

## The Interaction of Papain and Certain *S*-Alkylated Papains with Dextran and Polyacrylamide Gels used in Thin-Layer Gel Chromatography

Papain exhibits a strong interaction with Sephadex G-100, Superfine grade and Biogel P-100, -400 mesh. The movement of papain molecules through these gels was shown to be retarded during thin-layer chromatography, giving an apparent molecular weight much lower than the true molecular weight. Different solvents, having structural features related to Sephadex gels, decreased the interaction of papain with Sephadex G-100, yielding an increased apparent molecular weight which was still, however, lower than the true molecular weight. Thin-layer gel chromatography in 6 M guanidine hydrochloride gave a molecular weight near the expected value.

Papain prepared by the method of Kimmel and Smith (1) was purchased from Worthington Biochemicals and then further purified by affinity chromatography (2). *S*-Methylpapain (3) and *S*-aminoethylpapain (4) were prepared as previously described. Sephadex G-100, Superfine grade was purchased from Pharmacia Fine Chemicals. Methyl Cellosolve (2-methoxyethanol) and Biogel P-100, -400 mesh were purchased from Biorad. Guanidine hydrochloride was purchased from Schwarz/Mann. Molecular weight protein standards were purchased from Mann Research Labs. Maltose was purchased from Pfanstiehl Labs. All other chemicals were Baker Analyzed Reagents. Thin-layer gel chromatography was carried out in a Pharmacia TLG-apparatus on 20 × 40 cm plates by the procedures previously described (5-7). Chromatograms were run at 25°C at an angle of 10°. Proteins of known molecular weight were run as standards simultaneously with papain and the papain derivatives. Five standards (cytochrome *c*, ribonuclease, chymotrypsinogen A, oval-albumin, bovine serum albumin) were used during each determination. A replica of each chromatogram was made on Whatman 3MM filter paper and developed 10 min in 0.25% (w/v) Coomassie Brilliant Blue, R-250 in methanol/acetic acid (9:1). The apparent molecular weight of papain and its derivatives was determined from the linear plot of log molecular weight of the standards vs migration distance. The apparent molecular weight of papain and the papain derivatives in the various solvents tested are summarized in Table 1.

TABLE 1  
 SEPHADEX G-100, SUPERFINE

Buffer modification	$10^{-3} \times$ Apparent molecular weight (pH 7.6, 0.05 M potassium phosphate buffer)			
	Papain <sup>a</sup>	Papain <sup>b</sup>	<i>S</i> -methylpapain	<i>S</i> -aminoethylpapain
None	—	9.4	—	—
0.01 M maltose	10.7	10.7	10.7	10.4
1.0 M maltose	16.0	16.0	16.0	14.9
1.0 M methyl Cellosolve	14.0	—	13.0	12.0
10% Methanol	9.0	—	9.2	8.6
10% Dioxane	15.0	—	17.0	14.0
20% Dioxane	17.0	—	17.5	16.0
6 M guanidine hydrochloride	—	—	19.0	20.0
Biogel P-100, -400 mesh				
None	—	—	6.9	6.8

<sup>a</sup> Prepared by the method of Kimmel and Smith.

<sup>b</sup> Prepared by further purification by affinity chromatography.

Sephadex is a cross-linked dextran. In addition to the  $\alpha$ -D(1  $\rightarrow$  6) glycosidic linkages between glucosyl residues, there are also ether linkages formed between residues of adjacent chains during crosslinking. Solvents were chosen which had ether and glycosidic linkages to try to reduce the abnormal interaction of papain with the gel matrix.

Papain, which has a molecular weight of 23,406 (8) shows an apparent molecular weight of about 9000 determined by thin-layer chromatography on Sephadex G-100. From Table 1 we can see that adding 1.0 M maltose ( $\alpha$ -D(1  $\rightarrow$  4) glycosidic linkages) to the buffer increased the apparent molecular weight to about 16,000. A slight increase was seen in 0.1 M maltose. The ether, methyl Cellosolve (2-methoxyethanol), increased the apparent molecular weight but to a slightly lesser extent.

The cyclic ether 1,4-dioxane increased the apparent molecular weight of the papain derivatives to about 17,000. Papain retained full activity in 20% dioxane when assayed with *N*  $\alpha$ -benzoyl-L-arginine ethyl ester at pH 7.6. This result suggests that the reduced affinity of the papain derivatives toward Sephadex G-100 in 20% dioxane is not the result of denaturation of the native conformation.

The behavior of the two *S*-alkyl derivatives of papain parallels that of papain in all solvents studies indicating the interaction of papain with Sephadex does not require a free active-site sulfhydryl group.

Papain prepared by the method of Kimmel and Smith has a nonactive component having the same amino acid sequence as active papain except

for the active cysteine residue, Cys-25. Papain further purified by affinity, chromatography consists of only the active form. Since no difference is seen between the behavior of pure active and the roughly 1:1 mixture of active and nonactivatable papain, in thin-layer gel chromatography, the nonactivatable papain must interact with Sephadex to the same extent as active papain.

We can also see from Table 1 that *S*-methyl- and *S*-aminoethylpapain both interact with Biogel P-100.

Guanidine hydrochloride was seen to increase the apparent molecular weight to about 19,000–20,000. This value approaches the true value. Papain retains some enzymatic activity even in 8 M urea (9). This apparent difficulty in denaturing papain completely may explain why the expected molecular weight of 23,406 was not observed in 6 M guanidine hydrochloride.

This study demonstrates that a protein having no known specificity for the different monomeric units of Biogel and Sephadex can interact with both of these hydrophilic xerogels, giving rise to great errors in the molecular weight as estimated by thin-layer gel chromatography. It is apparent that the use of a denaturing solvent is essential in the determination of molecular weights by thin-layer gel chromatography. This limitation makes thin-layer gel chromatography an uncertain method in determining the molecular weight of proteins containing subunits.

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