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Evidence for the appearance of a reticulocyte population low in lipoxygenase mRNA during the recovery from a phenylhydrazine-induced anemia in rabbits

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It is shown that during recovery from a phenylhydrazine-induced anemia in rabbits a selective decrease in lipoxygenase mRNA takes place with a corresponding shut-off of the synthesis of the enzyme. It is suggested that a new population, 'recovery'-reticulocytes, makes its appearance in the peripheral blood. Their cells are more mature than the stress macroreticulocytes. A cell-free system prepared from the recovery-reticulocytes exhibits low endogenous synthesis of non-globin polypeptides, even without nuclease treatment, but retains full capacity to be stimulated by exogenous mRNA.

Lipoxygenase; mRNA; Phenylhydrazine; Anemia; (Reticulocyte)

1. INTRODUCTION

Reticulocytes, the penultimate stage of erythroid differentiation, are not a uniform population, despite their common feature, the presence of ribosomes [1]. The reticulocytes of normal peripheral blood, amounting to about 1% of the red cells, are highly mature as judged by their size and low respiration. Those produced by forced bleeding or phenylhydrazine administration, the so-called stress-reticulocytes, are distinguished by their large size. They synthesize intensively many proteins, the most abundant being erythroid 15-lipoxygenase in addition to globin chains. This enzyme plays a key role in the maturational degradation of mitochondria. The reticulocyte at the height of forced anemia may be subdivided into several fractions according to the presence of lipoxygenase and other features [1]. The most immature fraction contains the LOX mRNA in a masked form, followed by a class of reticulocytes characterized by sustained intensive synthesis of LOX. In the most mature reticulocyte the level of LOX is lower and the enzyme is absent in erythrocytes. In earlier studies it was suggested that during recovery from anemia different populations of reticulocytes appear based on the content of RNA and several enzymes [2].

Here, a comparison of the changes in intensity of LOX synthesis and LOX mRNA content was carried out in rabbits during the course of phenylhydrazine-induced anemia and recovery from it.

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2. MATERIALS AND METHODS

2.1. *Cells*

Reticulocytosis was induced in rabbits weighing 2-3 kg by injection of phenylhydrazine as in [3]. About 0.5 ml blood were taken from the ear vein every day over a period of 11 days (6 days phenylhydrazine treatment plus 5 days recovery period).

2.2. *Labeling of cells*

Labeling of cells was performed as in [4]. 10 μl cells were supplemented with 5 μl/ml $FeSO_4$, all amino acids (without methionine) and 20 μCi [^{35}S]methionine (50 Ci/mmol, Radiopreparat, USSR); cells were incubated for 1 h at 37°C. Cells were washed twice in isotonic NaCl solution and lysed for 10

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min with 3 vols ice-cold water. A clear supernatant was obtained by centrifugation (15 min at 16000 × g).

2.3 *Cell-free protein synthesis*

Preparation of the cell-free protein synthesis system and pretreatment of lysates with micrococcal nuclease were performed as described [5]. Rabbit reticulocytes from the 2nd and 5th days of the recovery period were used.

2.4. *SDS gel electrophoresis*

SDS gel electrophoresis was carried out according to Laemmli [6] except that 10–22% linear slab gels were used. For fluorography the gels were impregnated with 'Amplify' (Amersham). Hybridization and Northern blotting were performed as in [7].

3. RESULTS

The data in fig.1 show, in agreement with the literature, that the synthesis of proteins and the percentage of reticulocytes are approximately proportional. Fig.2 presents the pattern of newly synthesized polypeptides. It may be seen that in addition to globin chains a set of other proteins are found during both anemic and recovery periods. The most dramatic change takes places with respect to 15-LOX, which has been identified by a specific antibody [4]. Whereas no synthesis of LOX can be detected in normal blood and during the first days of phenylhydrazine administration, newly formed LOX is clearly visible after the 4th day and reaches its maximum on the second day of recovery. A good parallelism is observed between the intensity of LOX synthesis and reticulocytosis during the phase of increase in both parameters, whereas during the recovery period, selective shut-off of LOX synthesis takes places. Practically total cessation occurs at the fifth day despite the fact that at that

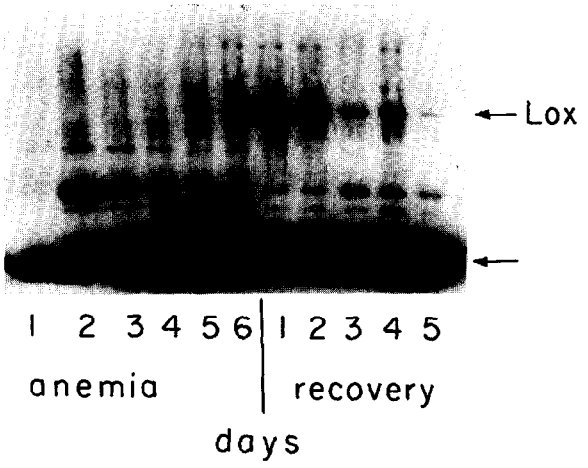

Fig.2. Fluorogram of SDS electrophoresis of proteins synthesized by rabbit reticulocytes in the course of anemia and recovery. Cells were incubated with [^{35}S]methionine for 1 h at 37°C. Each lane contains 60000 cpm [^{35}S]methionine-containing material.

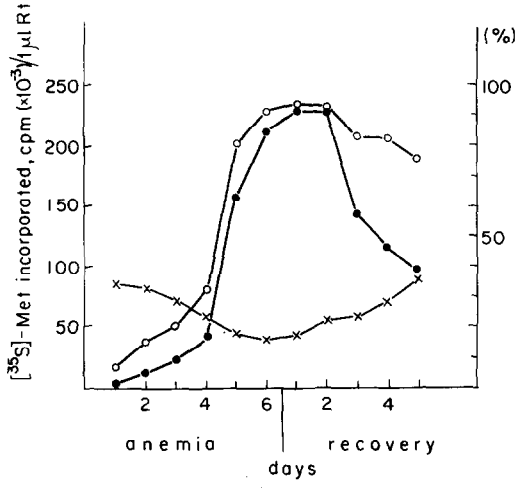

Fig.1. Changes of hematocrit, reticulocyte, percentage and [^{35}S]methionine incorporation in rabbit reticulocytes during the course of phenylhydrazine-induced anemia. (×—×) Hematocrit (%), (●—●) reticulocyte (%), (○—○) [^{35}S]methionine incorporation (cpm/µl).

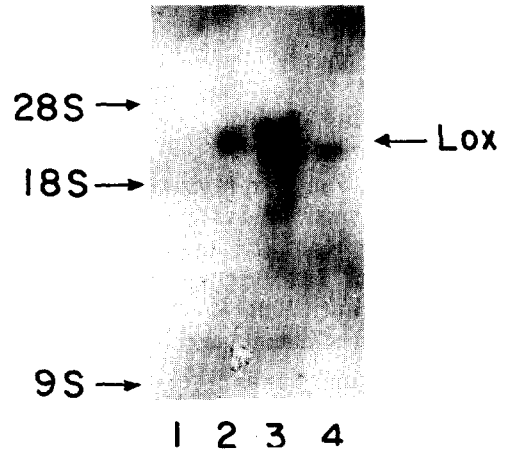

Fig.3. Northern blot analysis of total RNA isolated from red blood cell lysates during the course of anemia and recovery. Samples of RNA (20 µg) were electrophoretically separated, transferred to nitrocellulose and hybridized with cloned lipoxygenase cDNA. (1) Control, (2) 4th day of anemia, (3) 2nd day of recovery, (4) 5th day of recovery.

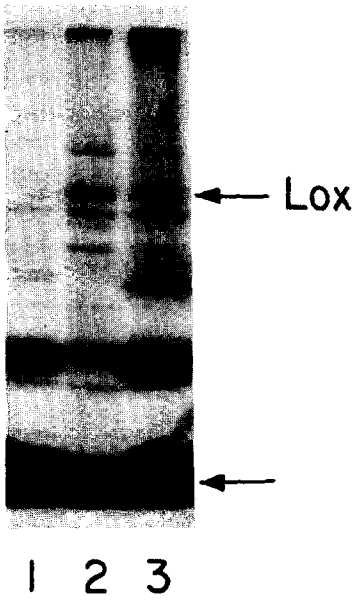

Fig.4. SDS gel electrophoresis of red blood cell lysates during the course of anemia and recovery, Coomassie blue staining. (1) Normal blood, (2) 2nd day of recovery, (3) 5th day of recovery. Each lane contains 3 μl lysate.

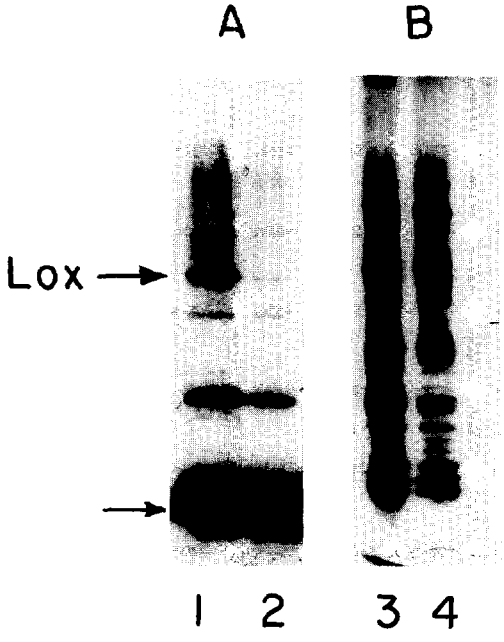

Fig.5. Fluorograms of SDS electrophoresis of proteins that were synthesized in cell-free systems from macro-reticulocytes (lanes 1,3) and recovery reticulocytes (2,4). (A) Products of endogenous mRNA translation. (B) Products of poly(A)$^+$ translation in nuclease-treated lysates. Each lane contains 100 000 cpm [^{35}S]methionine-containing material.

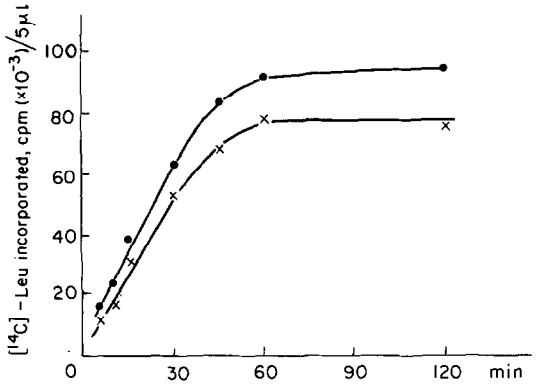

Fig.6. The kinetics of endogenous protein synthesis in cell-free translation systems from macro-reticulocytes (●—●) and recovery reticulocytes (×—×).

time the percentage of reticulocytes is still as high as 40%. It may also be noted that during the entire course of recovery globin chains and some other polypeptides were synthesized.

The amount of LOX mRNA, studied by Northern blot analysis with LOX cDNA, cloned recently in our laboratories [7,8], is presented in fig.3. The data indicate a close correlation between the amount of LOX mRNA and LOX synthesis. LOX mRNA was absent in lysates from non-anemic animals, maximal on the second day of recovery and again barely noticeable on the fifth day.

In fig.4, data on the total amount of lipoxygenase during the course of the anemia are plotted. The most important result is that the amount of enzyme present on the fifth day of recovery is about the same as that found on the second day. It appeared of interest to compare the translational characteristics of lysates from the recovery reticulocytes with those from the usual macro-reticulocytes. It may be seen that the lysate from recovery reticulocytes has a lower level of endogenous mRNAs, particularly for the non-globin proteins but is fully programmable by exogenous mRNA (fig.5).

The incorporation of labelled amino acids is linear for at least 1 h (fig.6). Other features, i.e., optimal concentration of K^+ of 80–120 mM, Mg^{2+} (0.3–2.0 mM), hemin and cAMP dependence, are similar to those of the standard system (not shown).

4. DISCUSSION

The present results on phenylhydrazine-induced anemia of rabbits are in general agreement with other work on the changes in lipoxygenase activity and its amounts during anemia produced by forced bleeding and recovery from it [9]. The new feature is the demonstration of a population of reticulocytes in the peripheral blood which is present on the fifth day of recovery. This is characterized by the nearly complete absence of LOX mRNA, corresponding to a low extent of synthesis of the enzyme. The main evidence for the conclusion that it represents a separate clone of reticulocytes is the disproportion between the high number of reticulocytes still present and the amount of mRNA demonstrable. Judging from the distribution of reticulocytes at the height of the anemia, which may be subdivided into four classes according to their maturity [10], the class reticulocytes described here must be more mature than macro-reticulocytes. It is still uncertain whether this type of reticulocyte represents a more mature state of the macro-reticulocyte or originates as a distinct population from the bone marrow in a more mature state and thus represents a stage of transition to the production of normoreticulocytes without superinduction of lipoxygenase, which is a characteristic of the macro-reticulocyte. Further studies are needed to resolve this question.

The properties of the lysate of recovery reticulocytes may make it suitable for some translation studies, but may also serve in further investigation of the maturation of red cells.

REFERENCES

[1] Rapoport, S.M. (1986) The Reticulocyte, CRC Press, Boca Raton, FL.
[2] Rosenthal, S., Künzel, W. and Wagenknecht, C. (1964) Acta Biol. Med. Germ. 13, 281–290.
[3] Adamson, J.D., Herbert, E. and Hemp, S.F. (1969) J. Mol. Biol. 42, 247–251.
[4] Thiele, B.J., Belkner, J., Andree, H., Rapoport, T.A. and Rapoport, S.M. (1969) Eur. J. Biochem. 96, 563–569.
[5] Pelham, H.R. and Jackson, R.J. (1976) Eur. J. Biochem. 67, 247–256.
[6] Laemmli, U.K. (1970) Nature 227, 680–685.
[7] Thiele, B.J., Fleming, J., Kasturi, K., O'Preg, J., Black, E., Chester, J., Rapoport, S.M. and Harrison, P.R. (1987) Gene 57, 111–119.
[8] Oleinikov, A.V., Evdokimova, V.M., Visozkaja, V.S. and Minich, W.B. (1988) Biokhimiya, in press.
[9] Ludwig, P., Höhne, M., Kühn, H., Schewe, T. and Rapoport, S.M. (1988) Biomed. Biochim. Acta 47, 593–608.
[10] Rapoport, S.M. and Schewe, T. (1986) Biochim. Biophys. Acta 864, 471–495.