than the one between fractions A and C, and amounts to 1.3 pH-units in the human enzyme (pH 6.5–5.2) and 1.6 in horse erythrocyte catalase (pH 7.3–5.7). Thus, additional factors other than SH-oxidation affect the IEP of erythrocyte catalase. Fraction A, although isolated under mild, non-oxidizing conditions, has a lower IEP than catalase in a fresh hemolysate and therefore does not correspond to the native enzyme. Since fraction A contains no irreversibly oxidized sulfhydryl groups[4], the difference cannot be attributed to the formation of acid derivatives of cystein. During the isoelectric focusing of hemolysates, hemoglobin is present in a large excess and might affect the result by protein-protein interaction. This possibility can however be ruled out, since catalase is always completely separated from hemoglobin. Furthermore, the addition of hemoglobin does not significantly influence the IEP of purified, hemoglobin-free fraction A. The observed difference is possibly due to conformational changes occurring during the purification of the enzyme.

The data on the IEP of different catalase preparations reported in the literature[8] correspond to the value obtained for fraction C. It is concluded that those catalase preparations must have contained a high percentage of irreversibly oxidized sulfhydryl groups[9].

Zusammenfassung. Mittels isoelektrischer Fokussierung wurde der IEP von nativer Erythrocytenkatalase bestimmt (Mensch pH 6,5; Pferd pH 7,3). Die im Verlauf der Reinigung auftretende Konformationsänderung ist von einer Verschiebung des IEP nach der sauren Seite begleitet (Mensch ΔpH $-1{,}3$; Pferd ΔpH $-1{,}6$).

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The Role of Neuraminic Acid in the Stability and Enzymic Activity of Acid Phosphatase of the Human Prostate Gland

Recently it was reported that acid phosphatase of the human prostate (EC 3.2.3.1) occurs in several fractions, which can be separated by chromatography on DEAE or CM-cellulose[1,2] and by isoelectric focusing[3]. The heterogeneity is a result of the presence of N-acetylneuraminic acid (NANA) in the phosphatase molecule, which can be split off by means of neuraminidase without loss of enzymic activity[2]. It was therefore of interest to compare some of the properties of native phosphatase, such as thermostability, optimum pH, K_m and K_i with neuraminidase-treated enzyme.

Acid phosphatase activity was assayed under standard conditions[4] with *p*-nitrophenylphosphate, disodium salt (*p*-NPP), product of Sigma (Biochem. Corp., St. Louis, Mo.) as substrate in 0.1 *M* citrate buffer, pH 5.5. Liberated *p*-nitrophenol was assayed spectrophotometrically (Uvispec, Hilger a. Watts, London) at 420 nm. Activity of the enzyme toward adenilic acid (pA, Koch-Light, Colnbrook, England) and deoxyguanosinediphosphate (d-pGp, kindly provided by Dr. M. Laskowski sr.) was also studied, in this case on the basis of Pi liberated, assayed by a modification of the Fiske-Subbarow method[5].

Acid phosphatase I was used, which represents about 70% of total activity of crude human hypertrophic prostate extract, from which it was obtained and purified by the method previously described[4]. The preparation was homogenous in ultracentrifuge and in disc electrophoresis on polyacrylamide gel. To about 1 mg of enzyme I in 1 ml of 0.05 *M* acetate buffer of pH 5.5 containing 1% NaCl and 0.1% $CaCl_2$, 30 U of neuraminidase from Vibrio cholerae (25,000 U/mg protein, Koch-Light) was added, and the mixture was incubated 20 h at 25 °C. The solution was dialyzed overnight against distilled water and stored in frozen state for further study. Phosphatase I treated with neuraminidase under these conditions, when examined by isoelectric focusing according to Svensson[6], exhibited a single symmetrical peak with isoelectric point at about pH 5.8, whereas the native enzyme gives 3 well-separated peaks at pH 4.82, 4.92 and 5.10 (Figure 1).

Treatment of phosphatase with neuraminidase causes a rise of optimum pH by ca. 0.4 U for pA and *p*-NPP as substrates. Thermostability of both enzymes was studied at 55 °C and different pH. Splitting off NANA had no effect on the sensitivity of phosphatase to heating. Figure 2 shows that both enzymes were inactivated at the same rate in the pH range 4.6–6.75.

The reaction velocities and K_m constants for various substrates of native phosphatase and phosphatase treated with neuraminidase indicate that the enzyme deprived of NANA has greater affinity for all substrates studied (Table). The values of V_{max} and K_m, determined according to the Lineweaver and Burk[7] and by the method of least squares, are always lower for the modified enzyme, indicating greater affinity for the substrate, presumably as a result of diminished surface charge of the protein molecule. Strongly polar substrate molecules possess greater ease of contact with molecules of the modified

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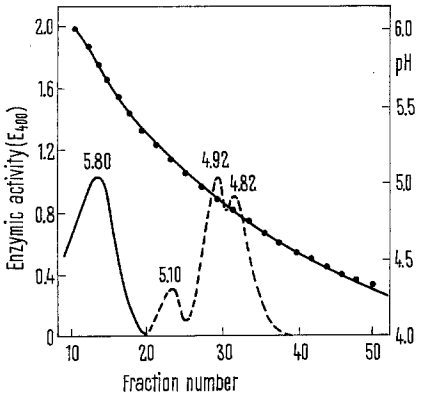

Fig. 1. Isoelectric focusing of native (---) and neuraminidase treated (—) acid phosphatase I. The diagram is a composition of 2 separate experiments carried out under the same conditions. The electrolysis (600 V/2 mA/48 h) was carried out according to SVENSSON[6] in a 26-ml apparatus[3] filled with 1% ampholine solution (LKB, Stockholm) in 0–50% sucrose gradient. ●—●, pH gradient. Protein concentration paralleled with enzymic activity.

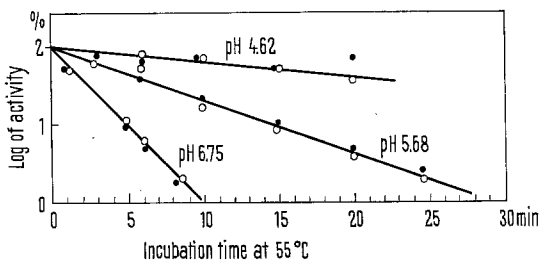

Fig. 2. Thermal denaturation of native (○—○) and modified (●—●) enzyme at various pH. The reaction mixture of 100 µl of buffer solution and 1 µl of enzyme solution (1 mg/ml) was heated at 55 °C for indicated periods of time, then cooled to 0 °C and activity determined with *p*-NPP as a substrate in standard conditions. For pH 4.62 and 5.68 citrate and for pH 6.75 *Tris*-maleic acid-NaOH buffer solutions were used.

Values of K_m, V_{max} and $\Delta(\Delta F°)$ of native phosphatase I (E) and treated with neuraminidase (En)

Substrate	K_m	V_{max}	$-\Delta(\Delta F°)$
pA	E 1.85×10^{-3}	9.5	
	E_n 1.05×10^{-3}	4.25	364
d-pGp	E 3.0×10^{-3}	10.5	
	E_n 2.35×10^{-3}	5.0	34
p-NPP	E 7.25×10^{-4}	4.0	
	E_n 7.05×10^{-4}	3.5	28

All the assays were carried out in 0.1 *M* citrate buffer of pH 5.5, with incubation at 37 °C for 10 min. The values of K_m are expressed in moles of substrate per liter and V_{max} in µg/ml of Pi or *p*-nitrophenol. Values of $\Delta(\Delta F°)$ are in cal/mole.

enzyme. This suggestion is supported by the changes in the decrease of free energy calculated for different substrates[8]:

$$\Delta F° = -RT \ln \frac{1}{K_m}$$

hence

$$\Delta(\Delta F°) = \Delta F°_{En} - \Delta F°_{E}$$

where E-native enzyme, and En-enzyme treated with neuraminidase. The Table shows that values of $\Delta(\Delta F°)$ decrease in proportion to increasing acidity of the substrate molecule, i.e. from pA, through d-pGp to *p*-NPP[9,10].

The phosphatase digested with neuraminidase was more strongly inhibited by L(+)-tartrate as competitive inhibitor[11], and the calculated value of K_i for p-NPP as substrate is 3.6×10^{-4}, compared with 6.2×10^{-4} for native enzyme. This is probably due to presence of neuraminic acid residues on the surface of the protein molecule presenting an obstacle to penetration of strongly polar molecules of the inhibitor into the active center of the enzyme.

It should be emphasized that, according to Moss[12] and SARASWATHI and BACHHAWAT[13], neuraminic acid in alkaline phosphatases from animal and human tissues plays a similar role in heterogeneity and activity as in prostatic acid phosphatase.

Résumé. On a comparé certaines propriétés physicochimiques et enzymatiques de la phosphomonoestérase acide native de la prostate de l'homme à celle traitée par la neuraminidase. L'enzyme dépourvue d'acide neuraminique atteint son optimum à un pH élevé, elle présente une plus grande affinité avec certains substrats et est inhibé d'une manière plus intense par le L(+)-tartrate que la phosphomonoestérase native.

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Molecular Architecture of Peptide Hormones Optical Rotatory Dispersion of Cholecystokinin-Pancreozymin, Bradykinin and 6-Glycine Bradykinin

The significance of secondary and tertiary structure of proteins with highly specific biological activity is fully recognized: the clefts in the molecule of enzymes accommodate the substrates and oxygen fits perfectly into the cavities of myoglobin or hemoglobin. A well-defined conformation for smaller molecules is less obvious and the general impression prevailing about shorter peptide chains, at least in neutral aqueous solution, is that of randomness. Peptide hormones with cycles formed by disulfide bridges (oxytocin, vasopressin, insulin) must have