J.RADIOANAL.NUCL.CHEM.,LETTERS 137 /4/ 259-264 /1989/

ON THE HETEROGENEITY OF ^{125}I-LABELLED PROTEINS USED AS TRACERS IN RADIOIMMUNOASSAY

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Received 21 July 1989
Accepted 7 August 1989

The distribution of mono- poly- and unlabelled protein molecules in the radiolabelling mixture was calculated from probability point of view as a function of the radioiodine/protein ratio.

INTRODUCTION

In the majority of cases ^{125}I-labelled compounds are used as tracers in radioimmunoassay /RIA/. When iodinating a protein the radioiodine label is incorporated via aromatic electrophilic substitution in one or several of the tyrosine residues at position 3 and/or 5 /Fig. 1/.

Even if the protein exhibits only a single tyrosine residue, which is a relatively rare case, the labelling reaction mixture contains, besides unreacted free radioiodine, three components: unlabelled, mono- and dilabelled molecules. The relative amount of these components depends on the radioiodine/protein (I/P) ratio according to Eq. /1/ /Ref. 1/:

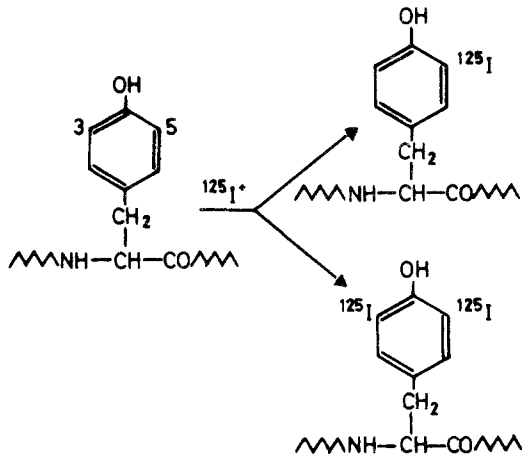

Fig. 1. General scheme of the formation of mono- and diiodothyrosine residue of proteins

$$F_n = \frac{m!\quad n}{n!\ /m-n/!\ r}\ (r/m)^n\ (1-r)m^{m-n} \qquad /1/$$

where m stands for the maximum number of reactable residues per protein molecule, n for the number of residues actually reacted, r for substitution degree, i.e., for the number of radioiodine atoms introduced per protein molecule and F_n for the fraction of radioactivity in the tracer contributed by molecules containing n radioiodine atoms.

The substitution degree, i.e., r can be calculated from the starting molar ratio of the reactants in the labelling mixture taking into account the efficiency of the labelling as well. The substitution degree thus calculated is an average, which is seldom a whole number. The fraction of mono- and polylabelled as well as unlabelled products can be obtained by dividing F_n by n. Eq. /1/ approaches the substitution degree from a proba-

bility point of view and does not take the heterogeneity of the potential sites of substitution into account. In fact, tyrosyl residues in proteins exist in different microenvironments and the unequal state of tyrosyls is reflected in their reactivity with iodine[2]. This renders the distribution of the mono-, poly- and unlabelled molecules more complicated as compared with that obtained by the use of Eq. /1/.

The aim of this paper is to demonstrate how the composition of the labelling reaction mixture and how the average specific activity of the components vary by changing the radioiodine/protein (I/P) ratio.

The specific activity of the tracer may influence the performance of the radioimmunoassay in two ways. Usually the higher the specific activity the higher the sensitivity of the assay. In this respect it seems to be advisable to increase the specific activity. On the other hand, polylabelled proteins usually lose their immunoreactivity and, in addition, with increasing specific activity the rate of radiolytic decomposition increases and thus the stability of the tracer decreases.

RESULTS

The distribution of the mono-, poly- and unlabelled protein molecules as a function of the I/P ratio in the labelling mixture was calculated by the use of Eq. /1/ for m = 2, m = 3, and m = 6 /Figs 2-4/.

From Figure 2 it turns out that even in the case of two reactable sites per molecule /e.g., one tyrosyl residue - apart from the marginal cases I/P≈0 and I/P≈2 - at any I/P ratio three components are present in the labelling reaction mixture. Since in the case of high molecular

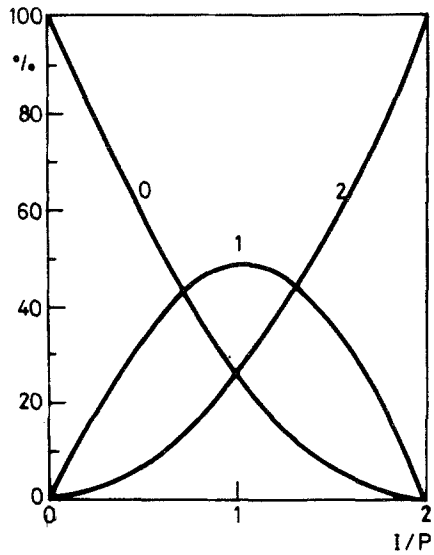

Fig. 2. The distribution of unlabelled /0/, monolabelled /1/ and dilabelled /2/ protein molecules as a function of radioiodine-protein ratio, m=2

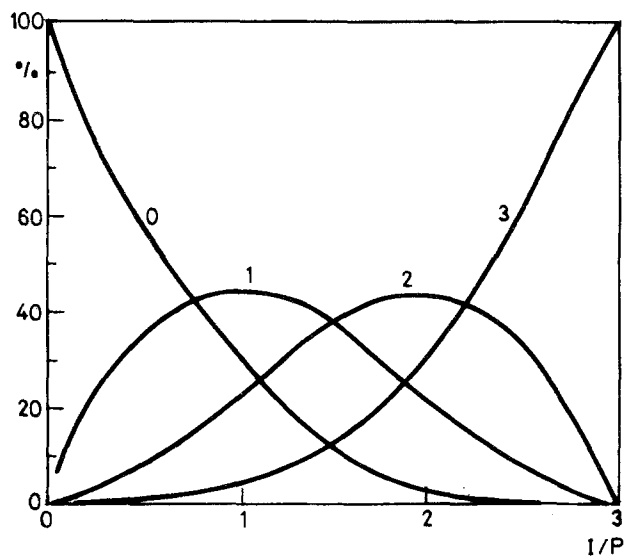

Fig. 3. The distribution of unlabelled /0/, monolabelled /1/ and polylabelled /2,3/ protein molecules as a function of the radioiodine-protein ratio, m=3

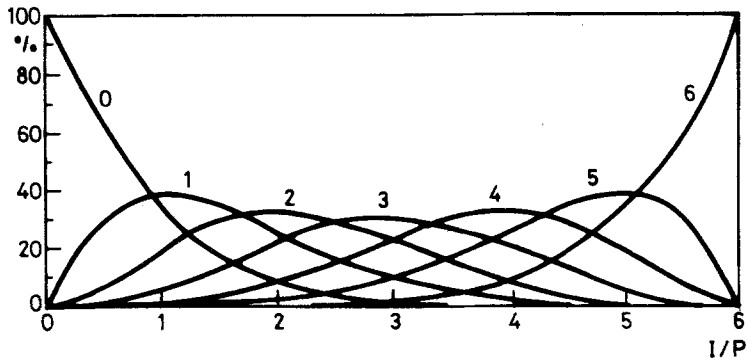

Fig. 4. The distribution of unlabelled /0/, monolabelled /1/ and polylabelled /2,3,4,6/ protein molecules as a function of the radioiodine-protein ratio, m=6

weight proteins no separation technique can distinguish unlabelled, mono- and dilabelled molecules, no uniformly labelled tracer can be produced at all.

For three reactable sites the distribution of unlabelled and labelled molecules is shown in Fig. 3 and for six reactable sites in Fig. 4.

CONCLUSIONS

From Figures 2-4 several conclusions can be drawn. The most important is that in the case of more than one reactive site no uniformly labelled protein can be produced at all. However, the specific activity of the tracer can be varied by changing the I/P ratio in the labelling reaction mixture from 0 to m, the optimum case, i.e., when each protein molecule contains one radioiodine tag and no unlabelled molecules remain in the reaction mixture, cannot be achieved. If m=6 and I=P, i.e., I/P=1 /see Fig. 4/ more than 30% of the protein molecules remain unlabelled, but in spite of this fact considerable amount of polylabelled molecules is also formed.

Since neither the increase of radiolytic decomposition nor the loss of immunoreactivity caused by the incorporation of more than one radioiodine atom into the protein molecule can be assessed on theoretical basis, the optimum specific activity is to be adjusted empirically.

REFERENCES

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