Eur Biophys J (1998) 27: 177–181

BIOPHYSICS LETTER

Gregory Barshtein · Ilana Tamir · Saul Yedgar

Red blood cell rouleaux formation in dextran solution: dependence on polymer conformation

Received: 8 August 1997 / Accepted: 28 November 1997

Abstract The velocity of rouleaux formation (RF), as previously shown, increases with increasing dextran concentration up to a critical concentration (C_a), beyond which the addition of dextran reduces the RF velocity (RFV). de Gennes' model for polymer solutions suggests that dextrans exist in two conformations: a coil structure at low concentrations, which changes to a network beyond a critical concentration (C^*). In the present study we examined the relation between C_a and C^* for dextrans of different molecular weight, and found that they coincide. This suggests that the change in dextran behavior, from increasing to decreasing RFV, occurs when their conformation changes from coil to network. In addition, it has been reported that in dilute dextran solutions the intercellular distance (D) between RBC in rouleaux increases with the molecular weight of the dextran. We found that D correlates with R_f, the end-to-end distance of the polymer molecule, and for all dextrans $D \leq 1.5\ R_f$. In accord with de Gennes' Model for polymers between surfaces, this corresponds to intercellular interaction with two overlapping surface-associated polymer layers, which may extend "tails" to interact with the opposing cells.

Key words Erythrocyte aggregation · Polymer conformation · Overlap concentration · Rouleaux · Intercellular distance

Introduction

Red Blood Cells (RBC) in the presence of high molecular weight polymers, such as plasma proteins or dextrans, aggregate to form rouleaux and rouleaux networks (Chien and Jan 1973). The mechanism of RBC aggregation in polymer solutions is complex and two processes have been proposed as inducers of the aggregation: the formation of bridges between the cells by the polymer molecules (the "bridging" mechanism) (Chien and Jan 1973; Chien et al. 1983), or condensation of the cells induced by osmotic gradients due to depletion of the macromolecules from the intercellular space (Brooks and Evans 1987; Evans and Parsegian 1983).

Dextrans have been widely used for induction of RBC aggregation and investigation of the mechanism of this process. Dextrans specifically have been shown to adsorb to the RBC cell surface. This has been documented in a number of studies, using diverse methodologies including dextrans with radioactive labels (Brooks 1973; Chien et al. 1977), or with fluorescent labels (Cudd et al. 1989; Avarez et al. 1996; Minetti et al. 1978). In addition, it has been predicted (de Gennes 1981, 1985) and experimentally documented (Kawaguchi and Takahashi 1983) that polymers adsorbed to the surface form a layer, with a thickness which is equal to the end-to-end distance (R_f) of the polymer molecule. de Gennes' model further suggests that when two surfaces interact in a polymer solution, the interfacial distance is $D \approx 2\ R_f$ when there is no overlap between the polymer molecules on the two surfaces, or $D < 2\ R_f$ when the two polymer layers overlap.

The velocity of dextran-induced rouleaux formation (RF) exhibits a biphasic behavior (Nash et al. 1987; Maeda and Shiga 1985): at low dextran concentrations the RF velocity (RFV) increases with the dextran concentration up to an optimal concentration, C_a, beyond which the addition of dextrans reduces the RFV (Nash et al. 1987; Maeda and Shiga 1985). Independently, de Gennes' model for polymers in solutions (de Gennes 1979) suggests that the polymer molecules may be in two conformations. At low concentration the polymer is in a coil conformation having a radius of gyration, R_g. Above a "critical overlap concentration", C^*, the polymer molecules form a polymeric network, with a characteristic mesh size ξ.

The present study was undertaken to examine the biphasic behaviour of RBC aggregation in dextran solution in the light of de Gennes' model for dextran conformation. For this purpose we used a novel cell flow-properties analyzer (CFA), developed in our laboratory, which enables the monitoring of RBC aggregation by direct visualization

G. Barshtein · I. Tamir · S. Yedgar (✉)
Department of Biochemistry,
Hebrew University-Hadassah Medical School,
Jerusalem, Israel 91120
(e-mail: Yedgar@CC.HUJI.AC.IL)

of the aggregation process in a narrow-gap (30 µm) flow chamber (Chen et al. 1994). As the CFA enables the visualization and recording of the continuous aggregation process, it was used here to determine the *velocity* of rouleaux formation, prior to formation of rouleaux networks. It was found that RFV increases with dextran concentration up to a critical concentration C_a, beyond which it decreases, and this inversion coincides with the change of the dextran conformation from coil to network.

Materials and methods

Materials

Dextran 70 (Dex-70, M. W. 73,000) and dextran-500 (Dex-500, M. W. 496,000) were purchased from Pharmacia (Stockholm, Sweden). Bovine serum albumin (Fraction V) was purchased from Sigma (St. Louis, MO).

RBC rouleaux formation (RF) was monitored by direct visualization of RBC in a narrow-gap flow chamber using a computerized cell flow-properties analyzer (CFA), which enables the continuous monitoring of the RBC aggregation process by direct visualization under the microscope (Chen et al. 1994).

Preparation of RBC suspensions

Blood was drawn from healthy volunteers into EDTA solution. RBC were separated by centrifugation (500 *g* for 10 min.), then washed twice with PBS buffer at pH = 7.4. For aggregation experiments the cells were suspended at 6% hematocrit, in PBS buffer supplemented with 1% albumin, with increasing concentration of dextrans.

RF kinetics

For determination of RFV, the RBC were singly dispersed by shear stress, and the spontaneous RF was monitored and videotaped starting at cessation of flow. The RBC images were analyzed for the distribution of the aggregate sizes (number of cells/aggregate) at the different stages. RFV was derived from the slope of the curve describing the change in average aggregate size (AAS) as a function of time. As shown in Fig. 1, measurements were taken during the first 15 seconds from cessation of flow, during which the process was linear.

Calculation of end-to-end distance of dextran molecules, R_f

For branched molecules the following relationship is given (Stockmayer and Fixman 1953):

$$R_f = 2.45\ g\ R_g$$

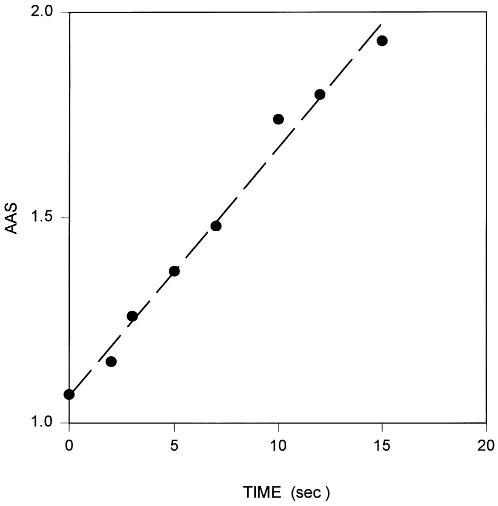

Fig. 1 Kinetics of RBC rouleaux formation in Dex-500 solution. For determination of RFV, RBC were singly dispersed by shear stress, and the spontaneous RF was monitored and videotaped starting at cessation of flow. The RBC images were analyzed for the aggregate size distribution (number of cells/aggregate) at the different stages of RF, from which the average aggregate size (AAS) was derived and plotted vs. time

For branched polymers such as dextrans,

$$g = (R_g^2)_b/(R_g^2)_l,$$

where b and l denote branched and linear polymers, respectively (Zimm and Stockmayer 1949).

The value of g decreases rapidly as the number of branches increases with increasing dextran M. W. (Nordmeier 1993). The value of g for the dextrans can be deduced from the viscosity average M. W. (M_v), using the equation (Granath 1958):

$$g = 6.8\ M_v^{-0.19}$$

M_v can be derived from the weight average molecular weight (M_w) using the relationship (Garg and Stivala 1978):

$$M_v = 1.27\ M_w^{0.97}$$

The radius of gyration, R_g, has been experimentally determined for a series of dextrans (Nordmeier 1993), and the R_g values used in the present study were obtained from this source by interpolation. These R_g values (Table 1) were practically identical to those derived by Snabre et al. (1985).

Calculation of “critical overlap concentrations”, C*

For a polymer solution C* follows the relation:

$$C^* = 0.77/[\eta]$$

when $[\eta]$ is the intrinsic viscosity (Graessley 1980).

The present study includes data obtained experimentally by us and previously published data (see Table 1). Accordingly, for solutions prepared for this study $[\eta]$ was de-

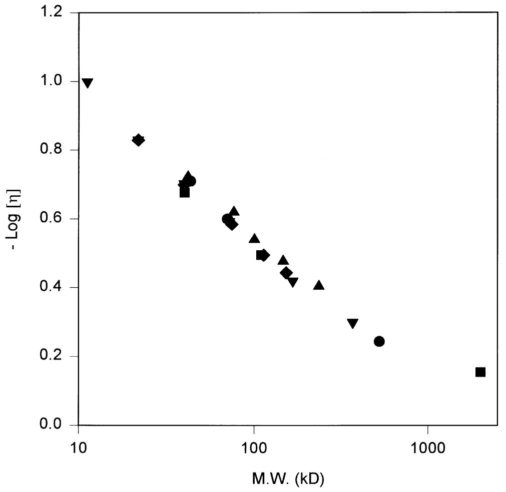

Fig. 2 Intrinsic viscosity of aqueous dextran solutions as a function of the polymer M. W. The values of [η] are experimental results obtained by us (●) or reported by: Kato et al. (1980) (◆), Stasiw et al. (1974) (■), Hawley and Dunn (1969) (▲) and Stankovic et al. (1991) (▼)

Table 1 C_a versus C* for dextrans

Polymer	Ca (g/dL)	C* (g/dL)
Dex-500	1.5[a]	1.3
	1.5[b]	1.1[c]
Dex-108	2.2[c]	2.6
Dex-70	3.0[a]	3.1
	3.0[b]	
	3.0[c]	

The values of C_a were either determined by us (a), or derived from Nash et al. 1987 (b) and from Maeda and Shiga 1985 (c). C* was calculated as described in Materials and methods

termined using an Ostwald capillary viscometer . For analysis of previously published data from the literature, [η] was interpolated from Fig. 2, which describes [η] as a function of dextran M. W.

Results

It has previously been shown, and further observed by us as shown in Fig. 3, that the RFV in dextran solution exhibits biphasic behavior: increasing with dextran concentration and reaching a peak at concentration C_a, beyond which it decreases.

Table 1 depicts the values of C_a, derived from Fig. 3 and from previous reports (Nash et al. 1987; Maeda and Shiga 1985), and C* for the corresponding dextrans. This table shows that C_a coincides with C*. This suggests that RFV increases with dextran concentration as long as the polymer is in a coil conformation. When the polymer changes to network structure it reduces the RFV.

Table 2 depicts the ratio, A, between the values of D, presented by Chien and Jan (1973) and R_f, calculated in the present study, for a series of dextrans with increasing M. W. As shown in this table, for all dextrans D is about 1.5 R_f or smaller. Assuming the bridging model for RF, the data of Table 2 may provide an explanation for the values of D as follows: According to de Gennes' model, the "bridging" between surfaces is formed by unimolecular layers of polymers, which are adsorbed to opposing surfaces and may extend "tails" to interact with the opposing surface (de Gennes 1981; Luckham and Klein 1990). As noted above this implies an overlap between the opposing polymer layers. When $D \geq 2 R_f$ there is no overlap and no cell-cell adhesion. When the two cell surfaces interact the polymer layers overlap, and $D < 2 R_f$. Table 2 shows that $D \leq 1.5 R_f$, implying considerable overlap of the polymer layer, for all dextrans. As noted in the Introduction, the objective of this study was to analyze the relation of RBC RF to dextran conformation (in the light of de Gennes' model for polymer solutions), and not to examine the two proposed models for RBC RF. Yet, it is agreed by all that dex-

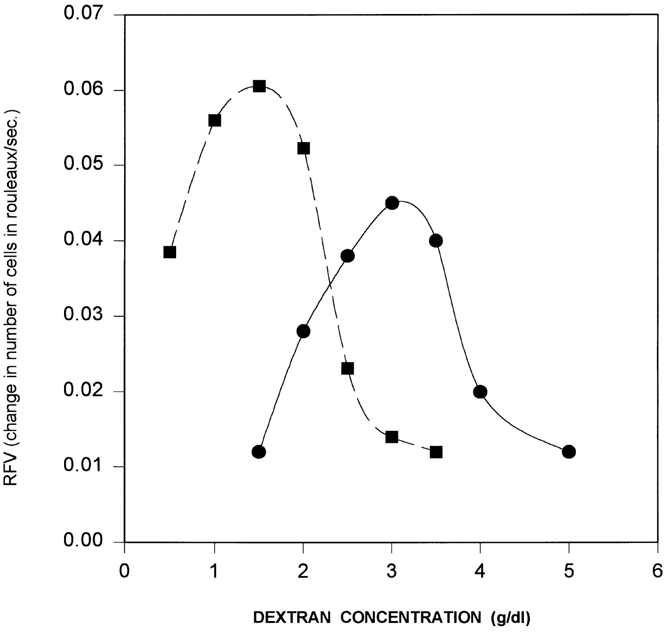

Fig. 3 RFV as function of the dextran concentration for Dex-70 (●) and Dex-500 (■): RFV was determined by monitoring RF process, from singly dispersed cells, during the first 15 s from cessation of flow, as described in Materials and methods

Table 2 Intercellular distance in rouleaux, and dextrans' molecular sizes for a series of dextrans of increasing M. W.

M_w (kDa)	D (nm)	R_g (nm)	R_f (nm)	D/R_f
20	"–"	4.7	6.9	"–"
42	19	5.8	13.2	1.4
74	22	7.4	14.9	1.5
140	25	9.8	17.6	1.4
450	30	16.2	23.6	1.3
2000	32	30.7	33.7	1.0

The values of the intercellular distance D were derived from the study of Chien and Jan (1973). The molecular sizes R_g and R_f were calculated as described in Materials and methods trans adsorb to the cell surface, and according to the "depletion" model the adsorbed dextrans repel the free dextran from the intercellular space. In this case the ratio D/R_f should be larger than 2, contrary to the findings reported here. Thus, the results presented here, are supportive of the "bridging" mechanism.

Discussion

The present study shows that for the dextrans examined here $C_a = C^*$, i.e. the inversion point in the RFV in dextran solution (the dextran concentration at which the RFV starts decreasing) occurs at the concentration at which the dextran conformation changes from coil to network. Accordingly, C_a decreases with increasing dextran M. W., as does C*. To understand the inversion in the dependence of RFV on [Dex] as the dextran changes to network configuration, we may consider the following: The studies of dextran adsorption described in the Introduction imply that the interaction between the dextran and the cell surface is stronger than the dextran-dextran interaction. To enable rouleaux formation, i.e. to bring the cells together, non-adsorbed, free dextran molecules have to be "pushed" away (depleted) from the intercellular space. The diffusion coefficient of free dextran molecules drops sharply at concentrations beyond C*, due to the change in its configuration (Luckham and Klein 1990). In addition, the viscosity of dextran solutions increases linearly with dextran concentration, up to C*, beyond which it increases parabolically (Furukawa et al. 1991). This implies that beyond C* it is harder (more time is required) to deplete the free dextran from the intercellular space, and RFV decreases accordingly. Since C* decreases with increasing dextran M. W., C_a decreases concomitantly (Nash et al 1987; Maeda and Shiga 1985).

Previous studies have presented disparate results as to the change of C_a with dextran M. W.: while some (Nash et al 1987; Maeda and Shiga 1985) showed that C_a decreases with increasing dextran M. W., others (Chien and Jan 1973; Chien et al. 1983) showed that RBC disaggregation starts at the same dextran concentration, independent of its M. W. However, these studies measured RBC aggregation at different stages and time scales. The former groups determined aggregation during the first seconds of the process (10–15 s), thus measuring the velocity of RF. The latter groups determined the aggregation after 1–2 min, thus looking at rouleaux networks or clusters. In accord with this, by monitoring the different stages of RBC aggregation by continuous visualization of the process in the CFA, we found that disaggregation of rouleaux networks and clusters starts at the same mass concentration, about 3%, for various dextrans, regardless of their M. W. It is plausible to conclude that the formation of rouleaux from single cells is dependent on the dextran M. W. (in addition to its concentration, of course), while formation of networks depends predominantly on the dextran mass concentration.

Acknowledgements This study was supported by a grant to S. Yedgar from the USA-Israel Binational Science Foundation (No. 91-00164), and from the Israel Ministry of Health (No. 2813).

References

Avarez FJ, Herraez A, Tejedor MC, Diez JC (1996) Behavior of isolated rat and human red blood cells upon hypotonic-dialysis encapsulation of carbonic anhydrase and dextran. Biotechnol Appl Biochem 23:173–179

Brooks DE (1973) The effect of neutral polymers on the electrokinetic potential of cells and other charged particles. III. Experimental studies on the dextran/erythrocyte system. J Colloid Interface Sci 43:701–713

Brooks DE, Evans E (1987) Rheology of blood cells. In: Chien S, Dormandi J, Ernst E (eds) Clinical hemorheology. Nijhoff, Amsterdam, pp 73–96

Chen S, Barshtein G, Gavish B, Machler Y, Yedgar S (1994) Monitoring of red blood cell aggregability in a flow-chamber by computerized image analysis. Clin Hemorheol 14:497–508

Chien S, Jan K-M (1973) Ultrastructural basis of the mechanism of rouleaux formation. Microvascular Res 5:155–166

Chien S, Simchon S, Abbot RE, Jan K-M (1977) Surface adsorption on human red cell membrane. J Colloid Interface Sci 62:461–470

Chien S, Sung LA, Simchon SS, Lee MM, Jan K-M, Skalak R (1983) Energy balance in red cell interactions. Ann NY Acad Sci 416:190–206

Cudd A, Arvinte T, Schulz B, Nicolau C (1989) Dextran protection of erythrocytes from low-pH-induced hemolysis. FEBS Letters 250:293–296

de Gennes PG (1979) Scaling concepts in polymer physics. Cornell Univ Press, Ithaca, NY

de Gennes PG (1981) Polymer solutions near an interface. 1. Adsorption and depletion layers. Macromolecules 14:1637–1644

de Gennes PG (1985) Model polymers at interfaces. In: Bongrand P (ed) Physical basis of cell-cell adhesion. CRC Press, Boca Raton, Florida, pp 39–60

Evans EA, Parsegian VA (1983) Energetics of membrane deformation and adhesion in cell and vesicle aggregation. Ann NY Acad Sci 416:13–33

Furukawa R, Arauz-Lara JL, Ware BR (1991) Self-diffusion and probe diffusion in dilute and semidilute aqueous solutions of dextran. Macromolecules 24:599–605

Garg KS, Stivala SS (1978) Assessment of branching in polymers from small-angle X-ray scattering (SAXS). J Polymer Sci: Polymer Physics Edn 16:1419–1434

Graessley WW (1980) Polymer-chain dimensions and the dependence of viscoelastic properties on concentration, molecular-weight and solvent power. Polymer 21:258–262

Granath KA (1958) Solution properties of branched dextrans. J Colloid Sci 13:308–328

Hawley SA, Dunn F (1969) Ultrasonic absorption in aqueous solutions of dextran. J Chem Phys 50:3523–3526

Kato S, Suzuki T, Nomura H, Miyahara Y (1980) Ultrasonic relaxation in aqueous solutions of dextran. Macromolecules 13:889–892

Kawaguchi M, Takahashi A (1983) Molecular weight dependence of the thickness of the polystyrene layer adsorbed onto a metal surface in good solvent conditions. Macromolecules 16:1465–1469

Luckham PF, Klein J (1990) Forces between mica surfaces bearing adsorbed homopolymers in good solvents: the effect of bridging and dangling tails. J Chem Soc Faraday Trans 86:1363–1368

Maeda N, Shiga T (1985) Inhibition and acceleration of erythrocyte aggregation induced by small macromolecules. Biochim Biophys Acta 843:128–136

Minetti M, Teichner A, Aducci P (1978) Interaction of neutral polysaccharides with human erythrocyte membrane: involvement of phospholipid bilayer. Biochem Biophys Res Comm 80:46–55

Nash GB, Wenby RB, Sowemino-Coker SO, Meiselman HJ (1987) Influence of cellular properties on red cell aggregation. Clinical Hemorheology 7:93–108

Nordmeier E (1993) Static and dynamic light-scattering solution behavior of pullulan and dextran in comparison. J Phys Chem 97:5770–5785

Snabre P, Grossman GH, Nills P (1985) Effects of dextran polydispersity on red blood cell aggregation. Colloid Polymer Sci 263:478–483

Stockmayer WH, Fixman M (1953) Dilute solutions of branched polymers. Ann NY Acad Sci 57:334–356

Stankovic RI, Jovanovic S, Ilic L, Nordmeier E, Lechner MD (1991) Study of aqueous dextran solutions under high pressures and different temperatures by dynamic light scattering. Polymer 32:235–239

Stasiw DM, Toth A, Cerny LC (1974) Dextran blends: theory and experiment. Biorheology 11:405–416

Zimm BH, Stockmayer WH (1949) The dimensions of chain molecules containing branches and rings. J Chem Phys 17:1301–1308