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Note

Application of a three-dimensional drawing procedure to the evaluation of series of protein samples after analysis by gel electrophoresis and other methods*

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Gel electrophoresis on polyacrylamide gels is widely used for assaying the purity or state of proteins, and is operationally simple when only a few samples are to be analysed[1]. However, evaluation of the data becomes difficult when large series of samples that differ in the concentration of various proteins are to be compared. One combined method for the investigation of heterogeneous samples is two-dimensional gel electrophoresis[2]. However, this method is difficult to perform and requires expensive equipment (a special gel system and two-dimensional scanner).

We have developed a graphical method that allows one to present and evaluate by a single three-dimensional plot the data obtained from two successive separation procedures, *e.g.*, chromatography followed by analytical electrophoresis. In contrast to two-dimensional electrophoresis, single samples are not subjected to a two-dimensional separation procedure. After the first separation step, however, the partially separated mixture represents a series of samples, and this is then subjected to the second separation step. Hence the standard equipment in a biochemical laboratory is sufficient for this kind of operation.

Similar drawing procedures have been already commonly applied to the evaluation of the data in other analytical methods that yield a series of single plots. Some related examples can be found in the recent literature, *e.g.*, for the presentation of chromatographic patterns[3], titration profiles[4], optical spectra[5] and neutron scattering profiles[6]. The application of the method to the purification of a hydrophilic protein (F_1ATPase) and a membrane protein (F_0F_1ATPase) and to the analysis of a series of samples that vary in the concentration of an effector (lithium chloride freezing of F_1ATPase) is demonstrated here.

EXPERIMENTAL

Electrophoretic experiments were carried out using a Desaga 1200/200 power supply unit connected with a gel chamber equipped for the use of 70 × 6 mm cyclindrical gels or a Desaga Desaphor flat gel electrophoresis system. After staining with Coomassie Brillant Blue G 250 (Serva, Heidelberg, G.F.R.) the gels were destained electrophoretically with a Pharmacia GD-4 gel destainer. Gel rods or strips were scanned optically at 580 nm with an ISCO 1310 gel scanner that was connected

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to an ISCO UA-5 absorbance monitor provided with a ISCO Type 6 dual-beam optical unit. Earlier studies were carried at 546 nm out using an Eppendorf 1101 photometer with a gel scanning system.

F_1ATPase from *Micrococcus luteus* ATCC 4698 was prepared according to Scheurich *et al.*[7] with an additional run on a Bio-Gel A-0.5 m (Bio-Rad Labs., Richmond, CA, U.S.A.) column. F_1ATPase from *Micrococcus* sp. ATCC 398 was prepared according to Risi *et al.*[8] and F_0F_1ATPase from *Rhodospirillum rubrum* FR1 according to Schneider *et al.*[9].

THREE-DIMENSIONAL DRAWING PROCEDURE

Aliquots of each of the samples belonging to an experimental series were subjected to electrophoresis on cylindrical gels or flat gels. The gels were stained and subsequently scanned optically at the same monitor sensitivity and scanning velocity. The resulting single plots were assembled as demonstrated by the following procedure (Fig. 1).

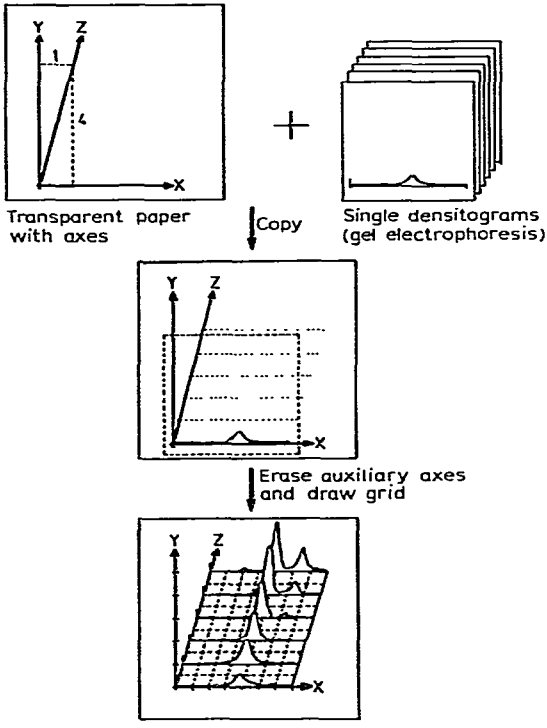

Fig. 1. Three-dimensional plot for a series of samples after gel electrophoresis.

(1) A system of three coordinates is drawn with a pencil on transparent paper. For the z-axis an x:y projection of 1:4 has been found to be optimal. The fraction numbers or concentrations of the solutes contained in the various samples are indicated on the z-axis. From these marks, parallels to the x-axis are drawn with a pencil.

(2) The transparent paper is attached to a drawing board equipped with a clamp. The single plots are placed underneath the transparent paper and subsequently copied with ink, starting at the origin of the coordinate system.

(3) The pencil lines are erased and the axes are drawn with ink. Subsequently, a basis grid is drawn where required. The basis grid is essential for the clear interpretation of the whole plot.

EXAMPLES

Fig. 2 shows the application of the three-dimensional drawing procedure during the purification of F_1ATPase from *Micrococcus luteus* ATCC 4698. Numbers 51–71 are chromatographic fractions subsequently separated electrophoretically under "native" conditions on cylindrical gels containing 5% of acrylamide[1]. The three-dimensional plot was used to select fractions 52–60 (F_I) of the highly purified enzyme for neutron and small-angle X-ray scattering experiments[10,11].

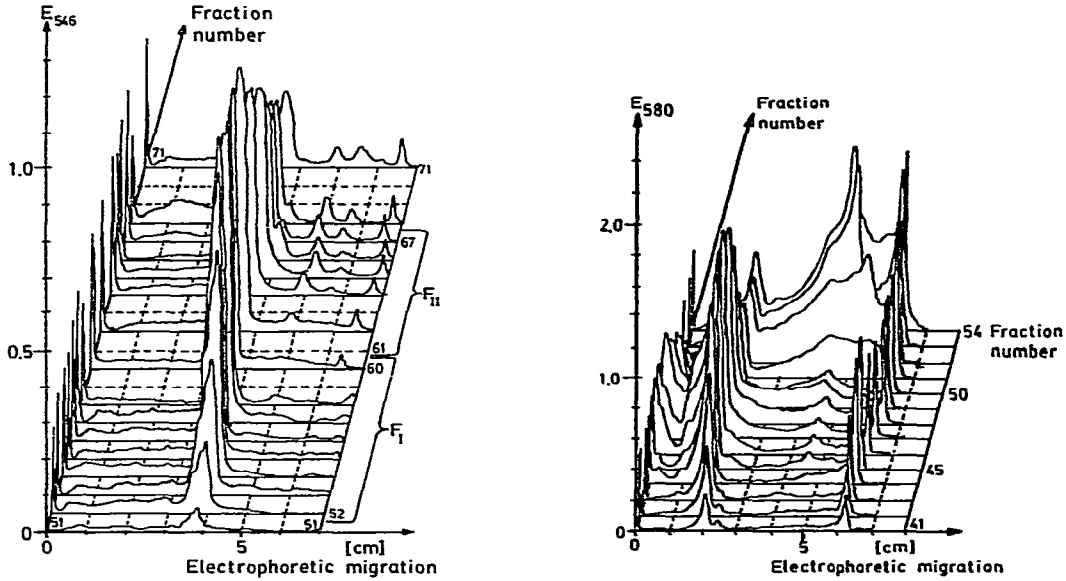

Fig. 2. Purification of F_1ATPase from *M. leuteus*: three-dimensional plot of chromatographic fractions after subsequent (native) electrophoresis (PAGE). According to this plot the fractions were combined to solutions of highly purified (F_I) and less highly purified emzyme (F_{II}).

Fig. 3. Purification of F_0F_1ATPase from *R. rubrum*: chromatography on a Sepharose CL-4B column and subsequent 5 m*M* TDOC analytical electrophoresis.

A three-dimensional plot of the gel chromatographic elution profile of a membrane protein (F_0F_1ATPase) after subsequent gel electrophoresis in the presence of 5 m*M* taurodeoxycholate (TDOC) is shown in Fig. 3. The gels contained 5% of acrylamide, 0.25% of bisacrylamide, 8 μl of tetramethylethylenediamine, 37.5 m*M* Tris–hydrochloric acid (pH 8.2) and 27 mg of ammonium peroxodisulphate per 30 ml of gel. This gel system is more suitable than the usually sodium dodecyl sulphate (SDS) gel systems for testing the purity of lipophilic proteins such as F_0F_1ATPases, their F_0

factors or cytochrome enzymes; *e.g.*, an F_0F_1ATPase preparation from *Rhodospirillum rubrum* FR1 that appeared to be pure according to SDS gel electrophoresis actually contained 5–10% of impurities according to 5 m*M* TDOC gel electrophoresis.

A more complex example of three-dimensional gel plotting is presented in Fig. 4. F_1ATPase from *Micrococcus* sp. ATCC 398 was dissociated by freezing and thawing in the presence of 0.5 *M* lithium chloride at pH 6.6. This procedure is similar to that introduced by Vogel and Steinhardt[12] for the dissociation of F_1ATPase from *E. coli*. Fig. 4 shows the dependence of the dissociation on the concentration of glycine that was used for protection against aggregation. The glycine concentration is plotted on the *z*-axis. The diagram shows that appreciable dissociation without substantial aggregation (polymers appear on the left part of the gels) is observed with 0.2 *M* glycine. At higher concentrations of glycine the enzyme becomes completely protected from dissociation and aggregation (see the rear part of the diagram). An experiment showed that the ATPase remains fully active at glycine concentrations above 0.2 *M*.

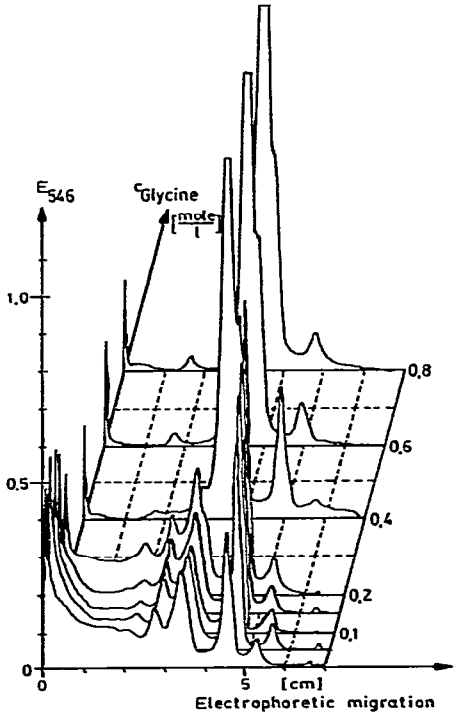

Fig. 4. Dissociation of F_1ATPase from *Micrococcus* sp. by freezing and thawing in the presence of 0.5 *M* lithium chloride at pH 6.6. Samples (0.5 mg of ml/enzyme) with different amounts of the protection agent glycine were subsequently analysed by polyacrylamide gel electrophoresis under native conditions. The gels contained 5% of acrylamide. The glycine concentration ($c_{glycine}$) is plotted on the *z*-axis.

CONCLUSION

The three-dimensional drawing procedure described here has proved to be the method of choice in the evaluation of series of protein preparations that have been

subjected to an analytical separation (*e.g.*, by gel electrophoresis). The examples presented include chromatographic fractions and protein samples subjected to varying conditions, *e.g.*, increasing concentrations of protection agent. The procedure may also be applied to the evaluation of other analytical data that yield single plots, such as optical spectra. Work is in progress to improve the procedure by using a microcomputer system.

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