# EFFECT OF INERT POLYMERS ON PROTEIN SELF-ASSOCIATION

Lawrence W. NICHOL
*Department of Physical Biochemistry, John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia*

Alexander G. OGSTON
*Department of Biology, University of York, York YO1 5DD, England*

Peter R. WILLS†
*Abteilung Wittmann, Max-Planck-Institut für Molekulare Genetik, 1000 Berlin 33, Germany*

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## 1. Introduction

In [1] polyethylene glycol (PEG) was reported to stimulate the self-association of the pyruvate dehydrogenase complex isolated from *Azotobacter vinelandii*. They state that the exact reasons for this phenomenon are not clear at present. It is our purpose to point out that this effect has a thermodynamic explanation in terms of the excluded volume interaction between the protein and the polymer. While we restrict our discussion to the consideration of protein self-association reactions, we refer to the detailed work in [2] on the effect of inert polymers on enzyme activity. The observation [2] that dextran promotes the inhibition of trypsin by serum albumin was interpreted in a manner consistent with the argument presented here. The biological significance of various aspects of polymer interactions has been reviewed in [3].

## 2. Theory

Consider a general macromolecular self-association reaction:

$$nA \rightleftharpoons C \tag{1}$$

The thermodynamic association constant $K_{ass}$ is a ratio of thermodynamic activities $a_X$:

$$K_{ass} = a_C/a_A^n \tag{2}$$

The thermodynamic activities are conventionally defined as equal to concentrations in the standard state of zero concentrations of all solute species. Consequently, all activity coefficients $y_X$ are unity in the standard state. Writing the association constant in terms of molar concentrations $m_X$:

$$K_{ass} = (m_C/m_A^n)(y_C/y_A^n) \tag{3}$$

we see that the ratio $m_C/m_A^n$, often identified as the association constant, approaches the value $K_{ass}$ in the limit of infinite dilution of all solute species. When we consider a situation away from the dilute limit, the virial theorem allows us to write the logarithm of each of the activity coefficients as a multinomial expansion in the concentrations of all solute species [4]:

$$\ln(y_X) = \sum_i \alpha_{Xi} m_i + \text{higher order terms} \tag{4}$$

Suppose now that we are dealing with a solution in which the protein species A and C are in low concentration (<0.1%) and that there is an 'inert' polymer P present at moderate concentration (1–5%). In this case, the higher order terms in the virial expansion can be ignored and the dominant terms $\alpha_{XP} m_P$ give reasonable estimates of the activity coefficients:

$$y_X = \exp(\alpha_{XP} m_P) \tag{5}$$

† To whom correspondence should be addressed

For a given polymer concentration, the self-association reaction still follows the thermodynamic law of mass action, albeit with a modified 'apparent' association constant, given by:

$$K_{app} = K_{ass} \exp[(n\alpha_{AP} - \alpha_{CP})m_P] = m_C/m_A^n \quad (6)$$

It is immediately evident that if $n\alpha_{AP} - \alpha_{CP}$ is positive, then the inert polymer will cause stimulation of the self-association reaction. Derivations based on statistical mechanics [4] or thermodynamics [5] indicate that the coefficients $\alpha_{XP}$ may be interpreted in terms of excluded volumes $U_{XP}$ between a molecule of species X and a molecule of the inert polymer P, which we suppose to be uncharged. The virial coefficient $\alpha_{XP}$ is in fact the 'excess excluded volume' [4]:

$$\alpha_{XP} = U_{XP} - V_P \quad (7)$$

The quantity $V_P$ is the thermodynamic volume (molecular weight times partial specific volume) ($M_r \times \bar{v}$) of a molecule of the polymer. Thus $\alpha_{XP}$ represents the volume of the solution excluded to a molecule of X by a molecule of P, over and above the volume which the polymer molecule actually occupies. It is in accordance with Le Chatelier's principle that addition of a volume-excluding polymer to an equilibrium mixture of A and C will cause the equilibrium to be displaced in the direction which opposes the change in excess excluded volume imposed on the system. When the quantity $n\alpha_{AP} - \alpha_{CP}$, which represents the difference in excess excluded volume between the dissociated and associated states of the protein, is positive, addition of polymer favours the association of A to form C.

## 3. Calculations

If we model all macromolecules as rigid spheres, then the excluded volumes are given by the formula:

$$U_{XP} = 4\pi N(R_X + R_P)^3/3 \quad (8)$$

where $N$ is Avogadro's number and $R$ represents a molecular radius. When the radii are expressed in cm, the units of $U_{XP}$ become $cm^3$/mol. There is a question as to whether a sphere or chain model is better for calculating excluded volumes between proteins and polymers such as PEG. The former has the advantage that intrinsic viscosity is likely to give a reasonable estimate of the radius of the equivalent rigid sphere and we shall adopt this convention here using eq. (8). A value of 3.0 nm was thus obtained [6] for the effective radius of PEG-6000 (species P) and this value is employed in subsequent calculations together with values of 8000 g/mol and 0.837 $cm^3$/g for av. $M_r$ and $\bar{v}$ [7]. Such approximations, as well as those which follow, are adequate in illustrating the basic effect under discussion, but it is appreciated that the actual numerical values which emerge should not be attributed undue significance. The pyruvate dehydrogenase complex from *A. vinelandii* (species A) has a Stokes radius of 13 nm and electron microscopy has been used to show that the associated form of this complex (species C) consists of a rather uniform population of spherical protein particles of larger radius [1]. Using this value as an impenetrable sphere radius for the monomer complex and making the assumption that the total volume occupied by the protein subunits is conserved during self-association to form the larger spherical units, we obtain values of $1.03 \times 10^7$ $cm^3$/mol for $U_{AP}$ and $6.15 \times 10^7$ $cm^3$/mol for $U_{CP}$. (These values correspond, respectively, to 1.3 and 7.7 litres/g PEG-6000.) Values of $K_{app}/K_{ass}$ for various concentrations of PEG-6000 are shown in table 1. These values also represent the factor by which the concentration of the associated form is increased by addition of polymer to the solution, should the concentration of the monomer complex be held constant.

## 4. Discussion

The rapid exponential increase in the ratio $K_{app}/K_{ass}$ with increasing polymer concentration suggests that a self-association reaction which is not evident in the absence of polymer, may appear as the dominant

Table 1
Effect of PEG-6000 on the self-association of pyruvate dehydrogenase

| Polymer concentration | | $K_{app}$ |
|---|---|---|
| % (g/100 ml) | mol/$cm^3$ | $K_{ass}$ |
| 0.1 | $1.25 \times 10^{-7}$ | 14 |
| 0.2 | $2.50 \times 10^{-7}$ | $1.9 \times 10^2$ |
| 0.5 | $6.25 \times 10^{-7}$ | $4.7 \times 10^5$ |
| 1.0 | $1.25 \times 10^{-6}$ | $2.2 \times 10^{11}$ |
| 2.0 | $2.50 \times 10^{-6}$ | $4.9 \times 10^{22}$ |
| 3.0 | $3.75 \times 10^{-6}$ | $1.1 \times 10^{34}$ |
| 5.0 | $6.25 \times 10^{-6}$ | $5.4 \times 10^{56}$ |

behaviour of the system at even moderate polymer concentrations. Precipitation of the protein will be induced at even higher polymer concentrations, but will not set in until the product of the temperature and the excess entropy of the excluded volume for one of the species is equal to the free energy of precipitation [8].

The self-association of the pyruvate dehydrogenase complex from *Escherichia coli* has been shown to be a slow equilibrium process [9]. If the same is true for the *A. vinelandii* enzyme, then areas under the Schlieren peaks observed in sedimentation velocity experiments [1] give a direct indication of the degree of self-association induced by PEG-6000. On this basis, the large value of $K_{app}/K_{ass}$ reported in table 1 for 3% PEG explains the observation that, at this concentration of polymer, a large proportion of the protein (50–90% depending on the preparation) was present as a rapidly sedimenting peak which was not observed in the absence of PEG. Thus, standard calculations (albeit approximate) readily show that introduction of an inert polymer at relatively high concentration, may cause the $K_{app}$ for a protein self-association reaction to become sufficiently large to ensure that the associated form of the protein predominates, even though the true thermodynamic constant $K_{ass}$ is small enough to prevent Schlierent optical detection of the larger species in the absence of polymer. In the cellular cytoplasm there exists a high concentration of macromolecular material which would mimic the effect of the polymer introduced into an in vitro preparation of the enzyme. Thus the associated form of the enzyme may predominate in vivo.

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