Macroamylasemia: Variation in the Response of the Macroamylase Complex to Acidification[1] (35745)

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The large amylase components in the sera of patients with macroamylasemia have been shown to differ when measured by several parameters (1–7). Among the behavioral differences noted is the response of the macroamylase to lowering of the pH (3, 5–7). This characteristic has important bearing on the nature and genesis of the macroamylase complex since susceptibility to pH 3.4 suggests an antigen–antibody complex (3). In order to learn more about the frequency of this feature, therefore, and its possible relationship to other characteristics of the macroamylase complex, we performed acidification dissociation studies on serum samples obtained from a series of patients with macroamylasemia.

Materials and Methods. Serum samples were obtained from 21 patients shown to have circulating macroamylases by either dextran gel (Sephadex G-200)[2] or polyacrylamide gel (Bio-Gel P-300)[3] chromatography at pH 7.2. Filtration studies were performed on these sera using columns (2.5 $\times$ 33 to 35 cm) of Sephadex G-200 or Bio-Gel P-300 and 0.05 *M* glycine hydrochloride buffer (pH 3.4) as the eluent. Polyacrylamide gel was preferred to avoid the effect of possible interaction between amylase and dextran gel. However, technical difficulty in maintaining an adequate flow rate with polyacrylamide led us to abandon this gel in favor of dextran gel. Polyacrylamide gel was used in seven cases (Cases 13, 14, 15, 16, 20, 21, 23) and Sephadex G-200 in 14 cases (Cases 1, 2, 5, 6, 8, 11, 17, 18, 19, 22, 24, 25, 26, 27).

Mixtures of 1 ml of serum and 3 ml of buffer were incubated at room temperature for 30 min. Following this, the mixtures were placed on the column. Flow rates were controlled between 15 and 30 ml/hr to minimize exposure of the amylase to low pH. The effluent was separated into 40 fractional parts of 5 ml each. The tubes in which the fractions were collected contained 0.3 ml of I *M* Tris–HCl buffer (pH 7.2) at room temperature. The final pH of the mixture of effluent and buffer in the collecting tubes was 6.9. Protein concentration in the eluted samples was measured spectrophotometrically at OD 280 mμ using a Beckman DU spectrophotometer. Amylase was assayed in 8 cases by a manual saccharogenic assay technique (8) and in 13 cases by an automated saccharogenic method (9).

Five acidification studies were performed by means of sucrose density gradient ultracentrifugation, using a Beckman Model L2-65B and a SW 65K head. These were done on four macroamylasemic serum specimens (Cases 1, 8, 21, 22) and one sample of normal serum. Serum (0.2 ml) was applied to 5 ml of linear gradient ranging from 10 to 50% sucrose in 0.05 *M* glycine hydrochloride buffer (pH 3.4). The tubes were centrifuged at 60,000 rpm for 16 hr at 4°. Following this, the tube contents were fractionated into 21 samples of 9 drops each (approx 0.25 ml/fraction). These were immediately neutralized by the addition of 2 ml of 0.01 *M* phosphate buffer (pH 7.0).

Previously determined sedimentation coefficients of these macroamylase complexes were compared with their responses to lowering of the pH to 3.4.

[1] Supported by U.S. Public Health Service Grant GM-11897.

[2] Pharmacia Fine Chemicals, Inc., Piscataway, N.J.

[3] Bio-Rad Laboratories, Richmond, Calif.

TABLE I. Extent of Reduction in Activity of the Macroamylase Complexes After Acidification Compared With Their Sedimentation Coefficients.

Case	Extent of reduction in activity after acidification	Sedimentation coefficient
6	+	Peak I > 19S
		Peak II-10.7S
1	—	10.2S
26	—	9.7S
15	±	9.4S
16	±	9.4S
20	±	9.2S
8	±	9.2S
24	+	8.6S
5	+	8.6S
19	+	8.5S
17	+	8.4S
21	+	8.4S
27	+	8.4S
2	+	8.4S
13	+	7.7S
14	+	7.7S
23	+	7.7S
11	+	Undeterminable
18	+	Undeterminable
22	±	Undeterminable
25	±	Undeterminable

Results. Exposure of known macroamylasemic sera to pH 3.4 and subsequent column chromatography at the same pH produced varied quantitative changes in the amylase components (Table I). As compared with the patterns obtained by similar chromatography of the same sera at pH 7.2, the macroamylase component completely disappeared and the amount of normal-size amylase simultaneously increased in 10 cases (Cases 2, 5, 6, 11, 13, 14, 18, 21, 24, 27). In three cases (Cases 17, 19, 23), acidification also caused complete disappearance of the macroamylase, but there was no corresponding increase in the amount of normal-size amylase. In six cases (Cases 8, 15, 16, 20, 22, 25), gel filtration at pH 3.4 resulted in partial disappearance of the macroamylase; the normal-size amylase simultaneously increased in four (Cases 8, 15, 16, 20). In two cases (Cases 1 and 26), acidification caused no alteration in either the macroamylase or the normal-size amylase.

The comparative ultracentrifugation patterns of normal serum and the four cases of macroamylasemia, which were additionally studied by ultracentrifugation at pH 7.0 and 3.4, also showed varied quantitative changes as a result of acidification. Recovery of amylase was almost 100% at both pH levels in all cases. As shown in Fig. 1, the amylase in normal serum was not altered by acidification. Similarly, the serum macroamylase complex and the normal-size amylase component in Case 1 were not affected by lowered pH (Fig. 1). By contrast, the macroamylase complex in Case 21 serum disappeared at pH 3.4 and was replaced by an amylase whose migration pattern resembled that of normal-size amylase (Fig. 1). In cases 8 and 22, the macroamylase components were still apparent but were markedly reduced in amount. Concomitantly, the amylase component of normal molecular weight increased in amount in both cases.

There appeared to be a distinct relationship between the sedimentation coefficients of the macroamylases and the response to acidification of the serum containing them (Table I). Macroamylases with the highest coefficients tended not to be affected by acidification; those with the lowest values tended to

be completely dissociated; those with intermediate coefficients tended to be partially dissociated. An exception to this tendency was Case 6. Even though the two macroamylases in the serum of this patient exhibited high sedimentation coefficients, both were completely dissociated when the pH of the whole serum was reduced to 3.4.

Discussion. Levitt and Cooperband (3) found that the macroamylase in one patient with 11S macroamylasemia dissociated when the pH was lowered to 3.4. In studies not yet published, they have extended their observations to include two other patients with 11S macroamylasemia and five patients with 7S macroamylasemia; both of the 11S macroamylases were unaffected by acidification, whereas all five 7S macroamylases appeared to dissociate at the lower pH. The macroamylase in the one patient examined by Wilding *et al.* (6) also exhibited a positive acidification response. In our series of 21 cases, the macroamylase complexes varied in their responses to acidification.

The question may be raised whether the macroamylase complexes that diminished in amount were actually dissociated or were selectively inactivated at pH 3.4. This question is largely answered by the ultracentrifugal studies on acidified sera. The data obtained from these studies strongly suggest that diminution in the macroamylase component after exposure to pH 3.4 represented dissociation that could be either partial (Cases 8 and 22) or complete (Case 21). On the strength of these observations, it would also seem reasonable to conclude that the macroamylase complex had dissociated even in those cases in which the amount of macroamylase diminished at pH 3.4 without there being a concomitant rise in the amount of normal-size amylase. Failure of the latter to increase may have resulted from inactivation of this component during the procedure.

In a series of studies on macroamylasemic sera involving sucrose density gradient ultracentrifugation at pH 7.0 (to be reported separately), there were seven cases (Cases 2, 5, 6, 17, 19, 21, 27) in which there were no discernible normal-size amylases. By contrast, earlier Sephadex G-200 filtration of these same sera at pH 7.2 had indicated the existence of a normal-size amylase component. We interpreted this observation as suggesting that the delayed appearance of

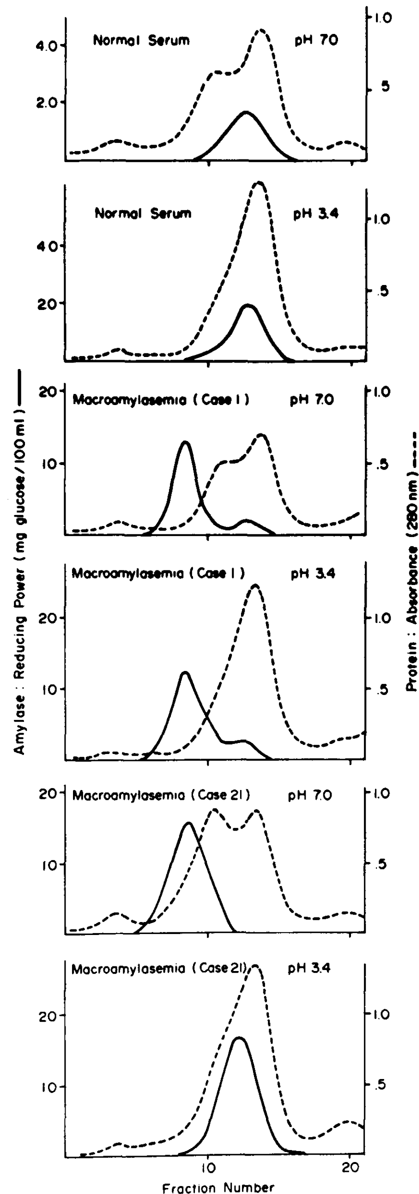

FIG. 1. Sedimentation patterns of normal serum and serum from two patients with macroamylasemia (Cases 1 and 21), before and after acidification. (Note that, by the technique used, the fractions were extracted from the bottom of the tube. Hence, the smaller the fraction number, the greater the distance of the fraction from the meniscus).

what seemed to correspond in molecular size with normal serum amylase was actually the result of delayed filtration due to interaction between amylase and the dextran in the gel. Supporting this interpretation was the observation that the normal-size amylase fraction (mol wt 45,000–50,000) in Case 21 corresponded in filtration position with the cytochrome *c* peak (mol wt 13,000). Another possibility is that dissociation of the macroamylase complex occurs during dextran gel filtration. This could occur if competitive affinity of dextran for amylase causes amylase to separate from the binding substrate to which it is attached in the serum.

The observations made in the present study, therefore, as well as the still unpublished ones dealing with the ultracentrifugal features of the macroamylases, suggest that these enzymatic macromolecules may be divided into four groups: (Group A) resistant to dissociation at pH 3.4; stable in dextran gel; sedimentation coefficient 9.7S to 10.2S; (Group B) completely dissociated at pH 3.4; tends to be altered in the direction of normal-size amylase during passage through a column of dextran gel; sedimentation coefficient 7.7S to 8.6S; (Group C) an intermediate mixture: partially dissociated at pH 3.4; sedimentation coefficient 9.2S to 9.4S; and (Group D) asymmetrical in pattern with a broad and irregular ultracentrifugal peak. The last two groups (C and D) may represent a variable mixture of groups A and B and normal-size amylase.

Using this classification schema, the 7S macroamylases reported by Levitt *et al.* (4) and by Wilding *et al.* (6) appear to be of Group B. The 11S macroamylase in the serum of the patient with malabsorption described by Levitt and Cooperband (3) has many of the characteristics of the Group A macroamylases but differs in its susceptibility to acid dissociation. Indeed, it resembles the macroamylase in our Case 6, a patient in whom malabsorption was also a feature. Sucrose density gradient ultracentrifugation of the serum of this patient showed two different amylase peaks; one was 10.7S and the other was heavier than 19S and both of them dissociated at pH 3.4.

Both Levitt and his associates (4) and Wilding and his co-workers (6) failed to demonstrate immunoglobulin binding in cases with 7S macroamylase. This form of macroamylase, which by our classification schema would be in Group B, may consist basically of a normal-size amylase that is weakly bound to serum protein. Hence, it lends itself to easy dissociation by acidification and by exposure to dextran gel. This possibility is exemplified by the fact that in the seven cases in our series showing a normal-size amylase component by dextran gel filtration but not by ultracentrifugation, the macroamylase complexes disappeared after exposure to pH 3.4.

The variation in responsiveness to lowered pH adds still another link to the chain of evidence pointing to heterogeneity of the macroamylase complex in cases of macroamylasemia. The suggestive correlation between acidification responsiveness and molecular weight is intriguing, but its precise significance remains to be determined.

Summary. Acidification had a variable effect on the integrity of the large amylase component in the sera of 21 patients with macroamylasemia. Reduction of pH to 3.4 resulted in complete dissociation of the macroamylase in 13 cases; partial dissociation occurred in six while two remained unchanged. The response to acidification could be correlated with the sedimentation coefficients of the complexes: macroamylases with the highest coefficients tended not to be affected by acidification; those with the lowest values tended to be completely dissociated, while those with intermediate values tended to be partially dissociated. The variable response to lowering of the pH provides further evidence pointing to heterogeneity of the macroamylase complexes.

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Received March 26, 1971. P.S.E.B.M., 1971, Vol. 137.