EFFECT OF CHARGE ON THE DETERMINATION OF MOLECULAR WEIGHT OF PROTEINS BY GEL ELECTROPHORESIS IN SDS

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SUMMARY. It has been found from a comparison of the electrophoretic mobilities on SDS-acrylamide gels of unmaleylated, maleylated, and demaleylated proteins that electrophoretic mobilities are affected by changes in charge as well as by changes in molecular size. Caution is therefore suggested in interpreting the values of molecular weight determined by electrophoresis in SDS-acrylamide gels.

Electrophoresis on acrylamide gels in the presence of SDS (1-3) has been widely hailed as a convenient and accurate method for determining the molecular weights of polypeptide chains. Generally it is not explicitly stated but it is almost universally assumed that the protein-bound SDS anions swamp out all charge effects so that proteins migrate in the gels almost entirely according to their molecular size.

We report here the results of some experiments which indicate that charge effects are not eliminated by electrophoresis in SDS-polyacrylamide gels and suggest that substantial errors in estimation of molecular size can result from unawareness of this possibility.

METHODS

Butler *et al.* (4) reported that at low temperature and at a pH of about 9, maleic anhydride reacts rather specifically with unionized amino groups of proteins to yield maleyl-derivatives which are stable above pH 5. The maleyl groups can be removed at pH 3.5, restoring the original

amino groups. Thus a system is provided in which the charges of proteins can be specifically altered with only slight changes in molecular size. The electrophoretic mobilities of unmaleylated and maleylated proteins can then be compared in order to assess the effect of charge; furthermore the mobility of demaleylated protein can be determined in order to test the possibility that important modifications other than those affecting charge might have resulted from maleylation. By use of ^{14}C-labeled maleic anhydride it is possible to determine the numbers of maleyl groups attached and to ascertain their removal.

In constructing a standard plot of molecular weight versus mobility the following proteins were employed: pepsin, two times crystallized from ethanol, Worthington, M. W. 35,000; pyruvate kinase, M. W. 57,000 and carbonic anhydrase, M. W. 29,000, from Calbiochem; cytochrome C, Boehringer Mannheim, M. W. 12,000; tobacco mosaic virus (TMV) protein, M. W. 17,500, prepared by us using the method of Fraenkel-Conrat (5). The molecular weights used were those determined previously by physico-chemical procedures (see Weber and Osborn (2)). Over the molecular weight range represented by these five proteins a linear relationship was observed when the logarithm of molecular weight was plotted against distance moved in the acrylamide gel. Also used in our studies, but not in construction of the standard curve, were alpha chymotrypsinogen A, six times crystallized, Sigma; potato virus X (PVX) protein prepared by us according to Reichmann (6); and cucumber virus 4 (CV4) protein prepared by us using the method of Fraenkel-Conrat (5).

The electrophoresis procedure was carried out according to Weber and Osborn (2) in 10% gel. The proteins were dissolved at about 2 mg/ml in 0.01 M sodium phosphate buffer, pH 7 containing 1% SDS and 1% mercapto-ethanol, and were incubated at 37° for two hours before electrophoresis. (Boiling for 5 minutes gave essentially the same results). After electrophoresis was completed (5 hours), the gels were stained in 0.25%

Coomassie brilliant blue for 2 to 10 hours at 25° and then destained electrophoretically in acetic acid-methanol-water (75:50:875). The distances that protein bands moved were used to calculate molecular weights from a standard plot in the usual manner.

The methods of Butler *et al.* (4) were used to prepare labeled and unlabeled maleyl proteins and to remove the maleyl group.

The results summarized in the table show that the molecular weights of the maleyl proteins estimated from electrophoretic runs with known markers (unmaleylated proteins) were in general higher than the values for the comparable unmaleylated or the demaleylated proteins. With the exception of maleyl-TMV protein, the added weights of the maleyl groups attached were obviously not great enough to explain the apparent molecular weight increases indicated by the results of the gel electrophoresis. For example, the increase in molecular weight of pepsin upon maleylation was found to be ten times the amount expected from the number of maleyl groups attached. Since the molecular weights of the demaleylated proteins were found to be very similar to those of the unmaleylated proteins, it seems that the observed changes in electrophoretic mobility can be attributed to the introduction of the maleyl groups.

The maleylation of amino groups of proteins increases the net negative charges of the proteins around neutral pH values. How this type of change produces the observed, altered electrophoretic mobilities in SDS-acrylamide gels is not yet clear. Possibly maleylation induces conformational changes in some proteins which are equivalent to increasing molecular size insofar as mobility in acrylamide gels is concerned. However this may be, it is clear that chain length (i.e., molecular size) is not the only factor governing electrophoretic mobility of proteins on SDS-acrylamide gels. Consequently, if absolute values for molecular weight are being sought, it would seem advisable to check the values obtained by gel electrophoresis by independent chemical or physical techniques.

Comparison of the Molecular Weights of Unmaleylated, Maleylated and Demaleylated Proteins as Determined by Electrophoresis on SDS-Acrylamide Gels

Protein	No. of Maleyl Groups Bound[1]	Expected Mol. Wt.	Observed Mol. Wt.
Pepsin	0	35,000	35,000
Maleylated-pepsin	7-8	~35,800	42,000
Demaleylated-pepsin	0	35,000	35,000
Chymotrypsinogen A	0	24,000[2]	24,000
Maleylated-chymotrypsinogen A	3-4	~24,400	29,000
Demaleylated-chymotrypsinogen A	0	24,000	25,000
PVX coat protein	0	27,000[2]	27,000
Maleylated-PVX coat protein	8	~27,800	31,000
Demaleylated-PVX coat protein	0	27,000	27,000
TMV coat protein	0	18,000[2]	18,000
Maleylated-TMV coat protein	4	~18,900	19,000
Demaleylated-TMV coat protein	0	18,000	18,000
CV4 coat protein	0	15,000[2]	15,000
Maleylated-CV4 coat protein	3-4	15,400	18,000
Demaleylated CV4 coat protein	0	15,000	14,000

[1]Calculated from radioactivity of maleyl protein formed by reaction of protein with ^{14}C-maleic anhydride.

[2]Mol. Wt. determined by use of a standard plot of log of mol. wt. against mobility in SDS-acrylamide gel of selected proteins of known mol. wt.

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