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Linkage effects in a model for cell survival after radiation

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A thermodynamic treatment for the effects of radiation on cell survival is proposed. The treatment is an extension of the linear-quadratic model (K.H. Chadwick and H.P. Leenhouts, Phys. Med. Biol. 13 (1973) 78) following the principles of linkage thermodynamics (E. Di Cera, S.J. Gill and J. Wyman, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 5077). Linkage effects between chemical binding to DNA and radiation action are considered, along with the synergism between different types of radiations. A simple mathematical condition is found for the additivity of radiation doses that result in an isoeffect. The resolvability of the model parameter is investigated by simulations and statistical analysis of the distributions obtained.

1. Introduction

The quantitative analysis of the effects of radiations on living cells has resulted in several attempts to develop suitable models for the description of the dose-response curves observed experimentally [1,2]. It is generally accepted that among the possible molecular lesions induced by radiation action on living cells, those involving the DNA molecule are of critical importance. These lesions arise from radicals produced by the passage of the radiation through the aqueous medium surrounding the DNA. In particular, the double-strand break (dsb) of the DNA molecule represents a most critical lesion which easily leads to cell death [3,4]. On the other hand, the single-strand break (ssb) is a molecular lesion which is efficiently repaired [5] and is not of great importance for the biological effect. Consistent with these experimental facts, Chadwick and Leenhouts [6,7] have proposed a model, referred to as the linear-quadratic (LQ) model, which relates cell survival, S, to the number of dsbs induced by radiation action in the DNA molecule. This number is assumed to be a linear-quadratic function of the dose of radiation, D, so that

$$S = \exp(-\alpha D - \beta D^2) \tag{1}$$

where α and β are two parameters to be determined experimentally.

The LQ model postulates that the dsb of the DNA molecule can be produced according to two different mechanisms. The first is breakage induced by a single ionizing particle and is proportional to D. The second is breakage induced by two separate ionizing particles and is proportional to D^2. Combination of these two possible mechanisms of cell damage yields eq. 1 for the probability of cell survival, after assuming that the lethal events follow the Poisson distribution [7].

The LQ model has successfully been applied to the analysis of a substantial body of experimental data [7]. Its beauty stems from both the mathematical simplicity and the underlying physical mechanisms invoked to explain the observed biological effects. The model parameters α and β have been measured for several cell types and

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tissues and, in some cases, under a variety of experimental conditions. This provides a considerable data base which allows for a deeper understanding of both the molecular theory and the significance of its physical parameters. It is the purpose of this paper to give the LQ model a broader theoretical basis, using considerations derived from the law of mass action. The approach we take here is cast within the framework of linkage thermodynamics [8,9] and is focussed on the phenomenological treatment of linkage effects. These effects have been documented experimentally by studying, for example, the influence of ionic strength on cell survival after radiation [10], or the synergistic action of radiations of different nature [11]. However, their possible relevance in connection with a more general formulation of the LQ model has not been recognized. It is therefore of relevance to develop such a general formulation for the description of reciprocal effects in the interaction of radiation with living cells. The strength of this approach is revealed by the derivation of linkage relations that have an immediate practical application to the analysis of experimental data. These relations broaden our understanding of the detailed molecular mechanisms of radiation action and provide a quantitative basis for further theoretical investigation of the subject. Along with the theoretical extension of the LQ model, we consider here the problem of the resolvability of the model parameters and their correlation.

2. Theory

We start from the basic postulate of the LQ model and consider the elementary effect responsible for cell death, the dsb of the DNA molecule. This effect can be produced in two different ways: the first one is due to a single ionizing particle and is proportional to the dose D. The second one is produced by two independent hits and is proportional to D^2. In these basic assumptions we recognize a parallel with the law of mass action, in the sense that the relation between the dose D and the effect observed, i.e., the number of ionizing particles producing a dsb, can be formulated as in the case of chemical binding phenomena in a biological macromolecule. In this respect the dose D can be considered to play the same role as the activity of a ligand. With such a parallel in mind we write down a partition function, Z, as follows

$$Z = 1 + aD + bD^2 \tag{2}$$

Each term in the partition function is proportional to a given configuration relative to the elementary effect being considered in the interaction of the radiation with the DNA. The first term refers to the absence of dsbs, and hence of biological damage. This term may include ssbs followed by repair. The second refers to a dsb due to a single ionizing particle, and the third one refers to a dsb produced by two separate ionizing particles.

From the partition function we compute by differentiation the average number of ionizing particles, n, that have produced a dsb at a given dose, D, as follows

$$\frac{\mathrm{d} \ln Z}{\mathrm{d} \ln D} = \frac{aD + 2bD^2}{1 + aD + bD^2} = n \tag{3}$$

The coefficients a and b can be given a mass law interpretation as 'affinity constants' for the occurrence of a dsb induced in the two alternative ways. The coefficient a is the inverse of the dose which gives 50% of the ionizing particles that can induce a dsb by a single hit. The coefficient b is the inverse of the dose squared which gives 50% of the ionizing particles that can induce a dsb by two separate hits. Eq. 3 thus gives the average number of 'lethal ionizing particles' per dsb. If we assume that the lethal events are all independent, then the total number of lethal ionizing particles per DNA molecule, N, is given by

$$N = m \frac{aD + 2bD^2}{1 + aD + bD^2} \tag{4}$$

where m is the number of base-pairs per DNA molecule. One sees from eq. 4 that when $b = 0$ the maximum number of lethal ionizing particles per DNA equals the number of base-pairs, since they all produce a dsb by a single hit. On the other hand, for positive values of the coefficient b the

maximum value of N is $2m$, since dsbs induced by separate hits become predominant at high doses.

Since n is very small at doses that are of interest in the study of eukaryotic cells [12], the value of N can be approximated by

$$N = \alpha D + \beta D^2 \tag{5}$$

The coefficients α and β are now combinations of the constants a and b and the number of base-pairs m per DNA molecule. Experimental determination of α and β shows that they are of the order of 10^{-1} Gy^{-1} and 10^{-2} Gy^{-2}, respectively. Since m is of the order of 3×10^9 in eukaryotic cells, we conclude that the coefficients a and b must be of the order of 10^{-11} Gy^{-1} and 10^{-12} Gy^{-2}, respectively. Therefore, in the dose range from 0 to 30 Gy, which is the one usually employed in experimental studies of radiation action, the value of Z is practically equal to 1 and n is indeed negligible. This justifies the use of eq. 5 for practical purposes. Saturation effects, that are unlikely to occur at the doses used experimentally [12], can however be treated with the use of eq. 4, which is the exact mathematical form relating the dose D to the number of lethal ionizing particles N.

Although the occurrence of a dsb is intrinsically a rare event (see eq. 4), nevertheless the number of possible dsbs per DNA molecule is almost infinite (about $3 \cdot 10^9$), which makes it convenient to assume that the number of lethal ionizing particles per DNA, at a given dose, follows the Poisson distribution. This is a straightforward application of basic principles of statistics [13]. The probability that there are 0 lethal ionizing particles per DNA gives the probability of cell survival, S, at a given dose, D, as

$$S = \exp(-N) = \exp(-\alpha D - \beta D^2) \tag{6}$$

which is identical to the expression for cell survival of the LQ model [6,7].

This result, obtained from considerations based on the law of mass action, provides the theoretical basis for an extension of the LQ model along the principles of linkage thermodynamics [8,9], where the quantitative description of linkage effects dominates the picture.

3. Linkage effects

The probability of cell survival as a function of the dose of radiation can be affected by several factors. Some of them are linked to the cell cycle, mitosis being the most sensitive phase to radiation action [14,15]. Other factors that reduce cell survival are exposure to high oxygen partial pressures [16], or lowering the ionic strength of the medium [10]. From the point of view of linkage thermodynamics, which is the one we are concerned with here, any substance which binds to the DNA molecule may affect the occurrence of a dsb and vice versa. The mutual interference between radiation damage and the binding of substances to DNA can be considered as a linkage effect that can be explored experimentally.

Starting from the partition function (eq. 2) we describe the effect of ligand binding to DNA and radiation action as follows

$$Z = \sum_{j=0}^{p} A_{0j} x^j + \sum_{j=0}^{p} A_{1j} x^j D + \sum_{j=0}^{p} A_{2j} x^j D^2 \tag{7}$$

Here the A parameters are mass law coefficients describing both ligand binding of an arbitrary substance x, whose activity is x, and the effect of radiation. The constant A_{0j} is the overall equilibrium constant for the reaction $BP + jX = BPX_j$ (BP = base-pair) when no dsb has occurred. The constants A_{1j} and A_{2j} describe the same reaction when a dsb has occurred by a single hit or two separate ones, respectively. The parameter p is the number of binding sites for ligand X per base-pair. The number of ligand molecules bound per DNA, X, at a given dose D, is

$$X = m\left(\frac{\partial \ln Z}{\partial \ln x}\right)_D \tag{8}$$

and the number of lethal ionizing particles per DNA, at a given ligand activity x, is given by

$$N = m\left(\frac{\partial \ln Z}{\partial \ln D}\right)_x \tag{9}$$

From the relations above it is easy to derive linkage relations such as

$$\left(\frac{\partial N}{\partial \ln x}\right)_D = \left(\frac{\partial X}{\partial \ln D}\right)_x \tag{10}$$

or

$$\left(\frac{\partial N}{\partial X}\right)_D = -\left(\frac{\partial \ln x}{\partial \ln D}\right)_X \tag{11}$$

that give a measure of the mutual interaction between chemical binding and radiation effects.

The most important implication of the existence of a linkage between chemical binding and radiation effects is the possibility of expressing the coefficients α and β of the LQ model as a function of the ligand activity x as follows

$$\alpha = m \sum_{j=0}^{p} A_{1j} x^j / \sum_{j=0}^{p} A_{0j} x^j \tag{12}$$

$$\beta = m \sum_{j=0}^{p} A_{2j} x^j / \sum_{j=0}^{p} A_{0j} x^j \tag{13}$$

Hence

$$\frac{\mathrm{d} \ln \alpha}{\mathrm{d} \ln x} = X_1 - X_0 = \delta X_{10} \tag{14}$$

$$\frac{\mathrm{d} \ln \beta}{\mathrm{d} \ln x} = X_2 - X_0 = \delta X_{20} \tag{15}$$

The relations above have an immediate practical application. The dependence of $\ln \alpha$ and $\ln \beta$ on the logarithm of the ligand activity x gives the change in ligand molecules bound to DNA upon induction of a dsb. The term δX_{10} is a measure of the linkage when the dsb is induced by a single ionizing particle, while δX_{20} is the corresponding linkage for a dsb induced by two separate ionizing particles.

An example of the application of eqs. 14 and 15 is reported in fig. 1. Cell survival curves obtained at different Na^+ concentrations [10] show a significant change of the two coefficients α and β. The slope of the line interpolating the experimental points yields -0.2 mol Na^+ per dsb in the case of a single ionizing particle, and -0.44 mol Na^+ per dsb in the case of two ionizing particles. Interestingly, the two linkage coefficients δX_{10} and δX_{20} seem to scale with the number of lethal ionizing particles involved in a dsb, which suggests that 0.2 mol Na^+ are released per lethal ionizing particle. One sees how the linkage approach developed here can be used to unravel the quantitative aspects of the mechanism of radiation action. This approach is thus of critical importance in the analysis of experimental data revealing a mutual interference of chemical binding and radiation damage.

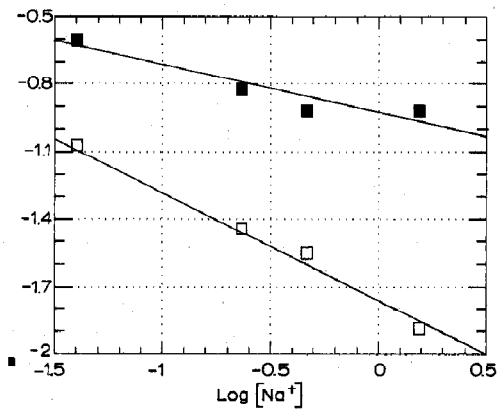

Fig. 1. Linkage between Na^+ binding and radiation action to DNA in the case of Chinese hamster cells [10], illustrated by the change of the logarithm of α (■) and β (□) as a function of the logarithm of Na^+ activity. One sees that cell survival increases with increasing Na^+ activity. This means that Na^+ binding to DNA stabilizes the configuration with no dsb with respect to the others (see eq. 7). The two lines are the best fits to the experimental data. The slope of each line gives the number of Na^+ released when a dsb is induced by a single ionizing particle (■) or two separate ones (□), as implied by eqs. 14 and 15. The values are: $\delta X_{10} = -0.21 \pm 0.06$, and $\delta X_{20} = -0.50 \pm 0.03$. Note how the ratio $\delta X_{20}/\delta X_{10}$ is practically equal, within errors, to 2. This suggests that the number of Na^+ released per lethal ionizing particle is constant.

4. Synergism as identical linkage

In the description given above concerning linkage effects between chemical binding and radiation action we have shown how experimental determination of the two coefficients α and β can be used to evidentiate chemical phenomena that accompany the occurrence of a dsb. Another class of linkage effects to be considered here is the one involving two different types of radiations that both lead to dsbs. This phenomenon, usually referred to as synergism, is of extreme relevance in both biological and medical fields [7]. It is here that the strength and elegance of the approach

based on linkage thermodynamics can be appreciated best. From the standpoint of linkage thermodynamics the synergistic interaction of two radiations of different nature is an example of identical linkage [9,17,18]. In this case the partition function is given by

$$Z = 1 + aD + bD^2 + sE + tE^2 + cDE \qquad (16)$$

where E denotes the dose of the second radiation, and the coefficients s and t have the same significance as a and b in eq. 2, and refer to the second type of radiation. The coefficient c refers to the configuration where a dsb is produced by two separate ionizing particles, one for each type of radiation.

The 'identical' nature of this kind of linkage arises from the fact that where a dsb has occurred due to the action of one type of radiation, there is no possibility of producing a dsb by means of the other type of radiation. In other words, the effects of the two types of radiations are mutually exclusive, as seen in the binding reactions of oxygen and carbon monoxide to the heme site of human hemoglobin [18], which has led to the definition of identical linkage [17]. In this respect, the identical linkage is quite different from the linkage described by eq. 7, where the two effects under consideration, i.e., ligand binding and radiation action, are not mutually exclusive. Notwithstanding, they are identically linked, and hence mutually exclusive, the two types of radiation lead to the same molecular lesion, the dsb. In such a feature one recognizes their synergistic interaction. The number of lethal ionizing particles per DNA due to the first type of radiation can be calculated as in the case of eqs. 3–5 as follows

$$N = m\left(\frac{\partial \ln Z}{\partial \ln D}\right)_E \approx \alpha D + \beta D^2 + \mu DE \qquad (17)$$

and similarly for the second type of radiation one has

$$Q = m\left(\frac{\partial \ln Z}{\partial \ln E}\right)_D \approx \sigma E + \tau E^2 + \mu DE \qquad (18)$$

The probability of cell survival is then given by

$$S = \exp(-N - Q)$$
$$= \exp(-\alpha D - \beta D^2 - \sigma E - \tau E^2 - 2\mu DE) \qquad (19)$$

which is identical to the expression derived by Chadwick and Leenhouts [7] without taking into account considerations based on the law of mass action. Eq. 19 shows that S is a function of the sum $N + Q$, and that the two types of radiation are synergistic in producing cell death.

From the partition function one can derive linkage relations such as eqs. 10 and 11. There is, however, a most important aspect in connection with the synergistic interaction of the two types of radiation. The ratio N/Q gives a measure of the partitioning between lethal ionizing particles due to the two types of radiation with respect to the DNA molecule. A partition law can be formulated as follows

$$N/Q = \phi D/E \qquad (20)$$

i.e., the ratio of lethal ionizing particles due to the two types of radiation is proportional to the ratio of the respective doses times a constant factor ϕ, the partition coefficient. This law is the exact parallel of the Haldane law [19] for the partitioning of two identically linked ligands such as oxygen and carbon monoxide to human hemoglobin [18]. The partition coefficient is equal to the ratio of the doses E/D when $N = Q$. The validity of the partition law given above implies that the survival curves obtained as a function of the logarithm of the dose of the two types of radiations must be parallel, the displacement of the two curves being constant and equal to the logarithm of ϕ. This simple condition can readily be tested from the analysis of experimental data. Failure of the partition law necessarily implies that ϕ is not constant.

An important consequence of the partition law is that if ϕ is indeed constant then the sum $N + Q$ is a function of $E + \phi D$ only. This means that in the domain where the partition law, eq. 20, holds the doses of the two types of radiation for which $N + Q$ is constant are additive. Demonstration is as follows. From the partition function, eq. 16, it results that [9]

$$\left(\frac{\partial \ln E}{\partial \ln D}\right)_{N+Q} = -\left(\frac{\partial N}{\partial Q}\right)_{\ln D - \ln E} \qquad (21)$$

This is a most important relation in the theory of identically linked ligands [9,17], and is even more

relevant in connection with the phenomenon of synergism because it describes an isoeffect. In other words, since S is a function of $N+Q$ only, when this sum is constant so is the effect. Therefore, the partial derivative on the left-hand side of eq. 21 gives the dependence of ln E on ln D which results in an isoeffect. This partial derivative is equal to the change of Q with respect to N which keeps the ratio D/E constant. Now, if the partition law, eq. 20, holds, then the right-hand side of eq. 21 is simply $\phi D/E$, so that

$$\left(\frac{\partial E}{\partial D}\right)_{N+Q} = -\phi \tag{22}$$

Hence,

$$\frac{\partial}{\partial D}(E+\phi D)_{N+Q} = 0 \tag{23}$$

i.e., the sum $E+\phi D$ is a function of $N+Q$ only and, conversely, the sum $N+Q$ is a function of $E+\phi D$ only. Eq. 20 thus represents a simple mathematical condition for additivity of doses of different types of radiation to yield an isoeffect. This result is of extreme practical importance as it establishes a quantitative criterion for the occurrence of synergistic isoeffects.

The condition of additivity, eq. 23, introduces constraints among the coefficients of eq. 19. The general form of ϕ is obtained from eq. 20 as

$$\phi = \frac{\alpha+\beta D+\mu E}{\sigma+\tau E+\mu D} \tag{24}$$

and the constancy of ϕ for any values of D and E necessarily demands that

$$\alpha = \phi\sigma \tag{25}$$

$$\beta = \phi\mu = \phi^2\tau \tag{26}$$

These are necessary and sufficient conditions for the validity of the partition law, eq. 20, and hence of eq. 23. Substitution of these conditions into eqs. 17 and 18 shows that $N+Q$ is a function of $E+\phi D$ only.

A partition coefficient surface can be constructed from eq. 24 by plotting ϕ vs. D and E. Validity of the partition law, eq. 20, demands the partition surface to be flat for arbitrary values of D and E. Experimental determination of the coefficients of eq. 24 has been reported for the case of the synergism between X-rays and ultraviolet radiation on Chinese hamster cells [11]. The corresponding partition coefficient surface is shown in fig. 2. One sees that the surface is flat only at relatively high doses, and therefore the partition law, eq. 20, and hence the condition of additivity, eq. 23, do not hold at low doses.

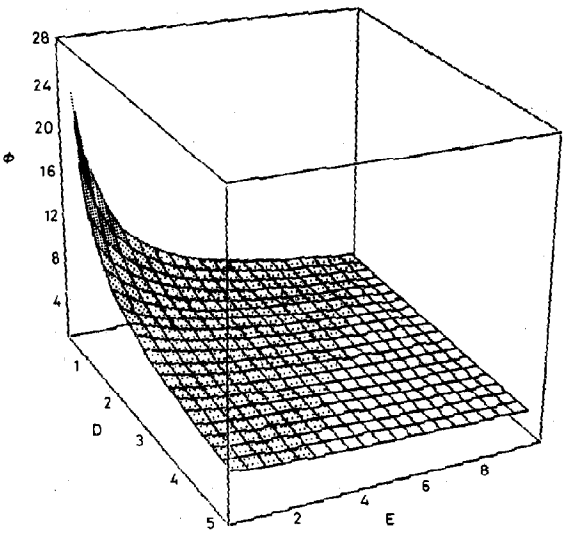

Fig. 2. Partition coefficient surface for the synergism of X-rays (the dose D is in Gy) and ultraviolet radiation (the dose E is in J/m^2) on Chinese hamster cells [11]. The surface was drawn according to eq. 24 with the parameter values [7,11]: $\alpha = 0.21$ Gy^{-1}; $\beta = 0.0266$ Gy^{-2}; $\sigma = 0$ (J/m^2); $\tau = 0.0102$ (J/m^2)$^{-2}$; $\mu = 0.016$ Gy^{-1} (J/m^2)$^{-1}$. One sees that the surface is flat only in the domain where the doses are relatively high. In this domain the partition law, eq. 20, holds, the partition coefficient ϕ is constant, and the doses that give an isoeffect are additive.

5. Discussion

The basic principles of linkage thermodynamics [8,9] have been used to extend the LQ model [6,7] in order to include linkage effects. We have shown how the mutual interference of chemical binding and radiation action, as well as the important phenomenon of synergism, can be rationalized with the help of simple considerations based on the law of mass action. The phenomenological treatment proposed here provides a more quanti-

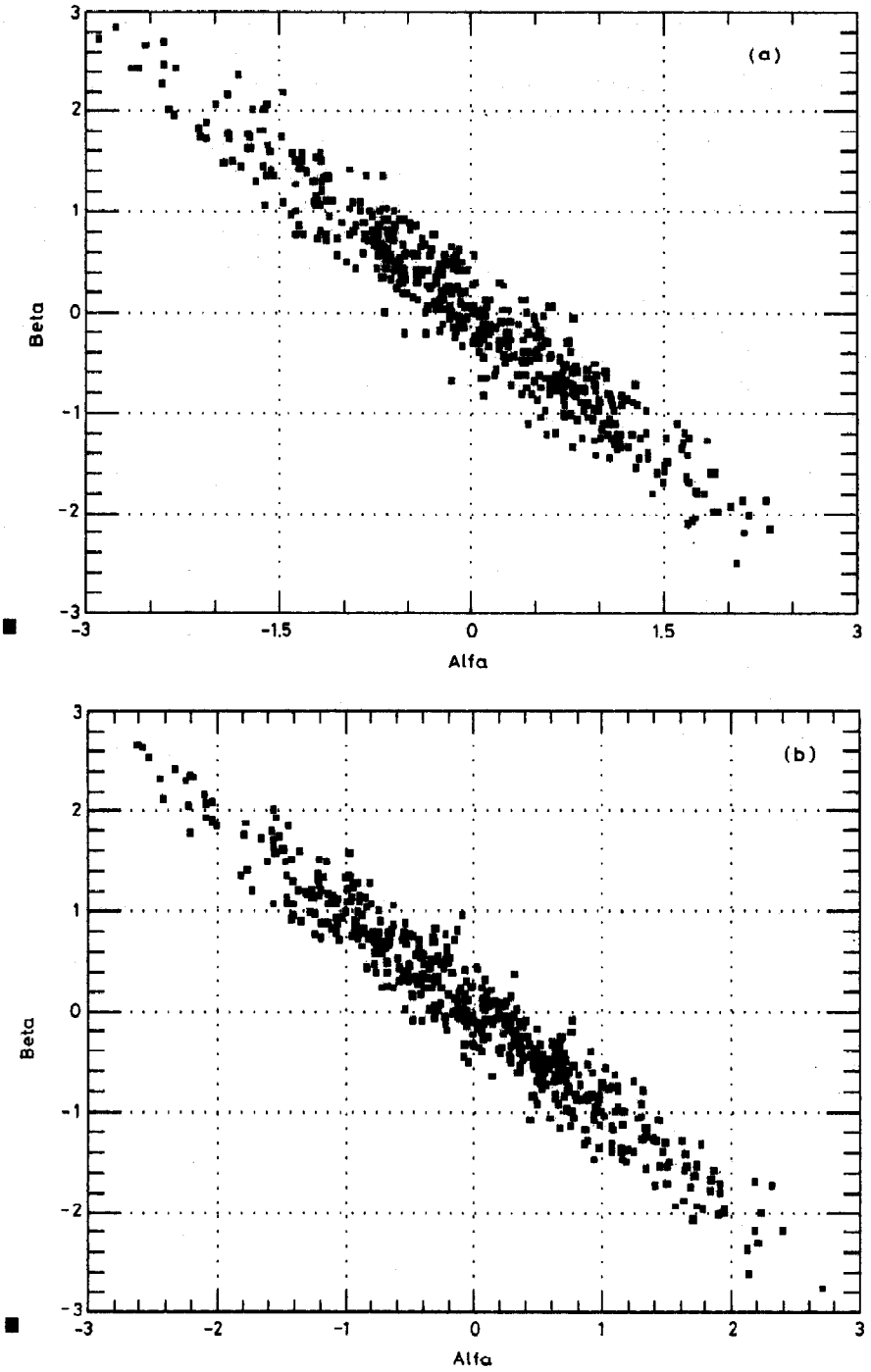

Fig. 3. (a,b) Correlation plot for the α and β coefficients of eq. 1 in the text. The plot depicts the best-fit values of α and β (in standard deviation units) obtained by analysing 500 data sets simulated as described in the text, with a pseudorandom error of 10% (a) and 50% (b). The values of the correlation coefficient are: -0.929 (a) and -0.939 (b).

tative basis to extensive application of the LQ model to the analysis of experimental data. Of particular relevance is the condition of additivity of doses which follows directly from the partition law, eq. 20. The importance of this law should be brought out especially in connection with the therapeutical use of radiations, where it is critical to establish alternative strategies of clinical treatment that guarantee an isoeffect. More generally, the theoretical approach to synergism and linkage effects proposed here can be most helpful in rationalizing experimental findings and making predictions on the effects of radiations on living cells. The approach considerably broadens the field of applicability of the LQ model [7] and its biophysical relevance.

The strength of any approach to the effect of radiation action on living cells cannot be exclusively based on the simplicity and biophysical significance of its principles. It necessarily also stems from the extent to which one can resolve the physical parameters involved in the theoretical treatment from analysis of experimental data. Since this is a basic issue in the rigorous application of biophysical theories [20–22], we have addressed the problem of the resolvability of the α and β coefficients of the basic eq. 1 of the LQ model by extensive simulation studies. Data points were simulated in the form usually taken experimentally, i.e., as the logarithm of S vs. the dose D, using eq. 1 and adding a pseudorandom error to all points (40 per data set). The resolvability of α and β was assessed by studying the distribution of the best-fit values of these parameters collected from analysis of 500 simulated data sets. Due to the linearity of the expression of the logarithm of S with respect to D, the parameters α and β were always normally distributed around the values used in simulating the data. We have also tested the intrinsic correlation between α and β as a function of the experimental error. The distributions of the two parameters are inversely correlated, as shown in fig. 3 for a typical case, but interestingly enough the correlation is barely affected by the added pseudorandom error in the range from 10 to 50%. This is the error range typically found experimentally. The thermodynamic treatment proposed here thus involves physical parameters which can be readily extracted from analysis of experimental data. The intrinsic correlation present between α and β in the error range of a typical experimental study does not seem to hinder their resolvability, and hence the validity of the conclusions drawn.

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