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Molecular Properties of a Galaptin and Its Activity in Normal and Leukemic Erythroid Tissue

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Recently, the generic term "galaptins" was proposed for the group of low molecular weight, acidic, β-galactoside-specific protein lectins that have been isolated from a wide variety of animal tissues and are thought to have a role in cell-cell recognition and adhesion. A molecule of this type, called erythroid developmental agglutinin (EDA), has been isolated from rabbit bone marow where it seems to mediate the intererythroblast adhesion seen in erythroblastic islands during erythropoiesis in vivo. Here, we show that after purification, EDA shows 95%–100% Coomassie blue staining as a single component on electrophoresis in native, urea, and SDS polyacrylamide gels and electrofocuses as a single band at pH 5.6. EDA has a subunit molecular weight of 13,000 in SDS gels and, unlike the majority of other galaptins, which are dimeric, native EDA is monomeric in solution. Another monomeric galaptin, chicken lactose lectin II, has been described recently, and it therefore seems that there may be two classes of galaptin distinguishable by their aggregation state in solution.

We have previously reported that EDA agglutinates rabbit erythroblasts in vitro and that this reaction is inhibited by β-galactoside-containing sugars and by anti-EDA Fab fragments suggesting that EDA bridges directly between cell surface glycoproteins. The insensitivity of this reaction to cooling, or to the disruption of cellular metabolism or the cytoskeleton demonstrated here further supports this hypothesis. EDA-mediated erythroblast agglutination was also shown to be independent of divalent cations.

Since galaptins are thought to be important in cohesion between normal cells, the possibility that EDA is not active in leukemic erythroid tissue was examined. The murine erythroleukemia cell line (MELC) provided an excellent system for this study since MELC are thought to be derived from an erythroid committed cell transformed at an early stage of development and can be induced by a number of chemical agents to differentiate terminally along the erythroid developmental pathway in culture. EDA of rabbit origin was found to agglutinate mouse erythroblasts in vitro and was used to investigate the response of MELC to EDA. It was found that the transformed cells were not readily agglutinated by EDA but on induction, and the concomitant loss of many of their transformed characteristics, MELC gained aggregation competence for EDA. The possible causes of these differences are discussed.

Key words: β-galactoside lectin, galaptin, erythropoiesis, murine erythroleukemia cells (MELC)

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Considerable interest is currently centered on the phenomena of cell-cell recognition and adhesion in higher animals, and a number of molecules that may be involved in these processes have now been isolated. Amongst these, a group of molecules with many properties in common has emerged, the 13,000–15,000 subunit molecular weight protein lectins, which can be extracted from the tissues of higher animals with lactose solutions and for which the collective term "galaptins" has been proposed [1]. Galaptins have been isolated from the electric organ of the electric eel [2], chick [3, 4] and chicken tissues [5], calf tissues [6], rat myoblasts [3], rat lung [7], human heart and skeletal muscle [8], and rabbit bone marrow and other tissues [9, and unpublished data FLH]. Galaptins from embryonic chick brain, muscle, and liver appear essentially identical by SDS-polyacrylamide gel electrophoresis, isoelectric focusing, and immunoassay [10], as do those isolated from calf liver, spleen, thymus, and heart [6], but a degree of species specificity has been observed [6, 8].

Although the functional significance of many galaptins remains uncertain as yet, it has been observed in several developmental systems that galaptin concentration is developmentally regulated and maximal at times of extensive tissue organization [3, 4, 7, 9] and galaptins have been identified at the surface of interacting cells [9, 11, 12]. These observations imply that galaptins might be involved in cell-cell recognition and adhesion processes.

Since it is well established that many tumor cells have severely altered adhesive properties, we were interested to compare the cell-cell adhesion mechanisms possessed by closely related normal and transformed cells. Erythropoietic tissue is an ideal subject for such investigation. During mammalian erythropoiesis, erythroblasts are clustered together around a macrophage nurse cell and investigation of these cellular interactions in rabbit bone marrow has strongly suggested that a developmentally regulated galaptin, termed erythroid developmental agglutinin (EDA), mediates the intererythroblast adhesion [9]. The murine erythroleukemia cell line (MELC), isolated by Charlotte Friend [13] has almost certainly been derived from an erythroid committed cell transformed at an early stage in erythroid development [14–17] and therefore represents a transformed counterpart of normal erythroid tissue. These transformed cells can be grown in continuous tissue culture but are particularly interesting because they can be induced by a number of chemical agents to terminally differentiate [18]. The activity of galaptins in normal and transformed cells has therefore been compared utilizing rabbit bone marrow tissue and the mouse erythroleukemia cell line (MELC).

MATERIALS AND METHODS

Cells

Erythroblasts were prepared from the bone marrow of anemic rabbits and mice on Percoll gradients [19], after washing the cells with lactose in MES (LMES: 0.3 M lactose in 0.15 M NaCl; 2 mM EDTA, pH 8.0; 1 ml/L β-mercaptoethanol).

Strain F4N murine erythroleukemia cells (MELC) were obtained from Dr. Ian Pragnell, Beatson Institute, Glasgow, and grown in suspension culture in a mixture of 50% Ham's F 12 and 50% modified Eagle's medium + 10% fetal calf serum + 50 IU/ml penicillin/streptomycin (Gibco) supplemented with 2 mM glutamine. MELC were induced to differentiate by the addition of 1.2% dimethyl sulfoxide (DMSO) to the culture medium. For agglutination experiments, MELC were grown

in standard medium for 24 h after three days induction, and both induced and noninduced cells were washed twice with LMES and resuspended in serum-free culture medium for agglutination assays. Approximately 40% of this cell population stained with benzidene, that is, were synthesizing hemoglobin. However, a higher proportion of the cells may be committed to erythropoietic differentiation.

Agglutination Assays

Cell suspensions, 0.25 ml of 10^7 cells/ml in either Hepes-buffered Basic Eagle's Medium with Hank's salts (erythroblasts) or serum-free culture medium (MELC), were mixed by end-over-end rotation (4 rpm) at room temperature in small plastic vials. Aliquots were taken throughout the incubation, diluted fivefold in 0.2% trypan blue in buffered medium (to check viability) and aggregation was assessed by counting the number of single cells present in a hemocytometer. The results presented represent the percent of single cells lost after 10 min incubation at various EDA concentrations.

The cellular compositions of the fractions used in the incubations were determined by cytocentrifugation and histological staining with benzidene-peroxide and Giemsa.

EDA

EDA was purified from anemic rabbit bone marrow as described previously [9]. The molecular weight of native EDA was determined using partially purified preparations from the gel filtration column.

Analytical Techniques

Polyacrylamide gel electrophoresis of purified EDA, 50 μg as determined by the Lowry method, was carried out in either a 10% native polyacrylamide disc gel prepared in 0.1 M phosphate buffer pH 7.1, a 12.5% gel containing 8 M urea or a 12.5% gel containing 0.1% SDS, loaded in MES or after preincubation with 0.08 M DTT and either 8 M urea or 0.1% SDS at 60°C for 30 min, respectively.

Velocity sedimentation of partially purified EDA was determined on 14-ml gradients of 5%–25% glycerol in 2× MES at 180×10^3 g for 63 h in the MSE 75 ultracentrifuge.

Gel filtration of partially purified EDA was carried out on a column (35 × 2.5 cm) of Sephadex G 75 equilibrated with MES.

EDA activity on the gradient and in the column eluate was detected by a standard hemagglutination assay [3]. Isoelectric focusing of purified EDA was performed in a 1 mm thick polyacrylamide gel (T = 5.5%, C ≐ 2.5%) at 2–4°C using pH range 4.0–6.5 Ampholines (Pharmacia) across 23 cm on an LKB 2117 Multiphor apparatus, at 1200 V, 15 mamp max for 4 h. pH gradient was determined after elution of the gel with freshly boiled distilled H_2O. Protein was detected by staining with fast green FCF [20].

RESULTS AND DISCUSSION

Molecular Properties of Galaptin Isolated From Rabbit Erythroid Tissue

EDA was purified from anemic rabbit bone marrow as previously described [9]. Briefly, the lectin is extracted from the cell surface with 0.3 M lactose and precipitated with ammonium sulphate. After purification by gel filtration on Sephadex

G-100, virtually homogeneous EDA is obtained by affinity chromatography on Sepharose 4B or asialofetuin derivatised Sepharose.

Purified EDA shows 95%–100% Coomassie blue staining as a single component after electrophoresis on native, urea, or SDS-containing polyacrylamide gels (Fig. 1) and electrofocuses as a single band at pH 5.6. In SDS polyacrylamide gels, EDA exhibits a subunit molecular weight of 13,000 by comparison with cytochrome c and lysozyme standards. Unlike other galaptins that have been detected as dimers and other multimers on gel filtration [6–8, 10], native EDA was detected only in the monomeric form in solution, with an approximate molecular weight of 13,000 estimated from its sedimentation velocity on glycerol gradients and elution profile from a Sephadex G 75 column in comparison with known molecular weight markers (Fig. 2). Recently, another monomeric galaptin has been isolated from chicken intestine [21]. This galaptin, termed chicken lactose lectin II, is clearly distinct from the other chick and chicken tissue lectin, termed chicken lactose lectin I, not only in its tendency to remain as a monomer in solution but also in its subunit molecular weight, isoelectric point, peptide map and immunological reactivity. Therefore, it seems that there may be two distinct types of galaptins that can be differentiated by their aggregation state in solution. The functional significance of this difference is not yet clear.

Activity of the Galaptin in Rabbit Erythroid Tissue

Rabbit erythroblasts are rapidly and specifically agglutinated by EDA in vitro at EDA concentrations ranging between 2 and 8 μg/ml [9] (Fig. 3). Previous studies have shown that this reaction is inhibited by low concentrations of β-galactoside-containing sugars and by anti-EDA Fab fragments [9], which suggests that EDA agglutinates erythroblasts by directly cross-linking cell surface glycoproteins. Here, the sensitivity of this agglutination activity to various agents that have been reported to

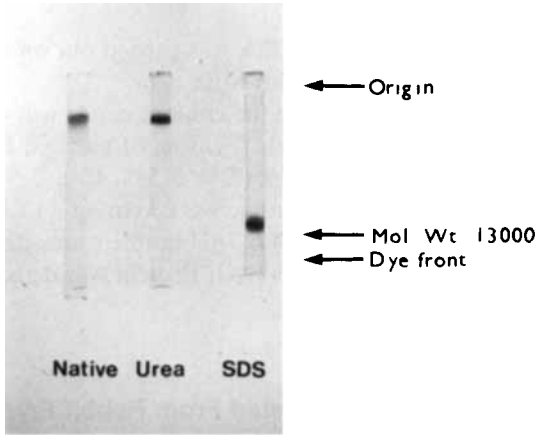
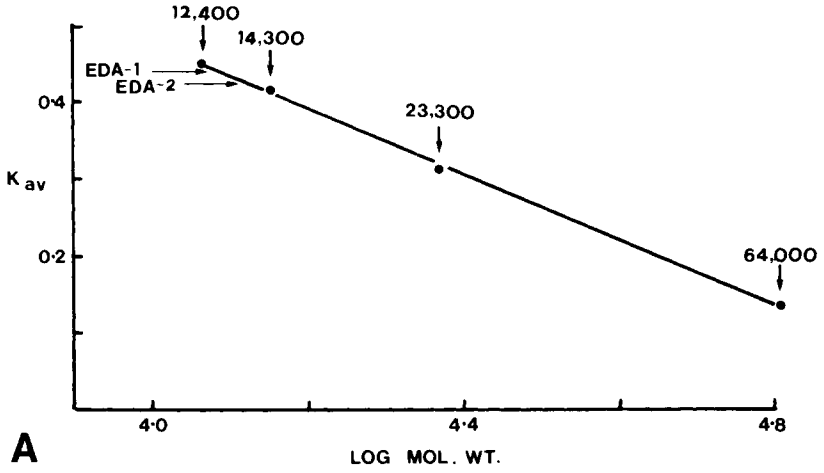


Fig 1. Purity of EDA as demonstrated by gel electrophoresis in polyacrylamide gels under nondenaturing condition and in the presence of urea or SDS.

disrupt aggregation in other cell types was also investigated. As shown in Table I, EDA-mediated erythroblast aggregation was unaffected by cooling to 8°C or by preincubating the cells with azide, indicating that cellular metabolism is not required for agglutination to be effected. Preincubation of the cells with neither colchicine nor cytochalasin B, which disrupt microtubule and microfilament structures, respectively, inhibited the agglutination, showing that these components of the cytoskele-



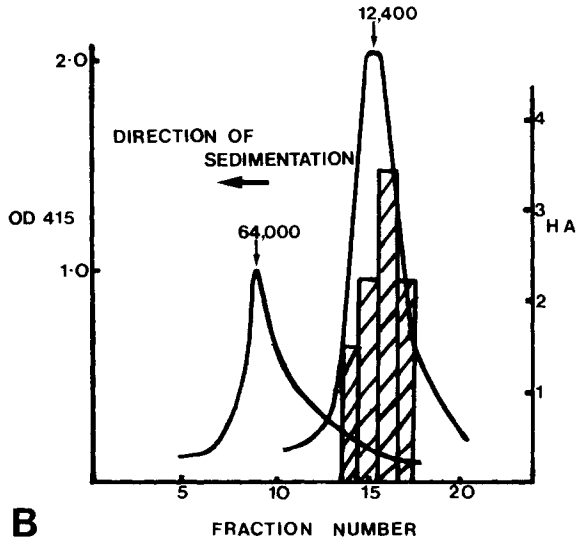


Fig 2. Determination of the molecular weight of native EDA: a) gel filtration on Sephadex G 75, b) velocity sedimentation. Histogram: hemagglutination activity (HA), serial dilution at last positive well. Molecular weight markers: cytochrome c 12,400; lysozme 14,300; trypsin, 23,300; hemoglobin 64,000.

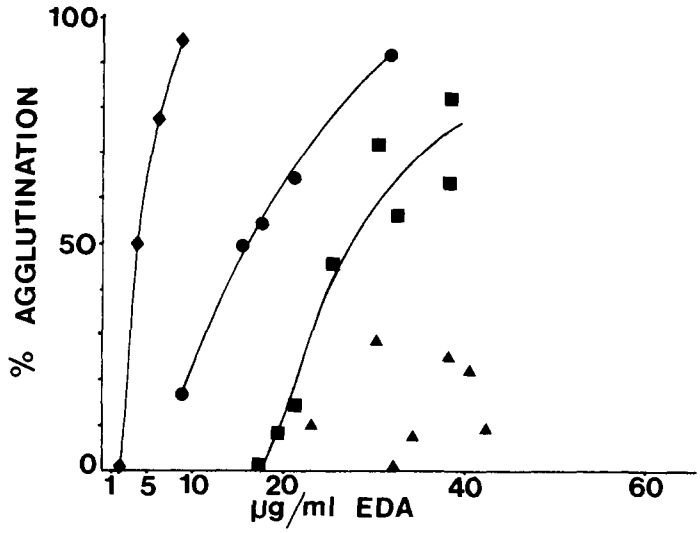


Fig 3. Cell agglutination mediated by EDA. Key: ◆ rabbit erythroblasts; ●, mouse erythroblasts; ■, 4-day-induced MELC; ▲, non-induced MELC.

TABLE I. The Sensitivity of EDA-Mediated Erythroblast Agglutination to Various Agents*

Incubation conditions	% Aggregation
Standard	78
Standard + 10 mM EDTA	75
Incubation in phosphate-buffered saline	65
Standard media at 8°C	74
Standard conditions with cells preincubated for 15 min in:	
standard media	78
standard media + 10 mM azide	77
standard media + 5×10^{-6} M colchicine	76
standard media + 20 μg/ml cytochalasin B	79

*The minimum concentration of EDA resulting in 70%–80% aggregation was used in these experiments (see Fig. 3). Each result represents the average of three incubations. The addition of colchicine or cytochalasin B caused an immediate clumping of the single cell suspension (16% and 20%, respectively); but in control incubations without the addition of EDA, no further agglutination was observed.

ton are not involved in regulating the cellular aggregation. These observations further support the thesis that EDA "bridges" directly between erythroblast cell surface EDA receptors.

Magnani and coworkers have recently reported that cell-cell adhesion in chick embryonic retina is mediated by two separate adhesive mechanisms, one Ca^{2+}-dependent and the other Ca^{2+}-independent [22]. Since a galaptin has been detected in this tissue [23], it was of interest to investigate the Ca^{2+}-dependence of EDA-mediated erythroblast agglutination. Equivalent agglutination was observed both in

standard medium (1.4 mM Ca^{2+}, 0.8 mM Mg^{2+}) and after the inclusion of 10 mM EDTA (to sequester divalent cations). Comparable agglutination was also observed in phosphate-buffered saline. EDA-mediated erythroblast aggregation is therefore clearly Ca^{2+}-independent, which is consistent with the well-established Ca^{2+}-independent HA activity of galaptins.

Activity of the Galaptin in Normal and Leukemic Murine Erythroid Tissue

Murine erythroblasts were found to be agglutinable by rabbit EDA in vitro (Fig. 3). However, a higher range of EDA concentrations is required for the aggregation of murine erythroblasts than for that of rabbit erythroblasts. Thus, EDA exhibits some species specificity in a functional assay, a finding that is compatible with the limited immunological cross-reactivity reported to exist between galaptins isolated from different species [6,8].

Since EDA of rabbit origin will cause the agglutination of murine erythroblasts, we were able to use this lectin in experiments on MELC. Agglutination tests showed that although the normal mouse erythroblasts are aggregated by 8–30 μg/ml EDA, little agglutination of the transformed cells is seen over the concentration range 20–40 μg/ml EDA (Fig. 3). Higher concentrations of EDA have been observed to cause more extensive agglutination of uninduced MELC, but there is variability between diferent batches of cells. The data shown in Figure 3 are the result of two separate comparisons of the agglutination of induced and uninduced MELC.

MELC are thought to be derived from an early proerythroblast cell [14–17] which, by analogy with rabbit erythropoiesis, we would expect to be sensitive to agglutination by EDA. These results suggest therefore that MELC are deficient in response to galaptin-mediated cell-cell adhesion in comparison with their untransformed counterparts. Indeed, after induction, which results in the loss of many of their transformed characteristics, MELC do become aggregation competent for EDA (Figure 3): 20–40 μg/ml will cause extensive agglutination. However, comparison of the agglutination curves shown in Figure 3 clearly shows that induced MELC remain less susceptible than normal murine erythroblasts to EDA-mediated aggregation. This may be due either to the increased cell size of MELC or to their in vitro growth in suspension culture.

Clearly, the differences between the aggregation competence of MELC before and after induction may result either from a loss of their transformed character on induction or from the resumption of their differentiation along the erythroid pathway. The former explanation is supported by the following observation. The agglutination of MELC by plant lectins has been reported to increase on induction [24], though the relevance of these observations to the specific aggregation mediated by an endogenous lectin as studied here is unclear. Nevertheless, further investigations are needed to clearly differentiate between these two possibilities.

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