Macronzymes: Biochemical Characterization, Clinical Significance, and Laboratory Detection

Alan T. Remaley and Peter Wilding

"Macronzymes" are enzymes in serum that have formed high-molecular-mass complexes, either by self-polymerization or by association with other serum components. Many enzymes in serum that are measured in clinical chemistry laboratories can occur in a macronzyme form. Macronzymes are interesting clinically because of their association with several diseases, including autoimmune diseases and liver disease, and are being investigated as possible diagnostic markers. Most importantly, macroenzymes frequently interfere with the interpretation of serum enzyme results, and as a result can cause diagnostic and therapeutic errors. We review the biochemical characterization, clinical significance, and laboratory detection of macroenzymes.

The existence of macroenzymes, or high-molecular-mass forms of serum enzymes, has been known for 25 years, since the first report of macroamylase (EC 3.2.1.1) by Wilding et al. (1). Subsequent reports of macro lactate dehydrogenase (EC 1.1.1.27; LD) in 1967 by Lundh (2) and Garrot (3), and macro alkaline phosphatase (EC 3.1.3.1; ALP) in 1975 by Nagamine and Ohkuma (4) established that the conversion of serum enzymes to high-molecular-mass forms is a general phenomenon. Most of the enzyme molecules routinely measured in clinical chemistry laboratories have now been described to exist in a macronzyme form (5-10b). Macronzymes are usually discovered in patients that have an unexplained persistent increase in an enzyme in serum. Biochemically, macroenzymes can be classified into two groups: immunoglobulin-bound and non-immunoglobulin-bound macroenzymes. Not much is known, however, about the reasons for the existence of macroenzymes, despite the investigation and characterization of hundreds of cases.

It is important for clinical chemistry laboratories to detect macroenzymes because of the frequent confusion that macronzymes cause in the interpretation of serum enzyme results (6-10), and because of the association of macronzymes with various diseases, particularly autoimmune disorders (5, 6). In this review, we discuss the biochemical characterization, clinical significance, and laboratory detection of macronzymes, with emphasis on general concepts and future areas of investigation.

Biochemistry of Immunoglobulin-Bound Macronzymes

Characteristics

Table 1 lists the immunoglobulin-bound macronzymes that have been reported to date and some of their characteristics. In the initial investigation of macroamylase (1), it was speculated to exist as an immune–enzyme complex because of its comigration with the gamma-globulin fraction during protein electrophoresis, and this was confirmed in 1967 by Levitt and Cooperband (24), who showed that macroamylase could be selectively precipitated with antisem to IgA. Although some proteins bind to immunoglobulins nonspecifically (25–27) and a few studies suggest a role for nonspecific binding in the formation of macronzymes (6, 28–30), most evidence to date indicates that immune–enzyme complexes form because of a specific interaction between circulating autoantibodies and serum enzymes. For instance, immune–enzyme complexes can be dissociated under acidic conditions (6, 8, 31), which dissociates other specific antigen–antibody complexes. The molecular mass of immune–enzyme complexes as determined by gel filtration and ultracentrifugation are consistent with a two-to-one ratio between enzyme and antibody (14, 23, 32, 33), which would be the expected ratio for a specific immune interaction. Papain (EC 3.4.22.2) treatment of purified anti-enzyme immunoglobulins produces Fab fragments that retain the ability to bind to enzymes, thus localizing the site of interaction between the antibody and the enzyme to the specific antigen-binding site on immunoglobulins (15, 16, 34). Finally, the affinity constants of antibodies involved in macronzyme formation (10^-9 to 10^-11 M/mole) are in the range of other high-affinity specific antigen–antibody complexes (35–37). An interesting biochemical feature of immune–enzyme complexes is the restriction of the heavy and light immunoglobulin chains that make up the complexes (Table 1).

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<thead>
<tr>
<th>Table 1. Immunoglobulin-Bound Macronzymes</th>
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<tr>
<td><strong>Enzyme</strong></td>
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<tr>
<td>Acid phosphatase (EC 3.1.3.2)</td>
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<td>Alanine aminotransferase (EC 2.6.1.2)</td>
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<td>Alkaline phosphatase (EC 3.1.3.1)</td>
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<td>Amylase (EC 3.2.1.1)</td>
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<td>Aspartate aminotransferase (EC 2.6.1.1)</td>
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<td>Creatine kinase (EC 2.7.3.2)</td>
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<td>γ-Glutamyltransferase (EC 2.3.2.2)</td>
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<td>Lactate dehydrogenase (EC 1.1.1.27)</td>
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<td>Lipase (EC 3.1.1.3)</td>
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2 Nonstandard abbreviations: LD, lactate dehydrogenase; ALP, alkaline phosphatase; CK, creatine kinase; and GGT, γ-glutamyltransferase.

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Except for amylase and LD, which are most often bound to IgA, the gamma heavy chain is involved in most other macroenzymes (5, 6, 38-42). IgM is rarely involved (43, 44), and we are unaware of any reports of IgE- or IgD-mediated macroenzymes. An example of light-chain restriction is IgA-mediated macro LD, which is almost exclusively made up of kappa light chains (45, 46). Light-chain restriction is especially interesting, because it suggests that the immune response against the serum enzymes is monoclonal. Other evidence in support of a monoclonal response is the finding of sharp, distinct bands on gel electrophoresis and isoelectric focusing of immune–enzyme complexes, the finding of linear Scatchard plots of the affinity of anti-enzyme antibodies (35, 29, 45), and the reports of patients with lymphoproliferative disorders who have monoclonal paraproteins that react with serum enzymes (43, 47). There are numerous reports, however, of patients with immune–enzyme complexes that contain both types of light chains or multiple types of heavy chains (5, 15, 18, 48-50), indicating that at least in some cases the immune response against serum enzymes can be polyclonal.

Antigen Specificity

Another interesting feature of immunoglobulin-bound macroenzymes is the antigen specificity of antibodies to enzymes. Some antibodies react with only certain isoenzymes, others react with all the isoenzyme forms of a given enzyme. For example, immune–enzyme complexes of amylase usually contain both salivary and pancreatic amylase (30, 51), probably because the two isoenzymes share common amino acid sequences and, therefore, common antigenic sites (52). Antibodies to ALP, in contrast, react only with specific ALP isoenzymes. ALP isoenzymes exist in two major antigenic groups, the placenta–intestine group and the liver–bone group (53, 54). An anti-ALP antibody will react with both members of an antigenic group but will not react with either member of the other group (16, 55, 56).

The antigenicity of LD, which is a tetramer of H and M subunits, depends not only on the composition of the subunits but also on the geometric arrangement of the subunits. Three kinds of binding specificities have been described for anti-LD antibodies (57-60): (a) antibodies that react with either the M or, more rarely, the H subunit and bind LD2-5 or LD1-4, respectively; (b) antibodies that react with either the H or M subunit and bind LD1-5; and (c) antibodies that react only with isoenzymes that contain both the H and M subunits, LD2-4. The IgA kappa antibodies that preferentially react with LD3 and to a lesser degree with LD2 and LD4 are members of the third class of antibodies (46, 60, 61). Kanemitsu (59) speculated that the epitope for the anti-LD IgA kappa antibodies is made up of peptide sequences from both the H and M subunit and is located in the region of the enzyme where the H and M subunit are adjacent, which would suggest that the intact protein and not the individual subunits is the stimulus for antibody formation. The same model has been proposed to explain why anti-histone antibodies in patients with systemic lupus erythematosus react with epitopes that are made up of multiple histone subunits (62, 63). However, the exact antigenic sites that anti-LD or other anti-enzyme antibodies react with are not fully known.

Antibodies to amylase have been proposed to bind near the active site of amylase, because antibodies to amylase preferentially interfere with access of high-molecular-mass substrates and inhibitors to the active site of amylase (64). The NAD+ binding site has been proposed to be the epitope for anti-LD antibodies of the IgG type, because NAD+ competes with IgG binding, and the addition of NAD+ dissociates the immune–enzyme complex (5, 29, 41, 43). Knowledge of the primary sequence of serum enzymes that is now available and the ability to produce peptides and monoclonal antibodies should make more precise localization of the antigenic sites more attainable in the future.

Effect on Activity

The impact of antibody binding on the activity of serum enzymes has been carefully examined, to determine the cause of the increased enzyme activity observed in many patients with macroenzymes. The mechanism for this increased activity is best understood for macroamylase. Amylase, a low-molecular-mass protein (~55 000 Da), is readily filtered and excreted by the kidney, whereas macroamylase has a molecular mass of approximately 210 000 Da and is not cleared by the kidney (6, 8, 64). It is the decreased renal clearance of macroamylase that accounts for the accumulation of amylase in the circulation. However, most serum enzymes are larger than amylase and are not significantly filtered by the kidney; these are thought to be removed instead by the reticuloendothelial system (65). It has been proposed that antibody binding to serum enzymes interferes with the reticuloendothelial-system clearance mechanism (17, 66, 67). Schifferli et al. (14) showed, in fact, that the half-life of radiolabeled human macro acid phosphatase was longer than that of free acid phosphatase in the circulation of rats. They also showed that the macro acid phosphatase was inactivated more slowly in serum than was the free enzyme. Both factors could account for the increased total enzyme activity frequently seen in patients with macroenzymes.

Despite the numerous reports of patients with macroenzymes having increased enzyme activity in serum, it has recently been appreciated that many patients with macroenzymes have normal activity concentrations of total enzyme in their serum. In a random screen, only two of 16 patients with macroamylase were found to have increased serum amylase (8). Less than 0.5% of patients with macro creatine kinase (CK) have increased total CK activity in their serum (38), although they may have an increase in the particular isoenzyme that the anti-CK antibody is directed against. There have even been reports of patients with macro LD who have decreased LD in their serum (68-70). Some anti-enzyme antibodies have been shown to block the activity of the bound enzyme (37, 56, 68) both in vivo and in vitro, presumably by interfering with substrate binding or catalysis, or both.

Concentration of Antibody

The amount of anti-enzyme antibody present compared with the concentration of the enzyme in serum has also been examined because of its potential clinical significance. In most cases, the concentration of the antibody exceeds the concentration of circulating enzyme (14, 15, 23, 68, 71), but it has been observed that the antibody concentration can decrease, and, at times, the decrease correlates with resolution of an underlying illness (1, 3, 23, 42, 43, 70, 72, 73). For some macroenzymes, however, the antigen appears to be in excess (46, 74). Fridhandler and Berk (8, 74) have identified three types of macroamylase (types 1-3), based on the relative amount of free and immunoglobulin-bound amylase. Patients with type-1 macroamylasemia usually
have very low concentrations of free (i.e., unbound) amylase, whereas patients with type-2 and -3 macroamylasemia have a substantial amount. The concentration of free enzyme in serum of patients with anti-enzyme antibodies depends on the relative amount of enzyme and antibody, as well as the affinity of the antibody for the enzyme. The lower the affinity of the antibody for the enzyme, the greater the amount of free enzyme likely to be present (35).

Why Do Antibodies to Enzymes Form?

Just why anti-enzyme antibodies form is not fully understood, but the two classic models of autoantibody formation, the "antigen-driven theory" and the "dysregulation of immune tolerance theory" (75), may partly explain their occurrence.

According to the antigen-driven theory, a self-antigen can become immunogenic by coincidently cross-reacting with an antibody initially formed against a foreign antigen, by being biochemically altered, or by being released from a sequestered site. Amylase has been proposed to become immunogenic because of a cross-reaction of amylase with antibodies that were initially formed against foreign antigens, such as animal amylase (35, 76). Exposure to non-human amylase could occur by the ingestion of animal and dairy products (35, 76). This would explain why the affinity of some antibodies to amylase is greater for animal amylase than it is for human amylase, and why such antibodies are usually IgA, because IgA is preferentially produced when antigen exposure occurs by the way of mucosal surfaces, such as the gastrointestinal tract (77).

The alteration-of-self-antigen theory has been used to explain the formation of autoantibodies directed against enzymes from damaged tissues, such as anti-CK and anti-LD antibodies in patients with myocardial infarctions (5, 15, 78–80). After a myocardial infarction, cardiac proteins, including cardiac enzymes, may be altered by proteases and other hydrolytic enzymes to become immunogenic. This mechanism has been proposed to explain the formation of anti-troponin, anti-myosin antibodies and other anti-cardiac antibodies in patients with Dressler's syndrome, an autoimmune disease of the heart that develops several weeks after a myocardial infarction (81, 82).

The sequestered-antigen theory may account for the formation of antibodies to CK-B in infants who have damage to the central nervous system and release of sequestered CK-BB from the brain (83–85).

The major evidence for the dysregulation of immune tolerance model is the association of anti-enzyme antibodies with other autoimmune disorders and other autoantibodies, including other anti-enzyme antibodies (18, 86). Macroamylase, for example, has been found in patients with rheumatoid arthritis (87), systemic lupus erythematosus (88), ankylosing spondylitis (89), cryoglobulinemia (90), AIDS (91), and inflammatory bowel disease (92). Macroamylase has been found associated with inflammatory bowel disease (93), drug-induced hemolytic anemia (72), and other autoimmune disorders (5). Some patients with macroenzymes also have laboratory evidence for altered immunity, such as increased ratios of T-helper cells to T-suppressor cells, increased immunoglobulin concentrations, and positive ANA tests (6, 42, 46). However, not all patients with anti-enzyme antibodies have either clinical or laboratory evidence of a general immune disorder (6, 17, 19, 46, 66, 69, 94). In an immunological screen of 14 patients with anti-CK antibodies, Schifferli et al. (42) found no significant increase in the incidence of immunologic abnormalities as compared with a control population, except for the interesting finding of an increased incidence of a certain HLA haplotype that is associated with autoimmune disorders.

In summary, although none of the theories on autoimmunity may fully explain immunoglobulin-bound macroenzymes, they do account for some of the observations and features of macroenzymes. A greater understanding of anti-enzyme antibody formation will probably not be forthcoming until autoimmunity in general is better understood.

Clinical Significance of Immunoglobulin-Bound Macroenzymes

Biological Significance

An unresolved question about anti-enzyme antibodies is whether they directly cause disease or are simply markers for disease. A causal role for anti-enzyme antibodies in disease has been proposed because the concentration of anti-enzyme antibodies reportedly fluctuates in patients according to the severity of an underlying disease (1, 3, 23, 42, 43, 70, 72, 73). Anti-enzyme antibodies could potentially cause disease by the same three mechanisms whereby other autoantibodies cause disease (75): (a) by immune-complex deposition, (b) by interfering with the function of the antigen, and (c) by a direct cytotoxic effect on the cells that express the antigen. Immune complexes of serum enzymes have been detected in patients with anti-enzyme antibodies (42), but patients with immunoglobulin-bound macroenzymes do not develop immune-complex disease unless there are immune complexes from another coexisting disease, such as systemic lupus erythematosus (88). It is unlikely that circulating anti-enzyme antibodies interfere with enzyme function, because the enzymes that form macroenzymes in serum do not have an extracellular catalytic role. A cytotoxic role of anti-amylase antibodies was proposed to explain the increased incidence of abdominal pain and malabsorption in patients with macroamylase and other macroenzymes (6, 24, 92, 93). However, it is not clear whether the association of anti-enzyme antibodies with disease is not simply ascribable to a bias in patient selection (6).

At any rate, any association between anti-enzyme antibodies and disease is at best weak, because many patients with macroenzymes are apparently healthy (6, 17, 19, 46, 66, 69, 94). Moreover, the correlation between the concentration of anti-enzyme antibody and clinical disease does not necessarily indicate a causal role for anti-enzyme antibodies in disease. This correlation could also occur if anti-enzyme antibodies form as a consequence of disease. Although various diseases have been reported to coexist with macroenzymes, no consistent pattern of disease associations has emerged, and at present there is no convincing evidence that anti-enzyme antibodies cause disease. At this time, the presence of an immunoglobulin-bound macroenzyme in a patient should be regarded as a benign phenomenon that does not necessarily portend any future disease or require any special treatment. Nevertheless, a cautious clinician may wish to be on the alert for any early signs of disease, especially in those patients who have a monoclonal immune response, because they may be at increased risk for developing a clinically significant autoimmune disorder (45, 46, 96).
Analytical Significance

Despite the lack of a clear role of immunoglobulin-bound macroenzymes in the pathogenesis of disease, they are clinically important entities because of the frequent confusion that they cause in the interpretation of serum enzyme results. Anti-enzyme antibodies can cause increases in most of the enzymes in plasma that are measured in the clinical laboratory, which can lead to misdiagnoses, as well as unnecessary and dangerous diagnostic tests and procedures (6, 14, 19, 66).

The best example of this is patients with macroamylase, who have been misdiagnosed as having pancreatitis and have had unnecessary laparotomies (6). The incidence of macroamylase in patients with hyperamylasemia is common enough—approximately 4.5% to 9.6% (71, 96)—that macroamylase should always be considered in the differential diagnosis of hyperamylasemia.

However, the macroenzyme that most frequently causes difficulty in the interpretation of diagnostic enzyme results is macro CK, which interferes with CK-MB measurements (10–12, 93, 97, 98). Determination of CK isoenzymes by immunoinhibition (11, 97, 99), immunoprecipitation (100), ion-exchange chromatography (101), protein electrophoresis (102–104), and immunoassays (11, 105) have all been reported to be affected by macro CK. In the immunoinhibition test, which is used as a screening test for the detection of CK-MB, B-subunit activity is measured after inhibition of the M subunit with an antibody to M (38, 99, 100). The immunoinhibition test is an accurate measurement of CK-MB if negligible amounts of CK-BB are present, as is normally the case (97). However, patients with macro CK type-1 have anti-CK-BB antibodies and have increased CK-BB immune complexes in their serum, which are resistant to inhibition by the anti-M antibody during the assay, and so will be mistaken for CK-MB. About 2% of the immunoinhibition tests performed in hospitalized patients yield falsely high values for CK-MB because of the presence of macro CK (38, 99, 106), and in some patients, such as elderly women, who have a higher incidence of macro CK, as many as 6% of immunoinhibition test results are affected (42, 99). Immunoprecipitation tests for CK-MB can also yield inaccurate results in the presence of macro CK if the results are not corrected for fluid displacement by the secondary antibody solution (100). Anti-enzyme antibodies can also affect the interpretation of CK and other enzymes during protein electrophoresis. For instance, a falsely negative diagnosis of a myocardial infarction can be made when anti-CK antibodies bind the MB isoenzyme and cause it to shift from its characteristic electrophoretic position (11, 86). Alternatively, anti-CK antibodies have also been reported to cause a false-positive diagnosis during protein electrophoresis, because macro CK-BB can sometimes migrate in the area of the gel where CK-MB typically migrates (102–104). Even the newer immunoassays for CK-MB, which were not initially thought to be affected by macroenzymes (103, 105, 107), may yield falsely decreased values for CK-MB in the presence of macro CK type-1, perhaps because of a competition between the patient’s anti-B antibody and the reagent anti-B antibody for CK-MB (11). Because results of numerous tests for CK-MB are affected by macro CK, Mifflin and Bruns (7) have recommended a combined immunological and electrophoretic approach in measuring CK-MB in the presence of macro CK type-1.

Finally, another potential problem of anti-enzyme anti-

bodies in serum enzyme measurements is the false decrease in measured enzyme resulting from the inhibition of enzyme activity by anti-enzyme antibodies (37, 69). Collectively, macroenzymes are not an infrequent cause of confusion in diagnostic enzyme testing, and because of the consequences of misinterpreting serum enzyme results, it is important for clinical laboratories to detect and identify macroenzymes in patients’ specimens.

Biochemistry of Non-Immunoglobulin-Bound Macroenzymes

Table 2 lists the non-immunoglobulin-bound macroenzymes and their mechanism of formation. Besides forming a complex with immunoglobulins, amylase can also form a complex with hydroxethyl starch (108, 109), which is given intravenously to patients for volume replacement. Amylase binds to hydroxethyl starch in the circulation because of its structural similarity to the natural substrate of amylase. The hydroxethyl starch–amylase complex has a high molecular mass and is not filtered by the kidney, and therefore it accumulates in the serum. This type of macroamylase is not frequently encountered, but it is important to be aware of this mechanism, because continually there are new drugs and other products that potentially could form complexes with enzymes in the serum.

Another unique class of macroenzymes is the complex of serum proteases, such as trypsin, with α2-macroglobulin, a high-molecular-mass protein in serum (109a). α2-Macroglobulin inhibits the activity of the bound protease and is thought to be important in controlling the damage that circulating active proteases can cause. Because trypsin and other proteases are not often assayed in clinical laboratories, this type of macroenzyme often is not detected, nor is its clinical relevance known.

Macro CK, besides occurring in an immunoglobulin-bound form, can also exist as a non-immunoglobulin-bound macroenzyme called macro CK type-2, which is found in up to 3.7% of hospitalized patients (106). Macro CK type-2 is a polymer of mitochondrial CK and has a molecular mass >300 000 Da (38, 111, 115). Mitochondrial CK is not related structurally to either the M or B subunit of CK and is produced by a separate gene (110, 116). In vitro, mitochondrial CK polymerizes in the absence of its substrates and magnesium (115), but whether similar factors control polymerization in vivo and whether polymerization has any functional significance are not known. The liver is a rich source of mitochondrial CK, and patients with liver disease have increased macro CK type-2 in their plasma,

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Mechanism</th>
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<tr>
<td>Amylase (EC 3.2.1.1)</td>
<td>Substrate complex</td>
<td>108, 109</td>
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</tr>
<tr>
<td>Trypsin (EC 3.4.21.4)</td>
<td>Protease-inhibitor complex</td>
<td>109a</td>
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<tr>
<td>Mitochondrial creatine kinase (EC 2.7.3.2)</td>
<td>Self-polymerization</td>
<td>110, 111</td>
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<td>Alkaline phosphatase (EC 3.1.3.1)</td>
<td>Lipid aggregate</td>
<td>112–114</td>
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<tr>
<td>γ-Glutamyltransferase (EC 2.3.2.2)</td>
<td>Lipid aggregate</td>
<td>9, 112, 113</td>
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<td>Leucine aminopeptidase (EC 3.4.11.2)</td>
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<td>5’-Nucleotidase (EC 3.1.3.5)</td>
<td>Lipid aggregate</td>
<td>112–114</td>
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probably from increased hepatocyte cell turnover and death (111, 117). Mitochondrial CK is also increased in the serum of patients with severe systemic diseases, such as metastatic carcinoma (106, 117, 118). Macro CK type-2 accumulates in the circulation, like the other macroenzymes, because the high-molecular-mass CK polymer is not readily cleared from the circulation. However, total CK activity in patients with macro CK type-2 is usually within normal limits or only slightly above (38, 106).

The remaining macroenzymes listed in Table 2 can be classified into one group because of their similar biochemical properties. These macroenzymes have various designations, such as "particulate enzymes," "high-molecular-mass bands," or "koinozymes." These macroenzymes are usually detected in clinical laboratories when electrophoresis on polyacrylamide gel is used for isoenzyme separation, and enzyme activity is noted at or near the origin. All of the enzymes in this group are amphiphilic and are located in the plasma membrane of the hepatobiliary system. These macroenzymes can be found in healthy patients, but amounts are increased in patients with liver disease (119–122). The two most frequently studied macroenzymes of this group are gamma-glutamyltransferase (GGT, EC 2.3.2.2) and ALP. On polyacrylamide gel electrophoresis, GGT can be resolved into three bands (9, 119, 121): a high-molecular-mass form (Mₐ > 1 000 000), an intermediate-molecular-mass form (Mₐ = 250 000–500 000), and (c) a low-molecular-mass form (Mₐ = 120 000). The last is believed to be the free hydrophilic form of the enzyme, produced in vivo after removal of the hydrophobic portion of the molecule by proteases (119, 123). ALP can be resolved into two bands on polyacrylamide gel electrophoresis (114), a high-molecular-mass form (Mₐ > 1 000 000) and a low-molecular-mass hydrophilic form (Mₐ = 220 000).

There are two theories concerning the mechanisms by which these macroenzymes are formed.

The first is that bile, by acting as a detergent, solubilizes plasma membrane-bound enzymes whenever there is obstruction to bile outflow (114, 119, 121, 122, 124). The hydrophobic domains or covalently attached phospholipids of these enzymes, which ordinarily enable these enzymes to anchor to the plasma membrane, are believed to cause the solubilized enzymes to bind to the hydrophobic lipoprotein carrier proteins in the circulation (22, 114, 119–122). If these enzymes are first modified in vitro by removal of hydrophobic domains or covalently attached phospholipids by protease or phospholipase treatment, they lose their affinity for lipoproteins (120, 123, 125). Likewise, removal of the associated lipids by treatment with detergents or extraction with organic solvents converts these macroenzymes into lower-molecular-mass forms (114, 126).

The other theory on the formation of these macroenzymes is that, during hepatocellular obstruction, the plasma membrane of cells lining the hepatobiliary system breaks down, releasing plasma membrane fragments into the circulation (112–114). In support of this model, De Broe et al. (112, 127), using gel filtration, isolated vesicles from the serum of patients with liver disease that have a trilamellar structure and centrifugal properties similar to that of plasma membrane fragments. These vesicles also contain enzyme activity for all of the enzymes ordinarily found in the bile canaliculi membranes, in roughly the same specific activity as in the plasma membrane (113).

Much of the experimental evidence, such as conversion of the macroenzymes to low-molecular-mass forms by detergents and proteases, actually can be explained by both models. For some macroenzymes, such as the intermediate form of GGT, the lipoprotein-carrier-binding theory may be correct, whereas for other macroenzymes, such as ALP, the plasma-membrane-fragment theory may be more accurate. The physical characterization of the hepatobiliary macroenzymes and their clinical significance are areas that clearly require further investigation.

**Clinical Significance of Non-Immunoglobulin-Bound Macroenzymes**

Except for macro CK type-2, which can cause a falsely positive diagnosis of a myocardial infarction in a manner similar to macro CK type-1 (128), these macroenzymes seldom cause difficulty in the interpretation of results for diagnostic enzymes. However, these macroenzymes are clinically interesting because of their potential role as markers for disease. Macro CK type-2 is strongly associated with several diseases, namely, severe liver disease and disseminated malignancies (5, 38, 106), and it has been proposed to be a useful tumor marker (129, 129a). In a study of 33 patients with adenocarcinomas of the gastrointestinal tract, Mercer and Talamo (129) found that measurement of macro CK type-2 had a sensitivity of 56%, which was slightly better than for carcinoembryonic antigen, a widely used tumor marker. Rogalsky et al. (118) found that tissue from colonic carcinoma had about the same macro CK type-2 activity as did adjacent normal tissue. Macro CK type-2 may increase in patients with malignancies because of increased cell turnover and death; however, its concentration does not appear to correlate closely with tumor burden (118, 130, 131). Further evaluation of macro CK type-2 is needed before it can be recommended as a routine tumor marker, but its detection in a patient should alert a physician to the possible presence of malignancy or liver disease in a patient.

The macroenzymes of the hepatobiliary system may also be useful as tumor markers, especially in detecting metastatic disease to the liver (132–135). The cholestasis and inflammation that a hepatic tumor causes appear to facilitate the formation of hepatobiliary macroenzymes. However, whether measurement of hepatobiliary macroenzymes offers any significant improvement in the detection of hepatic metastasis over conventional enzyme testing needs further investigation (133).

Hepatobiliary macroenzymes may also be useful in distinguishing extrahepatic from intrahepatic obstruction, for which there is currently no other satisfactory laboratory test (119, 136, 137). One of the more promising results has been with the electrophoretic measurement of an intermediate-molecular-mass form of GGT. Wenham et al. (119) found a sensitivity of 86% and a specificity of 96% in distinguishing extrahepatic from intrahepatic obstruction by measuring the concentration of an intermediate form of GGT. A subsequent study by Collina et al. (138) confirmed these findings, but they did not find the test to be as sensitive or as specific. Recently, using an improved cellulose acetate gel electrophoresis technique and a fluorescent stain, Sacchetti et al. (139–141) have been able to resolve five bands of GGT activity. Measurement of these five fractions of GGT currently appears to be the most sensitive macroenzyme marker for liver diseases.

It is to be hoped that further research into the formation and detection of macroenzymes will result in the develop-
Laboratory Detection of Macronzymes

Initial Testing

Evaluation of a suspected case of a macroenzyme should begin with a review of the patient's clinical history and results for routine laboratory tests. Patients with macroenzymes often have a persistently increased value for an enzyme in serum that either is not associated with any clinical symptoms or is atypical for the clinical course and routine laboratory tests of the patient. For example, patients with macroamylasemia characteristically have chronically increased amylase, often one- to fourfold normal, with no signs and symptoms of pancreatitis and a normal value for lipase (6, 142, 143). In tissues with multiple enzyme markers—such as the pancreas, liver, and heart—measurement of the other enzyme markers is often useful in evaluating a macroenzyme and will often reveal normal values for enzyme activity, except for the enzyme that is in the macroenzyme form. The degree of increase in the enzyme activity and the time course of the increase also are helpful in identifying macroenzymes, particularly macro CK. When immunoinhibition tests are performed on serum that contains either type-1 or type-2 macro CK, greater than 20% MB activity is typically found (11, 36, 59, 104). Myocardial infarctions usually result in less than 20% CK-MB activity, so a greater proportion should alert one to the possible presence of macro CK (38). Because macro CK has a long half-life in the circulation, the MB isoenzyme as measured by the immunoinhibition test will appear to remain constant, whereas true MB isoenzyme, which has a half-life of less than 12 h, decreases rapidly after a myocardial infarction (11, 38).

Whenever there is a suspicion of a macroenzyme from the clinical history or routine laboratory tests, it should first be evaluated by simple screening tests. One of the first screening tests used to identify a macroenzyme was the amylase/creatinine-clearance ratio (6, 8, 144), calculated by measuring the creatinine concentration and the amylase activity in a serum specimen and in a 24-h urine specimen. Because the amylase–immune complex is too large to be filtered by the kidney, this ratio will be smaller for patients with macroamylasemia, usually <1.0%, whereas it will be within normal limits or increased (0.8–4.5%) for patients with normal renal function and hyper-amylasemia from other causes (6). Unfortunately, this test is not positive in all cases of macroamylasemia (6, 8, 74, 145, 146), and it is not applicable to most other serum enzymes, which are larger molecules than amylase and so are not normally cleared by the kidney.

A more generally applicable test for macroenzymes that can be readily performed by most laboratories is protein electrophoresis (7, 8, 119, 126). Anti-enzyme antibodies can alter the normal electrophoretic pattern of enzymes by forming new enzyme bands, by altering the intensity of enzyme bands, and by causing band broadening (7, 8, 61, 71, 95, 131, 146). Macro CK can usually be recognized on CK electrophoresis because of the formation of new enzyme bands (Figure 1). Macro CK type-1 typically migrates between the MM and MB isoenzyme, whereas macro CK type-2, depending on the electrophoretic system used, often migrates cathodically to the MM isoenzyme (7, 38, 98). Macro CK and other macroenzymes can also migrate in less-characteristic positions, depending on the ratio of antibody to antigen (58, 147). Protein electrophoresis is also the principal test to identify the non-immunoglobulin-bound macroenzymes of the hepatobiliary system and, based on their electrophoretic mobility, several types have been identified for each enzyme (114, 119, 122, 126). Another simple screening test for immunoglobulin-bound macroenzymes is precipitation with polyethylene glycol (96, 148). Immunoglobulin-bound enzymes are less soluble than free enzymes in the presence of polyethylene glycol. Other tests that depend on the different physical characteristics of free vs bound enzymes include measurement of heat stability (149), energy of activation (150), and solubility in solutions of ammonium sulfate (89, 151).

Further Testing

If results of the preceding laboratory tests are inconclusive, then more-specific but more-complex tests, which depend either on showing the increased molecular mass of a macroenzyme or the physical association of the macroenzyme with immunoglobulins, should be performed. The increased molecular mass of immune–enzyme complexes is most readily determined by gel filtration (1, 7, 8, 15, 111). Figure 2 shows an example of gel filtration of serum containing both macroamylase and free amylase. Macroamylase, the first enzyme peak, is eluted from the gel-filtration column much sooner than free amylase because of its greater molecular mass. The molecular mass of most immune–enzyme complexes is consistent with a 2/1 ratio between the enzyme and the immunoglobulin (14, 23, 35,

Fig. 1. Diagrammatic representation of a typical creatine kinase isoenzyme separation by cellulose acetate gel electrophoresis
(1) normal serum; (2) serum with macro creatine kinase type-1 (mck-1); and (3) serum with macro creatine kinase type-2 (mck-2)

Fig. 2. Gel-filtration chromatogram of chromatography on Sephadex G-200 of serum from a patient with macroamylasemia
36). Non-immune–enzyme complexes on gel filtration often have an even greater molecular mass than do immune–enzyme complexes and appear in the void volume of most gel-filtration columns (114, 121, 122). Thin-layer gel filtration, which is less cumbersome to perform than column chromatography, has also been used to identify macroenzymes (35, 36, 142). The physical association between enzymes and immunoglobulins can be demonstrated by several immunological assays. Immune–enzyme complexes can be selectively precipitated with anti-human antibodies (7, 24, 60, 86), or with Protein A–Sepharose (14, 69). Antibodies that react with the apoprotein portion of lipoprotein carriers have been used in the immunoprecipitation of the hepatobiliary macroenzymes (22, 122). Finally, immunoelectrophoresis (15, 61), counter-immunoelectrophoresis (13), and immunofixation (59, 67) have all been used to show the physical association of an enzyme with an immunoglobulin.

Once a macroenzyme is identified in a patient, it is important to communicate this information to the physician who is caring for the patient and, when necessary, to educate the physician on the clinical significance of macroenzymes. Finally, macroenzymes often persist for long periods of time (1, 5–7, 17, 66, 69), so the finding of a macroenzyme in a patient should be clearly documented in the patient's medical records, to prevent any future misinterpretation of the patient's serum enzyme results. If at the end of the laboratory investigation a macroenzyme is not detected, then other causes for persistently high enzyme concentrations in serum—such as malignancy—should be explored (152–154).

Summary

Much has been learned about macroenzymes since the first report of macroamylase 25 years ago. It is now known that conversion of serum enzymes to higher-molecular-mass forms is a general phenomenon that can involve most of the serum enzymes that are routinely measured in clinical laboratories. In addition, the biochemical characterization of the interaction between immunoglobulins and enzymes has been carefully explored, as well as the association of the hepatobiliary macroenzymes with lipoproteins and plasma membrane fragments. There is, however, a general level of ignorance about macroenzymes, perhaps because of the lack of a clear role of macroenzymes in the pathogenesis of disease. A greater understanding of autoimmunity in general and anti-enzyme antibody formation in particular will, we hope, resolve the role of macroenzymes in disease. The investigation of macroenzymes as diagnostic markers is currently an active area of research, which may in the future result in the development of new markers for disease and may bring the subject of macroenzymes to the forefront of clinical laboratory testing. At this time, macroenzymes are important, at the very least because of their potential to interfere with interpretation of serum enzyme results. We encourage clinicians to consider macroenzymes in the differential diagnosis of elevated serum enzyme activity, and for clinical laboratory scientists to provide effective means for detecting macroenzymes.

References

27. Stanworth DR, Turner MW. Immunological and analysis of...


96. Levitt MD, Ellis C. A rapid and simple assay to determine if macroamylase is the cause of hyperamylasemia. Gastroenterology 1982;83:376–82.


