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Note

Electrophoresis of thiols in cellulose gels

IV. Estimation of molecular weight

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The electrophoretic mobility of a molecule in solution depends on its charge, size, shape and solvation. When a stabilizing support is employed the mobility is also dependent on adsorption, sieving effect and osmotic flow.

The sieving effect has been evaluated for the determination of the molecular weights of macromolecules[1], but for small molecules can be neglected.

Correction for adsorption involves division by R_F, the value of which must be determined chromatographically in the same system. This approach cannot be applied for gels. When however the electrophoretic zones are sharp and a distinct relationship between mobility and molecular weight can be established, one may assume that the influence of adsorption is not very large. This is the case for cellulose gels in neutral and alkaline solution.

The methods presented in this paper are based on evaluation of the relationship between molecular weight, charge and mobility. If the charge is known the molecular weight can be simply calculated. Neutral thiols can be converted into acids either by reaction with iodoacetic acid or carbon disulphide. In the latter case the resulting monoesters of trithiocarbonic acids must be resolved in alkaline solution to avoid decomposition.

If the charge of the investigated thiol is not known an alternative approach is based on esterification with propionic anhydride. From the decrease in mobility the molecular weight can be calculated.

EXPERIMENTAL

The measurements of electrophoretic mobility were carried out at 20°C using cellulose gel membranes in a xylene bath[2], and two buffers, *viz.*: pH 7.0, triethanolamine (10 g/l) + boric acid (20 g/l) + Na_2EDTA (2 g/l) + ethylene glycol (100 ml/l); pH 8.8, lithium hydroxide monohydrate (2 g/l) + boric acid (5 g/l) + Na_2EDTA (2 g/l) + ethylene glycol (20 ml/l).

Localization of thiols was performed by pressing the cellulose gel strip against filter-paper containing the complex *o*-hydroxymercuribenzoic acid–dithiofluorescein[2]. The strip was then placed for some minutes in a chamber containing dimethy-

lamine vapour. The thiol esters were then decomposed to form free thiols[3]. For detection of trithiocarbonic acid esters the strip was pressed against a strip of Whatman No. 1 paper previously soaked in a solution containing 20 mg/l of tetraacetoxymercuryfluorescein (TMF) in 0.02 M sodium hydroxide. The spots were visible in filtered UV light.

The correction for osmotic flow in both buffers was calculated by means of the equations

$$S_v = 1.24 S_v', \quad S = 0.24\ S_v' + S' \tag{1}$$

where S_v and S_v' are the corrected and uncorrected distances travelled by phenol red and S and S' those travelled by the thiol.

In order to correct the change in mobility resulting from any change in the cellulose gel, the relationship between the mobility of the substance, u, and mobility of phenol red, v, in the course of drying of cellulose gels has been established

$$\log u = A \log v + B \tag{2}$$

where $A = 0.80$ for substances of molecular weight less than 300 and B is a constant characteristic of the substance. For molecular weights $(M) > 300$, the constant A increases with increasing M, e.g., for captopril $(M = 217)$, $A = 0.80$; for tosylcysteine $(M = 273)$, $A = 0.84$; for glutathione $(M = 307)$, $A = 0.93$. No change in the relative osmotic mobility could be observed in the course of drying of cellulose gels.

Assuming at pH 7.0 as reference a constant mobility of phenol red equal to 4 cm^2 min^{-1} kV^{-1}, the corrected mobility, u, is calculated from

$$u = u' \left(\frac{v}{4}\right)^{-0.8} \tag{3}$$

where u' is the uncorrected mobility and v the mobility of phenol red. At pH 8.8 the mobility is corrected with regard to the mobility of phenol red $(= 10\ \text{cm}^2\ \text{min}^{-1}\ \text{kV}^{-1})$ by the equation:

$$u = u' \left(\frac{v}{10}\right)^{-0.8} \tag{4}$$

Procedures

Partial derivatization of thiols with propionic anhydride. A 0.05 M solution of thiol in 0.2 M triethanolamine was shaken with an equal volume of 0.2 M propionic anhydride in hexane for 2 min. The anhydride was removed by washing with hexane. A 0.002 M solution of phenol red was then added in a ratio 1:4 and the electrophoretic mobility determined at pH 7.0. The results are summarized in Table I.

Partial derivatization of dithiols with iodoacetic acid. One drop of 0.1 M iodoacetic acid and one drop containing excess of dithiol in 0.2 M triethanolamine were mixed, phenol red was added as above and the mobility of the product determined at pH 7.0. The results are shown in Table II.

Derivatization of thiols with carbon disulphide. To an aqueous solution of the thiol (0.05 M) were added some grains of barium hydroxide octahydrate and followed
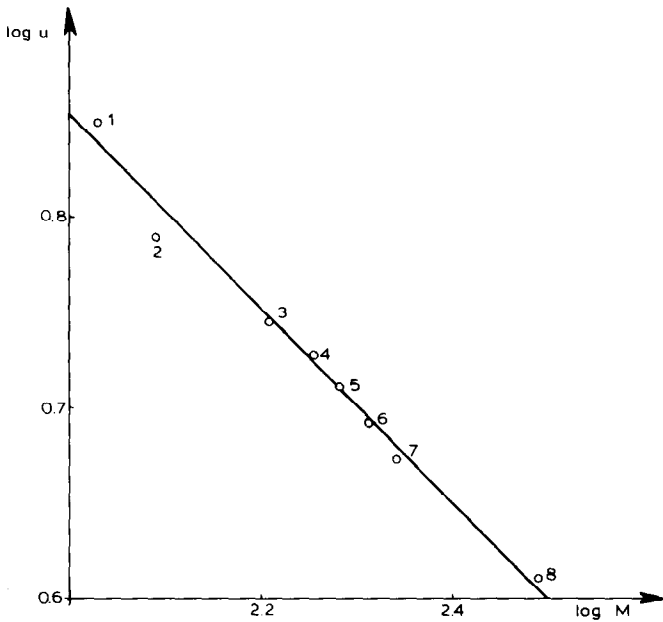
Fig. 1. The relationship between log mobility and log molecular weight. The slope of the straight line is −0.5. Compounds: 1 = 3-mercaptopropionic acid; 2 = mercaptolactic acid; 3 = N-acetylcysteine; 4 = N-propionylcysteine; 5 = N-propionylhomocysteine; 6 = N-propionylpenicillamine; 7 = Captopril; 8 = glutathione.

by shaking for 2 min with a few drops of a carbon disulphide solution in hexane (20%, v/v). The excess of carbon disulphide was removed by washing with hexane, phenol red was added as above and the mobility determined at pH 8.8. The results are given in the Table III.

RESULTS AND DISCUSSION

The general expression for electrophoretic mobility is

$$u = azM^b \tag{5}$$

where z is the apparent charge and a and b are constants. In order to estimate the constant b, mercaptoacids have been examined which may have nearly the same charge. The results presented in Fig. 1 as a plot of log u vs. log M indicate a slope of −0.50. Hence eqn. 5 becomes:

$$u = azM^{-0.5} \tag{6}$$

It is suggested that phenol red be used as reference with a charge, $z = -1$. Thus:

$$a = -v\,M^{0.5} = -4 \times 354.4^{0.5} = -75.3 \tag{7}$$
$$u = -75.3\,z\,M^{-0.5}$$

TABLE I

CORRECTED MOBILITIES, u IN cm^2 min^{-1} kV^{-1}, AND RELATIVE CHARGES OF THIOLS, Z_1, AND THEIR PROPIONIC ACID ESTERS, Z_2, AT pH 7.0 and 20°C

Thiol	u	$-Z_1$	$-Z_2$
Mercaptoacetic acid	8.71	1.11	0.99
3-Mercaptopropionic acid	6.12	0.97	0.95
Mercaptolactic acid	6.12	0.90	0.88
2-Mercaptoethanesulphonic acid	8.16	1.30	1.10
Thiomalic acid	10.5	1.71	1.66
Thiosalicylic acid	6.55	1.08	0.896
Acetylcysteine	5.57	0.94	0.93
Dimercaptosuccinic acid	9.55	1.71	1.67*
2,3-Dimercaptopropane-1-sulphonic acid	6.73	1.23	1.10**
Acetylpenicillamine	5.09	0.93	0.95
Captopril	4.71	0.92	0.91
N-Tosylcysteine	4.59	1.01	0.97
Glutathione	4.06	0.95	0.92***

     * For S,S-diester, $Z = -1.48$.
     ** For S,S-diester, $Z = -1.04$.
     *** For N-propionylglutathione, $Z = -1.57$; for N-propionyl-S-propionic ester of glutathione, $Z = -1.59$.

In the last equation z is the charge relative that of phenol red. The relative charges calculated on basis of eqns. 7 are summarized in Tables I and II. As is seen the relative charge is closely related to the acidic properties of the thiol. The aliphatic mercaptoacids have charges between -0.90 and -0.97. The introduction of a second carboxyl group increases the charge to -1.7. Glutathione behaves like a monocarboxyl acid. Thiols containing one carboxyl group and amide groups have charges of 0.93 ± 0.015. The relative error in the estimation of molecular weight on the basis of eqns. 7

$$\frac{\Delta M}{M} = \frac{2\Delta z}{z}$$

amounts to about 3%. As is seen from Table II, the molecular weights of compounds containing $-SCH_2COOH$ groups can be estimated with the same accuracy.

If the charge of the investigated thiol cannot be predicted, the method based on derivatization with propionic anhydride may be employed. As the increase in

TABLE II

CORRECTED MOBILITIES, u IN cm^2 min^{-1} kV^{-1}, AND RELATIVE CHARGES, Z, OF MONOSUBSTITUTED PRODUCTS OF DITHIOLS AND IODOACETIC ACID AT pH 7.0 AND 20°C.

Parent dithiol	Formula of the derivative	u	$-Z$
1,2-Dimercaptopropanol	$C_5H_{10}O_3S_2$	5.53	0.99
1,4-Dimercapto-2,3-butanediol	$C_6H_{12}O_4S_2$	5.00	0.97
1,4-Dimercaptobutane	$C_6H_{12}O_2S_2$	5.60	1.00

molecular weight resulting from conversion into the propionic ester amounts to 56, the molecular weight of the parent thiol is given by

$$M = \frac{56}{\left(\dfrac{S_1 Z_2}{S_2 Z_1}\right)^2 - 1} \tag{8}$$

where S_1 and S_2 are the distances travelled by the thiol and its ester on the same strip, corrected for osmotic flow, and Z_1 and Z_2 are the corresponding charges. The charge ratios for aliphatic mercaptoacids, with the exception of mercaptoacetic acid, do not differ much from each other and a mean value of 0.98 can be taken for calculation. The relative error in the estimation of molecular weight resulting from the measurements of distances is given by:

$$\frac{\Delta M}{M} = \frac{1.92 \dfrac{S_1}{S_2} \Delta \dfrac{S_1}{S_2}}{0.96 \left(\dfrac{S_1}{S_2}\right)^2 - 1} \tag{9}$$

The error $\Delta \dfrac{S_1}{S_2}$ results essentially from the error in measurement of the difference S_1 − S_2 and may be taken as not greater than 1 mm. Let S_1 be 80 mm and S_2 be 64 mm ($M = 112$). Then the error will be:

$$\Delta \frac{S_1}{S_2} = \frac{81}{64} - \frac{80}{64} = 0.016 \text{ and } \frac{\Delta M}{M} = 7.5\%$$

If $S_1 = 80$ mm and $S_2 = 70$ mm ($M = 221$), the relative error will increase to 12.7%. Experimentally found relative standard deviations are as follows ($n = 10$): thiomalic acid ($M = 150$), mean value 148, 8.1%; captopril ($M = 217$), mean value 214, 8.6%.

If two mercapto groups are present in a molecule the bands corresponding to

TABLE III

CORRECTED MOBILITIES, u IN cm^2 min^{-1} kV^{-1}, AND RELATIVE CHARGES, Z, OF MONOESTERS OF TRITHIOCARBONIC ACIDS AT pH 8.8 AND 20°C

Parent thiol	u	$-Z$
Methanethiol	13.85	1.13
Ethanethiol	12.54	1.12
Propanethiol	11.61	1.13
Butanethiol	10.57	1.11
Mercaptoethanol	11.38	1.12
N-Propionylcysteamine	8.75	1.12
S-Monocyanoethylated 1,4-Dimercapto-2,3-butanediol	6.92	1.14

the thiol, S-monoester and S,S-diester can be distinguished. The presence of an amino group results in additional bands. The total number of electrophoretic bands will be $(n + 1)(s + 1)$, where n and s are the number of amino and mercapto groups. Thus, glutathione yields four bans, two due to free thiols and two to thiol esters. As the bands are sharp and can easily be identified the method may be useful for estimation of the number of mercapto groups and amino groups.

The mobilities measured at pH 8.8 of trithiocarbonates produced from thiol and carbon disulphide are summarized in Table III. The results yield a linear $\log u$ − $\log M$ relationship with slope −0.85. Hence:

$$u = azM^{-0.85} \tag{10}$$

Assuming that the charge of phenol red in alkaline solution is −2, the constant a can be calculated

$$a = \frac{-10}{2} \cdot 354.4^{0.85} = -735$$

and the charge is given by:

$$z = \frac{-u\,M^{0.85}}{735} \tag{11}$$

The charges calculated from eqn. 11 are included in Table III. From the results obtained the reaction of thiols with carbon disulphide may be used for estimation of the molecular weights of neutral thiols, with an error of about 2%. Higher trithioalkyl carbonates must however be electrophoretically investigated between cooling plates because they are washed out from the membrane by the xylene bath.

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