Deoxyribonucleoside Triphosphate Pools in Vitamin B-12-Deficient Euglena gracilis

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The size of the deoxyribonucleoside triphosphate pools of vitamin B-12-deficient cells of Euglena gracilis, and of vitamin B-12-deficient cells repleted with the vitamin, were measured. We found that the pools were very small, if they exist at all, in deficient cells but expand rapidly with the addition of the vitamin. The sizes of the pools decrease when DNA synthesis is completed, and are very small when the cells begin to divide.

Euglena gracilis when grown in sub-optimal amounts of vitamin B-12 undergoes morphological and biochemical alterations (Carell, 1969; Carell et al., 1970) that are characteristic of cells in which the growth cycle and the DNA synthesis–cell division cycle have been uncoupled (Mitchison, 1971). The amounts of RNA and protein increase severalfold, the cells become enlarged, and the S phase of the cell cycle becomes extended (Carell et al., 1970). This unbalanced growth condition is reversed by the replenishment of the vitamin B-12-deficient culture (Johnston & Carell, 1973) with the vitamin (reversion). DNA synthesis resumes at normal rates, and the cells undergo two relatively synchronous cell divisions (Johnston & Carell, 1973; Goetz et al., 1974).

Studies in vitro have shown that Euglena gracilis Z strain has a vitamin B-12-dependent ribonucleotide reductase, though with extremely low activity (Gleason & Hogenkamp, 1972). Nevertheless, we reasoned that the diminishing rate of DNA synthesis and the lengthening of the S phase when the cultures progress into deficiency (Christopher et al., 1974) could be due to a shortage or absence of the deoxyribonucleoside triphosphates (dNTP). In the present paper we provide evidence that the dNTP pools are very small in vitamin B-12-deficient cells, but expand rapidly on the addition of vitamin B-12.

Materials and Methods

Euglena gracilis Klebs strain Z (Pringsheim) cells were grown in sub-optimal amounts of vitamin B-12 (25ng/l) until they reached advanced deficiency, as described previously (Carell, 1969). Vitamin B-12 was added to the cultures (10μg/l) at zero time, and samples were taken at indicated times for the different determinations. DNA was determined by the diphenylamine method of Burton (1955). Cell number was determined with a Coulter counter. The dNTP pools were extracted by using 0.8M-KOH and 0.16M-KH2PO4, rather than 0.8M-KOH and 0.12M-NaHCO3, as described by Bagnara & Finch (1972). The pool sizes were determined by using 3H-labelled deoxyribonucleoside triphosphates (New England Nuclear Corp., Boston, MA, U.S.A.) in the assay rather than 14C-labelled deoxyribonucleoside triphosphates as described by Solter & Handschumacher (1969). The DNA polymerase was purchased from Miles Laboratories (Elkhart, IN, U.S.A.).

Discussion

Table 1 shows that the pools of the dNTP are very small (if they exist at all) in deficient cells, and that they appear rapidly on the addition of vitamin B-12, reaching high values by the end of the first hour. The dATP, dCTP and dGTP reach their peak values at this time. The dTTP reaches its peak value after 3h, which corresponds to the completion of the S phase (Goetz et al., 1974). The dNTP pool sizes increase in an almost linear manner from 20min to 1h after vitamin B-12 replenishment. After the first hour they fluctuate, as they do in other systems (Nexo, 1975; Skoog et al., 1973).

The pool of dTTP is always the largest in reverting Euglena. This is also the case in Tetrahymena pyriformis (Nexo, 1975). The pool sizes we obtain during the completion of the S phase in reverting cells are larger than those of T. pyriformis (Nexo, 1975) or Chinese-hamster cells (Skoog et al., 1973). However, in Euglena the dGTP pool, as in Chinese-hamster cells (Skoog et al., 1973) and human bone-marrow cells (Hoffbrand et al., 1974), is always the smallest.

Table 1 also shows that the amount of DNA/ml of culture increases after the addition of vitamin B-12 and then levels off before the end of the third hour. At the end of S phase (3h) the pool sizes remain large, a phenomenon which was also reported to occur in
Chinese-hamster cells (Skoog et al., 1973) and in *T. pyriformis* (Nexo, 1975). However, after the completion of S phase, the pool sizes decrease and reach a minimum as the cells start to divide at 4h. By the fifth hour the dATP, dCTP and dGTP pools become undetectable, although with our method we could detect pool sizes as small as 2 pmol/10^6 cells. On the other hand there is a measurable pool of dTTP at that time. Such a rapid disappearance of the dNTP pools does not occur in *T. pyriformis* (Nexo, 1975) or in the Chinese-hamster cells of Skoog et al. (1973), although Walters et al. (1973) have observed such an event in their Chinese-hamster cells. They report that degradation begins in phase G2 and that cells in early phase G1 have very small or no dNTP pools.

Vitamin B-12 deficiency in *Euglena gracilis* is similar in many ways to megaloblastic anaemia in man. Megaloblasts have an extended S phase (Yoshida et al., 1968), as do vitamin B-12-deficient *Euglena* cells. Megaloblasts are large and have increased amounts of RNA and protein but less than the 4C (double) amount of DNA (Lajitha & Oliver, 1960; Davidson et al., 1948). Vitamin B-12-deficient *Euglena gracilis* shows the same characteristics (Carell et al., 1970). The chromosomes are abnormally elongated and diffuse in both cases. Vitamin B-12 replenishment corrects this condition.

The ribonucleotide reductase of humans is vitamin B-12-independent (Millard, 1972), whereas the ribonucleotide reductase of *Euglena* is vitamin B-12-dependent (Gleason & Hogenkamp, 1972). The pools of the dNTP in megaloblasts are at least of normal size (Hoffbrand et al., 1974), whereas the pools of dNTP in vitamin B-12-deficient *Euglena* are essentially non-existent, but appear within 20 min on the replenishment with the vitamin. These results suggest that under the experimental conditions described here there may be no vitamin B-12-independent ribonucleotide reductase in *Euglena* Z strain. The apparent absence of the pools of dNTP from the vitamin B-12-deficient cells, and their appearance on replenishing the cells with the vitamin, suggest that the ribonucleotide reduction may be the limiting step in DNA synthesis.

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**Table 1. Deoxyribonucleoside triphosphate pools in reverting *Euglena gracilis* cells**

Experimental conditions were as described in the text.

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<th>Time (min)</th>
<th>dATP</th>
<th>dCTP</th>
<th>dGTP</th>
<th>dTTP</th>
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References


