INTERPRETATION OF DATA ON SEQUENTIAL LABELING OF GROWING POLYPEPTIDES*

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Incubation of rabbit reticulocytes with tritiated leucine results in differential labeling at leucine positions in completed globin chains (Dintzis, 1961). Using amino acid sequence data, Naughton and Dintzis (1962) were able to draw curves (N-D graphs, e.g. Fig. 1) relating the position of leucines along the chain to their degree of labeling. These curves were used to demonstrate that synthesis of globin chains proceeds sequentially from the amino terminus. The curves also reveal differences in rates of synthesis along the growing polypeptide. This fact has been appreciated in a general way, but uncertainty seems to exist concerning the exact interpretation of the graphs in these terms. It can be shown that the slopes at various points of an N-D curve are inversely proportional to the rates of chain growth through those points.

Fig. 2 represents diagrammatically a possible steady state distribution of lengths of polypeptide chains growing on active ribosomes. The N and C termini are indicated. Fig. 1 shows the results obtained by Naughton and Dintzis for the α-chain after the indicated incubation times. Consider the 4-minute incubation. If it takes just 4 minutes for the chain to grow from leucine-11 (strictly, the leucine in N-D peptide-11) to completion (and to appear in soluble globin), then completed chains which had growing ends between leucine-11 and leucine-31 at zero time (addition of label) will have acquired label at leucine-31. Fewer chains, those which at zero time had ends between leucine-11 and

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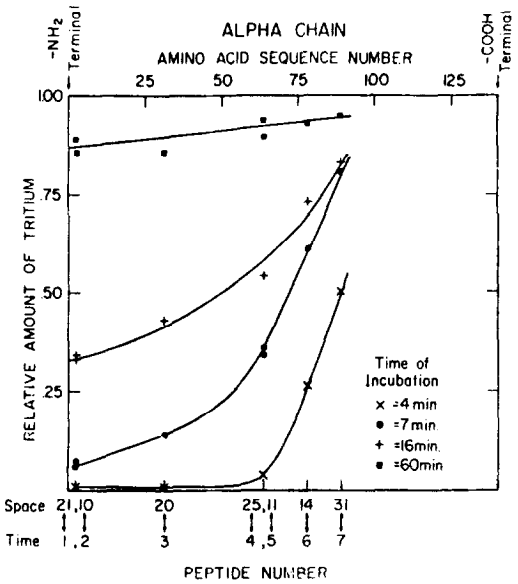


Fig. 1. Naughton-Dintzis curves for the α-chain of hemoglobin (Courtesy Proc. Nat. Acad. Sci.)

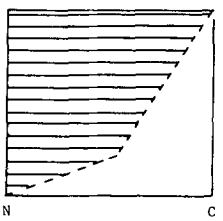


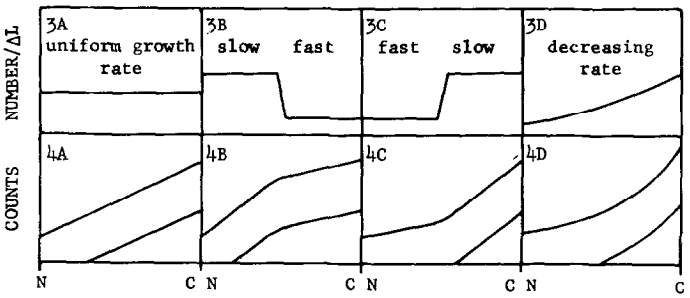
Fig. 2. A hypothetical distribution of lengths of nascent chains

leucine-14, will contribute to the observed radioactivity at leucine-14. Thus, the shape of the N-D curve, i.e. the increment of label between one leucine and another, reflects the number of nascent chains having their growing ends between those two leucines at zero time.

Differences in growth rates along the chain will result in a non-uniform distribution of lengths of the growing chains. Relatively more chain ends will be found in slow-growth regions (Fig. 2). Some possible distributions of ends (i.e. lengths) are shown in Figs. 3. A uniform distribution (Fig. 3A) results from uniform growth. If the chain grows at a uniform slow rate, then switches to a rapid rate, a distribution like that shown in Fig. 3B will result. The reverse - first fast, then slow growth - will generate distribution 3C. Distribution 3D will result from a continuously decreasing growth rate.

In the 4-minute incubation above, the label measured at a residue past leucine-11 indicates the number of chains having ends between leucine-11 and that residue. This number would be represented by the area

under an appropriate distribution curve between leucine-11 and the point in question. N-D graphs 4A-4D correspond to distributions 3A-3D. In



Figs. 3 and 4. Some nascent chain length distributions (Figs. 3) and the kind of Naughton-Dintzis graph each would generate (Figs. 4)

these, hypothetical curves for 4 and 8 minute incubations are shown for a protein which takes 6 minutes for total synthesis and stripping.

The results of Canfield and Anfinsen (1963) for lysozyme synthesis match the uniform growth situation of Fig. 4A (except at very early time, discussed below). The plots of Naughton and Dintzis (1962) (Fig. 1) for the α-chain of rabbit globin best match Fig. 4C. Fig. 3C and Fig. 2 represent this situation. It is useful to note that a line through the chain ends in a nascent chain diagram (Fig. 2) gives directly the shape of the N-D plot for that distribution. The N-D graph can also be considered a representation of the length of a growing chain (abscissa) vs. time (ordinate) (note reversal of the customary assignment of axes).

Only chains growing at zero time will be non-uniformly labeled. Once these are completed, chains produced will be uniformly labeled and will act to displace N-D curves uniformly upwards. Thus, curves for longer incubation times will be higher than, but parallel to, earlier curves. If these different curves are all normalized to the value 1 for their hottest peptide (e.g. Fig. 1), the later curves will appear flatter. Nevertheless, the ratio of slopes at any points on an N-D curve is always the inverse of the ratio of velocities of growth through those points (unless distorted by pool equilibration, discussed below).

Warner and Rich (1964) have concluded from their measurements of globin synthesis in rabbit reticulocytes that there is one growing peptide chain per active ribosome. In their calculations it was necessary to know the average length of the nascent, ribosome-bound chains. These authors interpreted the Naughton-Dintzis results as showing that the chains grow slowly at first, then more rapidly, giving an average nascent chain length of less than one-half chain. As shown above, however, the N-D graph for the α-chain suggests that the chain grows about 5 times faster through the first half of its length than through the second half.[1] The data for the β-chain are more equivocal, but the same picture of decreasing growth rate is seen. The average nascent globin chain length, then, is more than one-half chain. While the N-D graphs for hemoglobin do not specify the changing growth rates very precisely, the Warner-Rich calculation is rather insensitive to this uncertainty. A growth rate ratio of 5:1 (first half:second half) would give 0.56 nascent chains per ribosome by the Warner-Rich data. A ratio as low as 2:1 would give 0.65 chains per ribosome. As these authors point out, further correction for the skewing of the distribution of leucines towards the C terminus (Naughton and Dintzis, 1962; Diamond and Braunitzer, 1962) will lower the estimated number of ribosomes per chain. The exact correction which depends on the detailed (not on the average) position of the leucines and on the chain length distribution, cannot be specified for the present case but it would not be large enough to allow the Warner-Rich results to preclude more than one ribosome per chain.

It is worth noting that the type of experiment done by Dintzis can give no information on the kinetics of possible starting and finishing reactions which do not alter rates in other parts of the chain, since the N-D curve shape is sensitive only to the steady state distribution

[1] Lamfrom and Knopf (1964) find straight-line (cell-free) globin synthesis.

of chain ends between the first and last amino acid residues measured. A slow stripping reaction would delay the average time of appearance of residues by the average time required for stripping but would not change the ratios of slopes of different regions of an N-D curve. (Such reactions could, of course, affect the results of Warner and Rich.)

If the time for equilibration of added label with the operative amino acid pool is substantial, this can impress some upward concavity on N-D curves for relatively short incubation times. For incubation times longer than the equilibration time plus one total chain completion time, however, the equilibration effect will be simply to depress the entire curve and will not alter the ratios of slopes through the curve. Data of Warner and Rich (1964) showing leucine equilibration with the globin-synthesizing leucine pool to be essentially complete in about 1 minute indicate that equilibration effects are inconsequential to the results of Naughton and Dintzis, and the curves themselves support this conclusion. Canfield and Anfinsen (1963), working with hen oviduct minces in which impeded diffusion might will prolong pool equilibration, obtained upward concavity for their 3-minute incubation, while curves for longer times were accurately straight line.

Itano (1963) and Ames and Hartman (1963) have discussed the possibility that a particular "modulating" sRNA might act to slow the growth of a given polypeptide chain at some point along its length. Such a situation is depicted in Fig. 5. With respect to other possible mechanisms of modulation, it is noteworthy that the synthesis of lysozyme, a single chain enzyme, proceeds at a constant rate, while that of hemoglobin slows markedly as the chain lengthens. We perceive here, then, some process resulting in slowed growth and stripping. Such a growth-rate-limiting process, depending on interactions involving nascent chains, could account for such phenomena as altered rates of synthesis of mutant hemoglobins (Itano, 1957), polarity mutations, and the high percentage

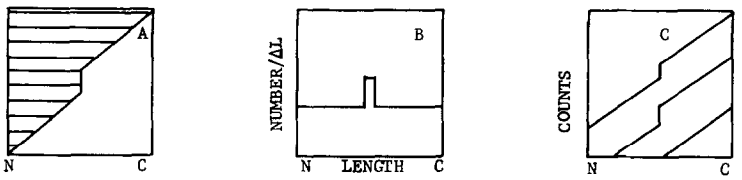


Fig. 5. Nascent chain length diagram (A), length distribution (B), and Naughton-Dintzis graphs (C) for a polypeptide growing at a constant rate except for a pause at one amino acid residue. "Backing up" of ribosomes, or its equivalent for membrane-bound ribosomes, might obscure this.

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of mutant (complementing) β-galactosidase subunits bound to ribosomes (Zipser and Perrin, 1963). A further dependence of folding and stripping on specific substrates, end products, and/or specific repressors (which might, then, have originally arisen as mutant complementary subunits of the regulated protein) could be the basis of enzyme induction and repression. (Repressor production itself might be analogously controlled.) In this scheme, nascent protein folding regulates the rate of production of more messenger by controlling the rate at which messenger is separated from the genome by ribosomes (see Byrne, et al., 1964; Stent, 1964; Bremer and Konrad, 1964). This scheme might be distinguishable from sRNA modulation by the type of experiment discussed above.

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