Immunological Identification of Human Plasma 3Sγ_1-Globulin as Carbonic Anhydrase

A low molecular weight plasma protein with an electrophoretic mobility, corresponding to the γ_1-globulin has been isolated by SCHMID et al.[1] and was referred to as 3Sγ_1-globulin. This globulin was reported to be present in low concentration in normal human plasma (1 mg/l)[1]. Its urinary excretion amounted to 0.02 mg/24 h in normal subjects while its level in kidney extract was relatively high (6 mg/100 g wet tissue)[2]. According to its low molecular weight, amino acid composition and electrophoretic mobility, SCHMID et al. have suggested an analogy of the 3Sγ_1-globulin with carbonic anhydrase B of erythrocytes[3]. The specific activity of the 3Sγ_1-globulin was found to be almost equal to that of carbonic anhydrase B[4]. Furthermore, the terminal amino acid sequence was established to be the same for both proteins[3]. However, no similar comparison has been made on the immunological behaviour of these 2 proteins.

Human erythrocytes were washed 3 times with cold saline solution and hemolysis was performed with distilled water. Carbonic anhydrase was prepared according to the procedure described by KELLER[5]. The 3Sγ_1-globulin was a gift of Dr. SCHMID. Agar gel electrophoresis and immunoelectrophoresis were performed using the procedure of WIEME[6] and SCHEIDEGGER[7] respectively. A specific rabbit antiserum against 3Sγ_1-globulin was prepared as previously described[2]. Immunofluorescent technique was made using the indirect fluorescent antibody reaction, with rhodamine-labelled goat anti-rabbit γ-globulins (Sylvania Co.). A Leitz fluorescent microscope was used with a mercury lamp (HBO200 W) and a blue filter BG 12, 1.5 mm.

The results have shown that the carbonic anhydrase preparation of erythrocytes has a heterogenous distribution on agar gel electrophoresis (Figure 1). According

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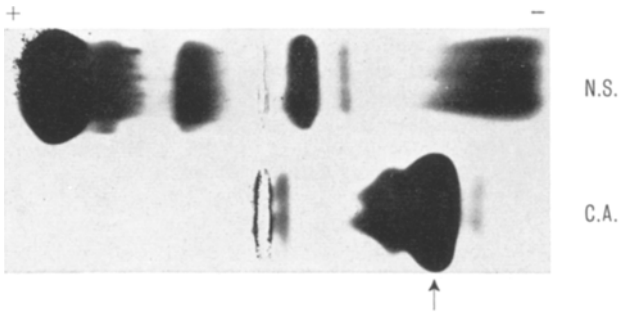

Fig. 1. Agar gel electrophoresis of carbonic anhydrase B. Agar gel electrophoresis was performed in 0.05 *M* veronal buffer (pH 8.4). The upper pattern shows a separation of normal human serum (N.S.) for comparison with the lower pattern which reveals the distribution of the carbonic anhydrase preparation (C.A.). The main component, indicated by an arrow, reveals the carbonic anhydrase B.

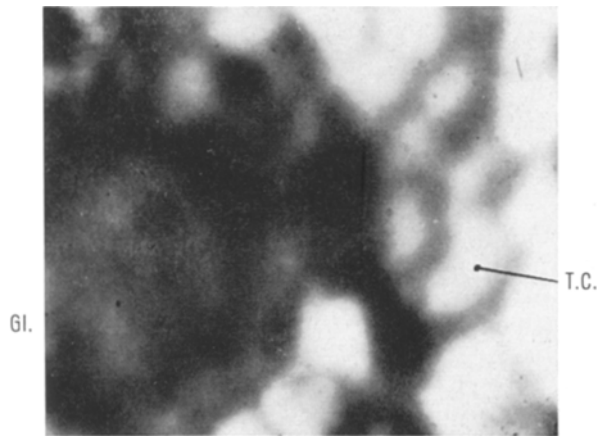

Fig. 3. Immunofluorescence pattern of kidney tissue. The fluorescence microscopy, using the indirect staining method, reveals that the 3Sγ_1 antibody activity is mainly located in the tubular cells (T.C.), while the glomerulus (Gl.) appears to be mostly non-fluorescent. × 250.

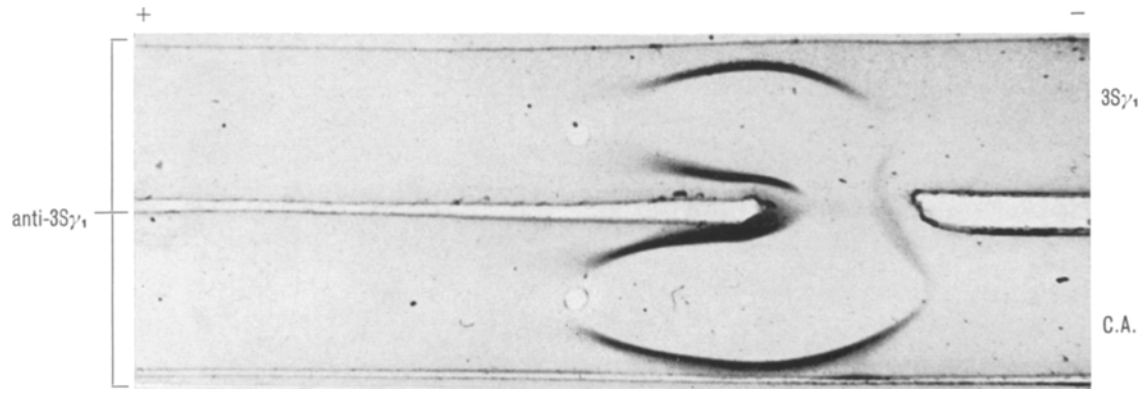

Fig. 2. Immunoelectrophoresis of 3Sγ_1-globulin and carbonic anhydrase B. Agar gel immunoelectrophoresis was performed in 0.025 *M* veronal buffer (pH 8.4). The following abbreviations are used: 3Sγ_1, 3Sγ_1-globulin; C.A., carbonic anhydrase; anti-3Sγ_1, anti-3Sγ_1-globulin. The interrupted groove shows the identity between the 3Sγ_1 and the C.A.B.

to LAURENT[8] the main component belongs to carbonic anhydrase B. By use of immunoelectrophoretic analyses, with interrupted groove, it was demonstrated that there was a complete reaction of identity between the $3S\gamma_1$-globulin and the carbonic anhydrase B (Figure 2).

Immunofluorescent technique using a rabbit anti-$3S\gamma_1$-globulin has revealed that labelling was distributed in the whole cytoplasma of all tubular cells, the glomerule being faintly fluorescent (Figure 3). A $3S\gamma_1$-antiserum of which the antibodies were precipitated by a pure $3S\gamma_1$-preparation did not show any further fluorescence.

As we know that carbonic anhydrase is commonly distributed in the kidney, the above data suggest an identity between this enzyme and the $3S\gamma_1$-globulin. Therefore, we may assume that on the basis of similarities on chemical structure, activity and immunological behaviour, the plasma $3S\gamma$-globulin and the carbonic anhydrase B are a unique component[9,10].

Résumé. La $3S\gamma_1$-globuline, précédemment isolée du plasma humain normal, a fait l'objet d'une comparaison immunologique avec l'anhydrase carbonique B isolée à partir d'érythrocytes. L'immunoélectrophorèse en gélose et l'immunofluorescence analysée sur le tissu rénal ont montré l'identité immunologique de ces 2 protéines.

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Detection and Identification of Pyridosine, a Second Lysine Derivative Obtained Upon Acid Hydrolysis of Heated Milk

Recently, a new basic amino acid, found by ERBERSDOBLER[1] in an acid hydrolysate of overheated milk, has been identified by HEYNS[2] and FINOT[3] as ε-N-(2-furoylmethyl)-L-lysine or 'furosine'[3]. This compound X can also be obtained by acid hydrolysis of ε-N-(1-deoxy-2-ketose)-L-lysine (derivative of glucose (I)[3,4]; derivative of lactose (II)[3]).

A second lysine derivative, compound Y, formed simultaneously with furosine during acid hydrolysis of I and II, has now been detected and identified in hydrolysates of overheated milk. This compound cannot be detected by applying the standard conditions of chromatography (SPACKMAN[5]), since it is eluted far after phenylalanine from the 'neutral' column, and well before lysine from the 'basic' column. Its migration is extremely sensitive to changes in pH; the peak can, however, be detected under special conditions (Figures 1 and 2).

Compound Y was isolated by chromatography on Dowex 50 (form H^+) as a peak eluted after furosine[3], and precipitated with methyl ethyl ketone from a methanolic solution of the dried chromatographic fraction. Its structure was determined by UV, IR, NMR and mass spectrometry (operating conditions as previously described[3]) and confirmed by synthesis.

The mass spectrum of the trifluoroacetyl-methyl ester derivative (molecular peak at m/e 364: $C_{15}H_{19}N_2O_5F_3^+$) shows that compound Y possesses the carbon skeleton of lysine and glucose from which it derives and that only 1 nitrogen can be trifluoroacetylated; the presence of ions at m/e 180, 166, 152, 138, and 124, obtained by elimination of the fragments $(CH_2)_n$–CH–(NH–CO–CF_3)–CO_2–CH_3 (n = 0, 1, 2, 3, 4) reveals that the α-nitrogen has been trifluoroacetylated; the last 2 fragments (n = 3, 4) are formed mostly by rearrangement of 1 hydrogen (m/e = 139, 125) or 2 hydrogens (m/e = 126). The great stability of the ion at m/e 125 indicates that the ε-nitrogen belongs to a ring ($C_6H_6NO_2^+$). The ring hypothesis is consistent with the IR bands at 1620 (s), 1550 (s), and 870 (m) cm^{-1} (γ-pyridone), and is confirmed by the UV-spectrum (maxima: 277 nm in neutral solution, 300 nm in basic solution, 246 and 275 nm in acidic solution) typical of the 3-hydroxy-4-pyridone structure. The UV-spectrum is similar to that of a natural compound, β-(1,4-dihydro-3-hydroxy-4-oxo-1-pyridyl)-alanine, namely mimosine or leucenol (maxima: 280 nm in neutral solution, 305 nm in basic solution, and 272 with shoulder at 250 nm in acidic solution) isolated by RENZ[6] and synthesized by ADAMS[7] and SPENCER[8].

The remaining substituents of the ring are given by the NMR-spectrum (Figure 3): singlets at δ (ppm) 8.16 and 7.20 (2 non coupled hydrogens), and at 2.65 (methyl). The methyl group must be either in position 5 or 6; the more probable position 6 has been confirmed by synthesis. The latter has been performed using the technique of HEYNS[9], by condensing α-N-formyl-L-lysine with the corresponding γ-pyrone, allomaltol, prepared according to BROWN[10]; the reference product, namely ε-(1, 4-dihydro-6-methyl-3-hydroxy-4-oxo-1-pyridyl)-L-lysine (V), has the same spectroscopic behaviour as compound Y. The trivial name pyridosine is proposed for this new amino acid.

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