To simplify the kinetics all these experiments were carried out with the enzyme saturated with glutathione. The presence of 3,4-dichloronitrobenzene did not alter the optimum concentration curve for glutathione when 4-nitropyridine *N*-oxide or 2,3,4,6-tetrachloronitrobenzene was used as the substrate (Fig. 1).

The low affinity of the second substrate indicates that the main requirement of the second substrate should be that it has an electrophilic site to accept the nucleophilic sulphur atom of the glutathione and that, in effect, the action of the enzyme is to give an 'activated' glutathione; in the cases described here the group displaced is different, but the actual conjugation takes place on similar carbon atoms. The exact nature of the specificity of the second substrate is not yet known.

SUMMARY

1. Rat-liver supernatant contains an enzyme that catalyses the replacement of labile aromatic nitro groups with glutathione.

2. Evidence is presented for its identity with the enzyme that conjugates 3,4-dichloronitrobenzene and glutathione with the liberation of chloride.

3. An explanation of the non-competitive inhibition of one second substrate by another is suggested.

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Improvements to the Ultraviolet-Absorption Optical System of an Ultracentrifuge

By ROY MARKHAM

Agricultural Research Council Virus Research Unit, Huntingdon Road, Cambridge

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In the original ultracentrifuges the course of the sedimentation was followed by means of light-absorption, but the general accuracy and ease of use of refractometric methods led to the disuse of this type of measurement until quite recently, when the renewed interest in nucleic acids and nucleoproteins revived it. The manufacturers of the Spinco model E ultracentrifuge (Beckman, Spinco Division, Palo Alto, Calif., U.S.A.) now provide ultraviolet-absorption optics for this machine, and, though they are usable, their design is by no means without defect. However, it is possible to

improve their performance by a few simple modifications, and the object of this paper is to indicate what can be done without a complete re-design of the instrument.

The modifications that have been made to our own instrument, and the reasons why they have been made, are as follows.

Lamphouse

This consists of a brass block enclosing the 85w medium-high-pressure mercury lamp; the block is cooled by being in metallic contact with the rest of

the centrifuge. We have found that this cooling is quite inadequate to prevent the quartz discharge tube from reaching its softening point (about 1665°) and bulging. This reduces the light-output and also causes the tube to devitrify. Eventually a steady state is reached when the brilliance of the discharge is reduced to about one-quarter of that of a new lamp. This defect may be remedied by soldering closely coiled copper tubing (6 mm. external diam.) to the rear and upper left-hand sides of the lamp-house, and circulating cold water at about 260 ml./min. through it. (The tubing is filled with solder before it is bent to fit the lamp-house.) There are two holes in the base of the centrifuge casing that are ideally situated to lead out plastic tubing from the lamp-house. The cooling coil could be plumbed in with the other water supplies to the centrifuge, but we use a completely independent supply for this purpose.

Light-source

The 85w source is a (General-Electric, U.S.A.) mercury discharge lamp. Contrary to general belief, this source forms an image on the plane of the cell so that any structure in the lamp is superimposed on the final image. The imperfections so introduced can be eliminated in two ways: The ideal solution would be to use a field lens as the apparent source and focus the lamp image on the camera lens. This would involve more or less complete reconstruction of the optical train. An alternative, reasonably satisfactory solution is to frost the quartz plate covering the lamp, on both faces, with Carborundum F. The top face of the plate should have a mask of aluminium with an aperture of 1 cm. $\times$ 1.5 cm. cut in it to form the actual light-source. The long axis of this aperture should be radial with respect to the centrifuge rotor.

Frosting the quartz plate reduces the amount of light available at the plane of the cell, but this is still ample. It also reduces contrast to a slight extent, an effect which results in slightly decreased definition of the meniscus line (Fig. 1), but the overall evenness of illumination is increased sufficiently to permit the recognition of very weak boundaries. In Fig. 1, and the tracing of its last boundary (Fig. 2), a solution of turnip crinkle virus in 0.1 M-NaCl is to be seen. The concentration was such (about 0.002 %) that the initial extinction in a 1.2 cm. cell was only 0.09.

Filter system

The filter system used in the centrifuge consists of two tubes 8.0 cm. long, containing Cl₂ gas and Br₂ vapour respectively. This system (Svedberg & Pedersen, 1940) transmits appreciable amounts (about 8 %) of the 365 m μ line and much of the visible spectrum; although it does not have a very

sharp cut-off in the short-wave ultraviolet region, it transmits only about 16 % of the 265 m μ line and 25 % of the 254 m μ line. (These figures are measured on our own filters and need not be precisely the same on other sets.) Moreover, the halogen filters are notoriously difficult to make vapour-tight, and we know that they have failed in a number of cases.

A much better filter is that described by Kasha (1948), which transmits 34 % of the 254 m μ line and 38 % of the 265 m μ line (including loss from all surface reflexions), and virtually no light in the long ultraviolet and visible regions (Fig. 3). This filter system has been used for chromatographic purposes (Lipkin, Markham & Cook, 1959) and a variant is in use in this Laboratory. We have adapted this filter for the Spinco ultracentrifuge,

Fig. 1. Ultraviolet-absorption record of the ultracentrifuging of turnip crinkle virus. Concentration about 0.002 % in 0.1 M-NaCl. Speed, 24630 rev./min. Temperature, 15.7°. Exposures, 15 sec. at 4 min. intervals. Kodak CF8 film (Kodak Ltd., London).

Fig. 2. Microdensitometer (Joyce-Loebl, Gateshead-on-Tyne) trace of last frame of Fig. 1. Bottom of cell is on the right.

and as a consequence the filters are rather larger than are actually necessary.

The filter system consists of a 7.5 cm. square of Chance OX7 glass (Chance-Pilkington Optical Works, St. Asaph, Flintshire), followed by a solu-

tion of $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ (Mallinckrodt Chemical Works, St. Louis, U.S.A.; A.R., Fe less than 0.001%) (63 g. to 100 ml. of water) in a cylindrical quartz cell (1 cm. internal diameter, 7.5 cm. external diameter; transparent Vitreosil cell no. T/E8 822, The Thermal Syndicate, Wallsend, Northumberland), in turn followed by 1 cm. of Cation X (25 mg./100 ml. of water; cat. no. 21938, California Corp., Los Angeles, Calif., U.S.A.) in a similar cell. Circular masks having central holes 6 cm. diam. are placed between the individual elements to reduce scatter. The whole combination is mounted in a tray made from sheet aluminium, having a hole about 7 cm. diam. in it. This tray (Fig. 4) is fastened to the bracket carrying the tensioning pulley for the chain driving the vacuum-chamber jacks, the bolt and washer holding the pulley wheel being used for fixing. As is seen from Fig. 4, provision is made for centring the tray. The liquid filters are closed by rubber 'policemen' to avoid internal pressure, and should be stable indefinitely. The Cation X is unstable to ultraviolet light, as is the OX7 filter, but under normal conditions of use the exposure is so small as to be negligible. Nevertheless, it is advisable to renew the Cation X solution occasionally, and to check occasionally that the OX7 glass does not increase its absorption at $260\text{ m}\mu$ appreciably. The liquid-filter system increases the optical path by about 0.6 cm. and this reduces the amount of defocusing of the light source to a negligible amount.

It should not be necessary to remove the original filter holder from the centrifuge, but care should be taken on first installing the new filters that they are not crushed when the vacuum chamber is lowered. If they are too high the aluminium tray can be lowered by bending the front part appropriately.

SUMMARY

1. Modifications to the Spinco model E ultracentrifuge-absorption optics are described. They include water cooling for the lamphouse, a diffuse light source and an improved filter system.

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Fig. 3. Ultraviolet-absorption spectrum of the complete filter system recorded on a Cary model 14 recording spectrophotometer (Applied Physics Corp., Monrovia, Calif., U.S.A.).

Fig. 4. Shape of filter tray before formation from sheet aluminium. The central square is 8.25 cm.^2 in area and the sides are 3 cm. high. The screw used for the fixing is about 8.5 cm. from the axis of the light-path and its approximate position is indicated as a dot.