The Molecular Weight of Sheep Plasma Erythropoietin

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SUMMARY

The molecular weight of sheep plasma erythropoietin, measured by gel electrophoresis, in the presence of sodium dodecyl sulfate is 46,000. The sedimentation coefficient of the biologically active material is 4.6 S.

Until pure erythropoietin was available, estimation of its molecular size depended on measurement of biological activity, with the inherent inaccuracy characteristic of bioassay methods. Previous measurements of molecular weight, by means of radiation inactivation (1), gel permeation chromatography (2, 3), and a combination of density gradient centrifugation and gel permeation (4) have ranged from 27,000 to 66,000. The availability of a limited amount of highly purified sheep plasma erythropoietin (5) has made it possible for us to determine its molecular weight directly by a micro-modification of the Weber and Osborn method (6).

Erythropoietin was iodinated with 125I by the method of McConahey and Dixon (7). Ten per cent polyacrylamide gels were formed in glass tubes with an inside diameter of 2.4 mm and a length of 33 mm. Along with the labeled erythropoietin, 1.0 µl of a mixture of bovine serum albumin, ovalbumin, and egg white lysozyme, each at a concentration of 2 µg per µl, was put on the gel. After running in the cold at 1.5 ma (22.5 volts) for 150 min the gel was removed from the tube and cut into 0.9-mm slices, each of which was put immediately into 100 µl of 20% sulfosalicylic acid. Radioiodine content of the slices was then determined on an automatic gamma counter. After overnight fixation, the sulfosalicylic acid was removed and the slices were stained overnight in 100 µl of 0.02% Coomassie blue (8) in 10% trichloroacetic acid. Radioiodine content of the slices was then determined on an automatic gamma counter. After overnight fixation, the sulfosalicylic acid was removed and the slices were stained overnight in 100 µl of 0.02% Coomassie blue (8) in 10% trichloroacetic acid. The slices were then washed free of unbound dye with several changes of a mixture of 7.5% acetic acid and 5% methanol in water. When washing was complete, the protein-bound dye was extracted from the gel slices with 200 µl of a solution containing 20% sulfosalicylic acid, 7.5% acetic acid, and 5% methanol in water. The dye solution was then read at 652 nm in micro-cells. Under the conditions used, native erythropoietin is not fixed to the gel by sulfosalicylic acid (or by any other fixative tried), although the marker proteins are, so that the radioactivity peak locates the hormone while the dye peaks locate the marker.

When native erythropoietin was run, the observed molecular weight was 37,000. Since there is a considerable number of sialic acid residues in erythropoietin, and since the Weber and Osborn method assumes that all proteins have the same net charge due to bound dodecyl sulfate ions, it seemed advisable to examine the effect of the sialic acid charges on the apparent molecular weight of erythropoietin. Removal of sialic acid was done by acid hydrolysis (0.01 M HCl at 80° for 30 min) instead of enzymically as previously (5) because the amount of enzyme protein needed was so large as to obscure the marker proteins. The desialated hormone (Fig. 1) had a molecular weight of 41,000 (Fig. 2). When this value is corrected for the known content of sialic acid (10%), the molecular weight of the original hormone is 46,000. The same value was found when the gel concentration was 12.5%.

The pattern of radioactivity shows appreciable heterogeneity, with the contaminants all moving faster than the main peak. Since desialation with neuraminidase yields a symmetrical peak with little evidence of heterogeneity (5) we conclude that the acid hydrolysis has caused the appearance of fragments smaller than the desialated erythropoietin.

We have determined, by use of the Yphantis and Waugh separation cell technique (9), that the sedimentation coefficient of erythropoietin, based on assay of the biological activity, is 4.6 S.

A molecule with a molecular weight of 46,000 and a sedimentation coefficient of 4.6 S would have a frictional ratio of 1.12, and, if it were a prolate ellipsoid, an axial ratio of 3. In contrast, the values derived by O'Sullivan et al. (4) by the Siegel and Monty method (10) are molecular weight, 32,000, sedimentation coefficient, 2.6 S, frictional ratio, 1.58, and axial ratio, 10. The hormone they studied was a crude preparation of human urinary erythropoietin. It is possible that the two hormones are distinctly different in their physical properties. When pure human urinary erythropoietin is available in sufficient quantity this question can be resolved.
FIG. 1. Gel electrophoresis of $[^{125}]$-erythropoietin in the presence of marker proteins. Two micrograms each of bovine serum albumin (mol wt 68,000), ovalbumin (mol wt 45,000), and egg white lysozyme (mol wt 14,300) were put on the gel along with 0.8 μg of labeled erythropoietin. The tracking dye was bromphenol blue.

![Diagram of gel electrophoresis]

FIG. 2 (left). Plot of the logarithm of molecular weight versus mobility relative to that of the tracking dye. These are the mean values from two separate experiments. The line was fit to the points by the method of least squares. The observed $R_F$ values were: serum albumin, 0.17, 0.20; ovalbumin, 0.34, 0.34; lysozyme, 0.76, 0.79; erythropoietin, 0.34, 0.37.

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