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Commentary

Measuring *levels* of proteins by various technologies: Can we learn more by measuring turnover?

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ABSTRACT

In routine experiments, scientists measure the *levels* of various substances such as proteins after various treatments. Detection of a change in levels suggests an impact of treatment on that particular protein. However, we sometimes forget the importance of turnover in this process. Proteins have half-lives that may change in response to treatments (which is in fact why *levels* may change), and an examination of half-lives may yield better clues as to how treatment affects the protein. After an exploration of the quantitative aspects of protein turnover, several interesting conclusions may be drawn. (1) Even though levels of some proteins may NOT change after treatments, their half-lives and turnovers do change, and these may be more sensitive indicators of the impact of treatments on the proteins of interest. (2) Treatments can affect protein *levels* because they alter either the synthesis or degradation of the protein or both. But, the rate of change of the levels depends on the half-life of the protein. If the experimenter waits only a fraction of a half-life of the protein after treatment, no significant change in level may be found since it can take up to 5 half-lives for the protein level to adjust to about 97% of its new level after treatment. (3) Half-lives of the same protein can vary in different species and experimental conditions may have to be altered if using different species. These factors suggest that a consideration of protein turnover and half-lives will be useful for future studies of this type.

1. Introduction

In many kinds of routine studies, pharmacologists and other scientists measure proteins by various technologies that include Western blotting, immunocytochemistry, mass spectrometry, etc. mRNAs have been measured by using microarray technology and RT-PCR. Proteins in living brains, for example, can be examined using PET scanning. These technologies have one thing in common, which is that they measure *levels* of proteins rather than some other index which might be a better measure of the utilization or activity of the protein. The proposal of this commentary is that a measurement of the *turnover or half-life* is a better indicator of the activity or utilization of these proteins. This is not a proven, general rule, but rather it is something worthwhile to consider.

It is known that proteins (and other molecules such as mRNAs) turn over, that is, they are degraded or removed and replaced by newly synthesized proteins. It is known that turnover rates of different proteins vary, and often depend on physiologic conditions. The concept of turnover is presented in Fig. 1, where there are two compartments. We can think of each of these compartments as being a brain region or a cell compartment or any entity where a measurement is made. Moreover, we can think of the dots in each compartment as protein molecules of interest. Note that if we measure the levels of the proteins in each box, we find 11 protein molecules, which is the same. However, there is a striking difference between the proteins in the top vs. the bottom boxes or compartments. The top compartment has one protein molecule that degrades per unit time, and it is replaced by one new molecule that is synthesized during that time. The second compartment is quite different. It degrades or loses 10 protein molecules per unit time, and 10 are synthesized to replace them. So, if we measure the levels of proteins, we see that there are 11 molecules in each compartment. However, that clearly does not provide an adequate description of the proteins in those compartments. The top compartment has proteins turning over much more slowly, while the bottom compartment has proteins turning over much more rapidly.

In the 1960s and 1970s, it was realized, in the field of neurotransmitter research, that simply looking at neurotransmitter levels alone did not adequately reflect the activity of the neurons containing those neurotransmitters. Rather, turnover of neurotransmitters became, and still is, a better measure, and

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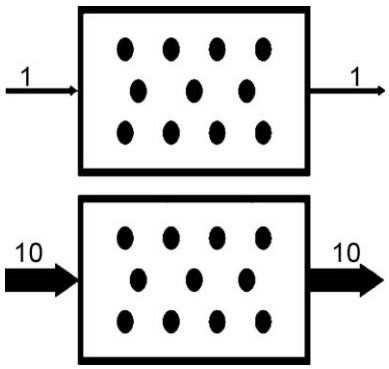

Fig. 1. Schematic illustrating the relative importance of turnover vs. levels of a protein. The concept and relative importance of turnover is illustrated by this figure, where there are two boxes or compartments. Either of these compartments could be a brain region or a cell compartment or any entity where a measurement is made. Let us think of the dots in each compartment as the protein molecules of interest. Note that if we measure the levels of the proteins in each box, we find 11 protein molecules, which is the identical. However, there is a striking difference between the top vs. the bottom boxes or compartments. The top compartment has one protein molecule that degrades per unit time, and it is replaced by one new molecule that is synthesized at about the same time. The second compartment degrades or loses 10 protein molecules per unit time, and 10 are synthesized to replace them. So, if we measure the levels of proteins, we see that there are 11 molecules in each compartment. However, that clearly does not provide an adequate description of the proteins in those compartments. The top compartment has proteins turning over much more slowly, while the bottom compartment has proteins turning over 10 times more rapidly. See text for further discussion.

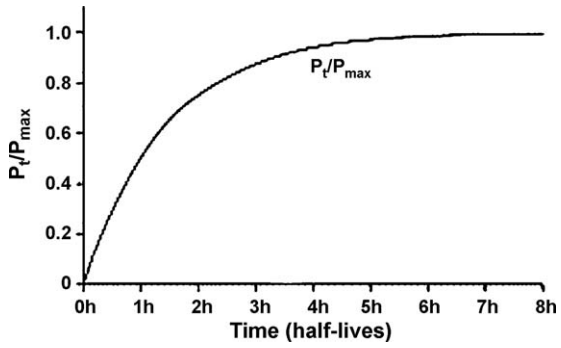

Fig. 2. The time for a protein to reach a new level after a change in synthesis rate or degradation rate depends on the half-life of the protein. The curve is generated from Eq. (3). If a treatment causes a change in synthesis or degradation, then the time it takes to reach about 97% of its new levels is 5 half-lives. See text for additional details. Adapted from Kuhar and Joyce [12].

perhaps the best measure, of neuronal activity and utilization of those neurotransmitters. Thus, ideas utilized in the past for other molecules and systems have relevance to current measurements of proteins and many other molecules such as mRNAs.

2. Half-lives of proteins

Examining Fig. 1 again, we can consider the idea of half-life which is well known to be the time that it takes for half the molecules in a compartment to be replaced. Half-lives have been measured for various substances for many years, and the quantitative formulation describing protein turnover and half-lives has been described in several places [12,14,16].

The equations relevant for describing these half-lives are as follows, and more of the details of the derivations can be found elsewhere. The basic equation for the change in protein levels over time is

$$P_t = (r/k)(1 - e^{-kt}) \quad (1)$$

where r is the rate of protein synthesis, and k is the degradation rate constant. P_t is the protein level at time t and P_{max} is the maximal level of the protein. t is the time after some event such as a change in synthesis or degradation, and as t becomes large, P_t reaches its maximal level, and we get

$$P_{max} = r/k. \quad (2)$$

Note that if either (or both) of the synthesis rate or degradation rate constant change, P_{max} will change unless the ratio of r/k remains about the same even after the change. This is really one of the points of this article. r and k can change together in the same fractional way without any significant or measureable change in P_{max} (levels). Therefore, simply measuring P_{max} does not provide more subtle information about the protein under study, which is how quickly it is synthesized or degraded.

Eq. (2) can be revised to

$$\frac{P_t}{P_{max}} = (1 - e^{-0.693t/h}) \quad (3)$$

where the half-life, h, is introduced. This is done because protein half-lives can be measured in the laboratory. The half-life of the protein is

$$h = \frac{0.693}{k} \quad (4)$$

It is intuitively clear that a faster turnover corresponds to a shorter half-life, while a slower turnover corresponds to a longer half-life.

A plot of P_t/P_{max} vs. h is shown in Fig. 2, where the exponential rise is evident. But if there is a sequence of proteins (say a total of "n" proteins in sequence) where the new level of a subsequent protein depends on the level of the previous protein, then we have:

$$\frac{Pn_t}{Pn_{max}} = (1 - e^{-0.693t/h1}) \times (1 - e^{-0.693th2}) \cdots (1 - e^{-0.693t/hn}), \quad (5)$$

where Pn_t is the level if protein "n" at time t, Pn_{max} is the maximal level of protein "n", $h1$ is the half-life of protein 1, and hn is the half-life of protein "n". After some thought and analysis of Eq. (5), the overall time for a change in levels of protein "n" will depend on the protein in the sequence with the slowest turnover or longest half-life [12].

Considering the case of a single protein as in Fig. 2, it is seen that after some treatment or change that alters synthesis or degradation, it will take 5 half-lives to reach about 97% of the new level. The change that alters synthesis or degradation could be the administration of some drug, either acute or chronic. Note that Fig. 2 suggests that the change observed is an increase because the curve rises, but it just as well could be a decrease with the level falling below the x-axis. The equation is the same but the sign of the change would be different.

This author realizes that reading equations can generate a strong urge to do something else. However, please read on.

3. Applications and examples

A well-known case where a protein level is changed and is associated with disease is the levels of D2 dopamine receptor proteins in brains of patients who have taken cocaine repeatedly for substantial periods of time. Fig. 3 shows that the levels of dopamine receptor proteins are decreased in cocaine abusers and return very slowly, over a period of months, towards normal levels [17]. Such a slow return suggests a relatively long half-life of D2 receptor proteins in humans, but of course other explanations are