BIOCHEMICAL LETTERS

Unpredictable behaviour of mucins in SDS/polyacrylamide-gel electrophoresis

We write this Letter in order to share our experiences in the use of SDS/polyacrylamide-gel electrophoresis (SDS/PAGE) for the analysis of mucins, and to draw attention to the fact that mobility of mucins (and indeed of many other glycoproteins) is not simply related to molecular mass, but rather depends on intrinsic charge.

SDS/PAGE has been used since the 1960s to determine molecular masses of proteins. Reynolds and Tanford [1] were able to explain the relationship between mobility and molecular mass by showing that in general SDS binds to polypeptides in a constant weight-for-weight ratio (1.4 g of SDS:1 g of protein at SDS monomer concentrations of above 0.8 mM), so that the charge unit mass is approximately constant. They also showed that the hydrodynamic properties of the protein/SDS complexes were a unique function of polypeptide length [2]. However, although these conditions held for many proteins, even at an early time exceptions were observed [3,4]. Often, although not exclusively, these concerned glycoproteins; the mobility of glycoproteins was often altered by removal of terminal sialic acid residues. However, these observations frequently seem to have been ignored. This may be in part because such gels have been very useful for studying the biosynthesis of glycoproteins. This has even included studies on mucins, glycoproteins which generally include at least 50% O-linked glycans [5]. Indeed, mucins from rat and human stomach, and human gallbladder, show large shifts in mobility on SDS/PAGE, on transition from precursor to mature mucins [6–9; Table 1], in keeping with the increases in molecular mass. Thus we used this procedure to study the colonic mucin MUC2 in humans and rats [10,11].

However, interpretation of our results on MUC2 was hampered for a long time by the fact that the ³⁵S-labelled mucin precursors and the mature mucins displayed virtually identical mobilities on SDS/PAGE [10,11; Table 1]. Interestingly, McCool and co-workers, studying biosynthesis of MUC2 in the

human colonic LS180 cell line, made very similar observations [12]. Size information (hydrodynamic volume) can also be obtained by using Sepharose CL-2B gel filtration in the presence of high concentration of guanidinium chloride. Reduced mature human and rat colonic mucins were eluted in the void volume under these conditions, whereas reduced mature human and rat gastric mucins were eluted in the included volume (Table 1), indicating that the latter are smaller. This supports the notion that the mobility of colonic mucin on SDS/PAGE, which is greater than the mobility of gastric mucins on SDS/PAGE, is not a reflection of size.

Buoyant density is a measure of relative amount of glycosylation of mucins, because glycans have a high density relative to polypeptides. Indeed, the buoyant density, and also the percentage of hydroxylated amino acids, which gives an indication of potential O-glycosylation sites, show a positive correlation (Table 1). Furthermore, there is a correlation between these parameters and the mobility of mature mucins on SDS/PAGE: human gastric mucin has a low buoyant density, a low T+S content, and a low mobility on SDS/PAGE, whereas human colonic mucin displays a high buoyant density, a high T+S content and has the highest mobility on SDS/PAGE. Most likely, the more abundant O-glycosylation provides more backbone for addition of negatively charged residues.

Most of the O-glycosylation of mucins is concentrated in the central part of the polypeptide [5], so this part is unlikely to bind much SDS and thus will not acquire substantial negative charge, unlike the peripheral parts. The mature colonic mucins from man and rat have the same mobility on PAGE in the absence or presence of SDS [10,11]. Moreover, removal of the proteinase-sensitive polypeptide regions only slightly increased the mobility on PAGE with or without SDS [10,11]. Thus the mobility of the mature colonic mucins seems to be determined to a very large extent by the intrinsic negative charge of the proteinase-resistant glycopeptides. This is in contrast with the major increase in mobility after proteolysis of rat gastric mucin [13], where the concomitant loss of both molecular mass and ability to bind SDS leads to a net increase in mobility.

Table 1 Published characterization of mucin preparations from various sources and from different laboratories

All mobilities were expressed relative to the mono-, di- and tri-meric forms of the rat gastric mucin precursor [6].

Mucin	Buoyant density (g/ml)*	Sialic acid (% of monosaccharides)	T/S (% of amino acids)	Sephadex CL-2B gel filtration in guanidinium chloride*	SDS/PAGE [relative mobility expressed as apparent M_r ($\times 10^{-3}$)]†			Reference
					Precursor	Mucin	Glycopeptide†	
Rat stomach	1.35	1%	T(16)/S(15)	V_1	300	> 500	> 200	6, 7, 19
Human stomach	1.30	1%	T(21)/S(14)	V_1	500	> 1000	> 1000	8, 19
Human gallbladder	1.46	3%	Not determined	V_0	470	> 1000	> 1000	9
Rat colon	1.50	8%	T(33)/S(14)	V_0	600	650	600	11
Human colon	1.48	20%	T(38)/S(8)	V_0	600	550	550	10

* All preparations were analysed under reducing conditions.

† i.e. protease part of the mucin.

The general effect of negative charge on the mobility of mature mucins can be seen from the recent experiments by Thornton and colleagues [14]. By separating heterogeneous mucin subunit preparations by anion-exchange chromatography, they were able to show that there was a clear correlation between the amount of negative charge on the mucins and mobility on agarose-gel electrophoresis in the presence of SDS [14]. This is also well illustrated by the study of MUC1, where it has been shown that the mobility of mature MUC1 glycoproteins depends partly on the amount of negatively charged sialic acid residues, since desialylation by neuraminidase leads to a significant decrease in mobility [15]. Nevertheless, allelic size differences due to variation in the number of 20-amino-acid tandem repeats in the central part of this peptide are detectable [16]. Presumably, these allelic variants are similar in charge density, so that the mobility difference reflects the length of the molecules, much like SDS-coated unglycosylated proteins.

The sialic acid content of mature colonic mucins is high, suggesting that they are likely to have a major impact on mobility. However, sialylation is generally low in gastric mucins (Table 1), although substantial amounts of sulphate residues are present. Gel filtration indicated that the glycopeptides of rat and human gastric mucin were very similar in size. However, the rat glycopeptide had a relatively high mobility on SDS/PAGE and a higher sulphate content, indicating that sulphate may determine the difference in mobility of these glycopeptides [13,17–19].

In summary, all the evidence suggests that the mobility of mature mucins on SDS/PAGE is largely determined by their intrinsic negative charge, imposed by sulphate and sialic acid residues, terminating the O-glycans. The nature of the terminal glycosylation may vary substantially between different cells which express the same MUC polypeptide, leading to considerable differences in mobility of the same mucin from one organ to the other, as seen clearly for MUC1 [20]. However, the electrophoretic mobilities of mucins from the same source are reasonably reproducible, but interpretations about molecular mass of mucins must be made with extreme caution, and molecular-mass markers should only serve as reference points on the gels. Use of specific antibodies which recognize the protruding, less glycosylated, protein parts helps to circumvent the difficulties in the interpretation of the results, since the mature mucin can be recognized, independent of its O-glycosylation and its mobility in electrophoresis [6–11]. However, great care must also be taken not to miss components which fail to migrate at all into the gel, due to the combination of large size and lack of charged residues. For such molecules, agarose-gel electrophoresis looks promising [14], and may become a valuable alternative to SDS/PAGE in the analysis of mature mucins. It is clear that electrophoresis in any support matrix is not a suitable method for determining

molecular masses of mucins, and that the problems in the analysis of mature mucins on SDS/PAGE should be given appropriate attention. Nevertheless, if interpreted with due caution, SDS/PAGE presently remains the technique of choice for studying mucin biosynthesis in metabolic labelling studies [5–13,15].

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