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Comparison of Amphibian Whole-Cell and Cell-Free Activity towards Different Transferrins

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1. A cell-free preparation from immature erythrocytes of the bullfrog (*Rana catesbeiana*) was about equally active towards iron bound to homologous, rabbit or human transferrin.
2. In contrast with the cell-free system, whole immature erythrocytes exhibited optimum, intermediate or low uptake of iron from the above and from other transferrins.

Mammalian erythroid cells optimally take up iron from transferrin or serum from the same species, whereas iron from different species of transferrins or sera is taken up more slowly or not at all (Paoletti *et al.*, 1958; Lane, 1972; Verhoef *et al.*, 1973). Since we have studied iron metabolism in the developing tadpole (Osaki *et al.*, 1974), I decided to see if the amphibian immature erythroid cell also exhibits a specificity in the uptake of iron from transferrins.

The removal of iron from transferrin by subcellular components has been a topic of wide interest. Studies with mammalian reticulocytes indicated that only intact cells could remove iron from transferrin (Morgan, 1971; Egyed, 1973). We have shown that the assay method used by Morgan (1971) and Egyed (1973) apparently could not efficiently detect iron removal from transferrin by a cell-free system; further, we showed that iron is removed from homologous transferrin by a cell-free preparation from *Rana catesbeiana* immature erythrocytes (James & Frieden, 1974, 1975). The present paper reports on the specificity of the *Rana catesbeiana* cell-free activity towards several transferrins compared with intact cellular iron uptake from different transferrins.

Experimental

Purification of *Rana catesbeiana* transferrin, preparation of apotransferrins or apo-sera, $^{59}\text{Fe}/^{56}\text{Fe}$ loading of apotransferrins or apo-sera, collection and washing of immature erythroid cells and their incubation with transferrin, preparation of lysates and the cell-free solution, and counting of radioactivity were all done as previously described (James & Frieden, 1975). All of the transferrins and sera were treated identically to produce the same iron content and protein concentration. Rabbit, pig, ox and horse transferrins were purchased from Miles Laboratories, Kankakee, IL, U.S.A., and human transferrin was from Behringwerke A.G., Marburg/Lahn, Germany.

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Serum was obtained from rabbits by heart puncture, from chickens by puncture of wing veins, from turtles by decapitation after exposure to ether, and from young frogs by scission of the conus arteriosus. Total iron-binding capacity and serum iron concentrations were determined as described by Ramsay (1957).

For studies on iron removal from transferrin by the cell-free component(s) obtained from immature erythrocytes, 10 μl of bipyridine and 10 μl of carrier bipyridine-iron was added to 100 μl portions of the cell-free solution such that the final concentrations would be 1.6 mM and 4 μM respectively. Then 25 μl of transferrin (10 mg/ml) was added. The transferrins were 50% saturated with iron and contained ^{59}Fe as in the whole-cell experiments. Incubation was carried out at 25°C until the desired time. Samples were either assayed immediately or were frozen in a solid- CO_2 /ethanol bath. Assay of iron transferred from transferrin to bipyridine was performed by gel filtration as described by James & Frieden (1975).

Results and Discussion

Whole-cell studies

The kinetics of iron uptake by cells in the homologous system of *Rana catesbeiana* transferrin and immature erythroid cells were similar to those reported earlier (Osaki *et al.*, 1974), i.e. uptake of ^{59}Fe or ^{59}Fe -transferrin was approximately linear with time for the first 2 h of incubation, then gradually reached a plateau at 4 h. In the present study, transferrins and sera of other species were also incubated with the *Rana catesbeiana* cells and gave qualitatively similar results, but the total amount or plateau value of iron taken up was less than from frog serum or transferrin. Relative to iron uptake from frog serum, cells took up 0.45, 0.40, 0.09 and 0.07 times as much iron from rabbit, chicken, human and turtle serum respectively. Relative to iron uptake from frog transferrin, cells took up 0.34, 0.30, 0.21, 0.05 and 0.03 times as much iron from pig, rabbit, horse, ox and human transferrin respectively. In all, these data

Fig. 1. Iron transfer from transferrins to bipyridine mediated by the amphibian cell-free solution as a function of time

Frog transferrin (▲), human transferrin (■), rabbit transferrin (●). Cell-free solution prepared from predominantly immature erythrocytes, solid symbols; cell-free solution from mature erythrocytes, open symbol. No detectable iron was removed from transferrin when buffer replaced cellular components (results not plotted).

indicate that the *Rana catesbeiana* intact cell can take up some species of ^{59}Fe -transferrins but not others, even within a particular vertebrate class. This principle is similar to that found in studies with mammalian intact cells (Paoletti *et al.*, 1958; Verhoef *et al.*, 1973).

Cell-free studies

Since intact cells took up some species of transferrin-iron but not others, it was decided to see if the cell-free activity would show some specificity in removing iron from different transferrins. Three iron donors, namely frog, rabbit and human transferrin, were selected on the basis of differences in whole-cell uptake of ^{59}Fe -transferrins (see above). The kinetics of iron removal and transfer to 2,2'-bipyridine by the cell-free system are shown in Fig. 1. The cell-free system transfers iron from frog and human transferrins to bipyridine at about the same rate and to the same extent, in contrast with a striking difference in whole-cell uptake of iron from these two transferrins. The cell-free removal of iron from rabbit transferrin was somewhat less than that from frog and human transferrin. This may be explained by the observation that rabbit transferrin contained some haemoglobin, as judged by its spectrum, showing a definite peak at 410nm; this impurity would lower the effective concentration of rabbit transferrin. Frog transferrin prepared in our laboratory was apparently pure (James & Frieden, 1975) and so was commercial human transferrin.

Incubation of transferrins with a cell-free system prepared from mature (rather than from immature) erythrocytes provided little or no release of iron (Δ, Fig. 1). Therefore the mature erythrocyte probably

Fig. 2. Maximal uptake or removal of iron from transferrins

Whole-cell uptake of ^{59}Fe in the absence of bipyridine (solid bars), whole-cell transfer of ^{59}Fe to bipyridine in the presence of bipyridine (open bars), and cell-free transfer of ^{59}Fe to bipyridine (hatched bars) from frog, rabbit and human transferrins. Whole-cell uptake of iron from all transferrins in the presence of bipyridine was nil (results not shown). Plateau values are given from plots of uptake or transfer versus time. Immature erythroid cells from *Rana catesbeiana* were used as the source of whole cells or the cell-free solution.

no longer synthesizes the as-yet unidentified component(s) which removes iron from transferrin. This would correlate with cessation of haemoglobin production in mature erythrocytes.

Results from both whole-cell and cell-free studies (all from *Rana catesbeiana* immature erythrocytes) with frog, rabbit, and human transferrins are given in Fig. 2. End-point or plateau values are shown from plots of iron uptake or transfer versus time. Solid bars indicate the uptake of ^{59}Fe or ^{59}Fe -transferrins by intact cells. In samples where bipyridine was included in the incubation, bipyridine- ^{59}Fe was found outside the cells (open bars) and cellular incorporation of ^{59}Fe was nil. This is consistent with the idea that bipyridine may enter the cell, accept ferrous iron after or during detachment of iron from transferrin, then diffuse back out of the cell (Morgan, 1971). It is not known whether reduction of ferric iron occurs simultaneously with its removal from transferrin. Hatched bars show the amount of iron incorporated into bipyridine from transferrins, mediated by the cell-free system rather than by whole cells. Thus, although the *Rana catesbeiana* intact immature erythrocyte recognizes some species of iron-transferrins but not others, the low-molecular-weight component (James & Frieden, 1975) which removes iron from transferrin appears to act without regard to the particular species of transferrin. Again, the mature erythrocyte and the cell-free preparation therefrom was ineffective in iron uptake or removal from the transferrins studied.

It should be noted that another cell-free study involved not iron removal from transferrins but iron mobilization from rabbit reticulocyte 'ghosts' after incubation with rabbit transferrin-⁵⁹Fe (Workman & Bates, 1974). This apparently represents a distinctly separate step in iron metabolism from the steps dealt with in the present paper. It was not reported whether the cell-free activity (Workman & Bates, 1974) was present in preparations from mature erythrocytes as well as from reticulocytes.

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