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SUPPRESSION OF PROTEIN BIOSYNTHESIS IN EHRLICH ASCITES CANCER CELLS BY CHEMICAL COMPOUNDS OF DIFFERENT CLASSES

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Abstract—IRP-1, IRP-2 and PG are shown to suppress protein biosyntheiss in Ehrlich ascites cells at concentrations of $10^{-4}-10^{-3}$ M. All oxyaromatic compounds investigated display the same mechanism of action which differs from that of ThioTEPA (alkylating agent).

The addition of ATP has no effect on the inhibiting action of oxyaromatic compounds while the addition of chloramphenicol (a specific inhibitor of protein biosynthesis) causes some suppression of the incorporation of L-C-14-amino acids.

When there is a shift of pH to the alkaline side within the range 6·4–8·0 and when the cells are incubated in the presence of oxygen the inhibiting effect of IRP-2 and PG becomes stronger, i.e. the suppression of protein biosynthesis occurs via intermediate products of inhibitor oxidations which is in agreement with earlier conclusions concerning isolated enzymes.

Measurement of incorporation of amino acids into cell proteins has recently become a universal method in investigations on protein biosynthesis.[1, 2]

It was shown earlier that propylgallate (PG), which is a typical inhibitor of free-radical processes (IRP), can suppress biosynthesis of cell proteins in the ascites hepatoma in rats.[3]

A number of investigations carried out at our laboratory showed that numerous oxyaromatic compounds display a marked antitumor effect.

Thus it has been considered desirable to study the effect of such compounds on protein biosynthesis in cancer cells as compared with the alkylating agents widely used in clinics.[4–8] The results of studies aimed at establishing the mechanism of action of the inhibitors of free-radical processes are also considered.

Ehrlich ascites cells taken from 10–15 mice on the seventh day after transplantation were used in each experiment. The ascites fluid taken from animals was added immediately to 4 volumes of salt solution cooled to 0°. The solution was prepared by mixing 0·9 per cent NaCl, 0·3 M Na_2HPO_4 and 0·3 M KH_2PO_4 in the ratio 5:4:I. The cells were separated by centrifuging (5 min, 800 rev/min) and washed three times in the cold with the salt solution. The washed cells were kept in a medium containing 0·04 M glucose in 0·05 M tris-buffer pH 7·4 at 30°. A mixture of uniformly labelled L-C-14 amino-acids (protein hydrolyzate of Chlorella) was added at a concentration of 0·25 μc/ml of the solution. Samples were withdrawn after certain intervals, the proteins were precipitated by adding equal volumes of 10% trichloracetic acid (TCA).

The precipitate was washed twice with cold TCA, heated for 20 min at 90° in a 5% solution of TCA and then washed twice in the cold with 5% TCA.

In order to remove all L-C-14-amino-acids that were not incorporated into proteins the centrifuged precipitates were washed 25–30 times with 70% alcohol (10 mg of the precipitate and 10 ml of alcohol for each washing). The washed precipitates were dissolved in 85% formic acid and plated on aluminium discs to determine their specific activity by means of an end-window Geiger counter using the routine equations.[9]

Kinetic curves for protein biosynthesis were plotted from the data obtained. The incorporation of amino-acids was usually completed in 60–90 min.

The maximum (limiting) incorporation of amino-acids in the control was taken as unity. In order to make sure that the L-C-14-aminoacids added to the cell suspension were bonded into the polypeptide chain and not added in any unspecific way (for example through NH_2, SH-groups or by hydrogen bonds) protein precipitates were treated with performic acid, ninhydrin and urea (6·2 M).[10] It was found that the specific activity of protein precipitates always remained unchanged.

The following compounds were studied: the hydrobromide of 4-oxy-3,5-di-tert-butyl benzylamine (IRP-1),[11] the water soluble hydrochloride of 4-oxy-3,5 di-tert-butyl-N-N (β-oxyethyl) benzylamine (IRP-2) (12), PG, thioTEPA and sarcolysine.

PG and sarcolysine had been dissolved in the buffer solution before the experiment, IRP-I, IRP-2 and tioTEPA were added to the mixture as powders since they dissolve readily in water.

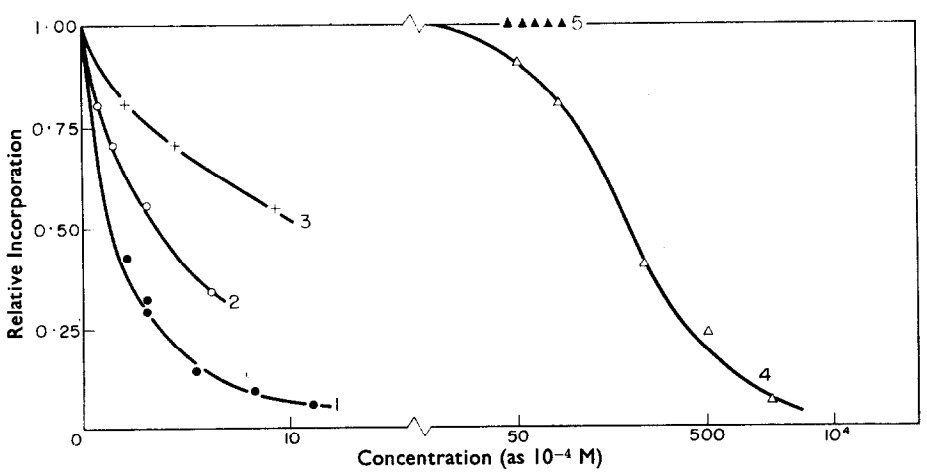

FIG. 1. Incorporation of L-C-14-amino acids as a function of the concentration of inhibitors and alkylating compounds. I-IRP-2; 2-IRP-I; 3-PG; 4-thioTEPA; 5-sarcolysin.

The incorporation of amino acids (the control being taken as unity) as a function of the concentration of the chemical compound is shown in Fig. 1.

The suppression of protein biosynthesis by all oxyaromatic compounds occurs at very low inhibitor concentrations, a 10^{-4} M concentration of IRP-2 ensuring almost complete suppression of the process.

To suppress, for example, 50 per cent of the incorporation into proteins the concentration of ThioTEPA should be 200 times higher than that of IRP-2. The concentration of sarcolysin which had been used in our experiments ($3{\cdot}4 \times 10^{-3}$–$1{\cdot}7 \times 10^{-2}$ M) proved insufficient for suppression of biosynthesis.

It will be pointed out that the nature of the incorporation dependence on the concentration of the preparation is different for oxyaromatic compounds and for ThioTEPA.

The limiting incorporation as a function of the concentration of any oxyaromatic compound used in our experiments may be expressed by the following equation:

$$\xi = \frac{1}{1 + KC + K^2C^2} \qquad (1),$$

where ξ is the relation of the limiting incorporation in the presence of the inhibitor to that of the control; C is the concentration of the inhibitor and K is a certain constant characteristic of the given inhibitor.

It is evident from the above that:

$$\sqrt{\left(\frac{1}{\xi} - \frac{3}{7}\right)} = KC + \tfrac{1}{2} \qquad (2),$$

$\sqrt{\left(\frac{1}{\xi} - \frac{3}{7}\right)}$ thus being a linear function of the inhibitor concentration.

It will be seen from Fig. 2 that there is a good fit between experimental data on the action of inhibitors, represented in respective coordinates, and the straight lines; the kinetic curve for the suppression of protein biosynthesis by ThioTEPA could not be

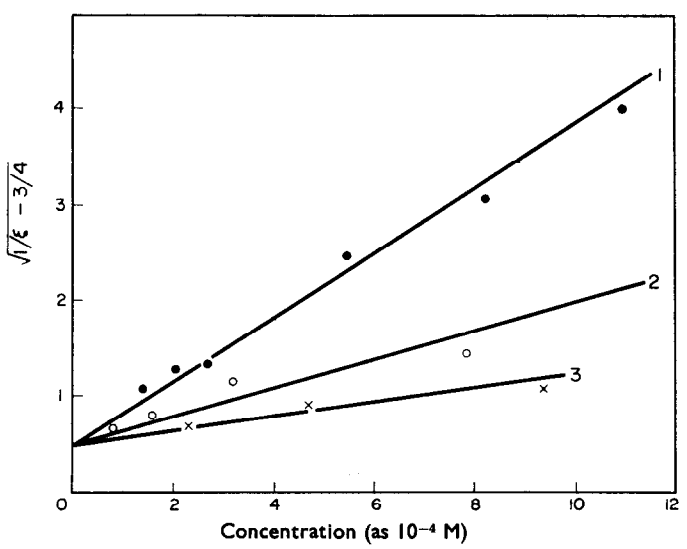

FIG. 2. Alternative expression (see text) for inclusion of amino acids into proteins as a function of the concentration of inhibitors.
(1)-IRP-2; (2)-IRP-I; (3)-PG.

straightened for these coordinates. This indicates that the mechanism of the protein biosynthesis suppression by oxyaromatic compounds is different from that for alkylating agents.

It was showed earlier[13, 14] that inhibitors of free-radical reactions suppress the activity of oxidative–reductive enzyme processes.

One of the results of the suppression of these enzyme process is the disturbance of biosynthesis of the adenosintriphosphate acid (ATP) which, in turn might account for the suppression of protein biosynthesis.

Figure 3 shows that in the presence of ATP (10 μM in the incubating medium) the incorporation of amino acids into cell proteins is increased by some 20 per cent.

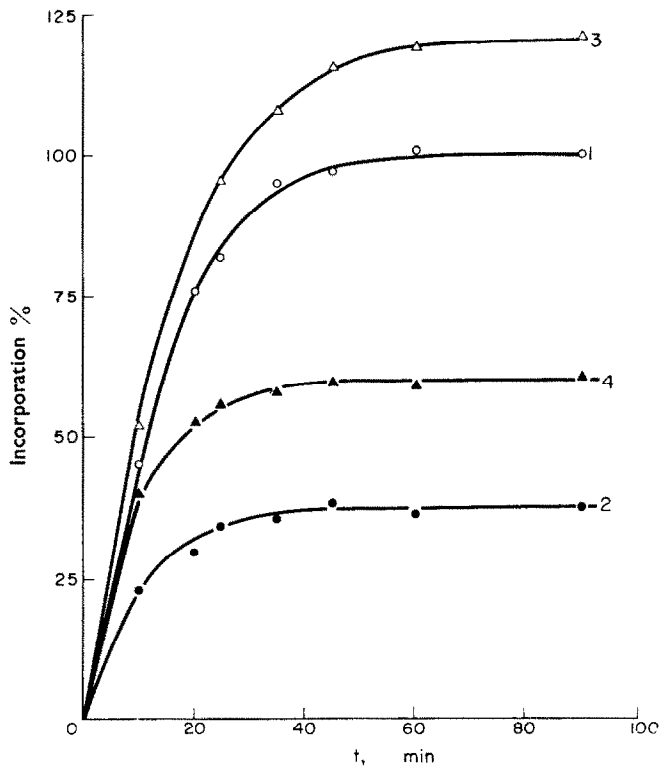

FIG. 3. The effect of ATP and IRP-2 on the incorporation of L-C-I4-amino acids into Ehrlich ascites cell proteins.
(1)-control; (2)-control + IRP-2 2·78 × 10^{-4} M;
(3)-control + ATP 10 μM;
(4)-control + ATP 10 μM + IRP-2 2·78 × 10^{-4} M.

In the presence of an inhibitor the decrease in incorporation with addition of ATP is the same as without ATP,i .e. the addition of ATP to the system does not affect the inhibiting action of IRP-2.

These data corroborate the conclusion that even if some disturbance of the ATP synthesis occurs in cells it is not sufficiently strong to inhibit the activation of amino acids.

The suppression of the incorporation of L-C-14-amino acids might be conceived as occurring at later stages of protein biosynthesis. Chloramphenicol is known to suppress protein biosynthesis specifically, inhibiting the transfer of amino acyl-RNA to ribosomes.[15] In our experiments a chloramphenicol concentration of 3·1 mM suppressed the incorporation of L-C-14-amino acids into Ehrlich ascites cells by 20 per cent that of 6·2 mM by 35 per cent and that of 9·3 mM by 80 per cent.

The results obtained show good agreement with published data.[16, 17]

In the presence of both chloramphenicol and IRP-2 the inhibiting effect is additive and this indicates that these compounds act independently (Fig. 4).

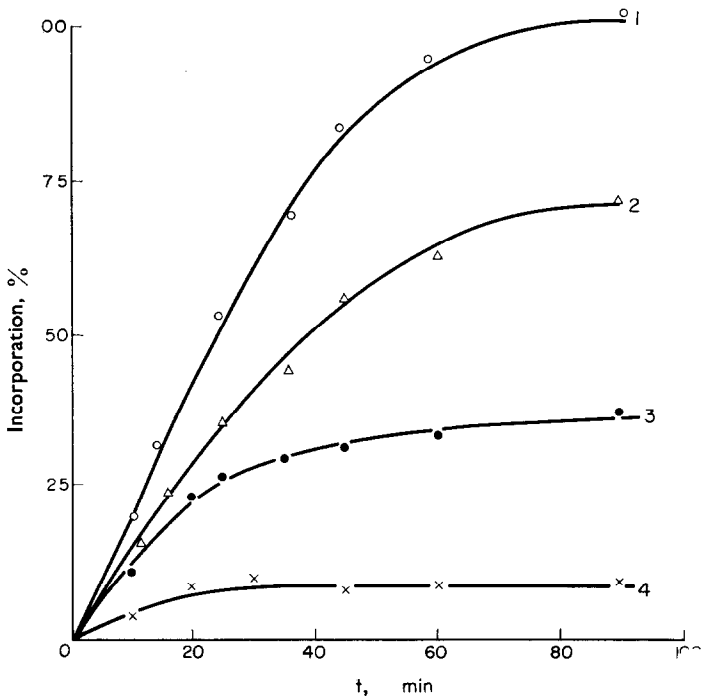

FIG. 4. The effect of IRP-2 and chloramphenicol (CP) on the inclusion of amino acids into proteins of Ehrlich ascites cells.
(1)-control; (2)-control + CP $6{\cdot}2 \times 10^{-3}$ M;
(3)-control + IRP-2 $2{\cdot}7 \times 10^{-4}$ M;
(4)-control + CP $6{\cdot}2 \times 10^{-3}$ M + IRP-2 $5{\cdot}27 \times 10^{-4}$ M.

Model systems should be used in order to find out where the chain of protein biosynthesis is broken. Oxyaromatic compounds may interact with SH-groups of enzymes participating in protein biosynthesis, or have an effect on the synthesis of RNA in cancer cells.[18, 19]

It was shown recently in our laboratory[20] that increase in the medium alkalinity of the medium results in a higher rate of inhibitor oxidation. It was shown earlier that the activity of the isolated lactate dehydrogenase is suppressed by intermediate oxidation products of PG.

It may be suggested that if intermediate products of inhibitor oxidation are active towards ascites cell suspensions in buffer solutions, shifting of pH to higher alkalinity would result in a higher suppression of protein biosynthesis. The experiments followed the ordinary scheme using a 0·05 M tris-buffer with 0·04 M glucose, the pH ranging within 6·4–8·0. Shifts of pH to the more acidic or more alkaline values beyond this range are impossible: in the first case the incorporation of L-C-14-amino acids practically terminates and in the second the cells are lysed.

The suppression of protein biosynthesis by IRP-2 inhibitor as a linear function of the pH of the medium is shown in Fig. 5. It will be seen that as pH of the medium shifts to alkalinity the suppression of protein biosynthesis becomes more intensive, which warrants the suggestion that intermediate products of the inhibitor oxidation display an inhibition even at the cellular level.

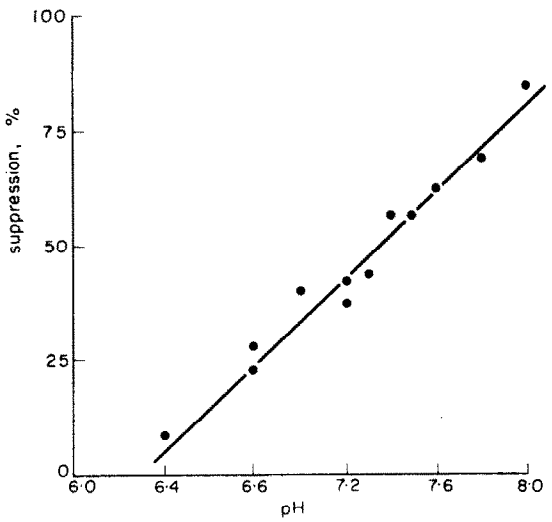

FIG. 5. The suppression of protein biosynthesis by IRP-2 inhibitor as a function of the medium (concentration of IRP-2 $2{\cdot}8 \times 10^{-4}$ M).

This is also corroborated by experiments on suppression of protein biosynthesis when the cells are exposed to a flow of oxygen.

In the presence of oxygen the oxidation of PG and IRP is more ready, and the suppression of protein biosynthesis becomes stronger than in the presence of air.

РЕЗЮМЕ

В данной работе было показано, что ряд оксиароматических соединений-ингибиторов свободно-радикальных процессов (ИРП-1, ИРП-2 и ПГ) подавляют биосинтез белка в клетках асцитного рака Эрлиха при концентрациях 10^{-4}–10^{-3} М. Все исследованные оксиароматические соединения обладают общим механизмом действия, отличным от такового для ТиоТЭФа (алкилирующего агента).

Добавление АТФ к системе не снимает ингибирующего действия оксиароматических соединений, в то время как добавка хлорамфеникола (специфического ингибитора биосинтеза белка) усиливает подавление включения L-C-14-аминокислот.

Показано, что при сдвиге pH в щелочную сторону от 6.4–8.0 и при инкубации клеток в атмосфере кислорода ингибирующее действие ИРП-2 и ПГ усиливается, т.е. подавление биосинтеза белка в клетках осуществляется промежуточными формами окисления ингибитора, что согласуется со сделанными ранее выводами при работе с изолированными ферментами.

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