Stabilisation of Proteins to Heat

This disclosure concerns methods for stabilising proteins such that they can be heated to inactivate potential viral contaminants without substantially destroying the biological activity of the protein.

A variety of stabilising methods have been previously developed for this purpose. Examples include the use of:


(iii) Sucrose to stabilise fibronectin (D.G. Wallace Biochemical Medicine 27 : 286-296, 1982).


In all of these examples the proteins are stabilised to the extent that they can be heated in solution for at least 10 hours at 60°C to inactivate hepatitis virus (S.S. Gellis et al. J. Clin. Invest 27 : 239-244, 1948).


In this invention sorbitol and glycine have been used to stabilise concentrates of Factor VIII, Factor IX and immunoglobulin. Fibrinogen, platelet factor 4, g-thromboglobulin and thrombospordin were also found to be stabilised by this method. Fibrinectin was stabilised using sorbitol alone.

**Factor VIII Concentrate.** In the presence of 1.7% w/w glycine and 64% w/w sorbitol an intermediate-purity factor VIII solution was heated for 10 hours at 60°C with a recovery of over 70% of the Factor VIII activity. (A.J. MacLeod et al. Thromb. Haemostasis 50 : 432, 1983). Further improvements have been achieved by the addition of calcium chloride to the stabilising mixture and by the use of either polypropylene or stainless steel containers for the heating process. A number of operating conditions are possible depending on the precise composition of the factor VIII solution to be heated. Sorbitol concentration can be in the range 50 - 75% w/w with about 65% w/w providing an appropriate balance between stabilisation and problems of high viscosity and high dilution which result at higher concentrations. Glycine can be used in the range 1-6% w/w depending on the fibrinogen content of the Factor VIII solution, as 2.1 molar glycine is known to precipitate fibrinogen. Calcium is added to maintain an excess of ionised calcium of about 3mM. The quantity of calcium to be added depends on the concentration of calcium binding ions present in solution, such as citrate, but will generally be in the range 1-10 mM. With these improvements factor VIII solutions can be heated more severely than in previous applications (60°C for 10 hours) thus enabling contaminants which may be more heat-resistant to be inactivated. For example, an intermediate purity factor VIII solution in the presence of 1.7% w/w glycine, 64% w/w sorbitol and 2.8 x 10^-6% w/w calcium chloride was heated at 60°C for 9.5 hours followed by 70°C for 0.5 hours with a 77%
recovery of factor VIII activity. This additional heating increased the
degree of inactivation of vaccinia virus from about 10^6 plaque forming
units/ml to over 10^7 pfu/ml (A.J. MacLeod et al. Proc. 18th Congress Int.

In further experiments factor VIII solution was heated at 60°C for 22
hours followed by 0.5 hours at 70°C or at 70°C for about 1 hour giving over
70% recovery of factor VIII activity in both instances. Hence these
stabilisers allow various combinations of heating times and temperatures to
be used within the broad range from a few minutes at about 70°C to many
hours at 60°C.

Factor IX Concentrate. A solution containing coagulation factors II,
IX and X has been stabilised in the presence of 2.1% w/w glycine and 55% w/w
sorbitol giving a 65% recovery of factor IX activity after heating at
60°C for 10 hours (A.J. MacLeod et al. Thromb. Haemostasis 50 : 432,
1983). Further experiments, with sorbitol concentrations and heating
conditions in the range described above for factor VIII, have given factor
IX recoveries of over 50% after heating.

Immunoglobulin Concentrate. Immunoglobulin products in clinical use
are generally considered safe despite the fact that they are not pasteurised to inactivate viral contaminants. However recent evidence of
hepatitis transmission by human immunoglobulin (R. Lane et al. Lancet ii :
974, 1983) together with concern about other potential viral contaminants
associated with human blood products (eg AIDS) suggest that heat treatment of
immunoglobulin may become of interest.

Immunoglobulin stabilised in the presence of 10% w/v glycine and 45%
w/v sorbitol was heated in solution at 60°C for 10 hours without loss of
specific antibody function (rubella) and without increase in either
aggregate content or anti-complementary activity (A.G. Welch et al. Lancet
ii : 1198, 1983). The concentrations of glycine and sorbitol required are
related to the protein content of the solution but are generally in the
range 5 - 15% w/w glycine and 30 - 70% w/v sorbitol. As with factor VIII,
heating conditions more severe than 10 hours at 60°C can be applied
including temperature up to 75°C for up to 4 hours. Heating can also be
carried out in the presence of up to 5% ethanol without loss of specific
antibody function (rubella). Similar results have been achieved using
sucrose in place of sorbitol.

Fibronectin Concentrate. Sorbitol alone is sufficient to stabilise
fibronectin and a concentration of about 20% w/v allows fibronectin to be
heated in solution at 60°C for 10 hours. Higher concentrations of sorbitol
can be used if more severe heating conditions are required.

This invention concerns the heat-stabilisation of human proteins some
of which are particularly labile or sensitive to heat induced damage
(factor VIII, fibrinogen). Therefore the method described has a wide and
general application for the stabilisation of virtually all proteins which
require to be exposed to heat treatment processes.

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