All hydrogen atoms were located. The results of the structure analysis for data set II are given in Fig. 1. The estimated standard deviations are 0.008 – 0.015 Å in bond lengths and 0.5 – 1.0° in angles. Apparently significant differences in the bond lengths and large thermal parameters of the oxygen atoms are probably due to the disorder in the structure.

Both of the independent molecules appear to be almost planar with respect to the heavy atoms; this is even the case for the "half" oxygen atoms. At the present stage of the refinement there are indications of a weak quinonoid character in the molecule.

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Antigenic Relationship between Lysozymes and its Use in Isolation and Purification of this Type of Enzyme

ARNE GROV

The University of Bergen, School of Medicine, The Gade Institute, Department of Microbiology, Bergen, Norway

Lysozymes or endo-β-*N*-acetylmuramidases (E.C. 3.2.1) have been isolated from a variety of sources including mammalian tissues, birds, insects, plants, and microorganisms.[1-3] These enzymes hydrolyze the glycan of the bacterial cell walls to oligosaccharides of the *N*-acetylglucosaminyl-*N*-acetylmuramic acid structure.

Immunological cross-reaction is a widespread phenomenon, and several cases of serological cross-reactions between related enzymes have been reported in recent years, *e.g.* between trypsin and chymotrypsin [4,5] and between papain and chymopapain.[6]

The many immunological studies carried out on hen egg-white lysozyme (E.C. 3.2.1.17), of which the primary as well as tertiary structures have been fully characterized, have given us a considerable knowledge of the antigenic determinants of this enzyme.[7-14] Comparative immunological studies of lysozyme from different sources have, in spite of the striking similarity in amino acid sequences and three-dimensional structures, detected few or no cross-reactions.[15-19] This is the case also for human lysozyme and hen egg-white lysozyme, which have also been found to differ in amino acid sequences at a considerable number of points near the catalytic site.[20,21] However, cross-reactions between human and chicken lysozymes have been detected by methods necessitating only one antigenic determinant in common.[17,19] Immunoadsorbents, therefore, are expected to be a useful tool in isolation and purification of related enzymes. In the present study rabbit anti-hen egg-white lysozyme has been used to isolate lysozymes from human milk and saliva.

Materials and methods. Antiserum to hen egg-white lysozyme (EL) (Sigma; Grade I, 3 × crystalline) was raised in rabbits given injections of a saline solution of the enzyme emulsified in an equal volume of Freund's complete adjuvant (Difco). Three injections (5 mg of antigen/dose) were given 3 weeks apart, the first one in a foot pad (0.5 ml), the two other intramuscularly (1 ml into 2 sites). IgG was precipitated from rabbit anti-EL sera at 1.33 M $(NH_4)_2SO_4$ concentration, chromatographed on a column of DEAE-cellulose (Eastman),[22] and coupled to Sepharose 4B (Pharmacia) and Sephadex G-15 (Sigma) activated with cyanogen bromide following the procedure described by Porath *et al.*[23] Casein-free milk whey was prepared by acidifying (to pH 4.6 with 1 N HCl) skimmed human milk (kindly provided by Dr. B. Haneberg, The University Hospital) which was subsequently centrifuged to sediment the precipitated casein. The whey

samples were then neutralized using 1 N NaOH. Portions of whey (5 ml) and human mixed saliva (5 ml) were applied to immunoadsorbent columns of anti-EL IgG (1.5 cm × 20 cm). The columns were washed thoroughly with 0.2 M borate-buffer, pH 8.0, and then eluted with 3 M NaSCN.[24] Fractions (2 ml) were collected on a Minirac connected with a Uvicord (LKB Produkter) reading at 280 nm. Enzymatic activity was measured either as reduction in turbidity at 600 nm of a suspension of *Micrococcus lysodeikticus* whole cells in 0.1 M phosphate buffer, pH 6.2, or as lysis of *M. lysodeikticus* mucopeptide, prepared according to the method described in Ref. 25, in agar plates (0.5 mg/ml of 1 % agar). Enzymatically active fractions were bulked, concentrated by evaporation *in vacuo*, desalted on a column of Sephadex G-15 and freeze-dried. Enzyme specificity was tested by digestion of mucopeptide followed by reduction with sodium borohydride (Merck),[26,27] hydrolysis in 3 N HCl (4 h at 95°C), and chromatography on thin layer (cellulose plates) in solvent systems BuOH:HOAc:H_2O (4:1:1) and pyridine:H_2O (4:1). Hydrolysate of non-reduced digests, standard glucosamine and muramic acid (Koch-Light) and their $NaBH_4$-reduced forms (glucosaminitol and muraminitol) were included as references. Ninhydrin was used as colour reagent. Electrophoresis in dodecyl sulphate-polyacrylamide gels was carried out according to the method of Weber and Osborn.[28] The serological tests; double diffusion in agar, ring test examinations, inhibition of precipitation, and indirect haemagglutination using tanned sheep erythrocytes (TSE), were carried out as described previously.[29]

Results and discussion. The anti-EL serum gave one single line in double diffusion in agar against homologous antigen. The line could be detected at a serum dilution of 1/4. The haemagglutination titre was 1/320 and the ring test titre (dilution of antigen) was 1/160.

Neither whey nor saliva gave precipitation with anti-EL serum. However, when whey and saliva were applied to immunoadsorbent columns, materials having the capability to lyse *M. lysodeikticus* were eluted with NaSCN. In contrast to Sephadex G-15, Sepharose 4B, without coupled antibodies, apparently bound small amounts of lysozyme which could be released by 3 M NaSCN.

Portions of *M. lysodeikticus* mucopeptide were digested with (1) EL, (2) the lytic material isolated from whey (ML), and (3) the lytic material isolated from saliva (SL). Reduction of digested materials by $NaBH_4$, followed by acid hydrolysis and chromatography, showed that in all the three portions muraminitol, the reduced form of muramic acid, had been formed. On dodecyl sulphate-polyacrylamide gel electrophoresis both ML and SL showed a single band with an electrophoretic mobility corresponding to that of EL. These results strongly indicate that isolated ML and SL are pure muramidases. Although untreated Sepharose fixed some EL, no difference could be observed in the corresponding preparations isolated on columns of Sephadex- and Sepharose-coupled antibodies.

Precipitation reaction could not be detected between anti-EL and ML or SL, but both enzyme preparations inhibited anti-EL to precipitate with EL. Thus, when equal volumes of anti-EL and ML or SL (approximately 2.5 mg/ml) had been incubated for 2 h at 37°C and overnight at 4°C, this mixture, in contrast to anti-EL (1/2 in saline), showed no line in agar diffusion against EL.

Rabbits were also immunized with ML and SL (a total of approx. 1.5 mg of enzyme preparation) according to the schedule described for EL. The antisera obtained were very weak. No precipitation was detected either with homologous or heterologous antigen. However, both antisera agglutinated TSE sensitized with homologous as well as heterologous antigen (Table 1). The cross-reactions between ML and SL were relatively strong, whereas that between EL and the two human lysozymes seemed to be rather weak. However, in spite of an apparently slight immunological resemblance it seemed possible to isolate pure lysozymes from human milk and saliva on a column of antibodies to egg-white lysozyme.

Table 1. Indirect haemagglutination titres (reciprocal values) of lysozyme antisera using tanned sheep erythrocytes (TSE) sensitized with the lysozymes.

TSE sensitized with	Antisera to EL	ML	SL
EL (from egg-white)	320	10	10
ML (from milk)	10	80	80
SL (from saliva)	20	160	160

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On the Structure of β-$Dy(NH_4)_3(SO_3)_3.H_2O$

LAURI NIINISTÖ* and
LARS OLOF LARSSON

Department of Structural Chemistry, Arrhenius Laboratory, University of Stockholm, Fack, S-104 05 Stockholm, Sweden

Several ammonium lanthanoid sulphites can be prepared in the system $NH_4^+ - Ln^{3+} - SO_2 - H_2O$ where Ln = Sm, Gd, or Dy.[1] Because of the low solubility of these phases the crystals are usually very small and cannot be studied by single crystal X-ray methods. After many unsuccessful attempts, however, crystals with suitable dimensions (approximately $0.02 \times 0.02 \times 0.15$ mm^3) were obtained of the high temperature polymorph of $Dy(NH_4)_3(SO_3)_3.H_2O$ by precipitating the compound at 75°C and then gradually warming to 90 – 95°C. The crystals were kept in a tightly sealed vessel for a week in this temperature range.

Weissenberg and rotation photographs indicated that the needle-shaped crystals have monoclinic symmetry, with the following unit cell dimensions: $a = 8.88$ Å, $b = 3.98$ Å, $c = 9.48$ Å and $\beta = 117.4°$. The powder pattern was satisfactorily indexed with these unit cell parameters. The measured density, 2.60 g cm^{-3}, corresponds to one formula unit in the cell (calculated density 2.65 g cm^{-3}). Single crystal intensity data were collected on a Philips PW 1100 computer-controlled four-circle diffractometer, using graphite monochromatized CuK radiation. 439 independent reflections were obtained, representing all observed ($\sigma(I_{net})/I_{net} < 0.25$) reflections with $\theta < 55°$. There were no systematically absent reflections. The net intensities were corrected for Lorentz and polarization effects as well as for absorption ($\mu = 338$ cm^{-1}).

The structure determination was started by assuming the centrosymmetric space group $P2/m$ (No. 10). A three-dimensional Patterson synthesis revealed presence of the dysprosium atom at the special position 1(g) ($\frac{1}{2}$,0,$\frac{1}{2}$), and the subsequent Fourier synthesis gave the positions of the

* Present address: Department of Chemistry, Helsinki University of Technology, SF-02150 Otaniemi, Finland.