УДК 577.112.543.52

THE USE OF A MULTISTEP AVALANCHE CHAMBER FOR ANALYSING THE DISTRIBUTION OF RADIOACTIVITY AND TWO-DIMENSIONAL SEPARATION OF A MIXTURE OF LABELLED COMPOUNDS. Analysis of ^{125}I-Labelled Proteins from Ribosomal 30S Subunits

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The possibility is considered of using a multistep avalanche chamber, operating on-line with a computer, for analysis of the radioactivity distribution after two-dimensional separation of a mixture of labelled proteins. A high sensitivity of the chamber allows one to identify reliably ~20 mcg of the total protein from E.coli ribosomal 30S subunits and ~1 mcg of the least iodized protein when the exposure takes ~30 min. The chamber makes it possible to decrease the analysis time by a factor of 100 as compared to conventional radioautography and to obtain information on label distribution among proteins without desctoying the sample.

The investigation has been performed at the Laboratory of High Energies, JINR, and IMB of the USSR Academy of Sciences.

Использование многоступенчатой лавинной камеры для анализа распределения радиоактивности после двумерного разделения смеси меченых соединений. Анализ ^{125}I-меченых белков 30S-субчастиц рибосом

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Рассмотрена возможность применения многоступенчатой лавинной камеры, работающей на линии с ЭВМ, для анализа распределения радиоактивности после двумерно-

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го разделения смеси меченых белков. Высокая чувствительность установки позволяет надежно идентифицировать ~ 20 мкг тотального белка 30S-субчастицы рибосомы E.coli и ~1 мкг наименее иодированного белка при длительности экспонирования ~30 мин. Установка позволяет более чем в 100 раз сократить время анализа по сравнению с традиционно используемой радиографией и получать информацию о распределении метки по белкам, не разрушая препарата.

Работа выполнена в Лаборатории высоких энергий ОИЯИ и ИМБ АН СССР.

1. *Introduction*

In order to reduce a required amount of protein, in the analysis of protein mixtures by means of electrophoresis technique the labelling by radioactive iodine is conventionally used. It is essential that when iodination is performed with the aid of chloramine /1/, iodine chloride /2/, and lactoperoxidase /3/, physical and functional properties of proteins remain unchanged /4/.

Industrial ^{125}I preparations with a specific activity up to 13-18 mCi/mcg allow one to detect several nanograms of protein in a polyacrylamide gel by radioautography. However, when the amount of protein is low (5×10^3 cpm/cm^2 gel), the exposure must be as long as several days what leads to some delay in obtaining results. Moreover, the radioautogram shows merely a qualitative distribution of radioactivity in the gel. Of course, by means of scintillation counters one can directly measure radioactivity in each region of the gel after its cutting. This is a quantitative and more sensitive procedure, but it also takes a lot of time and has low reproducibility.

An optimal method should combine the reliable localization of radioactivity in a sample inherent in radioautography with a high accuracy and sensitivity of measuring isotope amounts which are characteristic for scintillation counters. These requirements are met by a multistep avalanche chamber (MSAC) operating on-line with a computer. The chamber is used to analyze the distribution of ^{125}I-labelled proteins in a gel.

To illustrate the potential of this technique, we describe its use for analyzing quantitatively a complex mixture of ^{125}I-labelled proteins from E.coli ribosomal 30S subunits.

2. *Measuring System*

The system is composed of a MSAC on-line with an SM-4 minicomputer. The system allows one to get rapidly information on the spatial distribution of radioactive zones throughout the area of a sample and their relative activity. The operation principle of the system is described in refs.[5,6,7,8]. The developed programs make it possible to carry out the entire analysis in the dialogue mode. An alphabetic-digital display, a colour TV monitor, a plotter and an alphabetic-digital printer are used as computer terminals.

E.coli ribosomal 30S subunits were isolated as in ref.[9]. The total protein of 30S subunits was prepared according to[10]. The protein was iodinated using chloramine as in[1]. Two-dimensional gel electrophoresis of the 30S subunit protein was performed as in [11].

The characteristic radiation with a 4 keV energy produced in the decay of ^{125}I registered by means of the detector with a 160x160 mm^2 sensitive area.

The efficiency of the MSAC, (ϵ) (ϵ = A[cpm]/A[dpm] x x100%), was determined experimentally by measuring the radiation of a source with known activity. This efficiency was 3.5% for ^{125}I.

The spatial resolution of the system was measured by means of test samples with ^{125}I zones (1 mm in diameter). The distance between these zones was different. The neighbouring zones, the distance between the edges of which was 1.1 mm or more, were identified separately. The uniformity of the MSAC was measured by a radiation source (1x1 cm^2) moved along the sensitive area of the chamber, and the radiation was measured after each pitch. The data of such measurements were used to construct a map of nonuniformities for the MSAC area. A matrix of correction coefficients was made on the basis of this map. If this matrix is used to correct data, the nonuniformities in the MSAC sensitivity are automatically reduced to a value below 5%.

The MSAC proper background is 3-5 cpm/cm^2. Taking the efficiency of the detector into account, this value corresponds to a radioactivity of 40-60 pCi/cm^2. The count rate of the system is up to 2.5x10^5cpm.

The radiation of a label must be 3 times as high as that of the background for reliable localization of a radioactive zone. Therefore, the system allows one to detect reliable a radioactivity of 120-180 pCi/cm^2 or more. The time for information accumulation is determined by the activity of a sample zone which contains the smallest

isotope amount. To obtain a result with an error $R = N^{0.5}/N$ of no more than 5%, where N is the number of events per zone, it is necessary to accumulate at least 300 events. This takes about 25 min for a minimal recorded activity of 150 pCi/cm^2.

The system has very good reproducibility. Deviations in determining the radioactivity do not exceed 3-5% repeated analyses of the same sample.

3. *Treatment of the Results of Analysis*

Without desctroying a sample, the system allows one to make an accurate quantitative analysis of isotope distribution among the zones, to get information on their absolute and relative activity, the coordinates of each zone, the coordinates of their centres and so on. The results of the analysis are presented as a two-dimensional matrix, each element of which contains information on the number of events recorded from a chamber sensitive element of 0.5x0.5 mm^2. The number of elements in the matrix is 320x320. The size of the matrix elements can be changed for K (K = 1,2,3,...). The matrix is projected on the screen of the colour TV monitor as a semitonic map of the activity distribution throughout the sample area. There are 7 colours and 12 semitones in each colour, and the total number of map gradiations is 84. The transformation of the map with digital filters [12] allows one to get rid of random noise and to make the map more contrast.

Not only the map of the entire sample but also the maps of those regions which are of particular interest can be projected on the screen of the colour TV monitor. It is also possible to project the three-dimensional map or profiles of its sections along two coordinates on the screen of the TV monitor or the plotter (see Fig.1).This allows one to detect the presence of individual fractions even when their separation is far from being complete. The sections can be made either along one line (column) of the matrix or as a band of several lines (columns) up to the whole width of a sample.

In order to determine the quantitative isotope distribution among the zones, one has to outline regions of interest with a course recorded on the TV monitor screen. Then the computer performs a statistical treatment of the information. The result of the treatment is given as a table on the alphabetic-digital printer.

Fig.1. Three-dimensional map of the sample obtained by the plotter.

4. Discussion

E.coli ribosomal 30S subunits contain one molecule of 16S RNA and 21 protein molecules with molecular masses from 8000 to 61000 D. All these proteins can be separated by two-dimensional electrophoresis in a polyacrylamide gel /10/. A reliable detection of the proteins by staining the gel with Coomassie requires 200 mcg of the total protein from 30S subunits. Iodination of the proteins with ^{125}I prior to electrophoresis followed by radioautography allows one to reduce the amount of proteins required for the analysis. Using ICI /2/ or chloramine T /1/, one can incorporate up to 10^7 Bq per 1 mcg of protein at a specific radioactivity of ^{125}I 13-18 mCi/mcg. Since different proteins vary in the degree of iodination, 20 mcg of the total protein suffices for reliable detection of all the proteins in this case, i.e., 1 mcg of the least iodinated protein in a spot 3 mm in diameter when the exposure takes 70 h.

It is well known that radioautography makes it possible to determine the position and shape of spots and to estimate approximately the relative distribution of radioactivity in the spots. In order to measure the distribution of radioactivity in proteins, one has to find the spots, to cut the corresponding regions in the gel and to count them. This procedure takes a lot of time and may involve errors due to an inaccurate localization of the spots, the contribution of the background radioactivity of the gel and so on. Figure 2 shows a conventional radioautogram obtained after two-dimensional separation of ^{125}I-labelled proteins from E.coli ribosomal 30S subunits in a polyacrylamide gel. The X-ray film was exposed for 10 days, and only S1, S3/S4, S7, S9/S11 and S19/S20 proteins were observed. A longer exposure of the film or greater amounts of the analyzed material are required to localize the remaining proteins.

The same gel was analyzed using the MSAC system. The results are shown in Fig.3. The analysis took only 15 min.

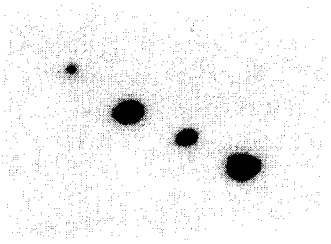

Fig.2. Autoradiogram of the film after two-dimensional separation of ^{125}I-labelled proteins from E.coli ribosomal 30S subunits in the gel.

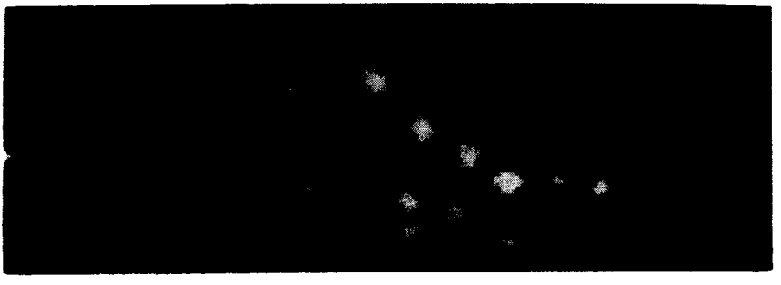

Fig.3. Digital autoradiogram from the same gel obtained by the MSAC system.

As one can see, this time sufficed to localize all the proteins from E.coli ribosomal 30S subunits. Hence, the method is highly sensitive.

The system has been used to determine the relative distribution of a label among proteins. These data are consistent with those on the radioactivity distribution in 30S subunits proteins labelled with ^{125}I using T chloramine that we have obtained with an "Intertechnic" counter. Hence, this approach is adequate for solving such problems. It should be noted that, in contrast to conventional analytical procedures, the gel is not to be cut and therefore it can be used in further studies.

5. *Conclusions*

The described automatic system for express-analysis of thin-layer samples can be used for examining a mixture of proteins and other compounds labelled with ^{125}I and separated by two-dimensional gel electrophoresis or two-dimensional chromatography. The system makes it possible to reduce the time of analysis by more than a factor of 100 as compared to conventional radioautography,

and to get information on label distribution among proteins without desctoying the samples.

The system is being successfully used for studying the structure and function of ribosom, and it may be applied to solve similar biochemical problems.

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Received on May 11, 1985.