immobilized lipoyl dehydrogenase in 30% (v/v) dioxan. The rate constants for in- 
activation in dioxan, like those for thermal inactivation at 90°C, increase with increasing 
distance from the matrix and eventually approach that for the inactivation of the native 
enzyme in 30% (v/v) dioxan, i.e. 0.01 min⁻¹. 

These data can be rationalized by the fact that the hydrophilic Sepharose matrix 
probably holds the enzyme in a rigid conformation, and thus the nearer the enzyme is to 
the matrix backbone, the greater its stability. These observations contrast markedly with 
the destabilization of proteins bound to the hydrophobic matrix polystyrene reported by 
Manecke (1962).


The Isolation of a New Warfarin-Sensitive Protein from Bovine Plasma

CHRISTOPHER V. PROWSE and M. PETER ESNOUF

Nuffield Department of Clinical Biochemistry, The Radcliffe Infirmary, 
Oxford OX2 6HE, U.K.

Mattock & Esnouf (1973) described a bovine protein, which had similar chromatographic properties and molecular weight on sodium dodecyl sulphate/polyacrylamide gels to bovine Factor X, but unlike Factor X this protein was composed of a single polypeptide chain. This protein which will be referred to as protein Z can only be successfully separated from Factor X by chromatography of the Factor X₂ pool (Esnouf et al., 1973) on DEAE-Sepharose. The purified protein gave a single precipitin arc in radial-immuno- 
diffusion experiments against an antiserum against protein Z and failed to react with a 
rabbit anti-(Factor X) antiserum.

The protein also moved as a single band on electrophoresis into sodium dodecyl sul-
phate/polyacrylamide gels with apparent mol.wt. 55000; however, equilibrium-sedi-
mentation experiments gave a lower mol.wt. of 44000. This difference in the molecular 
weights can be explained by the high carbohydrate content (20%) of protein Z. The 

The only biological activity that has so far been detected for protein Z is that it poten-
tiates the effect of adrenaline in causing platelet aggregation. This activity of protein Z 
resembles that found for protein C (Seegers, 1976), a γ-carboxyglutamic acid-containing 
protein present in bovine plasma (Stenflo, 1976), but unlike protein C, protein Z does not 
appear to inhibit blood coagulation, nor does it react with an antiserum prepared against 
protein C.
Protein Z causes platelet aggregation at high concentrations (1 mg/ml), an effect which is dependent on the presence of Ca\(^{2+}\). At lower concentrations (0.2 mg/ml), protein Z not only caused reversible aggregation, but enhanced the aggregation induced by ADP, collagen and most noticeably adrenaline, but did not potentiate ristocetin-induced platelet aggregation.

Platelet-aggregating activity is not a general effect of \(\gamma\)-carboxyglutamic acid-containing proteins, since neither prothrombin nor Factor X cause platelets to aggregate.


Cross-Reactivity in the Radioimmunoassay of Ferritin with Cells from High- and Low-Responder Mice

NICHOLAS J. DEACON and ALAN EBRINGER

Department of Biochemistry, Queen Elizabeth College, University of London, London W8 7AH, U.K.

Immunization with various antigens of synthetic (Pinchuck & Maurer, 1965) and biological origin (Playfair, 1968) evokes an immune response that varies in different strains of the same species of animals. Such responses have been shown to be linked to the histocompatibility antigens (McDevitt & Tyan, 1968) and are said to be under the control of autosomal immune-response genes (McDevitt & Benacerraf, 1969).

It has been suggested (Ebringer & Davies 1973) that such responses may be due to cross-reactivity between the antigen and self-determinants of the responder animals, whereby low-responder strains cross-react more than high-responder strains with the test antigen. We have determined the immune response to the antigen horse spleen ferritin in 16 strains of inbred mice (Young et al., 1976). A continuous distribution of responses was observed, ranging from the lowest in A.Thy 1.1 mice, to the highest in B10M and B10D2 mice.

In the present study, a radioimmunoassay for horse spleen ferritin has been established in order to determine the extent of cross-reactivity of cell-surface antigens from high- and low-responder mice with horse spleen ferritin. Ferritin antiserum was raised in a New Zealand White rabbit by repeated intraperitoneal injections of horse spleen ferritin (twice crystallized; Miles Laboratories), first in complete Freund's adjuvant and then in phosphate-buffered saline (0.15M-NaCl/0.01 M-sodium phosphate buffer, pH7.2). An antiserum-dilution curve was prepared by using ferritin labelled with \(^{125}\)I by the chloramine-T method (Greenwood et al., 1963). Briefly, 1 ng of \(^{125}\)I-labelled ferritin in 50 \(\mu\)l of phosphate-buffered saline containing 0.1 \% (w/v) gelatin and 0.02 \% (w/v) Na\(_2\)S, was added to 100 \(\mu\)l of anti-ferritin antiserum, diluted in the range 1/10\(^4\) to 1/10\(^5\) in phosphate-buffered saline, previously incubated with 50 \(\mu\)l of this saline for 1 h at 37°C. After further incubation for 3 h at 37°C, 100 \(\mu\)l of a goat anti-(rabbit immunoglobulin) antiserum was added to equivalence and incubated for 16 h at 4°C. Controls containing normal rabbit serum with the same range of dilutions were treated in a similar way. Total radioactivity in each sample was counted in a well-type gamma scintillation counter (Packard), then samples were centrifuged for 10 min at 2500 rev./min (MSE Multex centrifuge), precipitates were washed once with phosphate-buffered saline and counted for radioactivity. Results were expressed as percentage of antigen bound after subtraction of control from test readings.

In the assessment of cross-reactivity a 50 \(\mu\)l cell suspension containing \(2 \times 10^7\) twice-washed spleen cells/ml, obtained from low-responder A.Thy 1.1 and high-responder...