by cells

We have shown that the uptake of iron by DFe-T cells over a 24 h period is decreased in comparison with wild-type cells [21,31]. In the present study, ^{59}Fe uptake by cells was measured over a longer period by incubating cells with ^{59}Fe-NTA for up to 72 h. As shown in Figure 1, the uptake of iron by DFe-T cells over 24, 48 and 72 h of incubation was 42 %, 38 % and 33 % of that by wild-type cells respectively. Therefore iron accumulation in DFe-T cells was decreased relative to wild-type cells regardless of the duration of incubation with exogenous iron. These findings are consistent with our earlier report that showed that the uptake of ^{59}Fe-transferrin or ^{59}Fe-pyridoxal isonicotinoyl hydrazone, over a wide range of concentrations, is down-regulated in DFe-T cells [21]. It should also be noted in Figure 1 that after the first 24 h of incubation, wild-type cells displayed only modest further increases in their net accumulation of iron (the mean increases in iron taken up per 10^6 cells were 23 % and 18 % at 48 and 72 h respectively, relative to the 24 h uptake; $P < 0.02$ by Student's paired t test). In contrast, the net accumulation of iron in DFe-T cells did not change significantly beyond the initial 24 h of incubation.

CDP reductase activity

To determine the impact of the decrease in iron uptake by DFe-T cells on ribonucleotide reductase activity, enzyme activity was measured with a CDP reductase assay. In DFe-T cells the specific activity of CDP reductase was decreased on average by approx. 40 % in preparations made from four individual pairs of cell cultures. The specific activity of the enzyme in wild-type cells was 192 ± 49 pmol/30 min per mg of protein, whereas the activity of the enzyme in DFe-T cells was 108 ± 33 pmol/30 min per mg of protein ($P < 0.05$, $n = 4$). An analysis of the enzyme kinetics of CDP reductase activity in these four pairs of enzyme preparations is shown in Table 1; it indicates that the K_m of wild-type enzyme was not significantly different from that in DFe-T cells. However, the V_{max} of the enzyme contained in wild-type cells was significantly greater than that in the DFe-T cells (Table 1). These results suggest that DFe-T cells contain smaller amounts of active enzyme than wild-type cells.

Ribonucleotide reductase mRNA and protein expression

To determine whether the decrease in ribonucleotide reductase enzyme activity in DFe-T cells was due to a quantitative decrease in enzyme, cells were analysed for the expression of R1 and R2 mRNA and R1 and R2 protein. Wild-type and DFe-T cells were analysed under similar conditions of growth after different durations in culture and were found to contain equivalent levels of R1 and R2 mRNA and protein. The cDNA probe for R1 mRNA detects a single 3.0 kb transcript [23]. As shown in Figure 2(A), wild-type and DFe-T cells expressed comparable levels of R1 mRNA after 24 and 48 h of incubation. Densitometry analysis revealed that the band intensities of the R1 mRNA transcript from wild-type and DFe-T cells varied by less than 10 % (Figure 2A; compare lane 1 with lanes 2 and 3, and lane 4 with lanes 5 and 6). In contrast with the R1 probe, the cDNA probe for R2 detects two transcripts, of 3.4 and 1.6 kb [23]. As shown in Figure 2(B), wild-type and DFe-T cells expressed comparable levels of R2 mRNA after 24 and 48 h of incubation. Densitometry analysis revealed that the band intensities of the R2 mRNA transcripts from wild-type and DFe-T cells varied by less than 6 % (Figure 2B; compare lane 1 with lanes 2 and 3, and lane 4 with lanes 5 and 6). Ethidium bromide staining of the gel

© 2000 Biochemical Society

Table 1 Kinetic analysis of CDP reductase activity

CDP reductase activity in wild-type and DFe-T cells was analysed as described by Cleland [29]. Results are means ± S.E.M. ($n = 4$). * $P < 0.02$, † $P > 0.05$.

Cells	V_{max} (pmol/min)	K_m (μM CDP)
Wild-type	1342 ± 149	7.2 ± 1.6
DFe-T	571 ± 346*	4.2 ± 1.3†

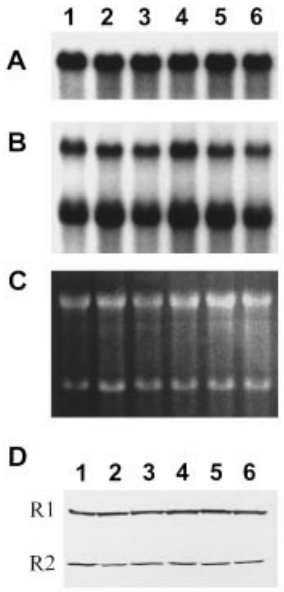

Figure 2 Ribonucleotide reductase expression in cells

(**A**–**C**) Northern blot analysis of R1 and R2 mRNA. Cells were analysed after 24 and 48 h of growth in culture. (**A**) R1 mRNA; (**B**) R2 mRNA; (**C**) ethidium bromide-stained gel to monitor RNA loading showing 18 S and 28 S RNA bands. Lanes 1–3, cells grown for 24 h: lane 1, wild-type cells; lanes 2 and 3, DFe-T cells. Lanes 4–6, cells grown for 48 h: lane 4, wild-type cells; lanes 5 and 6, DFe-T cells. (**D**) Western blot analysis of R1 and R2 protein. Cells were analysed after 24 and 48 h of growth in culture. Lanes 1–3, cells grown for 24 h: lane 1, wild-type cells; lanes 2 and 3, DFe-T cells. Lanes 4–6, cells grown for 48 h: lane 4, wild-type cells; lanes 5 and 6, DFe-T cells. Results are from separate Western blots for R1 and R2.

confirmed that equivalent amounts of RNA from each sample were loaded on to each lane (Figure 2C). In accordance with R1 and R2 mRNA levels, wild-type and DFe-T cells were found to contain equivalent amounts of R1 and R2 protein on Western blots (Figure 2D). Similar results were obtained when wild-type and DFe-T cells were compared after 72 h of growth in culture (results not shown).

ESR spectroscopy analysis

The above studies suggested that although the levels of ribonucleotide reductase mRNA and protein were similar in wild-type and in DFe-T cells, ribonucleotide reductase enzyme activity was significantly diminished in DFe-T cells. To gain further insight into ribonucleotide reductase activity in these cells, equivalent numbers of intact wild-type and DFe-T cells were analysed by ESR spectroscopy for the R2 subunit tyrosyl radical signal. Under these conditions, the amplitude of this signal is correlated with the activity of the enzyme. The ESR signal from

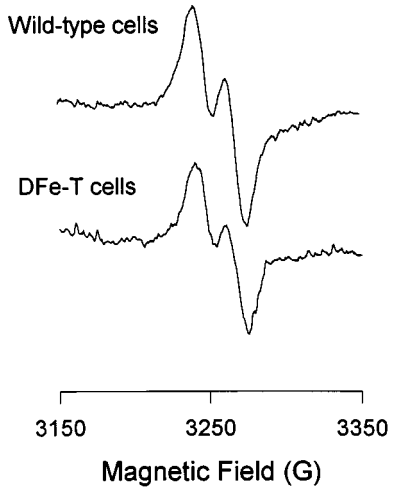

Figure 3 ESR spectroscopy of the R2 subunit tyrosyl radical of ribonucleotide reductase

Direct ESR measurements were performed at −196 °C on intact cells as described in the Materials and methods section. The amplitudes of the ESR signals from wild-type and DFe-T cells shown were 8825 and 6879 arbitrary units respectively.

equivalent numbers of wild-type and DFe-T cells (shown in Figure 3) was characteristic of the R2 tyrosyl radical signal previously reported by others [16]. Measurement of the ESR signals thus obtained showed that the amplitude of the signal in DFe-T cells was approx. 22% lower than in wild-type cells (Figure 3). These results suggest strongly that the activity of the R2 subunit is diminished in DFe-T cells; the results are also consistent with the decrease in CDP reductase enzyme activity in these cells.

DISCUSSION

The DFe-T cells were initially developed as a line of gallium-resistant CCRF-CEM cells. This resistance is related to a decrease in gallium uptake and is also associated with a down-regulation in iron uptake [21,31]. DFe-T cells are therefore faced with an interesting biological predicament. On the one hand, the decrease in gallium uptake serves to protect them from the cytotoxicity of gallium; on the other hand, the decrease in iron uptake potentially threatens their viability. The DFe-T cells seem to have adapted to the decrease in iron uptake by utilizing iron with a level of efficiency that enables them to maintain proliferation at a rate equivalent to that of wild-type cells.

The stable down-regulation of iron uptake by DFe-T cells provided us with a unique cell model system for examining the impact of changes in iron transport on the expression of the gene for ribonucleotide reductase. Our study suggests that the activity of ribonucleotide reductase is closely linked to the regulation of iron transport into CCRF-CEM cells. The iron-containing R2 subunit of ribonucleotide reductase has a half-life of 3–4 h and loses its iron spontaneously [11,19]. Proliferating cells therefore require the continuous delivery of iron to a kinetically active intracellular pool, from which iron is trafficked to R2 protein for deoxyribonucleotide synthesis. We have shown previously that inhibition of the R2 subunit by hydroxyurea in CCRF-CEM cells resulted in a decrease in their uptake of iron, suggesting a link between ribonucleotide reductase activity and iron transport into these cells [32]. In the present study we attempted to further elucidate the relationship between ribonucleotide reductase and

© 2000 Biochemical Society

iron transport by examining the expression of the gene for ribonucleotide reductase in cells that had constitutively down-regulated their uptake of iron. Our results show that an approx. 60% stable down-regulation of iron transport into DFe-T cells leads to a corresponding approx. 60% decrease in the V_{max} of CDP reductase without a diminution in cell proliferation. Our study also shows that, whereas ribonucleotide reductase enzyme activity is linked to iron transport into cells, the expression of its R1 and R2 subunits is not.

The discrepancy between R1 and R2 protein levels and ribonucleotide reductase enzyme activity in DFe-T cells can be explained by the spectroscopy studies, which showed that the tyrosyl radical ESR signal of the R2 subunit was diminished in these cells. The R2 tyrosyl radical is dependent on an intact iron centre, which, in turn, is maintained by the intracellular iron pool. When iron delivery is limited, the tyrosyl radical signal diminishes and the R2 subunit exists as a functionally inactive apo-R2 protein [33]. The ESR studies therefore suggest that, although wild-type and DFe-T cells contain equivalent amounts of R2 protein, a greater proportion of R2 protein in DFe-T cells probably exists as apo-R2 (R2 protein without iron, a functionally inactive form of the subunit). The decrease in the R2 subunit ESR signal in DFe-T cells is consistent with studies by others that have shown that the incubation of cells with iron chelators results in a loss of the tyrosyl radical, leading to the formation of apo-R2 [19].

A surprising finding in the present study was that the decrease in CDP reductase activity in DFe-T cells did not translate into a decrease in their rate of proliferation. Because DNA synthesis is correlated with the activity of ribonucleotide reductase [11,12], one would expect a decrease in ribonucleotide reductase activity to lead to a corresponding diminution in cell growth. However, our results suggest that ribonucleotide reductase activity can vary significantly in response to changes in iron transport without markedly affecting cell proliferation.

The results of our study might be relevant to the use of iron deprivation as a strategy to inhibit tumour cell growth. Several investigations have shown that the perturbation of cellular iron utilization, either by iron chelation or by monoclonal antibody blockade of the transferrin-receptor-mediated uptake of transferrin-iron, can inhibit malignant cell growth *in vitro* and in tumour-bearing animals [34–39]. The inhibition of ribonucleotide reductase is an important mechanism underlying the anti-proliferative action of iron chelators [17,19,20,40]. Therefore a block in iron-dependent ribonucleotide reductase activity should theoretically result in a uniform susceptibility of proliferating cells to a given iron chelator. However, malignant cells such as neuroblastoma seem to be more sensitive to iron deprivation than other cell types [41,42]. On the basis of our results it seems reasonable to speculate that it might be possible for some tumours to decrease their activity of ribonucleotide reductase in response to limited iron availability without actually decreasing their proliferative rate. This might partly explain why certain malignant cells are less sensitive than others to growth inhibition by iron deprivation.

We thank Dr. Yun Yen (City of Hope National Medical Center, Duarte, CA, U.S.A.) for providing us with the cDNA species for the R1 and R2 subunits, and Dr. Timothy Kinsella (Case Western Reserve University, Cleveland, OH, U.S.A.) for antiserum against human R2 protein. This work was supported by grant RO1 CA68028 from the United States Public Health Services.

Received 21 June 1999/7 October 1999; accepted 9 November 1999

REFERENCES

1 Richardson, D. R. and Ponka, P. (1997) Biochim. Biophys. Acta **1331**, 1–40
2 Cammack, R., Wrigglesworth, J. M. and Baum, H. (1990) in Iron Transport and Storage (Ponka, P., Schulman, H. M. and Woodworth, R. C., eds.), pp. 17–39, CRC Press, Boca Raton, FL
3 Brodie, C., Siriwardana, G., Lucas, J., Schleicher, R., Terada, N., Szepesi, A., Gelfand, E. and Seligman, P. (1993) Cancer Res. **53**, 3968–3975
4 Lucas, J. J., Szepesi, A., Domenico, J., Takase, K., Tordai, A., Terada, N. and Gelfand, E. W. (1995) Blood **86**, 2268–2280
5 Thelander, L. and Reichard, P. (1979) Annu. Rev. Biochem. **48**, 133–158
6 Nutter, L. M. and Cheng, Y.-C. (1989) in Inhibitors of Ribonucleoside Diphosphate Reductase Activity (Cory, J. G. and Cory, A. H., eds.), pp. 37–54, Pergamon Press, New York
7 Reichard, P. (1993) Science **260**, 1773–1777
8 Jordan, A. and Reichard, P. (1998) Annu. Rev. Biochem. **67**, 71–78
9 Brissenden, J. E., Caras, I., Thelander, L. and Francke, U. (1988) Exp. Cell Res. **174**, 302–308
10 Yang-Feng, T. L., Barton, D. E., Thelander, L., Lewis, W. H., Srinivasan, P. R. and Francke, U. (1987) Genomics **1**, 77–86
11 Eriksson, S., Gräslund, A., Skog, S., Thelander, L. and Tribukait, B. (1984) J. Biol. Chem. **259**, 11695–11700
12 Engstrom, Y., Eriksson, S., Jildevik, I., Skog, S., Thelander, L. and Tribukait, B. (1985) J. Biol. Chem. **260**, 9114–9116
13 Mann, G. J., Musgrove, E. A., Fox, R. M. and Thelander, L. (1988) Cancer Res. **48**, 5151-5156
14 Thompson, D. P., Carter, G. L. and Cory, J. G. (1989) Cancer Commun. **4**, 253–260
15 Gräslund, A., Ehrenberg, A. and Thelander, L. (1982) J. Biol. Chem. **257**, 5711–5715
16 Gräslund, A., Sahlin, M. and Sjöberg, B.-M. (1985) Environ. Health Perspect. **64**, 139–149
17 Lederman, H. M., Cohen, A., Lee, J. W. W., Freedman, M. H. and Gelfand, E. W. (1984) Blood **64**, 748–753
18 Hoyes, K. P., Hider, R. C. and Porter, J. B. (1992) Cancer Res. **52**, 4591–4599
19 Nyholm, S., Mann, G. J., Johansson, A. G., Bergeron, R. J., Gräslund, A. and Thelander, L. (1993) J. Biol. Chem. **268**, 26200–26205
20 Cooper, C. E., Lynagh, G. R., Hoyes, K. P., Hider, R. C., Cammack, R. and Porter, J. B. (1996) J. Biol. Chem. **271**, 20291–20299
21 Chitambar, C. R. and Wereley, J. P. (1998) Blood **91**, 4686–4693
22 Chitambar, C. R. and Sax, D. (1992) Blood **80**, 505–511
23 Yen, Y., Grill, S. P., Dutschman, G. E., Chang, C.-N., Zhou, B.-S. and Cheng, Y.-C. (1994) Cancer Res. **54**, 3686–3691
24 Kuo, M.-L. and Kinsella, T. J. (1997) Int. J. Oncol. **10**, 515–520
25 Laemmli, U.K. (1970) Nature (London) **227**, 680–685
26 Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4350–4354
27 Steeper, J. R. and Steuart, C. D. (1970) Anal. Biochem. **34**, 123–130
28 Cory, J. G., Russell, F. A. and Mansell, M. M. (1973) Anal. Biochem. **55**, 449–456
29 Cleland, W. W. (1979) Methods Enzymol. **63**, 103–138
30 Chitambar, C. R., Matthaeus, W. G., Antholine, W. E., Graff, K. and O'Brien, W. J. (1988) Blood **72**, 1930–1936
31 Chitambar, C. R. and Wereley, J. P. (1997) J. Biol. Chem. **272**, 12151–12157
32 Chitambar, C. R. and Wereley, J. P. (1995) Cancer Res. **55**, 4361–4366
33 Ochiai, E.-I., Mann, G. J., Gräslund, A. and Thelander, L. (1990) J. Biol. Chem. **265**, 15758–15761
34 Taetle, R., Honeysett, J. M. and Trowbridge, I. (1983) Int. J. Cancer **32**, 343–349
35 Sauvage, C. A., Mendelsohn, J. C., Lesley, J. F. and Trowbridge, I. S. (1987) Cancer Res. **47**, 747–753
36 Taetle, R., Honeysett, J. M. and Bergeron, R. (1989) J. Natl. Cancer Inst. **81**, 1229–1235
37 Kemp, J. D., Smith, K. M., Kanner, L. J., Gomez, F., Thorson, J. A. and Naumann, P. W. (1990) Blood **76**, 991–995
38 Richardson, D. R., Tran, E. H. and Ponka, P. (1995) Blood **86**, 4295–4306
39 Kemp, J. D. (1997) Histol. Histopathol. **12**, 291–296
40 Hoffbrand, A. V., Ganeshaguru, K., Hooton, J. W. L. and Tattersall, M. H. N. (1976) Br. J. Haematol. **33**, 517–526
41 Blatt, J. and Stitely, S. (1987) Cancer Res. **47**, 1749–1750
42 Kovar, J., Naumann, P. W., Stewart, B. C. and Kemp, J. D. (1995) Pathobiology **63**, 65–70

© 2000 Biochemical Society