and the redox state of NAD+/NADH system is again in the direction of reduction[18]. It must be pointed out that in this case the inductive response excludes the possibility that pyrazole by itself has some irreversible effect on the alcohol dehydrogenase molecule or some other inhibitory effect on the cell systems. The observed pattern of response with pyrazole is reasonably interpreted by assuming that the molecule of ethanol is not specifically required as a signal to induce this enzyme sequence. The change of significant intermediate is therefore to be searched for among the metabolic effects specifically deriving from the utilization of the C_2 fuels. Possibly the state of redox couples and adenine nucleotide systems are primarily involved.

In any case the response is concurrent for all three enzymes. This suggests a correlated regulation of level of enzymes catalyzing the ethanol → acetyl-CoA pathway, under the control of a common metabolic signal.

[18] P. K. Maitra and R. W. Estabrook, Arch. Biochem. Biophys. *121*, 140 (1967).

Antigenicity of Thermal Denatured Yoshida Glycoprotein

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Summary. The correlation between antigenic functionality and conformation of Yoshida glycoprotein upon heating has been investigated. Heating modifies the conformation of the antigen, but does not abolish the reaction with its specific antibody.

There has been a great deal of work in the past decade on the problem of determining the functionality of small synthetic antigen molecule[1], whereas for natural antigens detailed studies on the relation between structure and function are limited to few examples. Among these, convincing evidence has been reported for serum albumin and Yoshida glycoprotein[2-6]. In the course of extensive investigations on the possibility of substituting haptenic groups in the polypeptide chain of Yoshida glycoprotein, it has been observed that this protein behaves on heating in a very peculiar way and could represent an useful experimental model to study thermal denaturation of proteins. Because literature data concerning reversible thermal denaturation of proteins are scanty[7-11], in this report we give a preliminary account of the experiments undertaken in order to correlate antigenic functionality of Yoshida glycoprotein to conformational changes of the molecule upon heating.

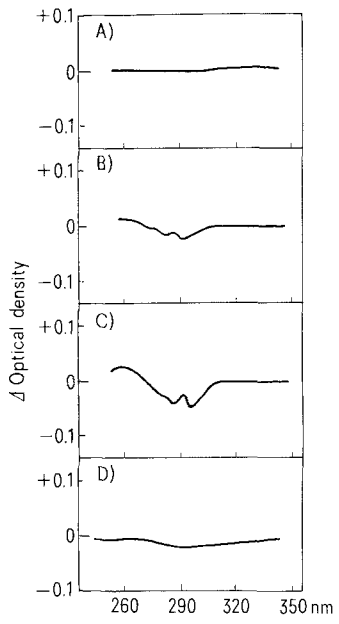

Fig. 1. Temperature difference spectra. A) Native Yoshida glycoprotein against native; B) Yoshida glycoprotein heated at 70 °C against native; C) Yoshida glycoprotein heated at 90 °C against native; D) Yoshida glycoprotein heated at 90 °C and cooled at room temperature against native. The protein concentration both in sample and reference cell was 1 mg/ml. The spectra were recorded 30 min after that temperature was reached. The pH of solutions was 7.0.

Materials and methods. Glycoprotein was isolated from Yoshida ascites tumor fluid as previously reported[12,13]. The purity was ascertained by ultracentrifugation and electrophoresis, during which it behaves as homogeneous monodisperse system as confirmed by the occurrence of one single precipitine arc in immunoelectrophoresis.

Thermal denaturation was carried out in a Colora thermostat and the temperature was measured by thermistored apparatus to an accuracy of ± 0.1 °C. Spectroscopic measurements were performed with a Beckman DK1 recording spectrophotometer equipped with a thermospacer apparatus.

Protein solutions were prepared by diluting 1 ml stock solution (5 mg/ml) to 5 ml in a volumetric flask. The concentration of protein was determined spectrophotometrically using an $E\,{}^{1\ \mathrm{cm}}_{1\ \mathrm{mg/ml}}$ of 0.700. The antigenic activity of the glycoprotein was measured in its capacity

[1] M. Sela, Science *166*, 1365 (1969).
[2] P. H. Maurer, Ann. N.Y. Acad. Sci. *103*, 549 (1963).
[3] C. T. Liu, B. R. Das and P. H. Maurer, Immunochemistry *41*, 1 (1967).
[4] A. Caputo, A. Floridi and M. L. Marcante, Biochim. biophys. Acta *181*, 446 (1969).
[5] R. Zito, M. L. Marcante, A. Floridi and A. Caputo, Biochim. biophys. Acta *194*, 74 (1969).
[6] A. Caputo; 10th Int. Cancer Congr. Houstons Commun. *372*, 231 (1970).
[7] M. L. Anson and A. C. Mirsky, J. phys. Chem. *35*, 185 (1939).
[8] A. C. Poland and H. A. Sheraga, Biopolymers *3*, 401 (1965).
[9] J. F. Brands, J. Am. chem. Soc. *86*, 401 (1965).
[10] C. Tanford, J. Am. chem. Soc. *85*, 2050 (1964).
[11] M. Brunori, E. Antonini, P. Wyman and A. Rossi-Fanelli, J. molec. Biol. *34*, 497 (1968).
[12] M. L. Marcante, Clin. chim. Acta *8*, 799 (1963).
[13] A. Caputo and M. L. Marcante, Arch. Biochem. Biophys. *105*, 193 (1964).

to combine with a specific antibody occurring in blood serum of rabbits immunized as previously reported[14]. Quantitative measurements were carried out according to the standard procedure of precipitine curves, and the precipitates were measured, after dissolution in NaOH 0.2 *M*, for their absorbance at 280 nm in a Beckman DU spectrophotometer.

Results and conclusions. When heated, the Yoshida glycoprotein does not change its solubility even if the heating is prolonged up to 3 h. Moreover the analysis in the ultracentrifuge reveals that the rise of temperature determines a loss of the homogeneity. A heavier component, which represents about 20% of the principal peak, has been observed and, from calculations of the sedimentation coefficient, it was concluded that, following heating, the Yoshida glycoprotein undergoes aggregation and the size of the aggregated molecule may be evaluated as the double of the native one.

The changes in spectroscopic properties of Yoshida glycoprotein upon heating are shown in Figure 1. Marked modifications in the spectrum are observed in the range between 310 and 260 nm. The modifications became apparent at 70 °C (Figure 1, B) and increased with temperature (Figure 1, C). Thermal denaturation is almost reversible because, after cooling (Figure 1, D), the difference spectrum of Yoshida glycoprotein is quite similar to that of native (Figure 1, A).

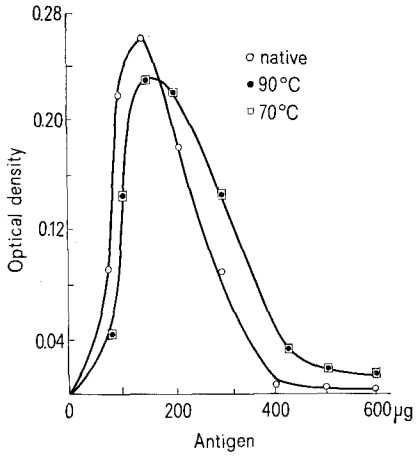

Fig. 2. Quantitative precipitin reactions of native (○—○) and heated (□—□, 70 °C; ●—●, 90 °C) Yoshida glycoprotein with rabbit antibodies to native Yoshida glycoprotein.

After heating, most proteins in solution soon lose their ability to interact with antibodies elicited by their undenatured counterparts. In the case of Yoshida glycoprotein, as observed in Figure 2, the original antigenic specificity has not been lost. The figure clearly indicates that quantitative precipitin reaction of heated Yoshida glycoprotein is similar to that of the native one. The only exception is represented by the descending portion of the curve which is broad and therefore less homogeneous, thus confirming the occurrence of differently sized antigenic determinants. The antigenic specificity did not change with the increase of the temperature. The precipitin reaction carried out with the protein heated at 90 °C was in fact identical with that of protein at 70 °C.

It has been previously demonstrated[5] that chemical modification of tyrosyl groups suppresses the capacity of this antigen to combine with specific antibody, thus indicating that tyrosyl residues are of fundamental importance for the immunospecificity of the glycoprotein.

Nevertheless, the fact that heating does not abolish this capacity is not surprizing if one considers that the changes in temperature difference spectra can be attributed not only to tyrosyl but also to a contribution of tryptophanyl and phenylalanyl residues.

Moreover the spectral changes, essentially due to modifications both of intrinsic solvent effect and of degree of ionization of certain groups, are reversible.

These observations indicate that the conformational changes of Yoshida glycoprotein, which occur upon heating, do not modify the small areas of the surface of the molecule which are involved in the reaction with antibody.

[14] P. P. GAZZANIGA, F. R. SONNINO and A. CAPUTO, in *Protides of the Biological Fluids*, Proc. 11th Colloq., Bruges 1963 (Ed. H. Peters; Elsevier, Amsterdam 1964), vol. 11, p. 338.

Wirkung von 6β-Methoxy-9β,10α-pregna-4,6-dien-3,20-dion (Ro 6-1963) auf die Steroidreduktasen in der Rattenleber[1]

Effect of 6β-Methoxy-9β,10α-pregna-4,6-diene-3,20-dione (Ro 6-1963) on the Steroid Reductases in Rat Liver

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Summary. 6β-Methoxy-9β,10 α-pregna-4,6-diene-3,20-dione inhibites the Δ^4-3-oxosteroid-5α-reduction in microsomes and the Δ^4-3-oxosteroid-5β-reduction in the soluble fraction of male rat liver. The 3α- and 3β-hydroxysteroid dehydrogenase are not inhibited by this substance.

6β-Methoxy-9β,10α-pregna-4,6-dien-3,20-dion (Ro 6-1963) zeigte sich wirksam als Inhibitor der Δ^4-3-Oxosteroid-5α-Reduktase der Rattenprostata in vitro[2]. Uns interessierte die Wirkung dieser Substanz auf die Enzyme des Steroidstoffwechsels in der Rattenleber, und zwar auf die Δ^4-3-Oxosteroid-5α-Reduktase und die 3α-Hydroxysteroid-Dehydrogenase in der Mikrosomenfraktion und die Δ^4-3-Oxosteroid-5β-Reduktase im Cytoplasma.

Methoden. Wir benutzten die Leber männlicher Wistar-Ratten. Die Präparation der Mikrosomen erfolgte in der schon beschriebenen Weise[3]. Die enzymatischen Aktivitäten wurden berechnet aus den gaschromatographisch bestimmten Produkten. Die enzymatischen Ansätze zur Bestimmung der Aktivität der Δ^4-3-Oxosteroid-5α-Reduktase enthielten in einem Volumen von 3,0 ml: 0,2 *M* Kaliumphosphatpuffer, pH 7,0, 0,8 m*M* NADPH, bis zu