INTRACELLULAR AMINO ACIDS AND PROTEIN SYNTHESIS IN HELA CELLS

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ABSTRACT. (1) When the intracellular amino acid pool is prelabelled and subsequently chased in non-radioactive medium, the radioactivity of the amino acid pool is not found to have been incorporated into protein.

(2) Leucine transport into Hela cells is reduced in the presence of 10 mM valine in the medium. This results in a lower specific radioactivity of leucine in the intracellular amino acid pool. However, neither the overall rate of protein synthesis nor the incorporation of radioactive leucine into protein is affected.

From these experiments it is concluded that incoming amino acids entering the intracellular amino acid pool are not used for de novo synthesis of protein.

I. INTRODUCTION

It is generally assumed that amino acids for protein synthesis are selected from the intracellular pool of free amino acids [1–6]. This assumption however seems to be incompatible with the reported observations of several workers [7–12].

Firstly, incorporation of radioactive amino acids into protein is linear from almost the beginning of the incubation, suggesting a precursor pool of constant specific radioactivity. However, the specific radioactivity of the amino acid pool increases in a curvilinear manner. Secondly, chase experiments in which the intracellular pool of amino acids was prelabelled indicated that the radioactive amino acids from the pool were not incorporated into protein.

In the present paper we describe that lowering the specific radioactivity of the intracellular pool of leucine by competitive inhibition of leucine transport, does not affect the rate at which this amino acid is incorporated into protein, suggesting again that incoming amino acids are directly used for the synthesis of cellular proteins, bypassing the intracellular pool.

II. MATERIALS AND METHODS

l-leucine ^{3}H [G, s.a. 19 Ci mmole^{-1}], l-leucine ^{14}C [U, s.a. 342 mCi mmole^{-1}] and l-lysine ^{3}H [G, s.a. 11 Ci mmole^{-1}] were obtained from the Radiochemical Centre, Amersham. Cycloheximide was a product of Koch-Light Lab.

Hela-cells (mycoplasma-free) were grown as a monolayer in Tc 199 medium supplemented with 10% newborn calf serum (Flow) in the presence of a penicillin and streptomycin mixture.

In the labelling experiments we used medium A which contained per liter: 100 ml dialysed

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newborn calf serum, 300 ml Tc 199 medium, 9 ml 0.77 M phosphatebuffer and 60 ml of a Hanks solution (20 times concentrated) with a lowered NaCl content (149.3 g l^{-1}) to maintain a physiological osmolarity.

The final amino acid concentration in this medium (a.o. leucine about 0.17 mM) allowed maximal rates of protein synthesis (data not shown). After incubation the medium was discarded and the cells were washed with icecold Hanks solution containing 0.25 mM leucine [or 0.5 mM lysine in the lysine-experiment] in order to remove extracellular amino acids. After 5 washings (each 5 min) the intracellular pool was extracted with 5% trichloracetic acid [6]. From the pellet the DNA was extracted with 0.5 N perchloric acid (15 min at 70 °C) and determined according to Burton [3]. The radioactivity remaining in the pellet was assumed to have been incorporated into protein.

III. RESULTS AND DISCUSSION

In a first series of experiments we confirmed earlier findings that the incorporation of radioactive leucine into protein is linear from almost the beginning of the incubation (Fig. 1B), suggesting a precursor pool of constant specific radioactivity. However, the specific radioactivity of the leucine in the amino acid pool increases in a curvilinear manner, similarly as is shown in Fig. 1A (first 60 min). When radioactive amino acids are allowed to accumulate into the intracellular pool of free amino acids, one can measure to what extent these radioactive amino acids used for protein synthesis by chasing the prelabelled cells in non-radioactive medium [7–10]. Prelabelling of the cells was performed with cycloheximide in the medium. The presence of cycloheximide during the prelabelling period enabled us to detect even low levels of incorporation during the non-radioactive chase.

Fig. 1A shows that the radioactive leucine, accumulated in the intracellular pool during the incubation in radioactive medium, effluxed very rapidly and almost completely into the medium

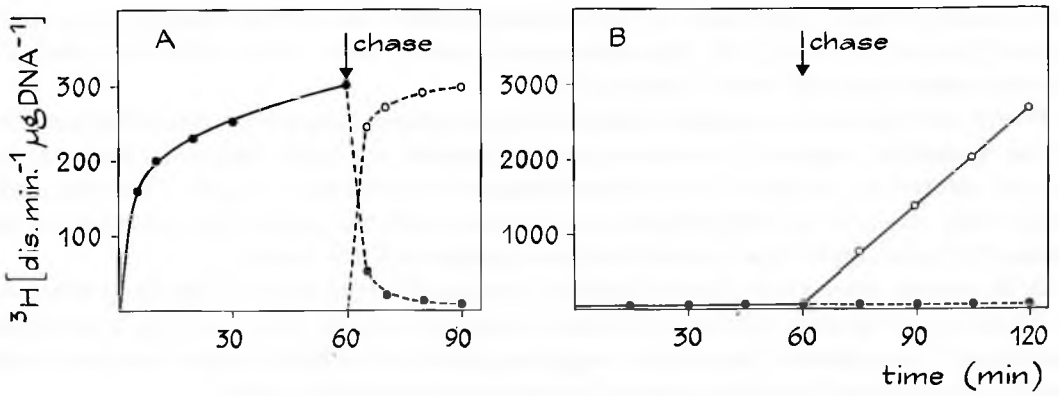

Fig. 1. Effect of a non-radioactive chase on intracellular ^{3}H-leucine content and incorporation. – The cells were incubated at 37 °C in medium A containg ^{3}H-leucine (1 µCi ml^{-1}) and cycloheximide (0.1 mg ml^{-1}). After 60 min the medium was removed and the cells were washed as usual with icecold Hanks solution containing 0.25 mM leucine (5 × 5 min) to remove residual ^{3}H-leucine and cycloheximide. Subsequently the cells were incubated for another 60 min in unlabelled medium A. – To control flasks [in Fig. 1B] ^{3}H-leucine was added (1 µCi ml^{-1}). A. ●——● acid soluble intracellular ^{3}H-leucine influxed into the cells during incubation in radioactive medium A with cycloheximide. ●-----● acid soluble intracellular ^{3}H-leucine remaining in the cells during non-radioactive chase (without cycloheximide). ○-----○ acid soluble ^{3}H-leucine accumulating in the medium during non-radioactive chase (without cycloheximide). – B. ●——● incorporation of ^{3}H-leucine into protein during incubation in radioactive medium A with cycloheximide. ●-----● incorporation of ^{3}H-leucine into protein during nonradioactive chase (without cycloheximide). ○——○ control incorporation of ^{3}H-leucine into protein during chase with ^{3}H-leucine (1 µCi ml^{-1}) present.

during the nonradioactive chase. During the chase, in the absence of cycloheximide, no detectable amount of intracellular radioactive material was incorporated into protein.

When the prelabelling was performed in the absence of cycloheximide we found essentially the same pattern of influx and rapid efflux as is illustrated in Fig. 1A (results not shown). Another control experiment showed that the rate of incorporation of leucine reaches normal levels again very shortly after the cycloheximide has been removed by washing the cells (Fig. 1B).

However, this result is not very conclusive because of the rapid release of the intracellular amino acids. In order to test our hypothesis [9, 10] we performed another kind of experiment, in which the specific radioactivity of the intracellular leucine pool was influenced selectively by a partial inhibition of leucine transport.

Our reasoning was as follows: when the influx of amino acids into the cells decreases, it is probable that the transfer RNA system would bind their normal amount of amino acids in order to maintain a constant rate of protein synthesis. Then the overflow of amino acids into the intracellular pool would be smaller giving rise to changes in size and specific activity of this pool.

We found that addition of 10 mM valine to the medium influences the leucine transport into the cells significantly (Fig. 2A). However the rate of incorporation of leucine into protein is unchanged (Fig. 2B).

We also measured the specific radioactivity of the leucine in the intracellular pool and in the protein. Table I shows that the specific radioactivity ($^{14}C/^{3}H$ ratio) of leucine in the intracellular pool is significantly lowered when valine is present in the medium. There are only small differences in the amount of incorporated counts and in the specific radioactivity of leucine in the synthesised protein between control and valine treated cells.

When lysine is used as the radioactive precursor no effect of valine can be observed, showing that the overall rate of incorporation of amino acids into protein is not altered by the presence of an excess of valine (Fig. 3).

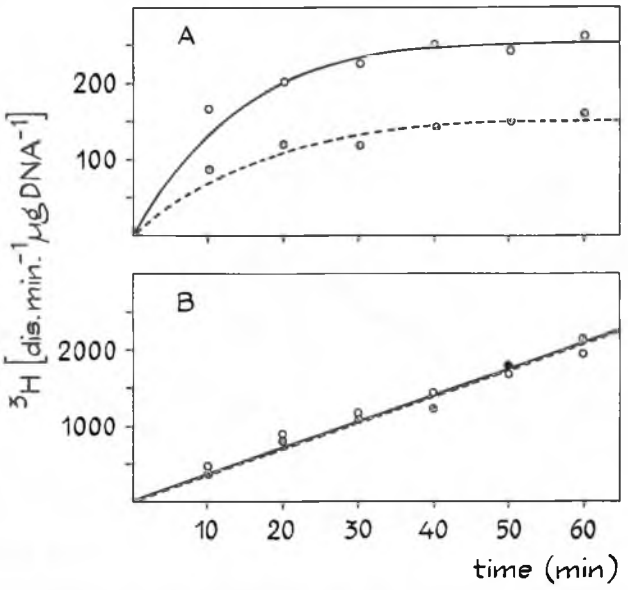

Fig. 2. The effect of 10 mM valine on influx (A) and incorporation (B) of ^{3}H-leucine. – Cells were incubated in medium A containing ^{3}H-leucine (1 µCi ml^{-1}) with (●-----●) and without (○——○) 10 mM valine. After the time periods indicated the cells were washed as usual and the radioactivity per cell in the intracellular pool (A) and in protein (B) was determined. – The data shown are the average of at least three determinations.

TABLE I

The effect of 10 mM valine in the medium on specific radioactivity of intracellular leucine

		^3H DPM/μg DNA		^{14}C DPM/μg DNA		$^{14}C/^3H$ ratio
Experiment 1						
medium	−valine	–		–		1.20
	+valine	–		–		1.20
pool	−valine	287± 17	100%	273±11	100%	0.95
	+valine	163± 5	57%	110± 1	40%	0.68
protein	−valine	11561±580	100%	576±29	100%	0.050
	+valine	12256±550	106%	578±26	100%	0.047
Experiment 2						
medium	−valine	–		–		1.050
	+valine	–		–		1.049
pool	−valine	315± 3	100%	215± 1	100%	0.680
	+valine	218± 2	72%	113± 1	55%	0.516
protein	−valine	17224±444	100%	560±15	100%	0.033
	+valine	15988±529	92%	482±13	85%	0.030

The cells were grown for 3 to 5 days on medium A containing ^3H-leucine (0.5 μCi ml^{-1}). Every second day and 1 hr before the start of the experiment the medium was replaced by fresh medium A containing ^3H-leucine. – The experiments were started by adding 0.5 μCi ^{14}C-leucine ml^{-1} of medium to the flasks. To some flasks 10 mM valine was also added. At appropriate times (Experiment 1, 20 min; Experiment 2, 30 min) the incubation was stopped by removing the medium and washing the cells as described in Section II. The data shown are the average of at least six determinations.

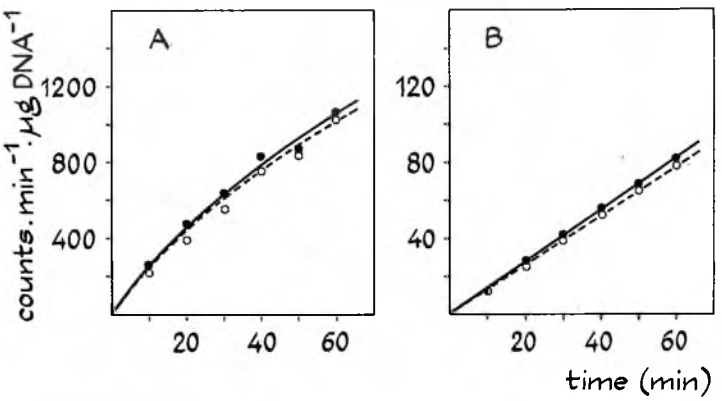

Fig. 3. The effect of 10 mM valine on influx (A) and incorporation (B) of ^3H-lysine. – Cells were incubated in medium A containing ^3H-lysine (1 μCi ml^{-1}) with (○-----○) and without (●——●) 10 mM valine. After the time periods indicated the cells were washed with Hanks solution containing 0.5 mM lysine. – Then the radioactivity per cell in the intracellular pool (A) and in protein (B) was determined. – The data shown are the average of at least three determinations.

Therefore our experiments clearly show that a change in specific radioactivity of leucine in the intracellular pool has no effect on the specific radioactivity of leucine incorporated into protein. Thus they confirm the hypothesis [7–11] that for amino acids entering the cell two roads are open. First the amino acids necessary for protein synthesis are selected. The 'spillover' of amino acids is funneled into the intracellular pool (or part of it) and from there effluxed into the extracellular space. This model may be applicable to several other cell-types and tissues with the possible exception of liver tissue [5] because of the amino acid-storage function of this organ.

What does this mean for studies in which the amount of incorporation of radioactive amino acid is used as a measure of the rate of protein synthesis? Our studies and the finding that a considerable amount of amino acids resulting from intracellular proteolysis is present in the acid soluble pool (Table I, ref. 14) justify the conclusion that measuring the specific radioactivity of the intracellular pool probably is of no value in this respect.

Furthermore, it has been suggested that the specific radioactivity of the extracellular precursor may not be constant [8, 10]. Another complicating factor is that reutilisation of amino acids probably occurs [15].

Therefore we think that the specific radioactivity of the aminoacyl-tRNA needs to be known. Only then can valuable conclusions about the rate of protein synthesis *in vivo*, as well as *in vitro*, be drawn.

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