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Order from Chaos: Observing Hormesis at the Proteome Level

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We report on an observed regularity in the overall response of cell proteome under influence of different doses of a peroxisome proliferator. Our analysis for the first time demonstrates presence of hormesis at the cellular level. It is shown that despite the fact that the response of individual proteins remains unpredictable, the perturbation of proteome as a whole (measured as the average departure of protein abundances from the corresponding values of the control group) shows regularity: It initially decreases, reaches a minimum, and then increases as the concentration of the used proliferator continue to increase. The work is based on the available data of Anderson at al. on the effects of peroxisome proliferator LY171883 on protein abundances in mouse liver when administrated at different concentrations.

Keywords: proteome perturbation • proteomics maps • hormesis • peroxisome

Introduction

It is well-known that changes produced by external factors in a cell proteome, such as the administration of toxins in the animal diet, cause unpredicted perturbations in the content of individual proteins.[1,2] This apparently chaotic response is reflected, for instance, in the fact that when a cell is exposed to various concentrations of an exogenous chemical, the response of individual proteins is not linear.[3,4] Thus, it has been difficult to establish reliable dose−response relationships for a proteome as a whole from individual proteins.[5] Here, we quantify the degree of perturbation of a proteome after the administration of an exogenous chemical by measuring the degree of departure of protein abundance from their nonperturbed positions in a proteomic map. Remarkably, we observe regularity in apparently chaotic data: the overall proteome perturbation depends on the toxin concentration and is moreover characterized by a J-shaped curve. The dose−response relationship reveals the existence of *hormesis*[6−8] in the proteome response to external perturbations. There is a low-dose region showing improved protein function, which is preceded by an initial inhibitory response and followed by the dysfunction response region.

Computational Method

We investigate the effects produced by the peroxisome proliferator LY171883 on the abundance of a large number of proteins in the livers of treated mice, data available from an earlier study of Anderson et al.,[9] who investigated the effects of administration LY171883 (illustrated in Figure 1) at different doses on liver cells of mice. Anderson et al.,[9] analyzed cell samples by quantitative 2-D electrophoresis. In Figure 2 we illustrate the nonlinear response of the abundance of a large number of individual proteins in the livers of mice treated with

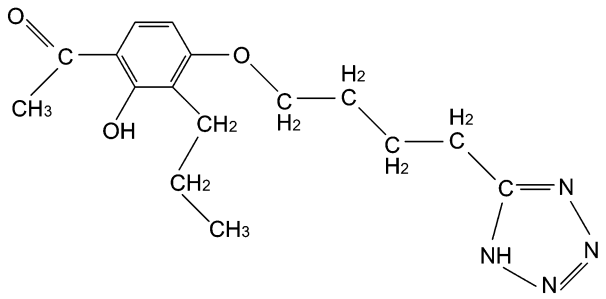

Figure 1. Peroxisome proliferator LY171883.

LY171883. The figure is based on their Table 1 in which variations of the abundance is given for about 100 protein spots. From Figure 2, it can be observed that some proteins increase their presence in cells with an increased toxin intake, some remain little affected, some decrease their concentration, and finally some show irregular changes. The dose response of individual proteins is clearly chaotic and unpredictable, but as we will see the overall effect on dose response curve does show some regularity.

To quantify the degree of perturbation of the whole proteome produced by the exogenous chemical we measure the scatter of the points s away from the line $z_C = z_0$ (that is $y = x$), after the application of each doses of LY171883:

$$s = \sqrt{\sum_i [z_0(i) - z_C(i)]^2}$$

where $z_0(i)$ is the abundance of protein i in the control, i.e., when no toxin has been added, $z_C(i)$ is the abundance of such protein in cells exposed to concentration C of the peroxisome proliferator and the sum is extended over all i proteins

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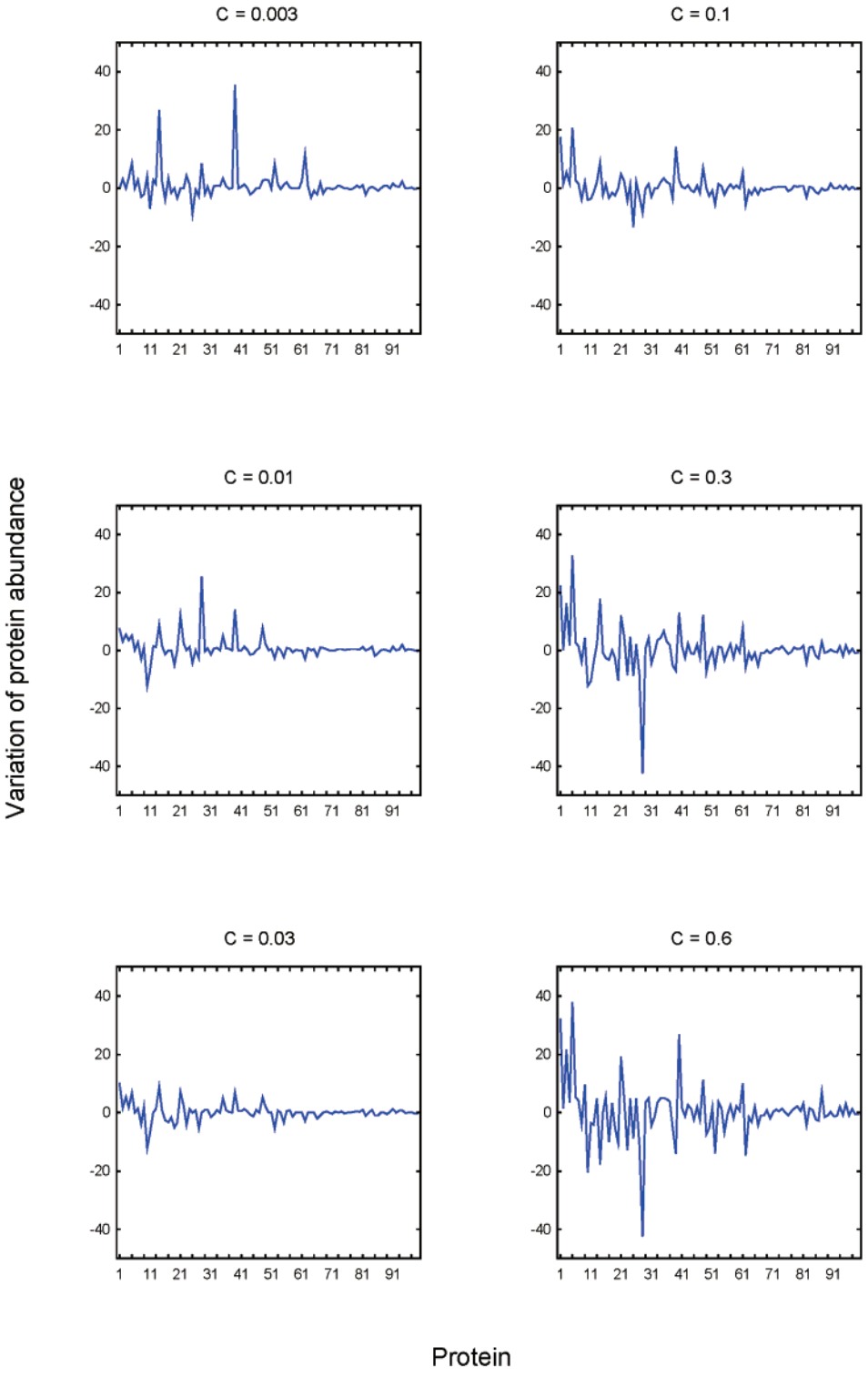

Figure 2. Variation of protein abundance for 99 proteins in the mice liver proteome after the administration of different doses of the peroxisome proliferator LY171883 respect to the unperturbed proteome.

identified in the proteomic map. Observe that in this analysis we have focused on cell proteome by simply considering only the information on the variation in the abundance of protein spots of the proteomics maps, and hence we are not trying to characterize the changes in the proteome maps, which would include information on the x and y coordinate of proteins spots. This appears to have been the objective of Anderson et al.[9] as well as of more recent analysis of the same data based on the construction of special distance matrix for a set of most intensive protein spots.[10]

Results

The idea behind the current approach is that the proteome as a whole produces an ordered response to the external factor despite apparent chaotic response of individual proteins. We consider that a proteome as a whole is more perturbed by an

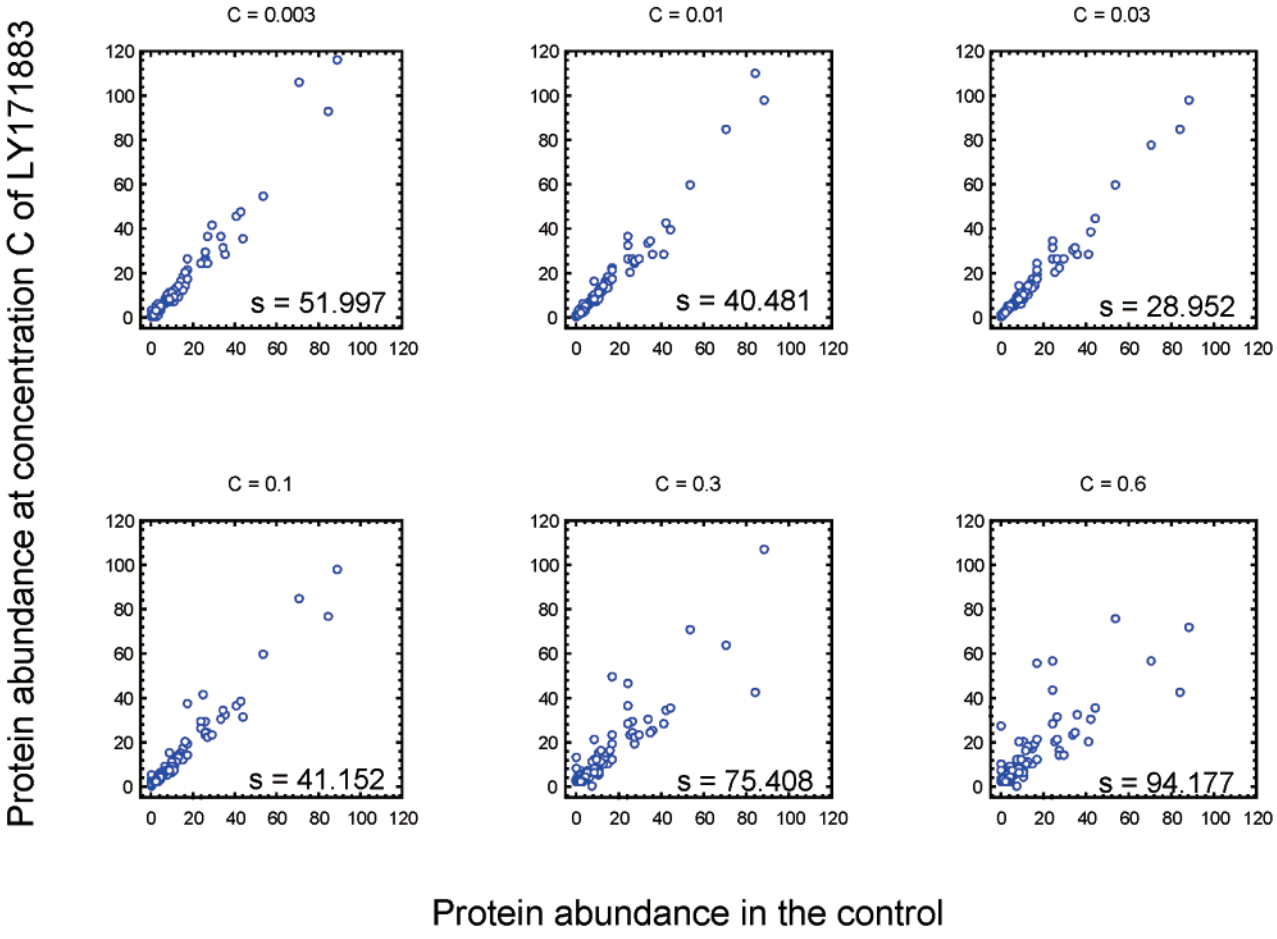

Figure 3. Perturbation of mice liver proteins after the administration of different doses of the peroxisome proliferator LY171883 measured by the degree of dispersion respect to the unperturbed proteome.

external factor when the abundance of individual proteins deviate more from the line $y = x$, in which x and y are the corresponding number-labels for the same protein spot selected for display. The ideal line $y = x$ means that there is no change in the protein abundance before and after incident. In Figure 3, we illustrate plots for the six different concentrations of LY171883 added to mice diet: $c = 0.003$; $c = 0.01$; $c = 0.03$; $c = 0.10$; $c = 0.30$; and $c = 0.60$ or 0.60% of the diet. The proteins on the line $y = x$ (which is for clarity not shown in Figure 2 but can be imagined to connect the origin with the corner at the opposite site of each of the six diagrams) have not changed their abundance. The proteins above the line $y = x$ have increased their abundance in the proteome, while proteins below the line $y = x$ have decreased their abundance in the proteome. Observe the rather chaotic response of individual proteins to variations of the concentration of LY171883 in the diet. Observe also that in each case there are proteins spots that have increased and decreased their abundance and that often these are not the same proteins.

As we can see from the values of s (see Figure 3), the proteome perturbation does not linearly increases with the increase in the toxin dose concentration but rather displays a characteristic initial "dip" which tells that at a window of rather small concentrations proteome is less perturbed than it was initially (see Figure 4). This type of J-shaped curve is known as *hormesis*,[6–8] which is an adaptive response to low levels of stress or damage resulting in improved fitness for some physiological systems for a finite time. The hormesis is defined as overcompensation to a disruption in homeostasis.[6,11] It is characterized by the disruption of homeostasis, the modest overcompensation, the reestablishment of homeostasis, and the adaptive nature of the process. Our current findings shows that in a cell hormesis can represents the advantage gained by the proteome

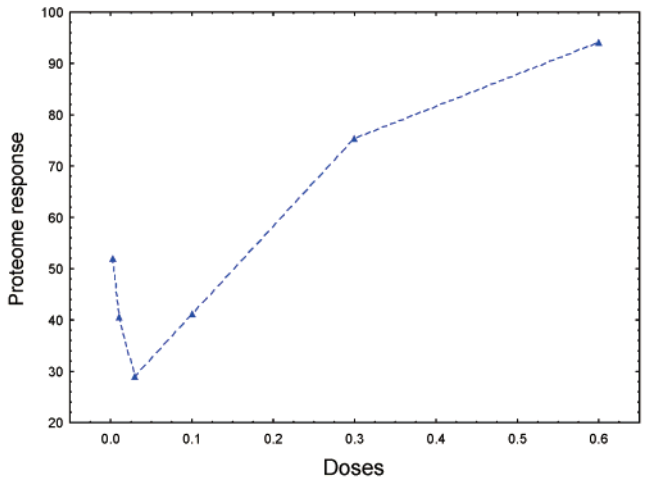

Figure 4. Dose−response relationship for the whole proteome of mice liver after the administration of different doses of the peroxisome proliferator LY171883. Response of the whole proteome is measured by the degree of perturbation respect to the unperturbed proteome.

from resources initially and principally allocated for repair activities but modestly in excess of that needed to repair the immediate damage produced by an external chemical agent.

Discussion

It should be worth commenting that our findings do not indicate any clear relation with possible beneficial effects of low doses of toxic substances. It is difficult to estimate possible beneficial effects from a dose−response study due to considerable biological complexity and because beneficial effects are very dependent on individual settings. As Calabrese and Baldwin[6] have remarked even though hormesis is considered an adaptive response the issue of beneficial/harmful effects should be left to a subsequent evaluation of the biological context of the response. However, what our current findings make clear, it is that in studying dose−responses relationships in proteomic analysis, such as in the emergent area of toxicoproteomics,[12−14] hormesis is an important factor that should be taken into account in providing risk assessment for toxic substances in biological and ecological environments. We should add that already Andersen at al.[9] recognized different behavior of the response curve for small concentrations and large concentrations and concluded that the low-dose and high-dose effects of peroxisome proliferates could be qualitatively different. Similarly in a subsequent analysis of the same data, the work of Randić et al.,[10] one can notice a larger scatter of points in the dose−response curve for small concentrations of LY171883. Now it is clear that at least in the case of the later study, the information on proteome was obscured to some degree by including data on proteomics maps, that is, data on the proteins mass and the relative charge, which was incorporated in multivariate statistical analysis, including use of principal component analysis (PCA) but which is not essential for evaluation of proteome as such.

The relationship between the here introduced s-index, which measures the average perturbation of the proteome, and the PCA components in the work of Andersen et al.[9] is less clear. Anderson et al. used PCA as a surrogate for the equivalent dose to examine the effects of different compounds on a common scale. Subsequently, they focused on specific protein responses. Principal components combine entry data in order to find novel axes so that PC1 shows maximal variance, and PC2 shows the next maximal variance in data. It represents multivariate analysis of the same input data but expressed from different points of view. It thus may offer new information but does not creates novel data. Our approach deals with the average variations in data, regardless of the orientations of coordinate axes. It used novel data, the average global abundance, which has led to a novel insight on the cell proteome.

Could the s-index be affected by a few outliers, and thus give the effect described as hormesis? We think this is not possible not only because we are considering the average change for a total of 99 protein spots, each of which contributes 1/99 part of its contribution but also because it is unlikely that bad gel or some other experimental cause will affect all the seven gel plates in the same manner.

Finally, we would like to indicate the relationship between the hormesis effect and the obvious difference between different proteins, some of which are induced at low doses and some at high doses. Andersen et al. have in fact identified several proteins and followed their different behaviors under different concentrations of LY171883. Thus, for example, cytosolic epoxide hydrolase (spot IEF: 22) shows evidence for induction at a low dose followed by a plateau at higher doses. In contrast, peroxisomal bifunctional enzyme (spot BASO 76) from an almost undetectable level continues to steadily increase without plateau. The history of each protein case has been plotted in Figure 2, and while individually, they appear chaotic, though each on its own being very important, we have demonstrated that globally, they show a collective effect—hormesis: initially, the overall perturbation decreases with an increase of the concentration of LY171883, reaches the minimum, and then continues to increase not showing plateau. Whether a similar response will accompany other toxins remains to be seen, but at least now we have an advantage in knowing that hormesis is possible at the cellular level, and hence we should look for its presence or absence in other cellular systems.

Concluding Remarks

Hormesis has been known for many years at the level of whole organisms reflected in the enhanced growth of plants exposed to low doses of selected compounds or as better survival of experimental animals exposed to small doses of radiations as compared to control animals. The result presented here for the first time clearly demonstrates the J-shape regularity at the cellular level in the dose−response of the domain from small to large concentrations.

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